UNIVERSIDADE D COIMBRA FACULDADE DE MEDICINA

MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

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Genetic Landscape of Primary Myelofibrosis and its role in pathogenesis and prognosis

REVIEW ARTICLE

ÁREA CIENTÍFICA DE HEMATOLOGIA

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ABRIL/2019

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ABSTRACT

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) characterized by stem cell-derived clonal myeloproliferation associated, often but not always, with driver mutations, abnormal cytokine expression, bone marrow fibrosis, anemia, extramedullary hematopoiesis (EMH), splenomegaly, constitutional symptoms, cachexia, leukemic progression and short survival. Somatic mutations in three driver genes, namely *JAK2*, *CALR* and *MPL*, represent major diagnostic criteria in addition to hematologic and morphological abnormalities. The MPN-restricted driver mutations abnormally activate the JAK-STAT signaling pathway. *JAK2*^{V617F} is the most frequent mutation found in PMF and is activated by the three main myeloid cytokine receptors (erythropoietin receptor, granulocyte colony-stimulating factor and MPL) whereas *CALR* and *MPL* mutants are restricted to MPL activation.

The others mutations, the nondriver mutations, affect mainly epigenetic regulator, splicing and signaling genes. They cooperate with the three drivers and play an important role in disease progression and leukemic transformation.

This document updates the molecular basis of PMF pathogeny, its impact in prognosis and the therapeutic approach based on the new prognostic models. The recently published prognostic models include clinical, unfavorable karyotype and nondriver mutations, which were identified to have prognostic impact, independent of conventional prognostic factors. *CALR* mutations appear to be a favorable prognostic factor although mutations in *ASXL1*, *SRSF2*, *EZH2*, *IDH1/2* and *U2AF1* seem to have impact on survival in PMF patients.

Keywords: Primary myelofibrosis, driver mutations, nondriver mutations, pathogenesis prognosis

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LIST OF ABBREVIATIONS

AlloSCT	Allogeneic hematopoietic stem cell transplantation
AML	Acute Myeloid Leukemia
ASXL1	Addition of sex combs like 1
Bp	Base pair
CALR	Calreticulin
CBL	Casitas B-lineage lymphoma
CMML	Chronic myelomonocytic leukemia
DIPSS	Dynamic International Prognostic Scoring System
DNMT3A	DNA methyltransferase 3A
EMH	Extramedullary hematopoiesis
EPO	Erythropoietin
ER	Endoplasmic reticulum
ET	Essential thrombocythemia
EZH2	Enhancer of Zeste Homologue 2
FLT3	FMS-like tyrosine kinase-3
GIPSS	Genetic only-based prognostic system
HMR	High molecular risk
HSC	Hematopoietic Stem Cell
IDH	Isocitrate Dehydrogenase
IL	Interleukin
IPSS	International Prognostic Scoring System
G-CSF	Granulocyte colony stimulating factor
JAK	Janus kinase
JH1	Janus kinase homology 1
JH2	Janus kinase homology 2
KG	Ketoglutarate
MAPK	Mitogen-activated protein kinase
MDS	Myelodysplastic syndrome
MIPSS70	Mutation-enhanced international prognostic scoring system for transplant-age patients

MPL	Myeloproliferative leukemia virus
MPN	Myeloproliferative Neoplasms
NGS	Next-generation sequencing
PRC	Polycomb repressive complex
PRE-PMF	Prefibrotic/early stage
PMF	Primary Myelofibrosis
PV	Polycythemia vera
RCB	Red Blood Cell
SF3B1	Splicing factor 3B subunit 1
SRSF2	Serine/arginine-rich splicing factor 2
STAT	Signal transducer and activator of transcription
TET	Encodes ten-eleven translocation
TPO	Thrombopoietin
U2AF1	U2 Small Nuclear RNA Auxiliary Factor 1
VAF	Variant allele frequency
VHR	Very high risk
WHO	World Health Organization
WT	Wild-type

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INTRODUCTION

PRIMARY MYELOFIBROSIS

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) with an incidence estimated from 0,1 to 1 per 100 000 persons per year in the European Union and generally occurs in advanced-age adults, with a median age of 70 years(1).

The last revision of World Health Organization (WHO) classification of MPNs in 2016 divided the MPN in 7 groups: chronic myeloid leukemia, BCR-ABL1^{+}; chronic neutrophilic leukemia; Polycythemia vera (PV); Primary myelofibrosis (PMF), prefibrotic/early stage (pre-PMF) and overt fibrotic stage; essential thrombocythemia (ET); chronic eosinophilic leukemia, not otherwise specified ; and MPN, unclassifiable.(2) PV, ET and PMF are classically grouped in BCR-ABL^{-} /Philadelphia-negative MPNs – their clinical and morphological features are summarized in table 1. Boundaries between those three disorders are difficult to delimit and in many cases a continuum can be observed by the progression of ET and PV to secondary myelofibrosis.

PMF is a clonal disorder arising from the neoplastic transformation of early hematopoietic stem cells (HSC) that is associated with abnormal cytokine expression, atypical megakaryocytic proliferation and a histology of bone marrow that displays a gradual reactive fibrosis, osteosclerosis and neoangiogenesis which result in prominent extramedullary hematopoiesis (EMH). Clinical manifestations are heterogeneous, varying from asymptomatic in one fourth of patients, to hepatosplenomegaly, constitutional symptoms, manifestations of portal hypertension, bone pain and, more rarely, microvascular symptoms, thrombohemorrhagic complications, pruritus and non-hepatosplenic EMH that might lead to cord compression, ascites, pleural effusion or pulmonary hypertension.(3)

Laboratorial analysis usually shows progressive anemia, leukoerythroblastosis, leukocytosis, and thrombocytosis, though leukopenia and thrombocytopenia may also occur.(4)

Diagnosis is based in 2016 WHO criteria that are reported in table 2.

PMF is a potentially aggressive disease with shortened overall survival and causes of death include leukemic progression in around 20% of patients, cardiovascular comorbidities and consequences of cytopenias like infection and bleeding.(5)

Table 1. BCR-ABL$^-$ /Philadelphia-negative MPNs: clinical and morphological features

MPN	CLINICAL AND MORPHOLOGICAL FEATURES
PV	Erythrocytosis frequently combined with thrombocytosis and/or leukocytosis (that is, polycythemia) and typically associated with suppressed endogenous erythropoietin production. Bone marrow hypercellularity for age with trilineage growth (that is, panmyelosis)
ET	Thrombocytosis. Normocellular bone marrow with proliferation of enlarged megakaryocytes
PMF	**Prefibrotic PMF** • Various abnormalities of peripheral blood •Granulocytic and megakaryocytic proliferation in the bone marrow with lack of reticulin fibrosis **Overt PMF** • Various abnormalities of peripheral blood. Bone marrow megakaryocytic proliferation with atypia, accompanied by either reticulin and/or collagen fibrosis grades 2/3. Abnormal stem cell trafficking with myeloid metaplasia (extramedullary hematopoiesis in the liver and/ or the spleen

Table adapted from Rumi, E. & Cazzola, M. (2016)(6)

PREFIBROTIC/ EARLY PMF

Prefibrotic PMF or early PMF is a prodromal phase of PMF which was included in the 2008 WHO classification of MPN. However, the detailed criteria only emerged in the last revision of the WHO classification in 2016 - reported in table 2.

It is very important to be able to classify an early PMF because of their close similarities with ET, mainly in the clinical presentation with marked thrombocytosis(7), since prePMF has a high probability of progression to overt PMF and has worse prognosis than “true” ET. They are distinguished by, among other features, the morphologic findings in the bone marrow biopsy, including the lack of reticulin fibrosis at onset.

