



Pathogenesis and Mutations of Myeloproliferative Neoplasms: An Overview

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Authors' contributions

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ABSTRACT

Chronic myeloproliferative neoplasms (MPNs), clonal disorders of hematopoietic system resulting in abnormal proliferation of one or more hematopoietic cell lineages, express different clinical, hematologic and biological features. The majority of classical MPNs -95% of polycythemia vera (PV) and half of essential thrombocythemia (ET) and myelofibrosis (MF) patients- display alterations in the JAK2 gene. JAK2 wild-type ET, and MF patients lack features of PV. Driver mutations in pathogenesis of Philadelphia-negative MPNs - JAK2, MPL and calreticulin (CALR)- all play pivotal roles in cytokine signaling in hematopoiesis. Negative regulators of signaling pathways, LNK and CBL are infrequently targeted genes in MPNs. Mutated or deleted transcription factors such as IKZF1, EZH2, Tp53 and RUNX1, are present in a low proportion of patients with MPN. Some of these factors, such as IKZF1 and Tp53, were associated with transformation to acute myeloid leukemia (AML), yet their prognostic impact needs to be elucidated. Mutations of CHEK2, the tumor suppressor gene against uncontrolled cell growth, were associated with susceptibility to

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ET. Mutations of NRAS gene, a member of MAPK signaling pathway frequently mutated in MPNs, seem to affect expression of JAK2 target genes and are primarily associated with transformation to AML. Mutations in epigenetic regulators including TET2, DNMT3A, ASXL1, EZH2, and IDH1/2 were described in MPNs and other myeloid malignancies at variable frequencies. The somatic mutation of SRSF2, one of the RNA splicing machinery genes, was associated with worse survival and increased leukemic transformation in PMF. Alterations in DNMT3A, ASXL1, EZH2, and IDH1/2 are more frequent in PMF than PV and ET. Recent studies suggest that for Ph-negative MPNs, mutations affecting epigenetic regulation might be prognostically more relevant than mutations affecting JAK-STAT signaling. Mutations in CALR were newly discovered in a majority of JAK2V617F- and MPL-negative ET and MF patients. In MPNs, genetic abnormalities affecting epigenetic regulation are often expressed in patients carrying JAK2, MPL, or CALR mutations, indicating a cooperation between these two classes of mutations in MPN pathogenesis. This review summarizes pathogenesis and molecular events of MPNs.

Keywords: Chronic myeloproliferative neoplasms; driver mutations; epigenetic regulators; essential thrombocythemia; molecular events; polycythemia vera; myelofibrosis.

1. INTRODUCTION

Chronic myeloproliferative neoplasms (MPN) are a group of heterogeneous disorders characterized by excessive production of clonal myeloid cells and a frequent tendency to transform to acute myeloid leukemia (AML) [1]. Defective hematopoiesis is the main contributing factor to the pathogenesis of MPN. Hematopoiesis is a dynamic and strictly controlled process in which cells continue to mature and proliferate. Acquired mutations (point mutations or chromosomal rearrangements) may disrupt this process resulting in defects affecting the myeloid series. Although most of the somatic mutations occurring during division of the pluripotent hematopoietic stem cell (HSC) have no effect on phenotype, some mutations have been reported to cause proliferation. The myeloproliferative clone gradually ensues as the mutated stem cell leading to proliferation enters cell cycle more frequently. The resulting monoclonal hematopoiesis is a characteristic feature of all myeloid malignancies. In humans, development of MPN is the most common phenotypic expression of clonal hematopoiesis. This review focuses on classic MPN, which is one of the 9 disorders included in the current classification of MPN by World Health Organization (WHO) [1]. Classic MPN comprises of polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF). Dameshek W. proposed two highly speculative possibilities as the cause of trilinear PV of erythrocythemia, thrombocythemia, granulocytopenia: excessive bone marrow stimulation by an unknown factor or the lack or decrease of an inhibitory factor [2]. In 2005, James C et al. [3] who demonstrated that the somatic JAK2V617F

mutation was the driver cause of trilinear MPNs including PV, ET and secondary MF, confirmed the hypothesis of PV as a trilinear myeloproliferative disorder of megakaryocytic, erythrocytic and granulocytic myeloproliferation. MF is characterized by accumulation of excess fibrotic tissue in bone marrow induced by MPN of various molecular etiologies. In the last decades, genomic studies have identified various genes relevant to the pathogenesis of MPN, which imply that many different cellular pathways may be responsible for the clonal evolution of hematopoietic stem cells. MPN related somatic mutations were divided into two main functional categories: mutations that affect cytokine receptor signalling and mutations related to regulation of gene expression. This review discusses the molecular basis of classic MPN and developments regarding the clonal evolution of disease as well as the contribution of various lesions to disease progression and their use as prognostic markers.

2. MUTATIONS AS DRIVER CAUSES OF CLONAL MYELOPROLIFERATIVE NEOPLASMS

The World Health Organization clinical molecular and pathological (WHO-CMP) criteria define three phenotypes of JAK2V617F-mutated MPNs -normocellular ET, hypercellular ET due to increased erythropoiesis (prodromal PV), ET with hypercellular megakaryocytic-granulocytic myeloproliferation (EMGM) or masked PV and classical PV versus the JAK2 exon 12-mutated idiopathic erythrocythemia (IE) and PV. MPL515-mutated JAK2-wild type ET and MF are distinct thrombocythemias without features of PV in blood and bone marrow. The third

thrombocytopenia entity -calreticulin-mutated JAK2/MPL wild-type ET and MF- show characteristic features of primary megakaryocytic and granulocytic myeloproliferation in the bone marrow, findings not observed in JAK2 and MPL-mutated MPNs. The MPN disease burden of various molecular etiologies is best reflected by the degree of anemia and splenomegaly rather than mutated allele burden, bone marrow cellularity, increase in reticulin or collagen fibrosis. [4-8]

The majority (95%) of PV patients harbor the JAK2V617F mutation. The 5% JAK2V617F-unmutated PV patients are frequently heterozygous for JAK2 exon 12 mutations [9,10]. MPL, and CALR-mutated MPNs lack features of PV and provide a genetic marker for the majority of JAK2 wild-type MF and ET (91.9% and 85%, respectively). [11,12] Several genes including regulators of DNA methylation (TET2, DNMT3A, IDH1/2), chromatin structure (EZH2, ASXL1), MAPK signaling pathway (NRAS), regulators of transcription factors (including IKZF1, RUNX1, and Tp53) and negative regulators of signaling pathways (LNK, CBL), are also mutated in MPNs [13-26].

2.1 JAK2V617F Mutation

Over two decades, researchers have investigated the underlying genetic disturbances in PV. Defects in the signalling pathways of the erythropoietin receptor (EPO-R) was proposed as the underlying pathology. These pathways include transcription activators such as tyrosine kinase JAK2, transcriptional signal transducers, transcription 3 signal mediator and activator molecule (STAT3) and transcription 5 signal mediator and activator molecule (STAT5). JAK2V617F mutation was described in 2005 by Vainchenker et al. in France [3]. It was demonstrated that in PV, inhibition of JAK2 by a small molecule (AG490) or siRNA led to a decrease in EPO independent colony production by bone marrow mononuclear cells. This observation led the researchers to directly sequence JAK2 in hematopoietic cells of PV patients and to the eventual discovery of repetitive point mutations. Guanine-to-thymine mutation in JAK2 resulted in substitution of valine to phenylalanine codon 617 within the pseudokinase domain. These findings were rapidly confirmed by other groups. In another study including PV patients, researchers identified loss of heterozygosity (LOH) on chromosome 9p and a commonly shared 6.2

Mbp region [27]. This specifically identified region was shown to harbor the JAK2V617F mutation. DNA content analysis demonstrated that JAK2V617F mutation was only present in hematopoietic cells and that in all PV patients, erythroid precursor cells were homozygous for the mutation. Based on the quantification of the allele burden of JAK2V617F mutation in granulocytes by polymerase chain reaction (PCR), patients were stratified into two groups: those with low allele burden of JAK2V617F mutations (<50%) and those with high allele burden (>50%). The mitotic recombination and duplication of the mutant allele were shown to account for the homozygosity for the JAK2V617F mutation. Also, it has been demonstrated that the heterozygote mutation transforms to homozygous state as a result of mitotic recombination during the disease course. Median disease duration for patients with high allele burden and low allele burden was 48 months and 23 months, respectively. These findings support the notion that low allele burden disease transforms to high allele burden disease during follow up. Furtheron, the aforementioned results suggest that pathogenesis of PV is a multi-step process. In the first run, the acquired mutation in the JAK2V617F gene results in low allele burden. Later on, homologous recombination leads to the appearance of precursor cells and granulocytes showing homozygosity for JAK2V617F in the presence of a high allele burden. The JAK2V617F mutation was reported to be present in more than 95% of the patients with PV [28].

40-60% of ET patients carry the JAK2V617F mutation. [28] JAK2 plays an important role in the intracellular signaling pathway of a group of growth factors such as IL-3, EPO, G-CSF and thrombopoietin (TPO). JAK2V617F allele burden was found higher in ET patients showing clonal hematopoiesis compared to those showing polyclonal hematopoiesis (26% and 16%, respectively). It is reported that the heterozygous JAK2V617F clone in ET remains stable in the presence of both clonal and polyclonal hematopoiesis. 70% of PV patients harbor the homozygote JAK2V617F mutation with high allele burden (>50%) where as it is less common in ET patients. JAK2V617F mutation is homozygous in all PV patients, including those with low allele burden. On the contrary, JAK2V617F homozygosity is rarely expressed in the hematopoietic colonies of ET patients. It was reported in ET patients that a higher JAK2V617F allele burden existed in thrombocytes compared to granulocytes while the allele burden was

higher in the granulocytes of PV and PMF patients. Furthermore, JAK2V617F mutations were demonstrated in erythroblasts of ET patients. In one study which utilized mutant single nucleotide polymorphism (SNP) genotyping for mutant alleles of JAK2V617F, the presence of interdependent multiple abnormal clones was demonstrated in ET [29]. In the same study, the real prevalence of biallelic JAK2 mutations in ET was reported as 5-10%.

Approximately 50% of MF patients carry the JAK2V617F mutation and nearly 13% carry it in the homozygous state [30]. It is believed that the homozygosity of this mutation in MF is associated with the presence of cytogenetic abnormalities with poor prognosis.

One of the questions to be addressed regarding the biology of the JAK2V617F in MPNs is how different phenotypes are constituted in the presence of this common mutation. In PV, the amount of phosphorylated STAT3 and STAT5 increase in bone marrow cells. ET patients show an increase in the amount of phosphorylated STAT3 but a decrease in the amount of phosphorylated STAT5. In MF patients, both the expression of STAT3 and STAT5 decrease. These observations imply that alternative or additional molecular events may play a role in the pathogenesis of PV, ET and MF. Moreover, it is known that clonal hematopoiesis exists in a major proportion of ET and PMF patients not harboring the JAK2V617F mutation. Consequently, it may be considered that JAK2V617F mutation emerges as a result of different genetic events.

