Somatic Mutations In Philadelphia Chromosome-Negative Myeloproliferative Neoplasms

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Abstract

Myeloproliferative neoplasms (MPN) include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). MPN are characterized by clonal proliferation of myeloid progenitors leading to erythrocytosis, thrombocytosis and/or leukocytosis, and risk of hemorrhagic and thrombotic events, as well as myelofibrosis and blast transformation.

The discovery of somatic mutations in MPN, namely JAK2 V617F, JAK2 exon 12, MPL, and CALR mutations, has permitted a more specific approach to diagnosis and treatment.

The prevalence of JAK2 V617F mutations is higher than 95% in PV, 50-75% in ET and 40-75% in PMF. JAK2 exon 12 mutations are specific of PV. 20-30% of ET and PMF patients present a CALR mutation. The screening of mutations strengthens the diagnosis of MPN since 97% of MPN have at least one somatic mutation.

Interestingly, different mutations grant different phenotype and prognosis. Of particular importance, CALR mutations grant a favorable prognosis in ET and PMF, while ASXL1 mutations confer a poorer outcome. In fact, the use of CALR/ASXL1 status for the prognostication of patients has increased clinical value and was suggested for guidance of therapy in PMF.

The increasing importance of mutations in the management of MPN warrants a revision of current diagnostic criteria and prognostic models.

Resumo

As neoplasias mieloproliferativas (MPN) policitémia vera (PV), trombocitémia essencial (ET) e mielofibrose primária (PMF) são caracterizadas por proliferação clonal de progenitores mielóides, levando a eritrocitose, trombocitose e/ou leucocitose, risco de eventos hemorrágicos e trombóticos, mielofibrose e transformação blástica.

A descoberta de mutações somáticas, nomeadamente JAK2 V617F, JAK2 exão 12, MPL e CALR, tem permitido um diagnóstico e tratamento mais específico.

A prevalência da JAK2 V617F na PV ultrapassa os 95%, enquanto na ET é de 50-75% e na PMF de 40-75%. As mutações JAK2 exão 12 são específicas da PV. 20-30% dos doentes com ET ou PMF apresentam mutação na CALR. A identificação de mutações reforça o diagnóstico pois 97% das MPN apresentam pelo menos uma mutação.

É interessante notar que diferentes mutações conferem diferentes fenótipos e prognósticos. De particular relevo, as mutações da CALR conferem bom prognóstico na ET e na PMF, ao passo que mutações da ASXL1 atribuem piores desfechos. De facto, a utilização do estado da CALR/ASXL1 para a definição do prognóstico na PMF tem valor clínico e foi sugerida com ferramenta para guiar a terapêutica.

A importância crescente das mutações na gestão das MPN implica uma revisão dos atuais critérios de diagnóstico e modelos de prognóstico.

Myeloproliferative neoplasms: overview of the diseases

Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are diseases commonly referred as myeloproliferative neoplasms (MPN) and are included in the 2008 World Health Organization classification of myeloid neoplasms (Table 1).[1]

Table 1 The 2008 World Health Organization Classification of Myeloid Neoplasms

1. Myeloproliferative neoplasms (MPN)
- 1.1. Chronic myelogenous leukemia, *BCR-ABL1*-positive (CML)
- 1.2. Polycythemia vera (PV)
- 1.3. Essential thrombocythemia (ET)
- 1.4. Primary myelofibrosis (PMF)
- 1.5. Chronic neutrophilic leukemia (CNL)
- 1.6. Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS)
- 1.7. Mast cell disease (MCD)
- 1.8. MPN, unclassifiable

2. Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGRFB*, and *FGFR1*

3. MDS/MPN
- 3.1. Chronic myelomonocytic leukemia (CMML)
- 3.2. Juvenile myelomonocytic leukemia (JMML)
- 3.3. Atypical chronic myeloid leukemia, *BCR-ABL*-negative (aCML)
- 3.4. MDS/MPN, unclassifiable