The patients with thrombocytosis and a histology of bone marrow suggestive of prePMF but who do not have a minor criterion listed in table 2, should be provisionally classified with MPN, unclassifiable.(2)

Table 2. 2016 WHO diagnostic criteria for prefibrotic PMF and overt PMF

	Major criteria	*Minor criteria*	*Diagnosis requires*
Prefibrotic PMF	1. Megakaryocytic proliferation and atypia, without reticulin fibrosis>grade 1, accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis 2. Not meeting WHO criteria for BCR-ABL1$^+$ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms 3. Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker [a], or absence of minor reactive myelofibrosis [b]	Presence of at least 1 of the following, confirmed in 2 consecutive determinations: • Anemia not attributed to a comorbid condition • Leukocytosis ≥ 11 x10^9/L • Palpable splenomegaly • Lactate dehydrogenase level increased to above upper normal limit of institutional reference range	All 3 major criteria and at least 1 minor criterion.
Overt PMF	1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3 2. Not meeting WHO criteria for BCR-ABL1$^+$ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms 3. Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker [a], or absence of minor reactive myelofibrosis [b]	Presence of at least 1 of the following, confirmed in 2 consecutive determinations: • Anemia not attributed to a comorbid condition • Leukocytosis ≥ 11 x10^9/L • Palpable splenomegaly • Lactate dehydrogenase level increased to above upper normal limit of institutional reference range • Leukoerythroblastosis	All 3 major criteria, and at least 1 minor criterion.

Table adapted from Barbui et al. (2018)(2) and Rumi, E. & Cazzola, M. (2016)(6)
[a] In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (eg, ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, SF3B1) are of help in determining the clonal nature of the disease.
[b] Bone marrow fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic myelopathies.

GENETIC LANDSCAPE OF PMF

The genomic landscape of PMF is more complex than initially thought. High resolution genome analysis using microarray and next-generation sequencing (NGS) resulted in the discovery of several gene mutations, that are usually classify into those that are “driver mutations” and those that are “nondriver” mutations. Table 3 summarized the genetical landscape of PMF. The current driver mutations recognized in PMF are in Janus kinase 2 (*JAK2*), calreticulin (*CALR*), and myeloproliferative leukemia virus (*MPL*) genes. They confirm the clonal nature of MPNs and are supportive but not essential for diagnosis. Additionally, none of them can be used to distinguish among the various MPNs. Beyond their role in pathogenesis, driver mutations have distinct phenotypic, therapeutic, and prognostic implications in PMF.(4)

Around 90% of patients carry a driver mutation, (mutations in *JAK2* are by far the most frequent, followed by *CARL* and *MPL* mutations respectively). The patients who lack mutations in *JAK2*, *MPL*, or *CALR*, estimated in around 10%, are classified as “triple negative”.(8)

The other mutations, the nondriver mutations, affect genes involved in epigenetic regulation, splicing, and signaling. None of these mutations is restricted to MPNs and they are even more frequent in myelodysplastic syndromes (MDS) and in acute myeloid leukemia (AML), thus explaining some of the myelodysplastic features of PMF. They cooperate with the 3 MPNs drivers and play a key role in initiation, progression and prognosis of PMF.(9) Screening for them is not necessary for diagnosis but is a complement, mainly in triple-negative patients.

The knowledge of these mutations is very important to characterize the disease and infer about pathogenesis, phenotype, prognostic and possible target therapy.

All the mutations that will be discussed are not necessarily present in the same tumor subclones. Analyses of hematopoietic colonies in a cohort of 200 MPN patients, in order to evaluate clonal heterogeneity and clonal evolution, have revealed that tumors are often composed of multiple subclones that coexist and remain stable over long periods of time. However, the order of mutations that are acquired in some genes seems to have important clinic impact but until now just few are known and the role of the majority of genes is still unclear.(10)

Table 3. Genetical Landscape of PMF

GENE	PROTEIN FUNCTION	FREQUENCY	CONSEQUENCES
DRIVERS			
JAK2	Tyrosine kinase associated with cytokine receptors	60%	Increased RBC, platelet and granulocyte production
CALR	Mutant: activator of MPL	25-30%	Increased platelet production
MPL	TPOR	5%	Increased platelet production
NONDRIVERS			
EPIGENETIC REGULATORS			
TET2	a-Ketoglutarate–dependent dioxygenase Oxidation of 5mC into 5hmC and active 5mC demethylation	10-20%	Initiation Mutations on 2 alleles associated with progression
DNMT3A	DNA methylase, de novo methylation	5-10%	Initiation
IDH1/IDH2	Neomorphic enzyme, generation of 2-hydroxyglutarate blocking a-ketoglutarate–dependent enzymes		Initiation and progression
ASXL1	Chromatin-binding protein associated with PRC1 and 2	5-10%	Initiation Disease progression
EZH2	H3K27 methyltransferase, loss of function	25%	Initiation Disease progression
SPLICING GENES			
SRSF2S	Serine/arginine-rich pre- RNA splicing factor	20%	Progression
SF3B1	RNA-splicing factor 3b subunit 1, part of U2	rare	Phenotypic change (anemia)
U2AF1	U2 small nuclear RNA-splicing factor	10-15%	Phenotypic change (anemia and thrombocytopenia)
SIGNALING GENES			
LNK	Negative regulator of JAK2	2%	Synergy with JAK2V617FDisease progression
CBL	Cytokine receptor internalization	4%	Disease progression (progression to AML)
NRAS	ERK/MAPK signaling	Rare	Progression to leukemia (5%-10% in secondary AML)
NF1	ERK/MAPK signaling	Rare	Progression to leukemia (5%-10% in secondary AML)
FLT3	Cytokine receptor (FLT3-L)	MPN (<3%)	Progression to leukemia (10%-15% in secondary AML)

Table adapted from Vainchenker W. et al(9)

DRIVER MUTATION CONVERGE ON JAK-STAT SIGNALING

The formation of blood lineages is the result of intricately regulated signaling pathways mediated by cytokines and their receptors. Hematopoietic cytokine receptor signaling is largely mediated by a family of tyrosine kinases named JAKs and their downstream transcription factors, termed STATs (signal transducers and activators of transcription).(11)

The JAK-STAT pathway is a cascade used to transduce a multitude of signals for development and homeostasis. It is the main signaling mechanism for a wide variety of cytokines and growth factors, such as erythropoietin (EPO), thrombopoietin (TPO), granulocyte colony stimulating factor (G-CSF), and interleukin (IL)-3 and IL-5, as well as interferons.(12) JAKs are constitutively present in the close proximity of the cytosolic domain of receptors and their activation causes differentiation, proliferation, migration and apoptosis through binding, phosphorylation, and nuclear translocation of downstream STAT transcription factors as well as activation of mitogen-activated protein kinase (MAPK) and phosphoinositide 3 kinase/protein kinase B (PI3K-AKT) signaling pathways. (13)

In myeloid cells, the JAK2-STAT5 signaling pathway is a critical downstream effector of EPO signaling. TPO signaling via its receptor MPL also uses JAK2. However, instead STAT5, activation of the MAP kinase pathway and STAT3 has been shown to be important for megakaryocytic differentiation.(14,15) Granulocytic differentiation, via G-CSF and its receptor G-CSFR, occurs predominantly through JAK1, and to a lesser degree via JAK2.(16)

Several studies confirmed that mutations in *CALR* drives oncogenic transformation via MPL-dependent cytokine-independent constitutive activation of JAK/STAT signaling, which drives enhanced megakaryopoiesis and platelet formation.(17)

There are also additional targets within the JAK-STAT pathway that can also be infrequently affected by somatic mutations in PMF. Genes like *LNK* and *CBL* encode messenger proteins and loss-of-function mutations culminate in increased JAK-STAT signaling.(18)

Like it was described above, all driver genes are, in some way, associated to the JAK-STAT pathway and thus oncogenic lesions in these genes, that lead to a constitutive activation of this signaling pathway, result in deregulated myeloid cell proliferation, a phenomenon central to PMF and all MPNs pathogenesis.