2.2 JAK2 Exon 12 Mutations

JAK2 mutations other than JAK2V617F (eg, JAK2 exon 12 mutations) are related to erythrocytosis and misdirect the autoinhibitor domain activating JAK2 kinase activity. A few functional mutations targeting the JAK2 exon 12 lie in the vicinity of JAK2V617F mutation. JAK2 exon 12 mutations are present in 2.5-3.4% of all PV patients and in 30% of JAK2V617F-negative PV patients [31,32]. Up to date, 17 different mutations of the JAK2 exon 12 have been reported [27]. 2/3 of the patients harboring the JAK2 exon 12 mutations have isolated erythrocytosis, a distinguishing bone marrow morphology and decreased serum EPO levels. In the remaining cases, leukocytosis and/or thrombocytosis accompany erythrocytosis. Thus far, JAK2 exon 12 mutations have not been

reported in ET or MF while were rarely demonstrated in patients with refractory anemia and refractory anemia with ring sideroblasts (RARS-T). It is proposed that JAK2 exon 12 mutations result from long duration activation of JAK2 related intracellular signalling pathway. Microvascular transient ischemic and major thrombotic events are frequently encountered in patients with JAK2 exon 12 mutations. Transformation to MF and acute leukemia may be encountered in PV [33]. BaF3 cells that express the mice EPOR and the JAK2 exon 12 mutation may proliferate without the aid of IL-3 added to the medium. This mutation also increases the phosphorylation of JAK2 and extracellular signal-regulated kinase (ERK). As a result, myeloproliferative disease phenotype characterized by erythrocytosis is constituted. In some series, approximately 2.7% of patients with a clinical syndrome mimicking PV do not carry JAK2 mutations. Although only a minority of sporadic PV patients do not carry the JAK2V617F mutation, the proportion of familial cases of JAK2V617F-negative PV patients is definitely higher [34,35]. JAK2 exon 12 mutations seem to account for the majority of mutations in PV patients negative for JAK2V617F [36].

2.3 MPL Mutation

JAK2V617F mutation is expressed in only approximately 50% of ET and MF patients. In one study, it was reported that JAK-STAT pathway may be activated by the stimulation of the hematopoietic specific cytokine receptors including EPOR or myeloproliferative leukemia virus (MPL) [37]. Sequencing of the MPL gene, which encodes the TPO receptor resulted in the discovery of a new molecular abnormality in JAK2 mutation-negative MPN. MPL mutations are localized in the juxtamembrane region of the receptor. 60% of MPL mutations are W515L and the remaining 40% are W515K mutations, which result in the substitution of tryptophan with leucine and lysine, respectively [37-39]. In different series, the frequency of MPL mutation was reported to be between 0-10% in MF and 0-6% in ET [40-43]. In 50% of patients with W515K mutation and in 17% of those with W515L mutation, the allele burden is more than 50%. MPL mutations result in cytokine independent proliferation in cell lines and increase susceptibility to TPO. As a result, JAK-STAT signal pathway is activated. It was demonstrated that MPL W515L mutation induces myeloproliferation characterized by splenomegaly, leukocytosis, marked

thrombocytosis without PV features, and extramedullary hematopoiesis in the spleen and myelofibrosis. Moreover, it was shown that JAK kinase inhibition halts the proliferation of MPL W515L-transformed cells. Consequently, it can be deduced that inhibition of the JAK-STAT signal may be effective in treatment of a JAK2V617F and MPL W515L mutation-positive MPN [37]. MPL mutation is present in granulocytes and it has not been detected in non-hematopoietic DNA. These findings indicate that MPL W515L mutation is a somatic mutation of clonal hematopoietic cells. Also, the absence of MPL W515L allele in normal individuals points out that it is not a common nucleotide polymorphism. These data support the notion that MPL W515L is a pathogenic mutation for JAK2V617F-negative MPN with no features of PV at diagnosis and follow-up [37]. In a series of 994 ET patients by Vannucchi et al, MPLW515L/K mutation was demonstrated in 30 patients, 18 of whom (60%) expressed the W515L and 12 (40%) expressed the W515K allele [44]. In the aforementioned study, 8 of 30 MPL-mutated ET patients co-expressed the JAK2V617F mutation [44]. In ET, a correlation between W515L and low haemoglobin (Hgb) levels and between W515K alleles and high platelet counts were reported [44]. Also, bone marrow examination in W515L/K mutation-positive patients showed reduced total and erythroid bone marrow cellularity, whereas the number of megakaryocytes, megakaryocytic clusters, and small-sized megakaryocytes were all significantly increased [44]. These data show that MPLW515L/K mutations do not define a distinct phenotype in ET.

30% of MF patients positive for the MPL mutant allele also carry the JAK2V617F mutation [39]. The mutational load of MPL W515L, MPL W515K and JAK2V617F remain stable during the clinical course of MF patients. In a mouse model, it was demonstrated that MPL W515L mutation induced a lethal and rapidly progressive form of MPN characterized by severe thrombocytosis, leukocytosis, splenomegaly, hepatomegaly, megakaryocytic hyperplasia and fibrosis in the bone marrow. Erythrocytosis was not observed in the presence of MPL W515L supporting that MPL mutation causes thrombocytosis while JAK2V617F mutation induces erythrocytosis. MPL W515L/K mutation-positive MF patients are older patients with severe anemia and require more frequent transfusions [37].

2.4 Somatic CALR Mutations

Calreticulin (CALR) is a multifunctional protein best known for its role as a Ca²⁺ binding chaperone in the endoplasmic reticulum (ER) lumen [45]. The CALR-1 gene is located on human chromosome 19 and mouse chromosome 8, and contains nine exons which are highly similar between human and mouse at a sequence level. Mature CALR is a 46-kDa protein composed of three structurally and functionally distinct domains. The globular N-terminal domain is lectin binding and consists of a signal sequence targeted to the ER. The middle proline-rich or P-domain contains high affinity, low capacity, binding sites for Ca²⁺, and the highly acidic C-terminal domain contains a number of high capacity, low-affinity Ca²⁺ binding sites. The acidic C-terminal domain is involved in cellular calcium homeostasis—cells that are deficient in CALR have diminished calcium storage capacity in the ER and overexpression of CALR augments ER calcium uptake. The C-domain terminates in a KDEL sequence; KDEL receptors function in the retrieval of KDEL-containing proteins from the cis-Golgi back to the ER [46,47].

The mutations in the gene CALR was first discovered by Nangalia J. et al and subsequently confirmed by Klampfl T et al. [46,47] CALR mutations are all located in exon 9. The two most prevalent mutations accounting for about 80% of cases are del52 (CALRdel52/Type I; c.1092_1143del; L367fs*46) and ins5 (CALRins5/Type II; c.1154_1155insTTGTC; K385fs*47 [48]. CALR mutations were reported to be present in about 15-25% of patients with ET, MF and post-ET myelofibrosis, who are negative for the JAK2V617F and MPL exon 10 mutations [46,47]. In a recent study, the frequency of CALR mutations was 17.7% in ET and 14.8% in PMF. CALR mutations were not detected in PV [49]. Thus, CALR mutations represent an exclusion criterion of PV, that is JAK2 mutated by definition. Mutations in CALR were identified in 8% of patients with MDS. CALR mutations have not been identified in healthy controls, AML, CML, lymphoproliferative disorders and a variety of solid tumors [46,47].

Recent studies revealed that CALR-mutated ET is associated with a higher platelet count, lower Hgb and show no features of PV at time of diagnosis and during follow-up. CALR-mutated ET patients are characterized by lower leukocyte count compared to those carrying the JAK2

mutation [48,50]. Although CALR-mutated ET patients have a reduced incidence of thrombosis, no differences in survival have been thus far been demonstrated for different mutation subgroups in ET [48,50]. Presence of CALR-mutation in MF patients showed a favorable impact on survival independent of both DIPSS-plus risk and ASXL1 mutation status [47,51].

In summary, CALR mutations occur in the majority of JAK2V617F- and MPL-negative ET and PMF patients. Finally, 2014 WHO Clinical Molecular and Pathological (WHO-CMP) Diagnostic criteria suggested CALR mutations as a novel major criteria for ET and MF [4]. Approximately 85% of MF patients exhibit clonal hematopoiesis yet do not harbor JAK2, MPL or CALR mutations. It is not possible to explain the pathogenesis of MF solely by the presence of these driver mutations or mutant allele load. The genetic basis of MF likely stems from the combination of several genetic and epigenetic phenomena.

2.5 LNK Mutation

Lymphocyte specific adaptor protein (LNK) is a JAK-STAT inhibitor adaptor protein. It is considered that LNK plays a role in the pathogenesis of myeloproliferative diseases. It consists of three regions including proline rich amino region, PH domain required for insertion into the cell membrane and the SH2 domain which binds to MPL-JAK2. LNK is a negative regulator of TPO and MPL mediated JAK2 activation [52]. In LNK knock out mice models, increase in megakaryocytes and erythroid precursor cells as well as enhanced hematopoietic stem cell regeneration were documented [52]. Some studies demonstrated that LNK in vitro suppresses the signals generated by MPL W515L and JAK2V617F [13,53]. In a mouse model, LNK deficiency facilitated the development of myeloproliferative disease induced by TEL-JAK2 and JAK2V617F. These findings showed that an interaction between JAK2V617F and LNK is required to suppress PV/MF expression and that the SH2 domain of LNK is needed to suppress the myeloid expansion induced by TEL-JAK2. In chronic phase MPN, the presence of LNK mutation was reported as a rare finding [54]. Yet, a higher frequency (9.8%) was reported for MPN with blastic transformation [55]. LNK binds to the phosphorylated tyrosine kinases and inhibits the major cytokine receptor signal that comprises c-KIT, JAK2 and MPL-JAK2 [56]. In conclusion,

LNK as a negative regulator of cytokine signalling plays an important role in hematopoiesis.

2.6 Casitas B-cell Lymphoma Mutation

Casitas B-cell lymphoma (CBL) is a gene located on chromosome 11q23.3 and contains three related but distinct mammalian protooncogenes: c-CBL, CBL-b and CBL-c [14,57]. It is thought to play a role in the maintenance of hematopoietic stem cell homeostasis. CBL functions as an adaptor protein and also increases the E3 ubiquitin-protein ligase activity hence regulating tyrosine kinase activity. The greatest frequency of mutations is found in chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia [58,59]. In AML, transforming activity of c-CBL may be related to augmented FLT3 signaling [58]. In MPNs, CBL mutations were first studied during screening for acquired 11q mutation. Most CBL mutations were localized in the RING region causing a disruption in ligase activity. To date, 27 CBL variants associated with MPNs have been identified [15]. In a cohort of patients diagnosed with chronic phase MPN, CBL mutations were reported at a frequency of 6% among PMF patients whereas CBL mutations were not detected in a small series of PV and ET patients [14].

2.7 CHEK2 Gene Mutation

CHEK2 is a tumor suppressor gene that regulates cell division and hence protects cells against uncontrolled and rapid division. [60] CHEK2 is a key mediator of cellular response to various types of DNA damage. CHEK2 encodes the kinase at the G2 checkpoint control in the cell cycle. CHEK2 kinase is activated on ATM dependent pathways. In addition to DNA repair, it phosphorylates substrates including p53, BRCA1, CDC25A and CDC25C which are involved in cell cycle checkpoint control through coordination of DNA repair, cell cycle progression and apoptosis [61]. It is reported that germline CHEK2 mutations (ie.1100delC, I157T) were detected in Li-Fraumeni syndrome, some lymphoid malignancies and in myeloid malignancies such as myelodysplastic syndrome (MDS) and AML [61-65]. In one recent study, the frequency of CHEK2 mutations in ET was reported as 15.1% [60]. Thus, authors proposed that CHEK2 gene mutations contribute to the susceptibility to ET. The same study also pointed out that germline inactivation of CHEK2 has no direct influence on development of ET, yet it may

result in disruption of cell cycle checkpoints and trigger the cancerogenic process in ET at an earlier age.