4. Myelodysplastic syndromes (MDS)

5. Acute myeloid leukemia (AML)

Polycythemia vera is a condition characterized by a clonal proliferation of erythroid progenitors leading to erythrocytosis, often accompanied with leukocytosis, thrombocytosis, and bone marrow panmyeloid hyperplasia.[2-4] Patients may experience symptoms of fatigue, pruritus, night sweats, and bone pain and they can present splenomegaly.[4] Complications of this condition include thrombosis, hemorrhage, leukemic transformation and myelofibrosis.[4-6] Cumulative incidences for blast transformation and fibrotic progression are 6.8% and 21%, respectively.[7]

Thrombotic complication in PV can affect both the arterial and venous system, including the abdominal vessels (hepatic, portal, splenic, and mesenteric veins).[4]

Progenitor cells in PV are hypersensitive to erythropoietin (EPO) and other growth factors and are also able to form erythroid colonies in the absence of EPO.[3, 5, 8, 9]

Although PV is mostly an acquired condition, some patients report a familial prevalence of the disease, usually with an autosomal dominant inheritance pattern with incomplete penetrance.[10]

Median age of diagnosis is 60 years and the incidence of PV is nearly 2.3 per 100000 individuals.[2, 3] With the use of phlebotomy for treatment, survival is now longer than 10 years, with some studies reporting a median survival rate of 13.5 years.[2,7]

In essential thrombocythemia, there is a clonal proliferation of the megakaryocytic lineage resulting in an elevated platelet count and a higher risk of both thrombotic and hemorrhagic events. Although the molecular pathogenesis is not well understood, it is accepted that the affected cell lines exhibit a higher sensibility to proliferative and survival signals resulting in clonal cell expansion.[5]

The annual incidence of ET is 0.59-2.3 in 100000 individuals, with a prevalence of 30 in 100000 population.[11, 12] Age of diagnoses is typically between 20 to 40 years but ET can be found virtually in all ages.[11] Clinical presentation reflects the thrombotic and hemorrhagic profile of the disease. Occlusive events can be manifested by stroke, acute coronary syndrome, limb ischemia, deep vein thrombosis, pulmonary embolism, and microvascular events (e.g., erythromelalgia, digital arteries occlusions).[11] Neurologic manifestations, such as headaches, diplopia and blurred vision, might also be present.[11] Bleeding manifestations most commonly affect the skin and mucosa (ecchymoses, epistaxis, gum bleeding) but petechiae are never present.[11] Hemorrhagic symptoms are caused by an acquired Von Willebrand's disease when platelet counts reach values higher than 1000 $x10^9$/L.[11] Approximately 36% of the patients have no symptoms when the initial diagnosis is made.[11] Like in PV, ET patients may progress to myelofibrosis or undergo blast transformation, with cumulative incidences of 9.2% and 3.8%, respectively.[7] Median survival rate is estimated to be 19.8 years.[7]

Primary myelofibrosis is characterized by granulocytic, erythroid, and megakaryocytic monoclonal proliferation associated with reactive fibrosis of the bone marrow and extramedullary hematopoiesis.[13, 14] PMF has an incidence of 0.5-1.5 per 100000 individuals and a median age of presentation of 65 years, with a median survival of 5.9 years.[7-13] This condition is frequently diagnosed in asymptomatic patients but it can sometimes present with cachexia and marked splenomegaly.[13-14] Typically, laboratory results show anemia, altered white blood cell and platelet counts (either increased or decreased), and increased lactate dehydrogenase (LDH) levels.[14] Further work-up reveals myelophtisis of the blood (leukoerythroblasts and teardrop-shaped red cells), possibly due to the ectopic hematopoiesis.[13] Frequently, marrow aspiration is impossible ("dry-tap") and the biopsy shows fibrosis, dysplastic-megakaryocyte hyperplasia, osteosclerosis, angiogenesis, and intravascular hematopoiesis.[13,14] Complications include infection, bleeding, portal hypertension, splenic infarction, and leukemic-transformation, the latter with a cumulative incidence of 14.2%.[7, 13, 14]

Somatic mutations in myeloproliferative neoplasms

JAK2 V617F

Until 2005 no single genetic marker capable of explaining the pathogenesis of PV, ET, and PMF was yet identified. In MPN other than PV, ET, and PMF, tyrosine kinases are often involved in the pathogenesis and constitute important therapeutic targets.[15] One of these tyrosine kinases is the Janus kinase (JAK), which activates members of the signal transducer and activators of transcription (STAT) family.[5] It is therefore understandable why five independent investigation groups pursued the JAK2 gene as a potential candidate for a mutation capable of playing a key role in Philadelphia-negative MPN.