JAK2

JAK2 gene, located in chromosome 9p24, is ubiquitously expressed and encodes the JAK2 protein which belongs to a family of nonreceptor tyrosine kinase, constituted by four Janus kinases: JAK1, JAK2, JAK3 and tyrosine kinase 2. JAK2 plays a very important role as a critical mediator for effective erythropoiesis, megakaryopoiesis, and, to a lesser extent, granulopoiesis, once JAK2 is activated by a number of cytokine receptors, including MPL (the thrombopoietin receptor), the EPO receptor and G-CSF receptor. The vast majority of

chromosomal translocations of the *JAK2* gene lead to leukemias or lymphomas and activating point mutations, deletions or insertions in this gene lead to MPNs.(19)

JAK2 mutations involved in hematopoietic malignancies are located in or around the JAK homology 2 (JH2) domain of the protein, also known as the pseudokinase domain, which normal functions are to inhibit the JH1 kinase domain, in the absence of cytokines and is required for JAK2 activation in response to cytokines. Thus, loss of function mutations or deletions in JH2 domains result in a constitutive activation of the kinase domain JH2 and consequently an increased activation of downstream effectors and diminished cytokine dependency and response.(20)

JAK2^{V617F} is a loss-of-function mutation and consists in a guanine-to-thymine somatic mutation in JH2 domain that result in a substitution of valine to phenylalanine at codon 617 (V617F) within the pseudokinase domain, resulting in the constitutive activation of JAK2, conferring a proliferative advantage on these cells.(20)

The *JAK2*^{V617F} is the most frequent somatic mutation in BCR/ABL^{-} MPNs, being found in 65% of PMF patients, 96% and 55% in PV and ET, respectively.(21) It has also been detected in a small frequency (less than 1%) in normal population, being associated to the clonal hematopoiesis associated with aging.(20) *JAK2*^{V617F} appears in all myeloid lineages and can be also found in lymphoid cells, suggesting that *JAK2*^{V617F} occurs in multipotent hematopoietic progenitor cells, although the phenotype of MPN is related to a selective proliferative advantage of the myeloid lineages.(22)

The question of how a single mutation can origin diseases with different phenotypes could be in part explained by the specific patterns of activation of different receptors in different patients. Even though upregulation in JAK-STAT signaling has been demonstrated in all MPNs, differential STAT signaling corresponds to specific MPN phenotypes, however, it remains unclear the way that which STATs are differentially activated. (23) Using a mouse genetic strategy it was proven that STAT5 plays an essential role in PV development.(24) Otherwise, in clonal analyses were shown that the balance between STAT1 and STAT5 activation determines the phenotype expressed – increased STAT1 and decreased STAT5 activation produces an ET-like phenotype although increased STAT5 and decreased STAT1 activation produces an PV-like phenotype. (25) Immunostaining of bone marrow biopsies from MPNs patients mutated with *JAK2*^{V617F} also shown that there is an increased STAT3 and STAT5 activation in PV; increased STAT3 and reduced STAT5 activation in ET; and uniformly reduced STAT3 and STAT5 activation in PMF.(26)

Another variable in *JAK2*^{V617F}, that has phenotypic impact, is the variant allele frequency (VAF). VAF is usually low in ET, is higher in PV and almost 100% in myelofibrotic transformation. A study found a significant proportion of patients with *JAK2*^{V617F} mutated in ET progressing to PV, while none of *CALR* mutated ET progressed. Also, some patients with PV have history of

isolated thrombocythemia, which could be a masked ET. The proportion of homozygosity is a key factor in determining the degree of erythrocytosis, white cells count, and marked splenomegaly. Thus, the study suggests that “ET, PV, and myelofibrosis most likely represent different phenotypes in the evolution of *JAK2*^{V617F}-mutated MPNs”.(27) The factors that influence the degree of expansion of homozygous *JAK2*^{V617F} clones are not fully understood; loss of heterozygosity by acquired uniparental disomy of the short arm of chromosome 9 (9p) seems to be the main cause, but the order in which *JAK2*^{V617F} is acquired relative to other somatic mutations has been shown to be important. (28) Whereas evidence of higher *JAK2*^{V617F} allele burden in PV than ET have been reported in a few studies, data on allelic burden in PMF is variable and remains to be validated.

On the other hand, individual factors, such as iron status, age, gender, renal function/Epo levels and timing of presentation, may all influence if a patient harboring *JAK2*^{V617F} presents more erythrocytosis and/or thrombocytosis.(29)

JAK2^{V617F} also has been described to have effects in the nucleus. It phosphorylates histone H3 and also protein arginine methyltransferase 5 causing reduced histones methylation and altered target gene expression.(30) How this dysregulation of histones influences the phenotype or is associated with acquisition of additional somatic mutations with phenotypic impact remains unclear.

JAK2 exon 12 mutations have also been found in MPNs but they are not usually associated with PMF despite being associated to the progress to secondary MF.

CALR

The *CALR* gene, located on chromosome 19p13.2, encodes calreticulin, a multifunctional calcio binding protein chaperone located primarily in the endoplasmic reticulum (ER). CALR participates within ER in quality control of protein folding and in Ca^{2+} storage and release, and outside of the ER participates in events such as cell proliferation, calcium homeostasis, cell adhesion/migration, antigen processing and presentation for the adaptive immune response and immunogenic cell death.(17)

Recent data suggest that wild-type (WT) *CALR* plays a role on hematopoiesis, in megakaryocytic and erythrocytic differentiation and HSC self-renewal, (31) which may explain the pathogenesis of *CALR* mutations in MPNs.

Until now, more than 50 *CALR* mutations have been reported in MNPs. Mutations in exon 9 of this gene occur in approximately 25-30% of patients with ET and PMF and are not found in patients with PV.(32,33) In PMF, 52-base pair (bp) deletion (or type 1 mutation) and 5-bp insertion (or type 2 mutation) are the most prevalent mutations, and have been found in 70% and 15% of CALR mutations, respectively. Moreover, alternative insertion or deletions or a

combination of both are also found. These indels lead to a novel C-terminus, in which the negatively charged amino acids, required for calcium binding, are variably replaced by neutral and positively charged amino acids. Thus, *CALR* mutations are classified as type 1-like or type 2-like according to the structural changes: type 1-like mutation lost most of the WT exon 9 sequence and calcium-binding sites; type 2-like mutation is closer to the WT sequence and kept around 50% of negative charges.(32) Both types have been shown in animal observation that induce an ET-phenotype, marked by megakaryocyte hyperplasia and expansion of HSC, (34–37) however red blood cells and white blood cells counts did not increased significantly,(36) suggesting that *CALR* mutations possess megakaryocyte lineage-specific oncogenic property and confers a selective lineage specific growth advantage.(17) Moreover, several studies have demonstrated the necessity of the TPO receptor MPL for *CALR*-mediated cellular transformation. The novel C-terminus in *CALR* mutations enables the N-terminal domain of *CALR* to interact with the extracellular domain of MPL causing its activation and, thus, constitutive activation of JAK/STAT/MAPK signaling, which drives enhanced megakaryopoiesis and proplatelet formation.(31,34,36,38–42)