2.8 IKZF Deletion Mutation

Ikaros Family Zinc Finger Protein (IKZF1) gene localized on 7p.12 encodes the Ikaros transcription factor, which is of functional significance in normal hematopoiesis. In mouse models, it was demonstrated that RNA mediated IKZF1 deficiency led to an increase in cytokine sensitivity and expression of p-STAT5. In one study, IKZF1 deletions were detected in 6 out of 29 (21%) post-MPN leukemia patients, yet IKZF1 deletions were not detected in 526 MPN patients with no leukemic transformation [16]. In conclusion, a significant relationship was found between IKZF1 deletions and post-MPN leukemia. IKZF1 deficient patients showed complex karyotypes and del 7p was reported as a late event in the genetic evolution of the MPN clone. As a result, it may be deduced that loss of IKZF1 may play an important role in leukemic transformation in some MPN patients.

2.9 EZH2 Mutation

In the development of multicellular organisms, polycomb-group (Pc-G) genes encode proteins that regulate gene expression pattern [66]. PcG proteins function via three key multiprotein complexes: Polycomb Repressive complex 1 (PRC1), Polycomb Repressive complex 2 (PRC2) and PhoRC. Enhancer of Zeste Homolog 2 (EZH2) proteins are one of the four components of PRC2. EZH2 is the multiprotein enzyme complex responsible for trimethylation of lysine 27 on histone H3 (EZH2, SUz12, EED ve YY1). Also, PRC2 makes chromatin more compact by attracting other polycomb complexes to the gene region [17]. EZH2 shows oncogenic activity. Its increased expression prevents differentiation in epithelial tumors [67]. In MPNs, EZH2 gene expression is up-regulated. EZH2 mutations are most commonly reported in PMF. It may be suggested that EZH2 acts as a tumor suppressor in myeloid malignancies. In one study including 614 patients with myeloid malignancies, a total of 49 EZH2 mutations was detected in 42 patients [17]. Of the 30 MF patients included in this cohort study, 13% carried the EZH2 mutation. EZH2 mutations were absent in ET and reported at a ratio of 3% in PV [17]. This study could not determine the real prognostic significance of the EZH2 mutation due to the

limited number of MF patients and short follow-up period. Another study including 370 MF patients revealed reduced leukemia-free survival (LFS) and overall survival (OS) after a median follow-up of 39 months in the presence of EZH2 mutations [68]. In that study, researchers concluded that EZH2 mutations are independently associated with shorter survival in MF patients [68].

2.10 RAS Mutations

Ras proteins are members of the small guanosine triphosphatase (GTPase) superfamily. In all human cancers, RAS mutations that abnormally activate the RAF/MEK/ERK, PI3K/AKT and other pathways are commonly encountered [69,70]. Three oncogenic mutations attributed to the RAS gene are HRAS, NRAS and KRAS mutations. Although KRAS and NRAS mutations are common in myeloid diseases, HRAS mutation is rare [71]. RAS mutations are usually single amino acid substitutions such as G12V and G13R that decreases the sensitivity of RAS for GTPase-activating protein thus enabling high RAS-GTP levels [69,72]. NRAS activating mutations of the RAS family are present in approximately 30% of hematopoietic malignancies including AML, MDS and MPN. [18, 19] NRAS mutations are found in 7-13% of post-MPN AML [73]. NRAS/KRAS mutations are rare in PV, yet they have been associated with transformation from PV to acute leukemia [19].

2.11 Tp53 Mutation

p53 mutations are the most commonly observed genetic alterations in human cancers including lymphomas and leukemias. [20] p53 transduced mouse hematopoietic stem cells showed increased in vitro proliferative and differentiation capacity. Also, increased expression of the mutant p53 triggered in vivo transformation of immature mice hematopoietic cells. As a result, two types of hematopoietic diseases, MPN and MDS, were induced [20]. Although there is a genetic tendency for leukemic transformation in MPNs, the underlying mechanisms leading to this transformation are still unclear. Of a total of 22 patients who transformed to AML from MPN, 6 patients (27.3%) were found to carry somatic mutations in Tp53 gene [74]. Thus, it is considered that Tp53 plays an important role in leukemic transformation. Tp53 mutations although rare in PV have been associated with transformation from PV to acute leukemia [74].

2.12 RUNX1 Mutation

Runt transcription factor 1 (RUNX1) point mutations in MDS and AML were first identified in 1999 [75]. Some mutants of RUNX1 exhibit a negative effect on transactivation activity. These mutations both cause loss of function and also carry some oncogenic potentials as well. Some MPN patients develop leukemic transformation shortly after a few years of initial diagnosis. The underlying molecular pathogenesis resulting in leukemic transformation has not been elucidated. In one study, the frequency of RUNX1 mutation was reported to increase during leukemic transformation from MPN, but RUNX1 mutation was reported not to be present in chronic phase MPN [21]. In the aforementioned study, 5 out of 18 patients (27.7%) who transformed to acute leukemia were shown to carry RUNX1 mutation.

2.13 TET2 Mutation

Ten-Eleven Translocation (TET) gene family is made up of TET oncogene family member 1 (TET1), TET oncogene family member 2 (TET2) and TET oncogene family member 3 (TET3) [76-78]. TET2 gene localized on chromosome 4q24 encodes the hydroxylase enzyme that enables the hydroxylation of 5-methylcytosine (5mC) [76]. TET2 mutations are loss-of-function mutations, which increase DNA methylation and result in decrease of hydroxymethyl cytosine levels. TET2 mutations may occur before or after acquisition of JAK2V617F or MPL mutations and are encountered in approximately 15% of myeloid malignancies [22,79-82]. IDH mutations exert functionally similar effects on DNA methylation as TET2 mutations. Acquired somatic mutations in the TET2 gene suppress the catalytic activity of the α -ketoglutarate dependent enzyme responsible for the oxidation of 5-methylcytosine (5 mC) to 5-hydroxymethylcytosine (5 hmC) in DNA. Low 5hmC levels lead to hypermethylation of CpG islands in various DNA promoter regions. TET2 mRNA is highly expressed in the Lin⁻, Sca-1⁺ and c-Kit^{hi} multipotent progenitor cells isolated from the bone marrow and thymus of C57BL/6 mice. This pattern of expression is maintained in myeloid precursor cells, yet is low in mature granulocytes. Furtheron in one study, comparison of patient samples with a control group showed that low 5hmC levels resulted in significant decrease in DNA hypermethylation. Currently, it is assumed that TET2 mutations lead to loss-of-function in TET2. As a result, these mutations cause accumulation of 5hmC in

DNA and induce increase in DNA hypermethylation [83]. DNA hypermethylation inhibits cell differentiation. In vitro studies demonstrated that TET2 deficiency prevents normal differentiation process of hematopoietic cells. In transgenic animal models using conditional knock-out systems, increase in monocytic cell line and a disease phenotype mimicking CMML were demonstrated. TET2 mutations include frameshift mutations due to insertions or deletions, splice-site mutations and base substitutions. These mutations occur more frequently at advanced age. In familial MPNs, TET2 mutations are almost always acquired. The incidence of TET2 mutations in familial MPNs are similar to the incidence in sporadic MF. In clonal analysis studies held in patients with MPNs, no certain chronological line of events were observed between acquisition of TET2 and JAK2 mutations [84,85]. TET2 mutations in MPNs may manifest later at advanced disease stage. These two genetic events, TET2 and JAK2V617F mutations in MF patients, seem to be independent of each other. TET2 mutations have not been demonstrated to influence the frequency of thrombosis, leukemic transformation or OS. It is considered that TET2 mutation status is not a new prognostic marker in MF patients. In multivariable analyses in MF patients, there was no correlation between the presence of TET2 mutation and anemia (Hgb<10 g/dl). The frequency of TET2 mutation was reported as 26.3% in post-MPN AML, 17% in MF, 11% in ET and 7-16% in PV [86,87].

2.14 DNMT3a Mutation

DNA methyl transferase (DNMT) family consists of 3 genes: DNMT1, DNMT3A and DNMT3B. DNMT gene family encodes the DNA methyl transferase-the enzyme that catalyzes the addition of a methyl group to the cytosine remnants of CpG dinucleotides [88]. The frequency of DNMT3A mutations in AML was reported as nearly 20% [88-90]. DNMT3A mutations are believed to be loss-of-function mutations. DNMT3A mutations are usually heterozygous and are predominantly located in the methyl transferase domain. They often present as repetitive mutations of the R882 codon. DNMT3A mutations were reported to portend poor prognosis in AML patients [88]. In one study, DNMT3A mutations were detected in 3 of 62 (4.8%) patients with refractory anemia with excess blasts (RAEB) while in another study, the frequency of these mutations was 8% in MDS [91,92]. DNMT3a mutations are very rare

in MPN. In a study population of 135 MPN patients, only 2 (1.5%) were found to carry DNMT3A mutations [93]. Studies involving a larger patient population are needed to better assess the clinical and prognostic significance of these mutations in MPNs [23].

2.15 ASXL1 Mutation

Additional Sex Combs-Like 1 (ASXL1) gene, the human homologue of 'Drosophila Additional sex combs' (Asx), is located on chromosome 20q11.1. Asx deletion results in dysregulation of the genes encoding the Pc-G and Trithorax group (Tx-G) proteins [94]. It is hypothesized that Asx influences the suppression and activation of homeotic gene expression. Moreover, functional studies in Drosophila demonstrated that Asx encodes chromatin related protein with high similarity to Pc-G proteins [95]. It is not clearly known how ASXL1 mutations promote myeloid transformation. In vitro studies held on non-hematopoietic cells demonstrated the physical interaction of ASXL1 with heterochromatin protein 1a (HP1a) and lysine-specific demethylase 1 (LSD1) for suppressing retinoic acid receptor activity and with peroxysome proliferator activated receptor gamma for suppressing lipogenesis [96,97].

The three members of the human ASXL gene family (ASXL1, ASXL2 and ASXL3) consist of an amino-terminal homologous domain (ASXH) and a C-terminal plant homeodomain (PHD) [96,98]. Studies regarding the conserved domains of mammalian ASXL proteins showed that N-terminal region (10-100 aminoacid) of ASXL1 may contain a specific DNA binding site, namely HARE-HTH domain (HB1, ASXL1, restriction endonuclease helix-turn helix domain) [99]. The results of the aforementioned study indicates that the PHD domain is specific for ASXL family. It is proposed that PHD domain recognizes the methylated lysine on the tail of the histone H3 over the lysine on the N-terminal tail of histone H3. Additional functional studies are required to understand the effects of ASXL1 domains. ASXL1 mutations are frameshift mutations on exon 12 that lead to formation of truncated proteins.

Drosophila Asx forms a complex with the 'chromatin deubiquitinase Kalipso' that forms the Polycomb-repressor deubiquitinase complex (PR-DUB). PR-DUB complex monoubiquitinates nucleosomal histone H2A at lysine 119. The mammalian homologue of Kalipso, BAP1,

interacts directly with ASXL1. Also, it was demonstrated that the mammalian BAP-1 - ASXL1 complex shows in vitro deubiquitinase activity [100]. The exact role of ASXL1 function in hematopoiesis has not been clearly elucidated. In a mouse model, ASXL1 was shown to be a significant cause of perinatal mortality. A small number of surviving mice were analyzed for an overt, short-latency hematopoietic phenotype. In this model by Fisher et al. [98] a PGK promoter-drive neomycin expression cassette was placed into exon 5 of ASXL1 to interrupt the reading frame of ASXL1 (this allele is referred to as Asxl1tm1BC) and hence enabling truncation of nuclear interacting domains as well as the PHD domain. This enabled the expression of proteins without nuclear contact sites in the vicinity of the PHD domain. Asxl1tm1BC/tm1BC homozygote mice had incompletely penetrant perinatal lethality. Yet, they neither developed overt hematologic malignancy nor was there a decrease in number or function of multipotent progenitors. In the aforementioned study, homozygote Asxl1tm1BC/tm1BC allele was the cause of perinatal death in 75% of cases. Moreover, embryonic lethality was observed in C57BL/6J hybrid mice. In conclusion, the impact of ASXL1 on hematopoiesis needs to be assessed using knockout models specific to postnatal and hematopoietic deletion.