The sequencing of the JAK2 gene (derived from peripheral myeloid lineages of patients with PV, ET, and PMF) showed a mutation at nucleotide 1849 (G-T transversion) in exon 14 that changed a highly conserved valine for phenylalanine at position 617 of the protein (an autoinhibitory region - JH2).[5, 6, 8, 9, 15] The JAK2 V617F mutant was also detected in erythroid and granulocyte-macrophage colonies highlighting the fact that this mutation occurs in a multipotent progenitor.[6] JAK2 V617F is a somatic mutation since it is only detected in myeloid cells and not in other cell lines (T cells, peripheral blood mononuclear cells, or non-hematopoietic).[5, 6, 8]

The JAK2 V617F protein is constitutively active leading to hematopoietic cell proliferation and survival, causing a selection advantage to the mutant cells. Furthermore, it confers factor-independent growth and hypersensitivity to erythropoietin.[5, 15] When expressed in Ba/F3 cell lines the mutant protein is capable of inducing growth in the absence of growth factors at the same rate as non-mutated cells in the presence of growth factors.[5, 8]

The importance of the mutation in the pathogenesis of PV was confirmed in mice transplanted with bone marrow cells with JAK2 V617F. These subjects developed erythrocytosis as opposed to those transplanted with wild type JAK2.[8]

In polycythemia vera, most works report a prevalence of JAK2 V617F mutations higher than 95%, while in essential thrombocythemia the prevalence is usually between 50% and 75%, and in primary myelofibrosis between 40% and 75%.[5, 6, 8, 16-51]

The presence of a common mutation in these MPN emphasizes the idea of Dameshek (1951) that MPN are a spectrum of related diseases.[6, 52]

JAK2 exon 12

The detection of the JAK2 V617F mutation in MPN was an important advance on the understanding of their pathogenesis but the mechanisms of MPN in patients with a normal JAK2 gene persisted to be clarified. PV in JAK2 V617F-negative patients remained with no known genetic marker until Scott and co-workers (2007) identified four novel mutations in the exon 12 of the JAK2 gene, affecting amino acid residues between K537 and E543.[21] These mutation were not found in either ET or PMF nor were they detected in other cell lineages, emphasizing the somatic nature of exon 12 mutations. As with the V617F mutation, expression of JAK2 exon 12 mutants in Ba/F3/EpoR cell lines permitted them to proliferate with no need for an exogenous cytokine and was also associated with growth-factor hypersensitivity.[21] Accordingly, when cells with exon 12 mutations were transplanted in mice they developed an elevated hematocrit, reticulocytosis, leukocytosis, and thrombocytosis, which configures a phenotype compatible with a MPN.[21]

JAK2 exon 12 mutations appear to be specific of PV (albeit usually with a prevalence lower than 5%) as no study, to the extent of our knowledge, has found this somatic mutation in either ET or PMF.[21, 36, 37, 49] When investigated in JAK2 V617F negative patients, the prevalence of JAK2 exon 12 mutations rises to around 40%.[26, 53, 54]

The work of Scott *et al* was later confirmed by several other studies which in the same way showed the importance of JAK2 exon 12 screening in JAK2 V617F-negative PV patients.[54-57]

MPL W515L

Several patients with MPN have an identifiable JAK2 mutation, either V617F or exon 12, that explains their phenotype. However, contrary to PV, a significant proportion of ET and PMF patients are JAK2 V617F negative, which suggests that other mutations might be responsible for the occurrence of an MPN phenotype. Based on this premise and the previously discovery of the importance of type 1 cytokine receptor in the JAK2 V617F-mediated transformation of hematopoietic cells, Pikman et al (2006) sequenced the EPOR, MPL, and GCSFR genes of patients with JAK2 V617F-negative MPN and identified a tryptophan to leucine substitution at codon 515 of MPL in 9% (n=45) of the PMF patients.[58, 59] No patient with PV harbored the MPL W515L. This mutation was neither found in other cell types of PMF patients (confirming its somatic origin) nor in healthy individuals. UT7 and Ba/F3 cell lines with the MPL W515L mutation were found

to have a cytokine-independent growth and MPL W515L-transduced animals developed a lethal MPN, with marked thrombocytosis and leukocytosis.[59]