Type 1/1-like mutations in contrast to type 2/2-like mutations in mice was shown to be related more to myelofibrosis phenotype while type 2/2-like presented less oncogenic properties and a lower progress to PMF,(36) maybe because the *CALR* mutant type 2/2-like maintains some of the negatively charged amino acids. Direct comparison between type 1 and type 2 *CALR* mutations, in a study by Tefferi *et al.* shown that type 2 mutations are more often associated with higher score in prognostic systems, marked leukocytosis, and higher peripheral blast percentage compared with type 1.(43)

Phenotype variability inside the same type of mutation can be attributed to the differences in cell of origin and levels of *CALR* expression.(17)

MPL

The third, and least common driver mutation in MPNs, are missense mutations in exon 10 of *MPL* gene, located in chromosome 1p34 which are present is roughly 7% of patients with PMF.(4) The *MPL* gene encodes the TPO receptor, the main megakaryopoiesis-stimulating cytokine, thus explaining the lack of these mutations in PV and the fact that murine models bearing MPL mutations develop a disease marked by thrombocytosis and other features of ET and PMF.(44) *MPL* mutations have also shown to confer an increased risk of myelofibrotic transformation since excessive signaling via the MPL receptor is associated with bone marrow fibrosis.(45) Therefore, a diagnosis of prePMF should be considered in *MPL*-mutated ET.

The main type of *MPL* mutation found in PMF is a gain of function mutation located in exon 10. The residue W515 located at the boundary of the transmembrane and the cytosolic domains

of MPL are the most affected. Although several substitutions have been described, the two most frequent are *MPL*^{W515L} and *MPL*^{W515k}, present in around 5-10% of *JAK2* unmutated patients with PMF.(46,47) The juxtamembrane tryptophan residue W515 is required to maintain the receptor in an inactive state in the absence of TPO binding.(48) Thus, W515 mutations result in a constitutive activation of the TPO receptor in a cytokine-independent fashion, increasing JAK2, STAT3, STAT5 and PI3K-AKT signaling.(46)

The other mutation also found, *MPL*^{S505N}, is even rarer and is located in the transmembrane domain, stabilizing receptors in active dimeric orientations. It was described as germline change in hereditary form of thrombocytosis (49) and later as acquired somatic mutation in less than 1% of patients with ET, but was not described in PMF.

NONDRIVER MUTATIONS

MPNs have always been viewed as neoplasms with a relatively simple genomic landscape. However, the development of NGS, as well as other whole-genome analysis techniques, allowed the identification of multiple acquired mutations in MPNs (Table 3), that are often mutated in myeloid malignances such as MDS and AML. These mutations, unlike *JAK2*, *CARL* and *MPL* are not specific for MPNs but explain the continuum between the different myeloid malignancies, the phenotypic changes and the risk of disease progression– that's why they are usually called nondriver mutations. PMF is the MPN where more somatic nondriver mutations were found.(9)

These other mutations affect genes involved in DNA methylation regulators (*TET2*, *DNMT3A*, *IDH1/2*), histone modifiers (*Polycomb* repressor complex 1 and 2 members, *EZH2*, *ASXL1*, *IDH1/2*), transcription factors (*TP53*, *CUX1*, *IKZF1*, *FOXP1*, *ETV6*, *RUNX1*), proteins involved in signaling (*NF1*, *NRAS*, *KRAS*, *LNK*, *CBL*, *FLT3*), and splicing factors (*SF3B1*, *SRSF2*, *U2AF1*,).(23) Table 3 lists the frequencies of such mutations. As the majority of the mutations are loss of function it seems that most of the mutated genes are myeloid tumor suppressors. Single-gene mutations had low frequency to allow convincing evidence of association with disease progression. However, the number of detected mutations (as an indirect measure of genetic complexity or progression of clonal evolution) allowed the identification of high-risk patients who are likely to have an increased risk of leukemic transformation and/or reduced survival.(51)

The presence of mutations in others than the 3MPN-restricted driver genes increases the myelodysplastic features and the severity of the disease, explaining the continuity between MPN, MPN/MDS, and MDS.

It is beyond the goal of this review to cover all the nondriver mutations in detail. Therefore, it will be focused the main genes with prognostic impact and the ones whose role have been recently studied in detail.

Table 3. Genetical Landscape of PMF

GENE	PROTEIN FUNCTION	FREQUENCY	CONSEQUENCES
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IDH1/IDH2	Neomorphic enzyme, generation of 2-hydroxyglutarate blocking a-ketoglutarate–dependent enzymes		Initiation and progression
ASXL1	Chromatin-binding protein associated with PRC1 and 2	5-10%	Initiation Disease progression
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NF1	ERK/MAPK signaling	Rare	Progression to leukemia (5%-10% in secondary AML)

FLT3	Cytokine receptor (FLT3-L)	MPN (<3%)	Progression to leukemia (10%-15% in secondary AML)

Table adapted from Vainchenker W. et al(9)

MUTATIONS IN EPIGENETIC REGULATORS

Epigenetic regulator genes are subdivided in DNA methylation genes (*TET2*, *DNMT3A*, *IDH1* and *IDH2*) and histones modifiers genes (*ASXL1* and *EZH2*).

DNA METHYLATION GENES

DNA methylation genes (*DNMT3A*, *IDH1*, *IDH2* and *TET2*) are epigenetic regulator genes and are mutated most often in PV (0-50 mutated genes/patients) and less frequently in ET (0-13) and PMF (0-07).(52)

TET2

TET2, a tumor suppressor gene located on chromosome 4q24, encodes ten-eleven translocation (TET) proteins which regulate DNA methylation and gene expression, by converting 5-methylcytosine to 5-hydroxymethylcytosine, an epigenetic mark which has been shown to be important in the regulation of stem cell genes in hematopoiesis.(53) Somatic mutations in this gene were found in all types of MPN, MDS, chronic myelomonocytic leukemia (CMML) and AML. *TET2* mutation is the most frequent nondriver mutation detected in all MPN subtypes and are also typically associated with age-related clonal hematopoiesis. They are present in 10-20% of patients with PMF and in 50-60% of CMML.(54)

All *TET2* mutations are loss of function point mutations or deletions, usually in one allele, but more rarely, on both somatic alleles. The loss of *TET2* results in obvious myelomonocytic proliferation and phenotypic features resembling CMML.(55)

After genotyping individual hematopoietic colonies, it is recognized that the mutation acquisition order influences subclonal composition within HSCs and mature cell compartments, disease presentation and clinical outcome.(28,29) In *JAK2*-first patients, the HSC compartment is dominated by double-mutant cells, and such patients present at a younger age, often with PV.(28) In contrast, in *TET2*-first patients, the HSC compartment is dominated by single mutant cells, and such patients present at an older age, usually with ET.

The influence of *TET2* mutations on disease progression remains unclear with some studies reporting an association with disease transformation to leukemia (10) while another study did not find prognostic significance.(56) However, *TET2* mutations have clearly been shown to

increase HSC self-renewal in model systems (53) and when acquired with a *JAK2*^{V617F} mutation, *TET2* loss rescues the self-renewal capacity of the *JAK2*^{V617F} HSC (57,58) and produces a more severe MPN phenotype.

DNMT3A

As *TET2*, *DNMT3A* gene encodes a protein, a DNA methyltransferase, that plays a central role in the regulation of DNA methylation at cytosine guanine dinucleotide (CpG) sequences. The most common mutations are heterozygous and alter R882, within the catalytic domain (most commonly R882H) which is a loss of function mutation, although clear-cut loss of function mutations (e.g. frameshifts, indels and nonsense mutations) also occur.(59) They were found in all MPNs subtypes and in 5-10% of PMF.