Mutations in the epigenetic regulator region ASXL1 were identified shortly following the identification of TET2 mutations in myeloid cancers. Mutations in ASXL1 were determined by comparative genomic hybridization (aCGH) performed on MDS patients [101]. ASXL1 mutation was first described in MDS by Gelsi-Boyer V. et al, who noted deletions on chromosome 20q11 [101]. ASXL1 mutations were detected in 4 of 35 (11%) MDS patients. Further sequence analysis of ASXL1 enabled the identification of the frequency of ASXL1 mutations in MPN and other myeloid diseases. ASXL1 mutations were reported at a frequency of 10%, 17% and 49% for MPN, AML and CMML, respectively [24,101-103].

A number of studies have assessed the impact of ASXL1 mutations in MPNs. [24,104-108] Although ASXL1 mutations in MPNs are encountered in advanced ages, it is relatively more prevalent in MF and post-PV/ET myelofibrosis compared to PV or ET [24,87,104]. ASXL1 mutation was reported at a frequency of 2-5% in PV, 5-10% in ET, 13-26% in MF and 22-

38.5% in post-PV/ET myelofibrosis. A recent study reported a significantly shorter OS for ASXL1-mutated MF patients compared to those without the mutation. However, this study had a limited number of subjects including only 9 ASXL1 mutation-positive patients and 35 ASXL1-negative patients [105]. The study by Vannucchi AM et al assessed the impact of ASXL1 mutations in PMF in a relatively large study population [106]. Of the 279 MF patients from the Mayo Clinic enrolled in this study, ASXL1 mutation was detected in 85 (31%) patients. Vannucchi AM et al. [106] reported that ASXL1 mutations did not predict LFS, but correlated with shorter OS. In the European cohort of the same study including 483 MF patients, ASXL1 mutation frequency was reported as 21.7%. In the European cohort, the presence of ASXL1 mutation in MF showed correlation with shorter OS and leukemic transformation. A recent study by Tefferi A. et al. [107] reported that ASXL1 mutation showed a significant association with shortened survival in a total of 570 MF patients (277 patients from Mayo Clinic and 293 from the University of Florence). In another study, it was reported that ASXL1 mutations were neither more frequent in blastic phase MPN nor did this mutation result in an increase in the risk of leukemic transformation in chronic phase MPN [87]. Thus, it may be hypothesized that ASXL1 mutation may play a critical role in the early phase of MPN development and clonal evolution of MPNs.

2.16 IDH1/IDH2 Mutations

Isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) are the two mutated oncogenes commonly associated with gliomas and secondary glioblastomas [109]. No IDH1 and IDH2 mutations were detected in assessment of samples from approximately 500 non-central nervous system solid tumor tissue specimen, which support the notion that these oncogenes are tissue specific. Therefore, detection of repetitive IDH1 mutations in whole exome sequencing analysis of 16 of 188 AML patients was an unexpected finding [110]. IDH1 and IDH2 mutations have been identified in AML, rarely in MDS and MPNs with leukemic transformation [87,111-114].

In mammalian cells, three classes of IDH isoenzymes are present: IDH1, IDH2 and IDH3 [115]. IDH1 and IDH2 are nicotinamide adenine dinucleotide phosphate (NADP⁺) dependent homodimeric enzymes that convert isocitrate to

α -ketoglutarate (α -KG). IDH1 is found in cytoplasm and peroxisomes and IDH2 in mitochondria. The gene encoding IDH3 is yet to be discovered and the data regarding IDH3 mutations are inadequate. Identified IDH1 and IDH2 mutations are specific missense mutations involving conserved codons. Frameshift and nonsense mutations have not been identified and all mutations are heterozygote for the wild-type allele. It is proposed that the mutant protein inhibits the wild-type enzyme and that IDH mutations cause a dominant negative phenotype. Also, it is considered that the mutant protein acts differently from the wild type and cause a gain of function. It was demonstrated that cells expressing the mutant IDH1 contain high levels of 2-hydroxyglutarate (2-HG) [116]. 2-HG is also present in primary leukemia cells that carry IDH1 or IDH2 mutations [117]. Mutant IDH enzymes alter substrate specificity. Mutant IDH catalyzes the conversion of α -KG to 2-HG and depletes NADPH instead of producing NADPH molecule by conversion of isocitrate to α -KG. It is not yet known whether 2-HG has a direct oncogenic effect or decrease in NADPH and α -KG correlates with leukemogenesis [118].

IDH1 is located on chromosome 2q33.3 and IDH2 is on chromosome 15q26.1. IDH mutations are located on exon 4 and involve a change of a single aminoacid in the arginine residue. These mutations affect 3 specific arginine residues: R132 (IDH1), R172 (IDH2) and R140 (IDH2) [111]. It is known that mutant IDH decreases the affinity for isocitrate and possesses catalytic activity to convert α -KG to 2-HG [117,119-121]. The oncogenic features of mutant IDH are attributed to the decrease in α -KG or to the accumulation of 2-HG [119,122]. IDH mutations were identified at a frequency of 70% in low grade gliomas and secondary glioblastomas, 10-20% in AML, 3-5% in MDS, 1-4% in MPN, 9% in MDS/MPN including CMML, 15% in AML transformed from MDS, 22% in AML transformed from MPN, 10% in AML transformed from MPN/MDS, 22% in high risk MDS or AML associated with del(5q) and 4% in blastic phase chronic myeloid leukemia (CML) [110,111,113, 114,123-128]. Most studies about the phenotypic and prognostic implications of IDH1 and IDH2 mutations in AML reported a consistent relationship between NPM1 mutations and normal or intermediate risk karyotype [124,129-134]. Boissel N et al. [125] reported that mutant IDH2 was a poor prognostic factor in normal karyotype AML. In another study, the prognosis in patients harboring mutant R140 (IDH2) was

found to be more favorable than those carrying mutant R172 (IDH2) [135]. Compared to AML, data on prognostic importance of IDH mutations in chronic myeloid neoplasias involving MDS and MPN, are limited [111,113]. In one study examining the frequency of IDH1 and IDH2 mutations in a population of 1473 Ph-negative MPN patients, the frequency of IDH mutations were reported as 0.8% for ET, 1.9% for PV, 4.1% for MF, 1% for post-ET/PV myelofibrosis, 25% for blastic phase PV, 25% for blastic phase MF and none for blastic phase ET [111]. Since this study included only 111 chronic phase MF patients with complete available clinical data, detailed prognostic analysis could not be performed. In another study examining the impact of IDH1 and IDH2 mutations on phenotypic features and prognosis in 301 chronic phase MF patients, IDH mutation was detected in 12 patients (4%). 7 patients carried IDH2 mutations (5 R140Q, 1 R140W and 1 R172G) and 5 carried IDH1 mutations (3 R132S and 2 R132C). 6 of 12 patients (50%) positive for IDH mutation harbored JAK2V617F mutation, as well. These findings indicate that IDH mutations are independent risk factors for leukemic transformation in MF and that they increase the leukemogenic risk in the copresence of JAK2V617F mutation [136]. The last comprehensive study which focused on the correlation of IDH mutations with prognosis in PMF was by Vannucchi AM et al. [106] In this study which enrolled 374 MF patients from the Mayo Clinics, IDH1 mutations were identified in 10 (3%) patients and IDH2 mutations in 7 (2%). IDH mutations showed correlation with OS and leukemic transformation [106]. In the European Cohort of the same study, which included 483 MF patients, IDH1/IDH2 mutation was reported as 2.6%. Moreover, this study reported that IDH1/IDH2 mutations had no impact on OS but increased the risk of leukemic transformation [106]. It is known that IDH mutations may be accompanied by JAK2V617F, TET2 and MPL mutations. However, whether or not different IDH mutational variants portend different biological and prognostic features in MF is not yet clear.

2.17 SRSF2 Gene Mutations

Serine/arginine-rich splicing factor 2 (SRSF2) is located on chromosome 17q25.1. It plays an important role in splice-site selection, spliceosome assembly and both constitutive and alternative splicing [137]. In that study, the frequency of SRSF2 mutation in MDS patients was reported as 14.6% (34 in 233). SRSF2

mutation was found to be associated with older age and male gender. Also in the same study, SRSF2-mutated MDS patients showed inferior survival [138]. Mutations affecting mRNA splicing (SF3B1, U2AF1, SRSF2) are frequent in MDS (45-85%). SF3B1 mutations are highly recurrent in RARS and SRSF2 mutations show high frequency in CMML [139]. Lasho TL et al. [140] investigated the prevalence and prognostic significance of SRSF2 mutations in PMF. In that study, the prevalence of SRSF2 mutations was 17% in MF and they were associated with reduced OS and LFS. In patients with AML transformed from MPNs, the frequency of SRSF2 mutations was reported as 18.9%. This finding indicates that SRSF2 mutations contribute to pathogenesis of leukemic transformation and may guide novel therapeutic approaches for MPN patients undergoing leukemic transformations [141]. In the study by Vannucchi AM et al. [106] the frequency of SRSF2 mutations was reported as 8.5% in PMF. That study suggests that in PMF, the presence of mutations in SRSF2 is associated with worse survival and increased leukemic transformation.

3. CONCLUSION

JAK2, MPL and CALR are the driver mutations in pathogenesis of MPNs [4]. 2008 European Clinical, Molecular and Pathological (ECMP) criteria and 2014 WHO-CMP Diagnostic criteria distinguished five distinct clonal MPNs: JAK2V617F mutated-ET and PV; JAK2 exon 12 PV and the JAK2 wild-type ET and MF mutated with MPL or CALR, thus leaving a small group of triple negative group of MPN (Fig. 1) [4-8]. The diagnostic differentiation of JAK2, MPL and CALR-mutated MPNs should be made according to bone marrow morphology mainly based upon megakaryocyte morphology [4]. Bone marrow histological features of JAK2V617F-mutated ET and PV include medium sized to large (pleomorphic) megakaryocytes with only a few giant forms and increased cellularity (60%-90%) mainly due to increased erythropoiesis (Fig. 2). Bone marrow histology in MPL W515L/K-positive ET and PMF is characterized by clustered large, giant large and giant megakaryocytes with hyperlobulated stag-horn like nuclei in a normocellular bone marrow with no accompanying features of PV or primary megakaryocytic, granulocytic myeloproliferation (PMGM) (Fig. 3). Bone marrow histology in CALR-mutated ET and MF is featured by dense clusters of large dysmorphic immature megakaryocytes and bulky (cloud-like)

hyperchromatic nuclei, findings not demonstrated in JAK2V617F, JAK2 exon 12 and MPL-mutated MPN (Figs. 4 and 5).

In MPNs, somatic lesions have an impact on a wide variety of cellular functions, from cytokine signaling to histone modifications. JAK2 and MPL mutations are now regarded as highly specific gene mutations for MPN and were demonstrated to develop myeloproliferative phenotype in animals. Most other MPN-related genes are expressed in other myeloid malignancies suggesting that these genes are not specific to MPN. Further studies with mouse models expressing single or combinations of mutations will reveal how these mutations target hematopoietic stem cells and their effect on MPN development. Screening for CALR mutations is now also part of the diagnostic work-up of suspected JAK2 wild-type ET or MF. Taking into account the clonal evolution of the disease, it is evident that MPN has a very complex genotypical and phenotypical structure. Individualized diagnostic and therapeutic approaches are warranted to successfully treat this complex disease.