MPL mutations are found in less than 10% of ET or PMF patients but this prevalence rises slightly in JAK2 V617F negative patients.[16, 19, 26-31, 33, 35-38, 42, 43, 46-50, 55, 60- 64]

CALR

The discovery of MPL mutations in ET and PMF has brought an additional diagnostic tool but there is still approximately one third of patients who lack these known mutations, a feature reflecting the need for further genetic categorization of MPN patients.[59, 65] In 2013, two independent groups used an exome sequencing approach and were able to identify a novel somatic mutation in the CALR gene in ET and PMF.[66, 67]

Calreticulin is a protein implicated in several important cell functions such as the folding of glycoproteins, the modulation of calcium homeostasis, and the proliferation, apoptosis, and immunogenic cell death.[66] CALR mutations affect the exon 9 and consist of indels that result in a one base pair frameshift.[66, 67] The calreticulin mutant protein has an altered C-terminal resulting from the deletion of 27 amino acids and the insertion of a novel peptide with 36 amino acids.[66] Two mutations appear strikingly more common than all others: Type 1 (L367fs*46) and Type 2 (K385fs*47).[66,67] CALR mutant transfection into murine Ba/F3 cell lines confirmed a cytokine growth independence phenotype and hypersensitivity to IL-3, as observed with JAK2 and MPL mutations.[67]

Between 20% and 30% of ET patients present a CALR mutation.[27, 29, 35, 36, 42, 60, 68] This mutation is particularly important in JAK2 wild type patients as the proportion of patients with a mutated CALR rises to more than 50%.[42, 49, 64, 68] As for PMF, the prevalence of CALR mutations is higher than 20% and rises to more than 30% in JAK2 V617F negative patients.[19, 28, 35, 47-49, 60, 68]

Despite being present in some myelodysplastic syndromes (MDS), CALR mutations appear fairly specific of MPN since they are infrequent in MDS and are not found in lymphoid or solid cancers or other cell types.[66, 67] Moreover, CALR mutations are not present in healthy individuals.[66]

The finding of CALR mutations strengthens the molecular approach to the diagnosis of MPN since approximately 97% of MPN had a mutation in JAK2, MPL, or CALR.[66] In ET and PMF, 71% and 56% of the JAK2 and MPL negative patients had CALR mutations, respectively.[66]

Other less frequent somatic mutations

Some MPN patients do not present a known recurrent somatic mutation and their diagnosis is made exclusively according to clinical criteria. In ET, the frequency of triple-negative patients (negative for JAK2, CALR, and MPL mutations) is ranged between 14% and 32%.[19, 27-29, 37, 43, 64, 68] As for PMF, triple-negatives represent a prevalence of 10-35%.[19, 27, 28, 37, 47, 68]

Additionally, less frequent somatic mutations have been described in MPN patients, some of which appearing to have an important impact in patient management.

ASXL1 (additional sex combs like 1) mutations are present in several myeloid malignancies, including MDS and chronic myelomonocytic leukemia.[69] ASXL1 mutations in MPN were first described in 2009 and were detected not only in fully differentiated cells but also in CD34+ cells, suggesting an early occurrence in the disease evolution.[70] In PV and ET, the frequency of ASXL1 mutations is 7% and 8.4%, respectively, while in PMF it reaches 24.7%.[71, 72] ASXL1 mutation appear to have an important prognostic role in PMF as its occurrence is associated with poorer outcome.[73]

Mutations in the TET2 (ten-eleven translocation 2) gene were also found across all the classic MPN with a frequency of 12%.[74] TET2 mutations are frequently associated with the JAK2 V617F mutations and usually precede the latter in the disease evolution.[74] Apart for an association with older age, TET2 mutations do not appear to be related to any specific phenotype.[75]

Other genes known to be altered in some patients with MPN include LNK, IKZF1, EZH2, TP53, SRSF2, U2AF1, CBL, DNMT3A, IDH1, IDH2, NF1, SF3B1 and SUZ12.[71, 76] Although each of these mutations is not commonly associated with a specific phenotype or prognosis, the accumulation of 2 or more somatic mutations significantly reduces survival in MPN patients.[77]