A study of human AML, where mutations in *DNMT3A* were first discovered, has shown that *DNMT3A* mutations are acquired early in tumorigenesis, and that preleukemic HSCs harboring mutated *DNMT3A* display a self-renewal advantage over wild-type HSCs. (59) This results in a stem cell advantage by increasing and decreasing methylation at distinct loci, including substantial CpG island hypermethylation. *DNMT3A* mutated HSCs upregulate HSC multipotency genes and downregulate differentiation factors, and their progeny exhibit global hypomethylation and incomplete repression of HSC-specific genes.(60)

Despite being associated with poorer outcomes in myelodysplasia and *de novo* acute myeloid leukemia, no clear poor prognostic impact has been implicated in MPNs.

DNMT3A mutations also may occur early or late in patients with an MPN, and that mutation order influences phenotype: *DNMT3A* preceding *JAK2*^{V617F} is associated with an ET phenotype and if acquired after is associated a PV phenotype.(61)

IDH1 AND IDH2

Genes encoding for isocitrate dehydrogenases 1 (IDH1) and 2 (*IDH2*) are frequently mutated in multiple types of cancer and are predominantly found in patients with PMF (around 3%) as well as in 20% of blast-phase MPN patients. Mutations in *IDH1* and *IDH2* result in simultaneous loss of their normal catalytic activity, the production of α-ketoglutarate (α-KG), and gain of a new function, the production of 2-hydroxyglutarate. This protein, which is structurally similar to α-KG, acts as an α-KG antagonist, competitively inhibiting multiple α-KG-dependent dioxygenases, including both lysine histone demethylases and the TET family of DNA hydroxylases. Abnormal histone and DNA methylation are emerging as a common feature of tumors with *IDH1* and *IDH2* mutations and may cause abnormal stem cell differentiation and eventual tumorigenesis.(62) Mutant *IDH1* has also been linked to increased DNA damage via

downregulation of the DNA damage sensor ATM. Knock-in mice for *IDH1*^{R132H} suffer expansion of HSC, extramedullary hematopoiesis, and anemia in keeping with this mutation being found in advanced phases of MPN. (63)

Mutations in *IDH1/2* genes are associated with a poor prognosis, identifying patients who are at risk for premature death or leukemic transformation.(51)

HISTONE MODIFIERS GENES

In addition to DNA methylation, gene expression is regulated by histone modifications. The Polycomb repressive complex 2 (PRC2) is a transcriptional repressor that acts by methylating lysine 27 of histone H3, which leads to gene silencing and compaction of chromatin. Additional somatic mutations in *ASXL1* and *EZH2* perturb PCR2 and were founded frequently mutated in PMF patients (0-50 mutated genes/patients) and, to a significantly lesser extent, in ET (0-13) and PV (0-07).(52) They are a marker of poorer prognosis associated with overall survival and increased risk of leukemic transformation.(64)

ASXL1

ASXL1 (addition of sex combs like 1) encodes an epigenetic regulator, which binds to chromatin. ASXL1 recruits PCR2 complex to specific loci through a direct interaction between ASXL1 and EZH288 and can be also involved in the PRC1 complex by its association with the deubiquitinating enzyme (DUB) BRCA-1–associated protein (BAP1), a critical tumor suppressor in solid tumor. (65) This protein also binds to nuclear hormone receptors such as retinoic acid and estrogen receptors.

Germ line *ASXL1* mutations are responsible for a syndrome called the Bohring-Opitz syndrome.(66) Somatic mutations in *ASXL1* were first described in MDS and CMML and then in MPN (67,68) and are also found in age-related clonal hematopoiesis. In MPNs they are essentially associated with PMF with a frequency around 25%. In ET they are present in 3% and in 13% of all patients in blast phase MPN.(21)

These mutations are either focal deletion or nonsense mutation or insertion/deletion leading to frameshift.(69) They are associated with more severe anemia and inferior survival.(51,70–72)

ASXL1 deletion in murine models results in a phenotype with features of both MDS and MF: anemia, leukopenia, dysplasia, extramedullary hemopoiesis, and splenomegaly.(70) This model also displays HSC clonal expansion and a block in erythroid differentiation, features that are reminiscent of many patients with PMF.(70)

The *ASXL1* mutations are loss of function and are associated with a higher frequency of AML transformation. Thus, they are associated with a worse prognosis, despite the International

Prognostic Scoring System (IPSS) classification.(69) However, regardless of the individual mutational landscape, the presence of mutations in *ASXL1* requires the treatment of the patients, based on the most recent prognostic models and the current treatment algorithm published by Tefferi et al. in 2018. Thus excluding a watch-and-wait strategy even if the patients are asymptomatic.(73)

EZH2

EZH2 (Enhancer of Zeste Homologue 2) is one of the two histone methyltransferases of the PCR2 complex, as described above. It compacts chromatin and represses gene transcription. Loss-of-function mutations in *EZH2* and other PRC2 members (*SUZ12*, *JARID2*, *EED*) lead to depression of several target genes, such as the Hox gene family, which enhances HSC self-renewal, and Lin28b/Hmga2, which promotes bone marrow fibrosis and reduces erythropoiesis in a $JAK2^{V617F}$ context.(74,75) Loss of *EZH2* can also be a consequence of other genetic perturbations, such as loss of heterozygosity at chromosome 7q, as well as mutations in spliceosome components *U2AF1* and *SRSF2*.(76,77)

EZH2 have been described in all NPMs and across all myeloid malignancies. In PMF, somatic mutations in *EZH2* occurs in 5% to 10%, more particularly associated with $JAK2^{V617F}$, and represents an unfavorable prognostic factor with significantly reduced survival due to rapid leukemic transformation.(64)

Recent studies have showed that *EZH2* deletion/inactivation dramatically modifies the MPN phenotype in murine models of MPN, resulting in an MDS/MPN phenotype, increased megakaryopoiesis and overexpression of Hmga2, a gene that was previously found deregulated in human PMF and that appears to play an important role in this phenotype.(75)

SPLICING GENES

SF3B1, *SRSF2* and *U2AF1* are the three major spliceosome genes mutated in PMF and in in approximately half of MDS patients. The precise pathogenic mechanism is not fully understood but it is believed to involve global abnormalities in RNA splicing, resulting in reduced cell proliferation and increased apoptosis.(77) Spliceosome mutations tend to co-mutate more often with $JAK2^{V617F}$ and rarely with *CALR*, despite being more prevalent in triple-negative PMF patients.(23)

SRSF2

SRSF2 (serine/arginine-rich splicing factor 2) encodes for a protein, which is a member of the serine/arginine-rich splicing factor family that binds to exonic splicing enhancer sequences in

the pre–messenger RNA (mRNA). *SRSF2* dysfunction promotes defects in alternative splicing and leads to numerous functionally relevant misspliced events.