In JAK2, MPL and CALR-mutated MF patients, additional mutations in genes such as ASXL1, EZH2, IDH1/2 and SRSF2 were associated with a poorer survival and increased leukemic transformation [34,106,136,142]. In a study including 254 patients from the Mayo Clinic, triple-negative MF (JAK2, MPL and CALR-negative) was associated with inferior OS and

LFS [143]. The aforementioned study also showed the prognostically detrimental effect of CALR^{ASXL1} mutation status [143]. The prognostic advantage of CALR mutation, especially in the absence of a concomitant ASXL1 mutation was demonstrated in another large cohort of PMF patients (277 from Mayo Clinic and 293 from the University of Florence). [144] In that study, multivariable analysis showed CALR^{ASXL1} mutational status as the most significant risk factor for survival and was DIPSS-plus independent [144]. COMFORT-II study revealed that ruxolitinib (JAK1/JAK2 inhibitor) improved survival in MF patients harboring high-molecular-risk (ASXL1, EZH2, IDH1/2 and SRSF2) [145]. The high-molecular-risk mutations may guide novel therapeutic approaches for MF patients. Given the relative rarity of epigenetic mutations in PV and ET patients, larger sequencing studies with comprehensive mutational data are greatly needed. CALR mutations are associated with reduced thrombosis in ET [48,50]. This finding remains to be validated in future prospective clinical trials. The current era of epigenetic research is giving rise to the testing of many novel agents as part of clinical trials. CALR mutations in ET and MF result in a novel protein C terminus which holds the potential to be a target for development of tumor-specific therapy. Although certain mutations provide prognostic information in MF patients, several unresolved questions regarding the importance of these alterations as therapeutic targets still exist.

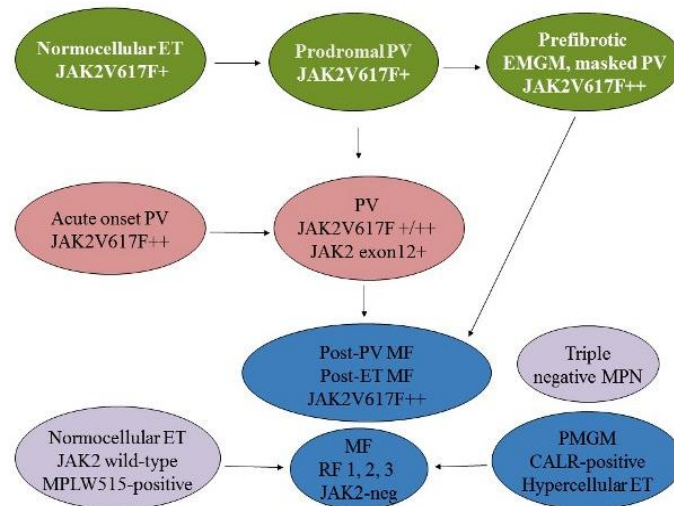


Fig. 1. WHO-CMP classification of five distinct clonal myeloproliferative Neoplasms (MPNs) and transitional states of JAK2V617F-mutated ET, prodromal PV, classical PV, post-ET and post-PV myelofibrosis and MPL and CALR-mutated ET and MF and triple negative MPN

Table 1. Mutations involved in the pathogenesis of Ph-negative MPN

Gene	Localization	Disease (frequency)	Prognostic significance	References
JAK2V617F	9p24	PV (>95%), ET (40%-60%), MF (50%)		[28,30]
JAK2 exon 12	9p24	PV (2.5-3.4%), JAK2V617F-negative PV (30%)		[31,32]
MPL	1p34	ET (0-6%), MF (0-10%)		[40-43]
CALR	19p13.2	ET (17.7%), MF (14.8%)	Mutations in CALR are associated with improved survival in MF and a reduced incidence of thrombosis in ET	[47,48,49,50,51]
CBL	11q23.3	PMF (6%), blastic phase MPN		[14,15]
CHEK2	22q12.1	ET (15.1%)		[60]
IKZF1	7p12	post-MPN AML (21%)		[16]
EZH2	7q35	PV (3%), MF (13%)	EZH2 mutations predict shorter LFS and OS in MF	[17,68]
RAS	NRAS 1p13.2	post-MPN AML (7-13%)		[73]
Tp53	17p13.1	post-MPN AML (27.3%)	Mutation in TP53 correlate with poorer outcome in post-MPN AML	[74]
RUNX1	21q22.3	post-MPN AML (27.7%)		[21]
TET2	4q24	PV (15%), ET (4%-11%), MF (19%), post-MPN AML (26.3%)		[86,87]
DNMT3a	2p23	PV (3%), PMF (4%)		[93]
ASXL1	20q11.21	PV (2-5%), ET (5-10%), MF (13-26%), post-PV/ET myelofibrosis (22-38.5%), post-MPN AML (19.3%)	Mutations in ASXL1 show correlation with short OS and leukemic transformation	[24,87,104,106]
IDH1	2q33.3	MF (3%), post-MPN AML (8%)	Mutations in IDH1 correlate with worse outcome in MF in terms of LFS and OS	[106,111,136]
IDH2	15q26.1	MF (2%), post-MPN AML (18%)	Mutations in IDH2 correlate with poorer LFS and OS	[106,111,136]
SRSF2	17q25.1	MF (8.5%-17%), post-MPN AML (18.9%)	Mutations in SRSF2 correlate with shorter LFS and OS and poorer outcome in MF and shorter OS in post-MPN AML	[106,140,141]
LNK	12q24	ET (5%), MF (5%), JAK2 mutation-negative erythrocytosis (25%), post-MPN AML (9.8%)		[55]

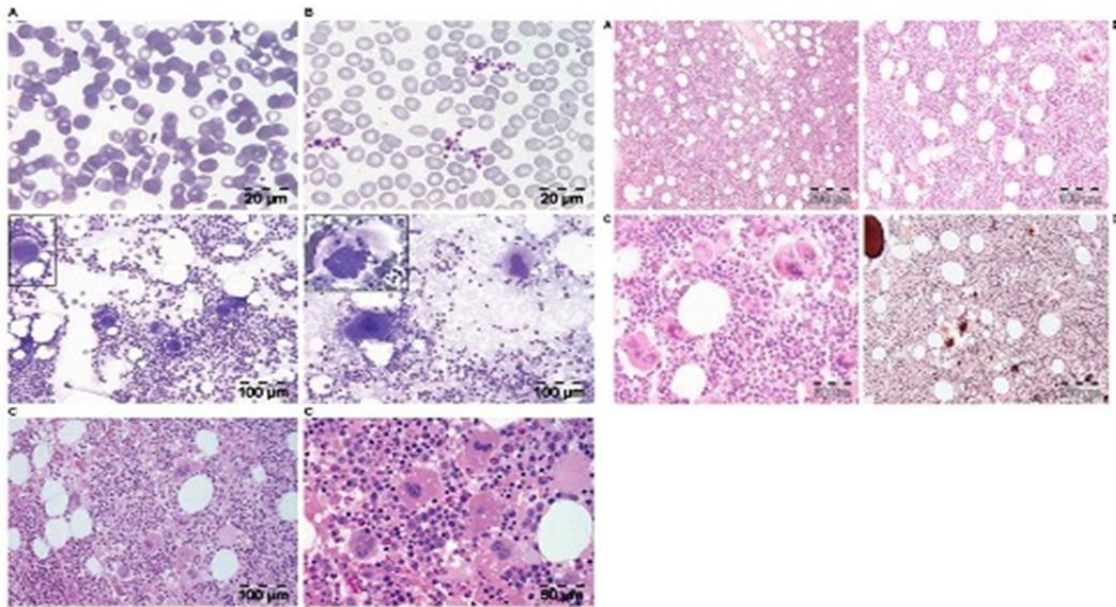


Fig. 2. Bone marrow findings of JAK2V617F heterozygous-positive ET for (left) and acute-onset JAK2V617F homozygous PV (right). Courtesy of Drs Michiels and De Raeye, University Hospital Antwerp, Belgium

Left. Appearance of normal platelets in a peripheral blood (a) and normal megakaryocytes in bone marrow (middle left). Presence of large platelets and increased number of platelet aggregates in peripheral blood (b) and large megakaryocytes with hyperlobulated nuclei in bone marrow of an ET patient (middle right). Bone marrow histology showing increased cellularity (c left), due to increased erythropoiesis and pronounced increase of large pleomorphic megakaryocytes with hyperlobulated nuclei without increase of bone marrow reticulin fibrosis (c right), diagnostic for JAK2V617F heterozygous-positive ET

Right. Hypercellular bone marrow due to increased megakaryopoiesis, erythropoiesis and granulopoiesis in JAK2V617F homozygous PV

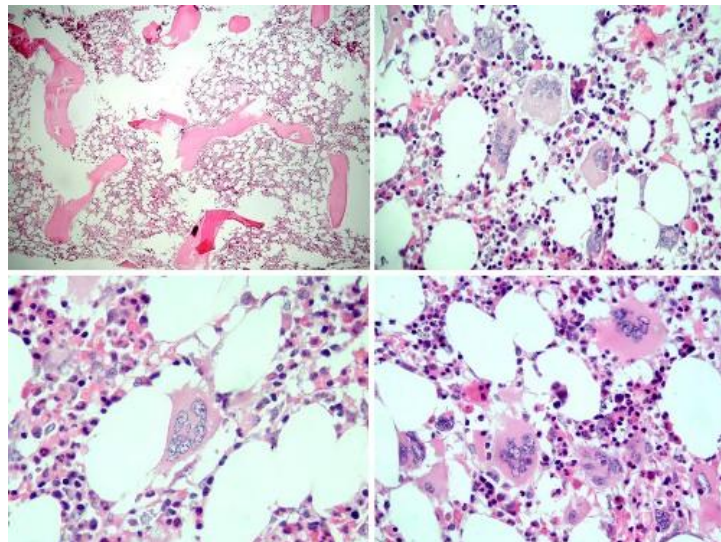


Fig. 3. Bone marrow histology in "True" ET consistent with WHO normocellular ET carrying MPLW515L mutation showing giant megakaryocytes with hyperlobulated stag-horn like nuclei characteristic for MPL W515L/K-positive ET. Courtesy of Dr Schwarz, University Hospital Prague, Czech Republic

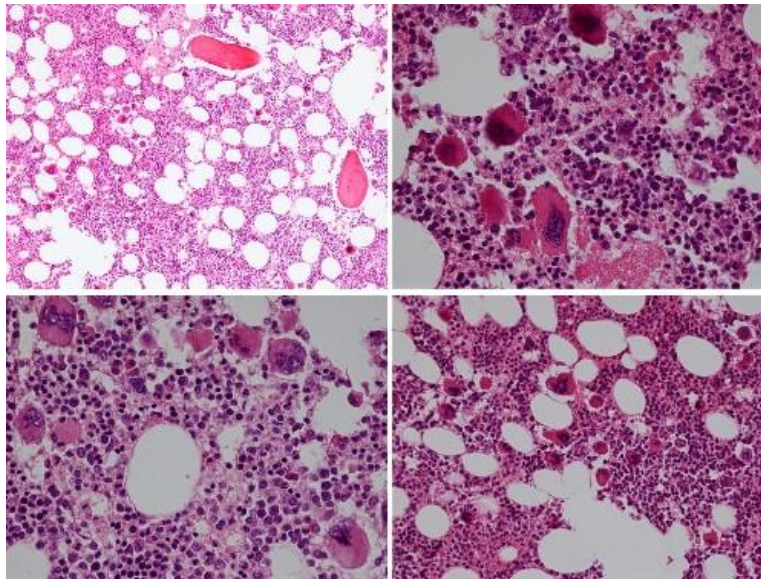


Fig. 4. Bone marrow histology in CALR-mutated ET showing hypercellular bone marrow with relative decrease of erythropoiesis, dense cluster of immature megakaryocytes with hypolobulated nuclei without increase of reticulin fibrosis, consistent with typical PMGM. Courtesy of Drs Valster and Schelfout, Lievensberg Hospital Bergen op Zoom, The Netherlands