Diagnostic impact of somatic mutations in MPN

Myeloproliferative neoplasms can be difficult to differentiate from reactive myelopoiesis based solely on clinical parameters. The discovery of acquired somatic mutations in MPN has permitted the diagnosis of patients that would otherwise be

excluded from older diagnostic criteria. The current diagnostic criteria in clinical use are those of the World Health Organization and are described in Table 2.[1]

The JAK2 V617F mutation has been found to be almost exclusive of MPN.[24, 78] In fact, in the general population, the JAK2 V617F mutation carries a higher risk of myeloproliferative neoplasm when compared to wild type JAK2, with a multifactorial adjusted hazard ratio of 97.12 (95% CI, 27.64-341.28).[79] When used in combination with clinical parameters, the presence of a JAK2 V617F mutation has a high diagnostic value for MPN.[79] In polycythemia vera in particular, JAK2 V617F detection was found to have a sensibility of 96% and a specificity of 100%.[17]

JAK2 analysis can also be useful for distinguishing different phenotypes of myeloproliferative neoplasms. JAK2 V617F allele burden has been shown to be highest among PV patients (72.66% in bone marrow specimens), and higher in PMF (59.04%) than in ET (24.95%).[80]

Besides JAK2 V617F, other acquired mutations have also added diagnostic accuracy in MPN. In patients with suspected PV but negative for the V617F mutation, seeking an exon 12 mutation of JAK2 gene might confirm the diagnosis.[26] Similarly, patients with an unclassifiable MPN presenting with thrombocytosis may be classified as having ET or PMF if an MPL W515 mutation is found.[26, 62]

Clinical phenotype with different somatic mutations

In polycythemia vera, patients with a JAK2 V617F mutation do not appear to have a distinct clinical phenotype nor appear to be demographically different than those with a non-mutated JAK2 gene, although some works show that patients with the mutation have a higher white blood cell count.[22, 40, 50] No difference in outcomes has been described between patients with and without the JAK2 V617F mutation.[22, 40]

Table 2 The 2008 World Health Organization Diagnostic Criteria for Polycythemia Vera, Essential Thrombocythemia, and Primary Myelofibrosis

		Polycythemia vera	Essential thrombocythemia	Primary myelofibrosis
Requirements		2 major criteria + 1 minor criterion or First major criterion + 2 minor criteria	4 major criteria	3 major criteria + 2 minor criteria
Major criteria	1	Hb > 18.5 g/dL (men) or 16.5 g/dL (women) or Hb > 17 g/dL (men) or 15 g/dL (women) if associated with a sustained increase of ≥ 2 g/dL from baseline that cannot be attributed to correction of iron deficiency or Hb or hematocrit greater than the 99th percentile of reference range for age, sex, or altitude of residence or Red cell mass > 25% above the mean normal predicted	Platelet count ≥ 450 x 10^9/L	Megakaryocyte proliferation and atypia* accompanied by either reticulin and/or collagen fibrosis or In the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased bone marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (i.e. prefibrotic PMF)
	2	Presence of JAK2 V617F or similar mutation	Megakaryocyte proliferation with large and mature morphology and no or little granulocyte or erythroid proliferation	Not meeting WHO criteria for CML, PV, MDS, or other myeloid neoplasm
	3		Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm	Demonstration of JAK2 V617F or other clonal marker or No evidence of reactive bone marrow fibrosis
	4		Demonstration of JAK2 V617F or other clonal marker or No evidence of reactive thrombocytosis	
Minor criteria	1	Bone marrow trilineage myeloproliferation		Leukoerythroblastosis
	2	Subnormal serum EPO level		Increased serum LDH
	3	EEC growth		Anemia
	4			Palpable splenomegaly

WHO, World Health Organization; PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; Hb, hemoglobin; CML, chronic myelogenous leukemia; MDS, myelodisplastic syndrome; EPO, erythropoietin; LDH, lactate dehydrogenase; EEC, endogenous erythroid colony