SRSF2 mutations have been reported in almost 20% of PMF(78) and have been found to have negative impact in leukemia-free survival and OS in PMF.(51)

SRSF2 mutation may be responsible for myelofibrotic transformation through downregulation of *EZH2*,(74) increasing bone marrow fibrosis, and therefore this abnormality is not infrequent in other myeloid neoplasms than PMF. The majority are missense mutations and a significant clustering was noted with both *IDH1* (P <.01) and *IDH2* (P < .01) mutations and a borderline association was also seen with $JAK2^{V617F}$ (P <.11). In contrast, none of the *SRSF2*-mutated patients expressed *MPL* mutations.(78)

SF3B1

SF3B1 (splicing factor 3B subunit 1) is also a component of the RNA spliceosome. Mutations in *SF3B1*, most frequently in K700 hotspot codon, are phenotypically characterized by ring sideroblasts and are associated to sideroblastic myelodysplastic syndrome and with progression to myelofibrosis.(79) They are also associated with clonal hematopoiesis only in more advanced decades of life.(80)

U2AF1

U2AF1 (U2 Small Nuclear RNA Auxiliary Factor 1) is a subunit of the U2 small nuclear ribonucleoprotein auxiliary factor involved in pre-mRNA processing. *U2AF1* is mutated in 10-15% of patients with PMF,(81) rarely in ET (003, P = 0037) and not mutated in PV (P = 0024).(52) They are also found in MDS, where has also been strongly associated with anemia and/or thrombocytopenia, both of which have been validated as negative clinical prognostic markers in PMF.(82) *U2AF1* mutations are usually classified into the two main mutations variants, Q157 and S34, which have different phenotype presentations and prognosis relevance. Q157 and S34 are present in 65% and 34% of PMF patients, respectively, although only those affecting the Q157 residue are prognostically relevant.(83)

SIGNALING GENES

Somatic mutations in genes that encode signaling proteins (*NF1*, *SH2B3/LNK*, *CBL*, *FLT3*, *KRAS*, *NRAS*) were also found mutated in a small frequency of patients in MPNs. In PMF, the most frequent mutations founded in signaling genes are in *LNK* and *CBL* genes.(9)

LNK

LNK (Lymphocyte adaptor protein) / *SH2B* adaptor protein 3 (SH2B3) gene encodes a LNK inhibitor adaptor protein which is a key regulator of normal hematopoiesis once it negatively regulates cytokine receptor mediated signaling in normal hematopoiesis.(84)

Loss-of-function mutations in the inhibitor adaptor protein LNK are present in 2% of PMF patients(9) and were described as a novel mechanism of JAK-STAT activation, playing an important role in MPN disease initiation and progression (84). *LNK* mutations appear more as predisposition mutations when germline or secondary mutations increasing the pathogenicity of *JAK2*^{V617F} and *CALR*, when acquired.(85)

Only few data about the pathogenicity of *LNK* mutations are available that suggest a potential moderate role in driving MPN phenotype in the absent of driver mutation, however it seems that this mutation synergize and cooperate with driver or nondriver mutations to induce MPN development or progression. Thus, more extensive molecular analyses of PMF patients are necessary to correlate genotype and phenotype.(84)

CBL

CBL (Casitas B-lineage Lymphoma) gene, localized in chromosome 11q23 encodes for a cytosolic protein that acts as a negative regulator of some signaling pathways by E3 ubiquitin ligase activity, promoting the ubiquitination of several signaling molecules including some receptor tyrosine kinases, e.g. MPL and FLT3 (FMS-like tyrosine kinase-3) and oncoproteins.(86,87) Loss-of-function mutations in either exon 8 or 9 of *CBL* gene are infrequent in myeloid malignancies other than CMML and juvenile monomyelocytic leukemia (87), being present in 6% of patients with PMF. These mutations in myeloid malignancies are frequently associated with 11q acquired uniparental disomy and leads to deregulation of downstream targets and an increase in cell proliferation rates. (86) A study of Francis H. Grand *et al* grouped patients with CML, PMF and CMML – subgroups in which *CBL* mutations were mostly found – and found differences in OS between *CBL* mutated and nonmutated patients (33 months vs 39 months) however the difference is not statistically significant. Likewise, there was not demonstrated differences in phenotypic presentations.(88)

PROGNOSTIC FACTORS

PMF carries the worst prognosis among BCR-ABL negative MPNs. Causes of death include leukemic transformation, disease progression without acute transformation, thrombosis, infections, bleeding, and complications of portal hypertension.(89) Since early clinical risk factors were found and more recently also molecular risk factors were described. These has allowed the improvement of the treatment in a way that therapy is now more targeted.

CLINICAL RISK FACTORS

A list of clinic risk factors is summarized in table 4. Advanced age, anemia, increase of blast cells, the presence of constitutional symptoms and leukocytosis were the first described and incorporated in prognostic models.

The huge breadth of cytogenetic abnormalities has been study since 1988, the year that was demonstrated that abnormal karyotype has an adverse impact on survival and nowadays unfavorable karyotype is also used in prognostic models and includes complex karyotype, monosomy 7, deletion 7q, trisomy 8, monosomy 5, deletion 5q, deletion 12p, inversion 3 or 11q23.(90) Other rearrangements are include in normal karyotype, considered “favorable”. Unfavorable karyotype occurs in 14% of patients with PMF and is associated with median survival of 2 years, versus 5,2 years in its absence, and with higher risk of leukemic transformation with reported 5-years risk of 46% versus 7% in patients with “favorable” karyotype.(91)

Bone marrow fibrosis, thrombocytopenia and dependence of transfusion were also identified as adverse prognostic factors and incorporated into prognostic scoring systems. Grades of bone marrow fibrosis correlate with clinical and molecular aspects. Clinically, patients with higher grade fibrosis (grade 2 or 3) had increased mortality (31.2% versus 13.1%), were older, had more frequent anemia and thrombocytopenia, higher prognostic scores and unfavorable karyotype, and less frequent leukocytosis.(92) However, the use the accuracy of fibrosis grading may impact its utility as a prognostic factor.

The effort to improve the prognostic factors has resulted in the discovery of new independent-risk factors in PMF that until now are not include in prognostic scoring systems. They are monocytosis,(93) markedly elevated serum lactate dehydrogenase,(94) increased serum levels of IL-8, IL-2R, free light chain and hepcidin.(95–97)

Table 4. Conventional Clinic Risk Factors

Age > 65 years
Hemoglobin < 10 g/L
Leucocyte count > 25.000/mL
Circulating blast cells ≥1%
Constitutional symptoms
Platelets count < 100.000/mL
Transfusion need
Unfavorable karyotype
Bone marrow fibrosis grade ≥ 2

MOLECULAR RISK FACTORS

The prognostic relevance of driver and nondriver mutation was carefully investigated in a series of collaborative studies and the findings indicate that genetic landscape of each patients is an independent predictor of clinical course and outcomes. Thus, analysis of the individual mutation status must be taken in account carefully due to its impact on clinical decision-making. In 2014, was published the firsts studies that evaluate the prognostic impact of driver mutations on survival in a large PMF cohort. A study by Rumi *et al.* of 617 PMF patients demonstrated that median OS was 17.7 years in *CALR*-mutated, 9.2 years in *JAK2*-mutated, 9.1 years in *MPL* mutated, and 3.2 years in triple-negative patients. Notably, triple-negative patients had much higher incidence of leukemic transformation compared with either *CALR*-mutated or *JAK2*-mutated patients. PMF patients with nonmutated *JAK2*, *CALR*, and *MPL* seems to be older and present a very aggressive myeloid neoplasm described with lower hemoglobin level, lower platelets count and higher scoring at prognostic models. Opposite, *CALR*-mutant patients had a better OS than *JAK2*-mutant, *MPL*-mutant and triple-negative patients. *CALR* mutations were also associated with younger age, less anemia, less blood transfusions, less leukocytosis and higher platelet count.(33) Thus, lower scoring in prognostic models and a favorable impact on survival which were further supported by another large cohort study. of 254 PMF patients conducted by Tefferi *et al*.(8)

A meta-analysis compared 435 *CALR*-mutated and 1116 $JAK2^{V617F}$-mutated PMF patients and found that *CALR*-mutated patients displayed a lower risk of splenomegaly and thrombosis but showed no significant difference in the risk of leukemic transformation when compared to *JAK2*-mutated patients.(98)

Regarding the two types of *CALR* mutation, a recent study by Li *et al.* on a cohort of 402 patients, 20.1% *CARL* mutated, in China, like the study by Tefferi *et al*., showed that type-2 or type-2 like *CALR* mutations had significantly worse OS compared to patients with type-1 or type-1 like *CALR* mutations.(99,100) However, comparing with patients with *JAK2* mutations, the study by Li *et al*. showed that patients with type-2 *CALR* mutations appeared to have a worse OS compared to patients with *JAK2* mutations on multivariate analysis, while the study by Tefferi *et al.* showed comparable OS in both.