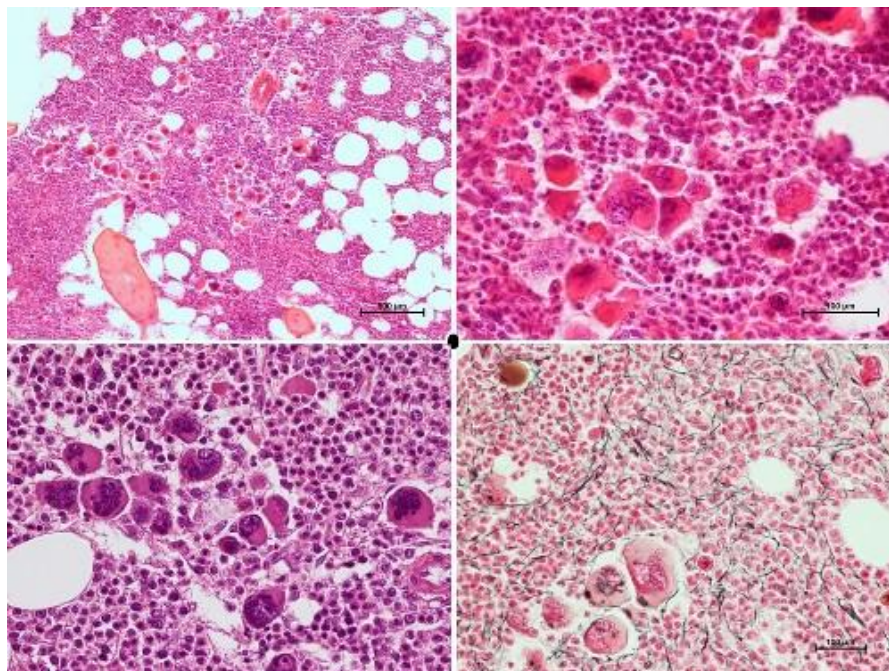


Fig. 5. Bone marrow findings of CALR-mutated MF. Hypercellular bone marrow with relative decrease of erythropoiesis, dense cluster of immature megakaryocytes with hypolobulated nuclei, and grade 2 reticulin fibrosis, consistent with bone marrow histological features similar to WHO-defined primary myelofibrosis (PMF), distinct from JAK2V617F-mutated ET and PV, and MPL- mutated ET. Courtesy of Drs Forstier and De Raeve, University Hospital, Brussels, Belgium

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009;114:937-51.
2. Dameshek W. Physiopathology and course of polycythemia vera as related to therapy. *J Am Med Assoc*. 1950;142(11):790-7.
3. James C, Ugo V, Le Couédic JP, Staerk J, Delhommeau F, Lacout C, Garçon L, Raslova H, Berger R, Bennaceur-Grisicelli A, Villeval JL, Constantinescu SN, Casadevall N, Vainchenker W. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005;434(7037):1144-8.
4. Michiels JJ, Forstier K, Valster F, Potters V, Schelfout K, De Raeve H. WHO Clinical Molecular and Pathological (WHO-CMP) Diagnostic Criteria for the Classification and Staging of Five Distinct JAK2, MPL and CALR Mutated Myeloproliferative Neoplasms. *J Hematol Thromb Dis*. 2014;2:172. DOI:10.4172/2329-8790.1000172
5. Michiels JJ, Berneman Z, Schroyens W, Hebeda K, Lam KH, Valster F, Potter V, Schelfout K, De Raeve H. The 2006 → 2008 European Clinical, Molecular and Pathobiological (2008 ECMP) Classification and the 2007 WHO Revised Criteria for Myeloproliferative Neoplasms. *J Hematol Thrombosis Dis*. 2015;3:1
6. Michiels JJ, Berneman Z, Schroyens W, De Raeve H. PVSG and WHO vs European Clinical, Molecular and Pathological Criteria for prefibrotic myeloproliferative neoplasms. *World J Hematol*. 2013;2(3):71-88.
7. Michiels JJ, Ten Kate F, Lam KH, Schroyens W, Berneman Z, De Raeve H. The European Clinical, Molecular, and Pathological (ECMP) Criteria and the 2007/2008 Revisions of the World Health Organization for the Diagnosis, Classification, and Staging of Prefibrotic Myeloproliferative Neoplasms Carrying the JAK2V617F Mutation. *Turk J Haematol*. 2014;31(3):239–254.
8. Michiels JJ, Berneman Z, Schroyens W, De Raeve H. Changing concepts of diagnostic criteria of myeloproliferative disorders and the molecular etiology and classification of myeloproliferative neoplasms: from Dameshek 1950 to Vainchenker 2005 and beyond. *Acta Haematol*. 2015;133(1):36-51. DOI: 10.1159/000358580.
9. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, Futreal PA, Erber WN, McMullin MF, Harrison CN, Warren AJ, Gilliland DG, Lodish HF, Green AR. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med*. 2007;356(5):459-68.
10. Pardanani A, Lasho TL, Finke C, Hanson CA, Tefferi A. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. *Leukemia*. 2007;21(9):1960-3.
11. Tefferi A, Lasho TL, Finke CM, Knudson RA, Ketterling R, Hanson CH, Maffioli M, Caramazza D, Passamonti F, Pardanani A. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. *Leukemia*. 2014;28(7):1472-7. DOI: 10.1038/leu.2014.3.
12. Nangalia J, Green TR. The evolving genomic landscape of myeloproliferative neoplasms. *Hematology Am Soc Hematol Educ Program*. 2014;2014(1):287-96. DOI: 10.1182/asheducation-2014.1.287.
13. Gery S, Gueller S, Chumakova K, Kawamata N, Liu L, Koeffler HP. Adaptor protein Lnk negatively regulates the mutant MPL, MPLW515L associated with myeloproliferative disorders. *Blood*. 2007;110:3360-4.
14. Grand FH, Hidalgo-Curtis CE, Ernst T, Zoi K, Zoi C, McGuire C, et al. Frequent CBL

- mutations associated with 11q acquired uniparental disomy in myeloproliferative neoplasms. *Blood*. 2009;113:6182-92.
15. Bacher U, Haferlach C, Schnittger S, Kohlmann A, Kern W, Haferlach T. Mutations of the TET2 and CBL genes: novel molecular markers in myeloid malignancies. *Ann Hematol*. 2010;89:643-52.
 16. Jäger R, Gisslinger H, Passamonti F, Rumi E, Berg T, Gisslinger B, et al. Deletions of the transcription factor Ikaros in myeloproliferative neoplasms. *Leukemia*. 2010;24:1290-8.
 17. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet*. 2010;42:722-6.
 18. Ayllon V, Rebollo A. Ras-induced cellular events. *Mol Membr Biol*. 2000;17:65-73.
 19. Scheele JS, Ripple D, Lubbert M. The role of ras and other low molecular weight guanine nucleotide (GTP)-binding proteins during hematopoietic cell differentiation. *Cell Mol Life Sci*. 2000;57:1950-63.
 20. Shounan Y, MacKenzie K, Dolnikov A, Miller M, Symonds G. Myeloproliferative disease and myelodysplastic syndrome induced by transplantation of bone marrow cells expressing mutant p53. *Leukemia*. 1997;11:1641-9.
 21. Ding Y, Harada Y, Imagawa J, Kimura A, Harada H. AML1/RUNX1 point mutation possibly promotes leukemic transformation in myeloproliferative neoplasms. *Blood*. 2009;114:5201-5.
 22. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Massé A, et al. Mutation in TET2 in myeloid cancers. *N Engl J Med*. 2009;360:2289-301.
 23. Abdel-Wahab O, Pardanani A, Rampal R, Lasho TL, Levine RL, Tefferi A. DNMT3A mutational analysis in primary myelofibrosis, chronic myelomonocytic leukemia and advanced phases of myeloproliferative neoplasms. *Leukemia*. 2011;25:1219-20.
 24. Carbuccia N, Murati A, Trouplin V, Brecqueville M, Adélaïde J, Rey J, et al. Mutations of ASXL1 gene in myeloproliferative neoplasms. *Leukemia*. 2009;23:2183-6.
 25. Pardanani A, Lasho TL, Finke CM, Mai M, McClure RF, Tefferi A. IDH1 and IDH2 mutation analysis in chronic- and blast-phase myeloproliferative neoplasms. *Leukemia*. 2010;24(6):1146-1151.
 26. Stegelmann F, Bullinger L, Schlenk RF, et al. DNMT3A mutations in myeloproliferative neoplasms. *Leukemia*. 2011;25(7):1217-1219.
 27. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*. 2005;352:1779-90.
 28. Levine RL, Wernig G. Role of JAK-STAT signaling in the pathogenesis of myeloproliferative disorders. *Hematology Am Soc Hematol Educ Program*. 2006;233-9.
 29. Lambert JR, Everington T, Linch DC, Gale RE. In essential thrombocythemia, multiple JAK2-V617F clones are present in most mutant-positive patients: a new disease paradigm. *Blood*. 2009;114:3018-23.
 30. Tefferi A, Lasho TL, Huang J, Finke C, Mesa RA, Li CY, et al. Low JAK2V617F allele burden in primary myelofibrosis, compared to either a higher allele burden or unmutated status, is associated with inferior overall and leukemia-free survival. *Leukemia*. 2008;22:756-61.
 31. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med*. 2007;356:459-68.
 32. Pietra D, Li S, Brisci A, Passamonti F, Rumi E, Theocharides A, et al. Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. *Blood*. 2008;111:1686-9.
 33. Passamonti F, Elena C, Schnittger S, Skoda RC, Green AR, Girodon F, et al. Molecular and clinical features of the myeloproliferative neoplasm associated with JAK2 exon 12 mutations. *Blood*. 2011;117:2813-6.
 34. Rumi E, Passamonti F, Pietra D, Della Porta MG, Arcaini L, Boggi S, Elena C, Boveri E, Pascutto C, Lazzarino M, Cazzola M. JAK2 (V617F) as an acquired somatic mutation and a secondary genetic event associated with disease progression in familial myeloproliferative disorders. *Cancer*. 2006;107(9):2206-11.
 35. Rumi E, Passamonti F, Della Porta MG, Elena C, Arcaini L, Vanelli L, Del Curto C,