* Small to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering

In contrast, in essential thrombocythemia having a JAK2 V617F mutation confers a significantly different disease phenotype and prognosis. ET patients with the mutation tend to be older, have higher hemoglobin and hematocrit, lower platelet counts, and higher white blood cell counts, in particular neutrophil counts.[22, 38-40, 81, 82] It is interesting to denote that a V617F mutation grants a PV-like phenotype to ET patients. Moreover, the risk of transformation to PV is higher in patients with this mutation.[81] Although there is no difference in most clinical outcomes, some works have shown a higher prevalence of thrombosis in patients with the V617F mutation.[22, 38, 81, 82] No consensus exists on which territory of thrombosis, arterial or venous, is more affected in ET patients with the JAK2 V617F mutation, with some studies indicating a higher risk of arterial thrombosis and others showing an odds ratio of 4.9 for venous thrombosis in mutated patients.[38, 81] Major clinical outcomes, such as major hemorrhage, transformation to acute myeloid leukemia, or death, do not appear to be different in JAK2 V617F or wild type ET patients.[38-40, 81]

In primary myelofibrosis however, patients with a JAK2 V617F mutation do not appear to be clinically different from patients without this mutation, although Guglielmelli and co-workers (2009) found these patients to have higher white blood cell and platelet counts and higher hemoglobin than JAK2 wild type patients.[22, 50, 51] In addition, they found that the V617F mutation might provide a clinical benefit since it was associated with fewer anemic patients and a longer time until the development of anemia or leukopenia.[51]

Mutations in the MPL gene appear to be related to different phenotypes in both ET and PMF. MPL mutations seem to be associated with older age and higher rates of arterial events and microvessel disturbances in ET.[38] When compared to JAK2 V617F patients, ET patients with an MPL mutation have lower hemoglobin and more frequent microvessel disturbances.[38, 41] This mutation does not appear to grant a different prognosis from that of JAK2 V617F.[38, 41] No consensus exists on the difference in clinical phenotype between ET patients with an MPL mutation and those with a CALR mutation. While one work reported a lower thrombosis-free survival in MPL-mutated ET, another study has found no difference in the same outcome.[42, 43] These disparities difficult the clinical use of somatic mutations as prognostic markers in ET and thus large multi-centric prospective studies are urgently needed.

MPL mutations grant similar characteristics in PMF, with patients being older, having lower hemoglobin and higher need for transfusions.[83] When compared to those with a CALR mutation, MPL mutated PMF patients have a worse prognosis, presenting higher

cumulative incidence of both anemia and thrombocytopenia as well as a lower overall survival.[48]

Regarding the presence of CALR mutations, ET patients with an altered CALR gene are usually younger, have lower hemoglobin and hematocrit, lower white blood cell counts, and higher platelet counts than patients with a JAK2 mutation.[37, 42, 43, 49, 64, 84] CALR mutations in ET appear to grant a lower risk for thrombosis and a better overall survival than the V617F mutation.[42, 43, 49, 84] No clinical difference appears to exist in ET between the two most frequent CALR mutations – type 1 and type 2.[49]

In PMF, patients with CALR have lower white blood cell counts, mainly due to low neutrophil counts.[37, 84] No difference in age or gender appears to exist between patients with CALR and JAK2 mutations.[37, 49, 84] Similarly to ET, PMF patients with CALR mutations appear to have a better prognosis than those with JAK2 V617F mutation. CALR-positive patients have lower incidence of anemia, thrombocytopenia, marked leukocytosis and thrombosis.[48] Although PMF patients with either type 1 or type 2 CALR mutation are younger, have lower leukocyte counts, and have lower cumulative incidence of thrombosis than patients with a JAK2 V617F mutation, type 2 mutation is associated with lower hemoglobin while only type 1 is associated with more frequent anemia and a better overall survival.[47, 48]

ASXL1 mutations appear to have an important impact on the prognosis of PMF patients. ASXL1 is associated with poor survival in PMF patients in a DIPSS-plus independent fashion, in particular when in conjunction with a negative CALR mutational status (CALR- ASXL1+).[73, 85] Based on these premises, Tefferi and co-workers (2014) developed a molecular prognostic model of PMF patients according to CALR and ASXL1 mutational status.[86]