Definitive conclusions regarding the impact of *MPL* mutations on prognosis are difficult given the relative low frequency of these mutations in all of studies.

Analyzing the nondriver mutations, studies have been shown that mutations in *ASXL*, *SRSF2*, *EZH2*, and *IDH1/2* represent unfavorable prognostic factors and identify patients who are at risk for premature death and leukemic transformation.(51) The presence of one of the five "prognostically detrimental" mutated genes (*ASXL1*, *SRSF2*, *EZH2* and *IDH1/2*) define a high-molecular risk category (HMR). Guglielmelli *et al.* demonstrated the additional value of the number of these prognostically-detrimental mutations in the prognostication of PMF,

representing an additional unfavorable prognostic factor *per se*, with 2 or more mutations being associated with shortened leukemia-free survival and OS compared to patients harboring only one mutation. Median survival can ranging from 12 years – nonmutated patients – to a 2 years median survival – in patients with HMR≥2 mutations.(101)

CARL/ASXL1 mutational status is also predictive of leukemic transformation. A study of 570 patients reported the longest survival in *CALR*$^{+}$*ASXL1*$^{-}$ patients (median 10.4 years) and shortest in *CALR*$^{-}$*ASXL1*$^{+}$ patients (median 2.3 years). *CALR*$^{+}$*ASXL1*$^{+}$ and *CALR*$^{-}$*ASXL1*$^{-}$ patients had similar survival (median survival 5.8 years). Tefferi *et al.* points to the *CALR*$^{-}$ *ASXL1*$^{+}$ status as the most detrimental mutations profile in PMF. (71) Another study of 709 consecutive Mayo Clinic patients with PMF confirms that survival was significantly longer with type1/1-like *CALR* mutation compared to all other driver mutations and the adverse survival effect of not carrying this mutation was independent of *ASXL1* or *SRSF2* mutations, while the presence of the particular mutation partially improved the detrimental effect of *ASXL1*/*SRSF2* mutations. (102)

A screening of samples of 491 patients specifically for *U2AF1*, published by Tefferi *et al*. in 2018 showed that 16% of patients have *U2AF1* mutations and 65% of that involved Q157 and 34% involved S34. Comparing phenotype and prognostic differences between the three *U2AF1* mutational categories (unmutated vs mutated for Q17 vs mutated for S34), this study disclosed the following significant associations: both Q157 and S34 variants with anemia, absence of marked splenomegaly and *JAK* and *MPL* mutations; Q157, but not S34, was also associated with thrombocytopenia, older age, *ASXL1* mutations and constitutional symptoms. Significance was lost between Q157 mutation and age and constitutional symptoms by multiple logistic regression analysis, and confirmed for anemia, thrombocytopenia, *ASXL1* mutations, driver mutational status and absence of marked splenomegaly. A median follow-up of 3.9 years shown that OS was significantly shorter in *U2AF1* Q157 mutations compared to the *U2AF1* unmutated and *U2AF1* S34 mutated patients. (83)

Furthermore, in the most recent NGS study of PMF patient, overall or leukemia-free survival were also adversely affected by *RUNX1*, *TP53*, *KIT*, *CEBPA*, *CBL* and *SH2B3*/*LNK* mutations.(102) However, these analyses did not include a multivariate assessment of all factors.

PROGNOSTIC MODELS

Although patients with PMF share some clinicopathological and molecular features, there is a huge interpatient variability in risk of disease complications and progression. Thus, in the last decade, several prognostic models of PMF have been created and have enabled clinicians to determine the most appropriated therapy for the individual patient.

In 2009, International Prognostic Scoring System (IPSS)(103) was designed by the International Working Group for Myeloproliferative Neoplasm Research and Treatment (IWG-MRT) to predict survival at diagnosis and one year later IPSS was upgraded to the dynamic IPSS (DIPSS)(104) in order to be used at any time during the clinical course of the disease. Both are based in 5 independent predictors of inferior survival that are described in table 5. In 2011 was created DIPSS-plus,(105) where for the first time was included cytogenetic information as independent clinic risk factor, which boosted the curiosity and the study of additional prognostic information, and also two others clinical variables (red blood cell (RBC) transfusion need and platelets count < $100x10^9$/L) were include in addition to the same 5 used in IPSS/DIPSS.

More recently investigators developed new contemporary prognostic models, focused on clinical variables, driver mutation status, high-risk mutations and karyotype.

One of those is the Mutation-enhanced International Prognostic Scoring System for transplant-age patients (MIPSS70), providing a complementary system for risk stratification and was developed specifically for patients with PMF with age bellow 70 years old in order to facilitate allogeneic stem-cell transplantation (alloSCT) decision. This system integrates prognostically relevant clinical and mutation data. It introduced the concept of HMR category (presence of a mutation in any of the following genes in a patient: *ASXL1*, *EZH2*, *SRSF2* or *IDH1*/*2*) and the absence of *CALR* type 1/1-like mutation as significant risk factors for OS. A total score of 0–1, 2–4, and ≥5 defined the three-tiered MIPSS70 low, intermediate, and high-risk categories. MIPSS70-plus was created to include cytogenetic information (unfavorable karyotype), in addition to mutations and some of the clinical risk variables included in MIPSS70. The overall score was reviewed, ranged from 0 to 12, and define the four-tiered MIPSS70-plus: low-risk, score of 0 to 2; intermediate-risk, score of 3; high-risk, score of 4 to 6; and very high-risk, score≥7.(106)

A study of 1002 Mayo Clinic patients with PMF by Tefferi *et al.* in 2018, considered three-tiered cytogenetic risk stratification: “very high-risk (VHR)” karyotype included single or multiple abnormalities of −7, i(17q), inv(3)/3q21, 12p −/12p11.2, 11q−/11q23, or other autosomal trisomies not including +8/9 ; “favorable” karyotype included normal karyotype or sole abnormalities of 13q−, +9, 20q−, chromosome 1 translocation/duplication or sex chromosome abnormality including −Y; and “unfavorable” karyotype included all other abnormalities, with corresponding median survivals of 1.2, 2.9, and 4.4 years.(107) Based on this cytogenetic risk stratification, MIPSS70-plus version 2.0 was created to include the additional prognostic contribution from VHR karyotype, the *U2AF1* Q157 as an additional HMR mutation and the new sex- and severity-adjusted hemoglobin thresholds. A total score of 0, 1-2, 3-4, 5-8, and ≥9 defined the five-tiered MIPSS70-plus version 2.0: very low-risk, low-risk, intermediate-risk, high-risk and high-risk categories. (108)

Most recently, was developed a genetic only-based prognostic system (GIPSS) which is exclusively dependent on karyotype and a limited number of mutations, including *ASXL1*, *SRSF2*, *U2AF1* Q157, and *CALR*. Contrary to what had previously been published, this study of 641 patients with PMF by Tefferi et al. with multivariable analysis restricted to genetic risk factors did not identify *EZH2* and *IDH1*/*2* as inter-independent risk factors for survival.(109) The overall score defines the four-tiered GIPSS: low-risk, score of 0; intermediate-1 risk, score of 1; intermediate-2C risk, score of 2; and high-risk, score≥3.