- Pietra D, Boveri E, Pascutto C, Cazzola M, Lazzarino M. Familial chronic myeloproliferative disorders: clinical phenotype and evidence of disease anticipation. *J Clin Oncol.* 2007;25(35): 5630-5.
36. Pardanani A, Lasho TL, Finke C, Hanson CA, Tefferi A. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. *Leukemia.* 2007;21(9): 1960-3.
 37. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med.* 2006;3:e270.
 38. Beer PA, Campbell PJ, Scott LM, Bench AJ, Erber WN, Bareford D, et al. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. *Blood.* 2008;112:141-9.
 39. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood.* 2006;108:3472-6.
 40. Lieu CH, Shen YJ, Lai WC, Tsai WH, Hsu HC. Prevalence of MPL W515L/K mutations in Taiwanese patients with Philadelphia-negative chronic myeloproliferative neoplasms. *J Chin Med Assoc.* 2010;73:530-2.
 41. Ruan GR, Jiang B, Li LD, Niu JH, Li JL, Xie M, et al. MPL W515L/K mutations in 343 Chinese adults with JAK2V617F mutation-negative chronic myeloproliferative disorders detected by a newly developed RQ-PCR based on TaqMan MGB probes. *Hematol Oncol.* 2010;28:33-9.
 42. Ma W, Zhang X, Wang X, Zhang Z, Yeh CH, Uyeji J, et al. MPL mutation profile in JAK-2 mutation-negative patients with myeloproliferative disorders. *Diagn Mol Pathol.* 2011;20:34-9.
 43. Schnittger S, Bacher U, Haferlach C, Beelen D, Bojko P, Bürkle D, et al. Characterization of 35 new cases with four different MPL W515 mutations and essential thrombocytosis or primary myelofibrosis. *Haematologica.* 2009;94: 141-4.
 44. Vannucchi AM, Antonioli E, Guglielmelli P, Pancrazzi A, Guerini V, Barosi G, Ruggeri M, Specchia G, Lo-Coco F, Delaini F, Villani L, Finotto S, Ammatuna E, Alterini R, Carrai V, Capaccioli G, Di Lollo S, Liso V, Rambaldi A, Bosi A, Barbui T. Characteristics and clinical correlates of MPL 515W>L/K mutation in essential thrombocythemia. *Blood.* 2008;112(3):844-7.
DOI: 10.1182/blood-2008-01-135897.
Epub 2008 Jun 2.
 45. Gold LI, Eggleton P, Sweetwyne MT, Van Duyn LB, Greives MR, Naylor SM, et al. Calreticulin: Non-endoplasmic reticulum functions in physiology and disease. *FASEB J.* 2010;24:665-83.
 46. Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundle G, Wedge DC, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med.* 2013;369:2391-2405.
 47. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med.* 2013;369:2379-90.
 48. Rumi E, Pietra D, Ferretti V, Klampfl T, Harutyunyan AS, Milosevic JD, et al; Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative Investigators. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. *Blood.* 2014;123:1544-51.
 49. Kim SY, Im K, Park SN, Kwon J, Kim JA, Lee DS. CALR, JAK2, and MPL Mutation Profiles in Patients With Four Different Subtypes of Myeloproliferative Neoplasms: Primary Myelofibrosis, Essential Thrombocythemia, Polycythemia Vera, and Myeloproliferative Neoplasm, Unclassifiable. *Am J Clin Pathol.* 2015;143(5):635-44.
 50. Rotunno G, Mannarelli C, Guglielmelli P, et al. Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocythemia. *Blood.* 2014;123(10):1552-1555.
 51. Tefferi A, Lasho TL, Finke CM, et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. *Leukemia.* 2014; 28(7):1472-1477.
 52. Tong W, Zhang J, Lodish HF. Lnk inhibits erythropoiesis and Epo-dependent JAK2

- activation and downstream signaling pathways. *Blood*. 2005;105:4604-12.
53. Gery S, Cao Q, Gueller S, Xing H, Tefferi A, Koefler HP. Lnk inhibits myeloproliferative disorder-associated JAK2 mutant, JAK2V617F. *J Leukoc Biol*. 2009;85:957-65.
54. Oh ST, Simonds EF, Jones C, et al. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. *Blood*. 2010;116(6):988-992.
55. Pardanani A, Lasho T, Finke C, Oh ST, Gotlib J, Tefferi A. LNK mutation studies in blast-phase myeloproliferative neoplasms, and in chronic-phase disease with TET2, IDH, JAK2 or MPL mutations. *Leukemia*. 2010;24:1713-8.
56. Gery S, Gueller S, Nowak V, Sohn J, Hofmann WK, Koefler HP. Expression of the adaptor protein Lnk in leukemia cells. *Exp Hematol*. 2009;37:585-92.e2.
57. Fu JF, Hsu JJ, Tang TC, Shih LY. Identification of CBL, a proto-oncogene at 11q23.3, as a novel MLL fusion partner in a patient with de novo acute myeloid leukemia. *Genes Chromosomes Cancer*. 2003;37:214-9.
58. Rathinam C, Thien CB, Flavell RA, Langdon WY. Myeloid leukemia development in c-Cbl RING finger mutant mice is dependent on FLT3 signaling. *Cancer Cell*. 2010;18(4):341-352.
59. Muramatsu H, Makishima H, Jankowska AM, Cazzolli H, O'Keefe C, Yoshida N, Xu Y, Nishio N, Hama A, Yagasaki H, Takahashi Y, Kato K, Manabe A, Kojima S, Maciejewski JP. Mutations of an E3 ubiquitin ligase c-Cbl but not TET2 mutations are pathogenic in juvenile myelomonocytic leukemia. *Blood*. 2010;115(10):1969-75.
DOI: 10.1182/blood-2009-06-226340.
60. Janiszewska H, Bak A, Pilarska M, Heise M, Junkiert-Czarnecka A, Kuliszkievicz-Janus M, et al. A risk of essential thrombocythemia in carriers of constitutional CHEK2 gene mutations. *Haematologica*. 2012;97:366-70.
61. Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, et al. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. *Science*. 1999;286:2528-31.
62. Rudd MF, Sellick GS, Webb EL, Catovsky D, Houlston RS. Variants in the ATM-BRCA2-CHEK2 axis predispose to chronic lymphocytic leukemia. *Blood*. 2006;108:638-44.
63. Hangaishi A, Ogawa S, Qiao Y, Wang L, Hosoya N, Yuji K, et al. Mutations of Chk2 in primary hematopoietic neoplasms. *Blood*. 2002;99:3075-7.
64. Sellick GS, Sullivan K, Catovsky D, Houlston RS. CHEK2*1100delC and risk of chronic lymphocytic leukemia. *Leuk Lymphoma*. 2006;47:2659-60.
65. Aktas D, Arno MJ, Rassool F, Mufti GJ. Analysis of CHK2 in patients with myelodysplastic syndromes. *Leuk Res*. 2002;26:985-7.
66. Schuettengruber B, Chourrout D, Vervoort M, Leblanc B, Cavalli G. Genome regulation by polycomb and trithorax proteins. *Cell*. 2007;128:735-45.
67. Kleer CG. Carcinoma of the breast with medullary-like features: diagnostic challenges and relationship with BRCA1 and EZH2 functions. *Arch Pathol Lab Med*. 2009;133:1822-5.
68. Guglielmelli P, Biamonte F, Score J, et al. EZH2 mutational status predicts poor survival in myelofibrosis. *Blood*. 2011;118(19):5227-5234.
69. Konstantinopoulos PA, Karamouzis MV, Papavassiliou AG. Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. *Nat Rev Drug Discov*. 2007;6:541-55.
70. Parikh C, Subrahmanyam R, Ren R. Oncogenic NRAS rapidly and efficiently induces CMML- and AML-like diseases in mice. *Blood*. 2006;108:2349-57.
71. Wang J, Liu Y, Li Z, Du J, Ryu MJ, Taylor PR, et al. Endogenous oncogenic Nras mutation promotes aberrant GM-CSF signaling in granulocytic/monocytic precursors in a murine model of chronic myelomonocytic leukemia. *Blood*. 2010;116:5991-6002.
72. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene*. 2007;26:3291-310.
73. Beer PA, Delhommeau F, LeCouedic JP, et al. Two routes to leukemic transformation after a JAK2 mutation-

- positive myeloproliferative neoplasm. *Blood*. 2010;115(14):2891-2900.
74. Harutyunyan A, Klampfl T, Cazzola M, Kralovics R. p53 lesions in leukemic transformation. *N Engl J Med*. 2011;364:488-90.
 75. Osato M, Asou N, Abdalla E, Hoshino K, Yamasaki H, Okubo T, et al. Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2alphaB gene associated with myeloblastic leukemias. *Blood*. 1999;93:1817-24.
 76. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science*. 2009;324:930-5.
 77. Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature*. 2010;466:1129-33.
 78. Ficiz G, Branco MR, Seisenberger S, Santos F, Krueger F, Hore TA, et al. Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. *Nature*. 2011;473:398-402.
 79. Tefferi A, Lim KH, Abdel-Wahab O, Lasho TL, Patel J, Patnaik MM, et al. Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML. *Leukemia*. 2009;23:1343-5.
 80. Langemeijer SM, Kuiper RP, Berends M, Knops R, Aslanyan MG, Massop M, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. *Nat Genet*. 2009;41:838-42.
 81. Jankowska AM, Szpurka H, Tiu RV, Makishima H, Aftab M, Huh J, O'Keefe CL, et al. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. *Blood*. 2009;113:6403-10.
 82. Abdel-Wahab O, Mullally A, Hedvat C, Garcia-Manero G, Patel J, Wadleigh M, et al. Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. *Blood*. 2009;114:144-7.
 83. Pronier E, Almire C, Mokrani H, Vasanthakumar A, Simon A, da Costa Reis Monte Mor B, et al. Inhibition of TET2-mediated conversion of 5-methylcytosine to 5-hydroxymethylcytosine disturbs erythroid and granulomonocytic differentiation of human hematopoietic progenitors. *Blood*. 2011;118:2551-5.
 84. Schaub FX, Looser R, Li S, Hao-Shen H, Lehmann T, Tichelli A, et al. Clonal analysis of TET2 and JAK2 mutations suggests that TET2 can be a late event in the progression of myeloproliferative neoplasms. *Blood*. 2010;115:2003-2007. DOI: 10.1182/blood-2009-2009-245381.
 85. Tefferi A. Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, CBL, IDH and IKZF1. *Leukemia*. 2011;24(6):1128-38. DOI: 10.1038/leu.2010.69. Epub 2010 Apr 29.
 86. Pronier E, Delhommeau F. Role of TET2 mutations in myeloproliferative neoplasms. *Curr Hematol Malig Rep*. 2012;7:57-64.
 87. Abdel-Wahab O, Manshouri T, Patel J, Harris K, Yao J, Hedvat C, et al. Genetic analysis of transforming events that convert chronic myeloproliferative neoplasms to leukemias. *Cancer Res*. 2010;70:447-52.
 88. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, et al. DNMT3A mutations in acute myeloid leukemia. *N Engl J Med*. 2010;363:2424-33.
 89. Yamashita Y, Yuan J, Suetake I, Suzuki H, Ishikawa Y, Choi YL, et al. Array-based genomic resequencing of human leukemia. *Oncogene*. 2010;29:3723-31.
 90. Yan XJ, Xu J, Gu ZH, Pan CM, Lu G, Shen Y, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. *Nat Genet*. 2011;43:309-15.
 91. Ewalt M, Galili NG, Mumtaz M, Churchill M, Rivera S, Borot F, et al. DNMT3a mutations in high-risk myelodysplastic syndrome parallel those found in acute myeloid leukemia. *Blood Cancer J*. 2011;1:e9.
 92. Walter MJ, Ding L, Shen D, Shao J, Grillo M, McLellan M, et al. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. *Leukemia*. 2011;25:1153-8.
 93. Brecqueville M, Cervera N, Gelsi-Boyer V, Murati A, Adélaïde J, Chaffanet M, et al. Rare mutations in DNMT3A in myeloproliferative neoplasms and