CALR+ ASXL1- patients were considered low risk and presented a median survival of 10.4 years, intermediate risk patients were CALR+ ASXL1+ or CALR- ASXL- and had a median survival of 5.8 years and patients with a CALR- ASXL1+ status were tiered into a high risk category (median survival of 2.3 years) (Table 3).[86] The molecular prognostic model was DIPSS-plus independent and showed an added value in identifying short- and long-

Table 3 Molecular prognostication in primary myelofibrosis

Risk Category	Mutational Status	Median Survival
Low	CALR+/ASXL1-	10.4 years
Intermediate	CALR+/ASXL1+ or CALR-/ASXL1-	5.8 years
High	CALR-/ASXL1+	2.3 years

term survivors in DIPSS-plus low and intermediate-1 categories and short-term survivors in intermediate-2 and high categories.[86] Conversely, DIPSS-plus stratification was capable of identifying different prognostic groups in molecular low and intermediate risk patients.[86]

The use of both DIPPS-plus and CALR/ASXL1 status for the prognostication of patients appears to have an increased clinical value over the use of a sole prognostic model and has been suggested for guidance of therapeutic approaches in PMF patients.[76]

The absence of recurrent mutations for JAK2, MPL, or CALR – triple negatives – in patients with ET or PMF appear to be associated with rather different characteristics according to the sub-type of MPN. While triple negative ET patients usually have a better phenotype, triple negative PMF patients tend to have a more aggressive disease.

Triple negatives ET patients have lower platelet counts, less frequent splenomegaly and a lower need of cytoreductive therapy than CALR patients.[42, 43] Despite the better phenotype, no difference appears to exist in the prognosis of these patients. Triple negative and CALR ET patients have similar rates of thrombosis, hemorrhage, myelofibrosis, leukemic transformation, and survival.[42, 43]

Inversely, in PMF patients, the absence of a recurrent somatic mutation seems to be associated with a worse clinical presentation and poorer prognosis. Triple negative patients have a higher risk of anemia and thrombocytopenia than patients with either JAK2 V617F or a CALR mutation.[47, 48] Moreover, a triple negative profile encompasses a higher incidence of leukemic transformation than JAK2 V617F and, when compared to patients with a CALR mutation, a lower age-adjusted overall survival.[48]

Conclusion

Since the introduction of the term myeloproliferative neoplasms by Dameshek in 1951, this group of hematologic malignancies has been considered as related diseases sharing similar pathophysiology.[52] The recognition of the Philadelphia chromosome and its molecular expression - BCR-Abl - has emphasized the role of chromosomal and molecular changes in the mechanisms of MPN and permitted a new categorization of the

individual entities: chronic myelogenous leukemia (BCR-Abl positive) and Philadelphia chromosome-negative MPN, including the classic PV, ET, and PMF.[87]

Despite the similarities among different MPN and the difficulty in distinguishing them from reactive disorders, the approach to a patient with a suspected MPN had been based solely in clinical parameters and laboratory and pathology data. The discovery of somatic mutation in Philadelphia chromosome-negative MPN, first the JAK2 V617F mutation, followed by the discovery of JAK2 exon 12 mutations and MPL and CALR mutations, has permitted a more specific approach both to the diagnosis and treatment of patients with MPN.[5, 6, 8, 9, 15, 21, 59, 66, 67] In fact, the introduction of somatic mutations in the diagnostic criteria of PV, ET, and PMF reflects the clinical importance of these scientific breakthroughs.

Molecular prognostication of patients is now possible and is capable of distinguishing which patients might benefit from more aggressive therapies. ET patients with a JAK2 V617F mutation are at more risk of a thrombotic event and PMF patients with an MPL mutation have worse survival.[22, 38, 48, 81, 82, 88, 89] Probably the most important somatic mutations in defining patient prognosis, CALR mutations are associated with a better survival in both ET and PMF.[48, 49] Moreover, when used in conjunction with ASXL1, testing for a CALR mutation in a PMF patients has permitted risk stratification beyond that of the DIPSS-plus scoring system.[86]

With the exception of ASXL1, somatic mutations other than the frequent JAK2, MPL, or CALR mutations, have not yet held significant impact on the management of patients with MPN and thus more studies are warrant to identify subsets of patients that might need specific treatment and follow-up approaches.[71, 77] Moreover, the increasing importance of somatic mutations in the management of MPN patients may justify revising diagnostic criteria and prognostic models.

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