The study revealed significant alignment of risk distribution between GIPSS and MIPSS70-plus version 2.0. In other words, a patient with GIPSS high-risk disease was most likely to also be in the MIPSS70-plus version 2.0 high or very high-risk category whereas a patient with GIPSS low-risk disease was almost certain to be in the MIPSS70-plus version 2.0 low-risk disease category.(109) Thus, additional prognostic information from MIPSS70-plus version 2.0 or other clinically derived prognostic models (e.g., IPSS and DIPSS) might not be necessary for GIPSS high or GIPSS low risk patients to decide the best therapy. However, the corresponding MIPSS70-plus version 2.0 risk allocation was not predictable for GIPSS intermediate-1- and intermediate-2 risk disease, thus in these cases is necessary to calculate directly the MIPSS70-plus version 2.0 score.

Table 5 described all the prognostic models mentioned above.

Table 5. Prognostic models for patients with PMF

Prognostic model	Risk groups (median survival)
IPSS(103)	
Risk factors (weight):	Low risk: 0 (11.3y)
• Age>65y (1 point)	Intermediate-1 risk: 1 point (7.9y)
• Constitutional symptoms (1 point)	Intermediate-2 risk: 2 points (4.0y)
• Hemoglobin < 10g/dl (1 point)	High-risk: ≥3 points (2.3y)
• Leukocyte count > $25x10^9$/L (1 point)	
• Circulating blast ≥1% (1 point)	
DIPSS(104)	
Risk factors (weight):	
• Age>65y (1 point)	Low risk: 0 (>20y)
• Constitutional symptoms (1 point)	Intermediate-1 risk: 1-2 points (14.2y)
• Hemoglobin < 10g/dl (1 point)	Intermediate-2 risk: 3-4 points (4.0y)
• Leukocyte count > $25x10^9$/L (1 point)	High-risk: 5-6 points (21.5y)
• Circulating blast ≥1% (1 point)	
DIPSS-PLUS(105)	
Risk factors (weight):	
• Age>65y (1 point)	
• Constitutional symptoms (1 point)	Low risk: 0 (15y)
• Hemoglobin < 10g/dl (1 point)	Intermediate-1 risk: 1 point (6.6y)
• Leukocyte count > $25x10^9$/L (1 point)	Intermediate-2 risk: 2-3 points (2.9y)
• Circulating blast ≥1% (1 point)	High-risk: 4-6 points (1.3y)
• RBC transfusion need (1 point)	
• Platelets count < $100x10^9$/L (1 point)	
• Unfavorable karyotype (1 point)	
MIPSS70(106)	
Risk factors (weight):	
• One HMR mutation (1 point)	
• ≥2 HMR mutations (2 points)	Low risk: 0-1 points (not reached)
• Type 1/like CALR absent (1 point)	Intermediate-risk: 2-4 points (6.3y)
• Constitutional symptoms (1 point)	High-risk: ≥5 points (3.1y)
• Hemoglobin < 10g/dl (1 point)	
• Leukocyte count > $25x10^9$/L (1 point)	
• Circulating blast ≥2% (1 point)	
• Bone marrow fibrosis grade ≥2 (1 point)	
MIPSS70-plus version 2.0(108)	
Risk factors (weight):	
• VHR karyotype (4 points)	Very low risk: 0 (not reached)
• Unfavorable karyotype (3 points)	Low risk: 0-1 points (16.4y)
• One HMR mutation (2 points)	Intermediate-risk: 3-4 points (7.7y)
• ≥2 HMR mutations (3 points)	High-risk: 5-8 points (4.1y)
• Type 1/like CALR absent (2 points)	Very high-risk: ≥9 points (1.8y)
• Constitutional symptoms (2 points)	
• Severe anemia (2 points) (man: Hb<8g/dl; woman: Hb<9g/dl)	
• Moderate anemia (1 point) (man: Hb 8-9.9g/dl; woman: Hb9-10.9g/dl)	
• Circulating blast ≥2% (1 point)	
GIPSS(109)	
Risk factors (weight):	
• VHR karyotype (2 points)	
• Unfavorable karyotype (1 point)	Low risk: 0 points (26.4y)
• Type 1/like CALR absent (2 points)	Intermediate-1 risk: 1 point (8y)

• ASXL1 mutation (1 point) • SRSF2 mutation (1 point) • U2AF1Q157 mutation (1 point)	Intermediate-2 risk: 2 points (4.2y) High-risk: ≥3points (2y)

RISK-ADAPTED THERAPY

Based on the most recent prognostic scoring systems showed above, Tefferi *et. al* proposed a step-wise prognostication approach that stars with simple-to-use GIPSS but also considers MIPSS-plus version 2.0 for confirming the appropriate treatment for the individual patient.(21) As described above, since GIPSS has a significant alignment of risk distribution with MIPSS70-plus version 2.0 in high and low-risk, might not be necessary to identify others prognostic factors to recommend alloSCT in patients with GIPSS high-risk disease and to recommend *watch-and-wait* strategy in asymptomatic patients with GIPSS low-risk. Unfortunately, despite alloSCT being the only potentially curative treatment of PMF, it is often ineligible by the high treatment-related mortality and by age-related comorbidities, and, in these cases, patients receive symptom-directed conventional therapy and are encouraged to participate in clinical trials.(73) On the other hand, GIPSS low-risk patients might require palliative therapy for anemia, splenomegaly, non-hepatosplenic EMH, bone pain, EMH-associated pulmonary hypertension or constitutional symptoms. Anemia is best managed by the use of androgens, danazol, thalidomide and prednisone. JAK inhibitor Ruxolitinib has been valuable in patients who have splenomegaly and/or constitutional symptoms.(73) Hydroxyurea should be used in symptomatic splenomegaly and if the treatment of the thrombocytosis was considered in patients with low or intermediate-1.(110) Interferon-α revealed improvements in constitutional symptoms, resolution of thrombocytosis, and leukocytosis.(111) Bone pain and symptomatic non-hepatosplenic EMH responds well to radiotherapy.

In contrast, the corresponding MIPSS70-plus version 2.0 risk allocation was not predictable for GIPSS intermediate-1- and intermediate-2 risk disease. Thus, in these cases, it is necessary to calculate MIPSS70-plus version 2.0 score and follow the treatment that is recommended for each of the risk groups, already presented. In MIPSS70-plus version 2.0 intermediate-risk clinical trials are the preferred option, otherwise should be used conventional therapy based on treatment indications.(73)

CONCLUSION

In the last decade there has been an impressive improvement in the understanding of the genetic basis of PMF and although mutation landscape is much more complex than initially thought, nowadays is known that abnormal activation of JAK2 pathway is a common feature of the disease caused by mutations in the three driver gene mutations. The genetic cause remains unknown in <10% of PMF, the triple-negative patients, whose pathogenic basis is far from completely understood. Despite similar clinical features (bone marrow fibrosis, abnormal stem cell trafficking and myeloid metaplasia), the remarkable differences in clinical course and outcomes seems to be mainly related to the combination of different genetic lesions and perhaps their order of acquisition.

Whole-genome sequencing allowed the knowledge of additional mutations whose pathogenicity was studied in order to infer about their phenotypic and prognostic impact. Some of these mutations, such as those in *DNTM3A* or *TET2*, have not been shown to correlate with OS, contrary to the mutations in *ASXL1*, *EZH2*, *SRSF2*, *IDH1/2* and *U2AF1* genes that have shown to be predictive of inferior survival. Also, the absence of type 1/like *CALR* mutations and the *CALR*$^{-}$*/ASXL1*$^{+}$ profile predicted short survival. These discoveries allowed to create contemporary prognostic scoring systems more accurate than the classical ones and thus, based on the overall survival given by each risk group, the therapeutic decision is now more appropriate for the individual.

However, further studies are necessary to discover the precise role of each mutation and therefore use this knowledge to test alternative therapeutic strategies and improve the management of the disease.

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