- myelodysplastic syndromes. *Blood Cancer J.* 2011;1:e18.
94. Gaebler C, Stanzl-Tschegg S, Heinze G, Holper B, Milne T, Berger G, et al. Fatigue strength of locking screws and prototypes used in small-diameter tibial nails: a biomechanical study. *J Trauma.* 1999;47: 379-84.
 95. Sinclair DA, Milne TA, Hodgson JW, Shellard J, Salinas CA, Kyba M, et al. The Additional sex combs gene of *Drosophila* encodes a chromatin protein that binds to shared and unique Polycomb group sites on polytene chromosomes. *Development.* 1998;125:1207-16.
 96. Fisher CL, Lee I, Bloyer S, Bozza S, Chevalier J, Dahl A, et al. Additional sex combs-like 1 belongs to the enhancer of trithorax and polycomb group and genetically interacts with Cbx2 in mice. *Dev Biol.* 2010;337:9-15.
 97. Park UH, Yoon SK, Park T, Kim EJ, Um SJ. Additional sex comb-like (ASXL) proteins 1 and 2 play opposite roles in adipogenesis via reciprocal regulation of peroxisome proliferator-activated receptor $\{\gamma\}$. *J Biol Chem.* 2011;286:1354-63.
 98. Fisher CL, Pineault N, Brookes C, Helgason CD, Ohta H, Bodner C, et al. Loss-of-function Additional sex combs like 1 mutations disrupt hematopoiesis but do not cause severe myelodysplasia or leukemia. *Blood.* 2010;115:38-46.
 99. Aravind L, Iyer LM. The HARE-HTH and associated domains: novel modules in the coordination of epigenetic DNA and protein modifications. *Cell Cycle.* 2012;11:119-31.
 100. Scheuermann JC, de Ayala Alonso AG, Oktaba K, Ly-Hartig N, McGinty RK, Fraterman S, et al. Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. *Nature.* 2010;465:243-7.
 101. Gelsi-Boyer V, Trouplin V, Adélaïde J, Bonansea J, Cervera N, Carbuccia N, et al. Mutations of polycomb-associated gene ASXL1 in myelodysplastic syndromes and chronic myelomonocytic leukaemia. *Br J Haematol.* 2009;145:788-800.
 102. Carbuccia N, Trouplin V, Gelsi-Boyer V, Murati A, Rocquain J, Adélaïde J, et al. Mutual exclusion of ASXL1 and NPM1 mutations in a series of acute myeloid leukemias. *Leukemia.* 2010;24:469-73.
 103. Boulwood J, Perry J, Pellagatti A, Fernandez-Mercado M, Fernandez-Santamaria C, Calasanz MJ, et al. Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia. *Leukemia.* 2010;24:1062-5.
 104. Abdel-Wahab O, Pardanani A, Patel J, Wadleigh M, Lasho T, Heguy A, et al. Concomitant analysis of EZH2 and ASXL1 mutations in myelofibrosis, chronic myelomonocytic leukemia and blast-phase myeloproliferative neoplasms. *Leukemia.* 2011;25:1200-2.
 105. Brequeville M, Rey J, Bertucci F, Coppin E, Finetti P, Carbuccia N, et al. Mutation analysis of ASXL1, CBL, DNMT3A, IDH1, IDH2, JAK2, MPL, NF1, SF3B1, SUZ12, and TET2 in myeloproliferative neoplasms. *Genes Chromosomes Cancer.* 2012;51: 743-55.
 106. Vannucchi AM, Lasho TL, Guglielmelli P, Biamonte F, Pardanani A, Pereira A, et al. Mutations and prognosis in primary myelofibrosis. *Leukemia.* 2013;27:1861-9.
 107. Tefferi A, Guglielmelli P, Lasho TL, Rotunno G, Finke C, Mannarelli C, Belachew AA, Pancrazzi A, Wassie EA, Ketterling RP, Hanson CA, Pardanani A, Vannucchi AM. CALR and ASXL1 mutations-based molecular prognostication in primary myelofibrosis: an international study of 570 patients. *Leukemia.* 2014 Jul;28(7):1494-500.
DOI: 10.1038/leu.2014.57. Epub 2014 Feb 5.
 108. Shen H, Chao H, Ding Z, Feng Y, Cen J, Pan J, He J, Zhou M, Chen Z, Chen S. CALR and ASXL1 mutation analysis in 190 patients with essential thrombocythemia. *Leuk Lymphoma.* 2015 Mar;56(3):820-2.
DOI: 10.3109/10428194.2014.939963. Epub 2014 Aug 13.
 109. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med.* 2009;360:765-73.
 110. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med.* 2009;361:1058-66.
 111. Tefferi A, Lasho TL, Abdel-Wahab O, Guglielmelli P, Patel J, Caramazza D, et al.

- IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis. *Leukemia*. 2010;24:1302-9.
112. Paschka P, Schlenk RF, Gaidzik VI, Habdank M, Krönke J, Bullinger L, et al. IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. *J Clin Oncol*. 2010;28:3636-43.
 113. Thol F, Weissinger EM, Krauter J, Wagner K, Damm F, Wichmann M, et al. IDH1 mutations in patients with myelodysplastic syndromes are associated with an unfavorable prognosis. *Haematologica*. 2010;95:1668-74.
 114. Kosmider O, Gelsi-Boyer V, Slama L, Dreyfus F, Beyne-Rauzy O, Quesnel B, et al. Mutations of IDH1 and IDH2 genes in early and accelerated phases of myelodysplastic syndromes and MDS/myeloproliferative neoplasms. *Leukemia*. 2010;24:1094-6.
 115. Plaut GW, Cook M, Aogaichi T. The subcellular location of isozymes of NADP-isocitrate dehydrogenase in tissues from pig, ox and rat. *Biochim Biophys Acta*. 1983;760:300-8.
 116. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2010;465:966.
 117. Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Collier HA, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell*. 2010;17:225-34.
 118. Bejar R, Levine R, Ebert BL. Unraveling the molecular pathophysiology of myelodysplastic syndromes. *J Clin Oncol*. 2011;29:504-15.
 119. Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, et al. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. *Science*. 2009;324:261-5.
 120. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2009;462:739-44.
 121. Gross S, Cairns RA, Minden MD, Driggers EM, Bittinger MA, Jang HG, et al. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. *J Exp Med*. 2010;207:339-44.
 122. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell*. 2010;18:553-67.
 123. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science*. 2008;321:1807-12.
 124. Chou WC, Lei WC, Ko BS, Hou HA, Chen CY, Tang JL, et al. The prognostic impact and stability of Isocitrate dehydrogenase 2 mutation in adult patients with acute myeloid leukemia. *Leukemia*. 2011;25:246-53.
 125. Boissel N, Nibourel O, Renneville A, Gardin C, Reman O, Contentin N, et al. Prognostic impact of isocitrate dehydrogenase enzyme isoforms 1 and 2 mutations in acute myeloid leukemia: a study by the Acute Leukemia French Association group. *J Clin Oncol*. 2010;28:3717-23.
 126. Abbas S, Lugthart S, Kavelaars FG, Schelen A, Koenders JE, Zeilemaker A, et al. Acquired mutations in the genes encoding IDH1 and IDH2 both are recurrent aberrations in acute myeloid leukemia: prevalence and prognostic value. *Blood*. 2010;116:2122-6.
 127. Pardanani A, Patnaik MM, Lasho TL, Mai M, Knudson RA, Finke C, et al. Recurrent IDH mutations in high-risk myelodysplastic syndrome or acute myeloid leukemia with isolated del(5q). *Leukemia*. 2010;24:1370-2.
 128. Soverini S, Score J, Iacobucci I, Poerio A, Lonetti A, Gnani A, et al. IDH2 somatic mutations in chronic myeloid leukemia patients in blast crisis. *Leukemia*. 2011;25:178-81.
 129. Caramazza D, Lasho TL, Finke CM, Gangat N, Dingli D, Knudson RA, et al.

- IDH mutations and trisomy 8 in myelodysplastic syndromes and acute myeloid leukemia. *Leukemia*. 2010;24:2120-2.
130. Thol F, Damm F, Wagner K, Göhring G, Schlegelberger B, Hoelzer D, et al. Prognostic impact of IDH2 mutations in cytogenetically normal acute myeloid leukemia. *Blood*. 2010;116:614-6.
 131. Schnittger S, Haferlach C, Ulke M, Alpermann T, Kern W, Haferlach T. IDH1 mutations are detected in 6.6% of 1414 AML patients and are associated with intermediate risk karyotype and unfavorable prognosis in adults younger than 60 years and unmutated NPM1 status. *Blood*. 2010;116:5486-96.
 132. Green CL, Evans CM, Hills RK, Burnett AK, Linch DC, Gale RE. The prognostic significance of IDH1 mutations in younger adult patients with acute myeloid leukemia is dependent on FLT3/ITD status. *Blood*. 2010;116:2779-82.
 133. Marcucci G, Maharry K, Wu YZ, Radmacher MD, Mrózek K, Margeson D, et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol*. 2010;28:2348-55.
 134. Wagner K, Damm F, Göhring G, Görlich K, Heuser M, Schäfer I, et al. Impact of IDH1 R132 mutations and an IDH1 single nucleotide polymorphism in cytogenetically normal acute myeloid leukemia: SNP rs11554137 is an adverse prognostic factor. *J Clin Oncol*. 2010;28:2356-64.
 135. Green CL, Evans CM, Zhao L, Hills RK, Burnett AK, Linch DC, et al. The prognostic significance of IDH2 mutations in AML depends on the location of the mutation. *Blood*. 2011;118:409-12.
 136. Tefferi A, Jimma T, Sulai NH, Lasho TL, Finke CM, Knudson RA, et al. IDH mutations in primary myelofibrosis predict leukemic transformation and shortened survival: clinical evidence for leukemogenic collaboration with JAK2V617F. *Leukemia*. 2012;26:475-80.
 137. Long JC, Caceres JF. The SR protein family of splicing factors: master regulators of gene expression. *Biochem J*. 2009;417(1):15-27.
 138. Wu SJ, Kuo YY, Hou HA, Li LY, Tseng MH, Huang CF, Lee FY, Liu MC, Liu CW, Lin CT, Chen CY, Chou WC, Yao M, Huang SY, Ko BS, Tang JL, Tsay W, Tien HF. The clinical implication of SRSF2 mutation in patients with myelodysplastic syndrome and its stability during disease evolution. *Blood*. 2012;120(15):3106-11. DOI: 10.1182/blood-2012-02-412296.
 139. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, Sato Y, Sato-Otsubo A, Kon A, Nagasaki M, Chalkidis G, Suzuki Y, Shiosaka M, Kawahata R, Yamaguchi T, Otsu M, Obara N, Sakata-Yanagimoto M, Ishiyama K, Mori H, Nolte F, Hofmann WK, Miyawaki S, Sugano S, Haferlach C, Koeffler HP, Shih LY, Haferlach T, Chiba S, Nakauchi H, Miyano S, Ogawa S. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature*. 2011;478(7367):64-9. DOI: 10.1038/nature10496.
 140. Lasho TL, Jimma T, Finke CM, Patnaik M, Hanson CA, Ketterling RP, Pardanani A, Tefferi A. SRSF2 mutations in primary myelofibrosis: significant clustering with IDH mutations and independent association with inferior overall and leukemia-free survival. *Blood*. 2012;120(20):4168-71. DOI: 10.1182/blood-2012-05-429696.
 141. Zhang SJ, Rampal R, Manshouri T, Patel J, Mensah N, Kayserian A, Hricik T, Heguy A, Hedvat C, Gönen M, Kantarjian H, Levine RL, Abdel-Wahab O, Verstovsek S. Genetic analysis of patients with leukemic transformation of myeloproliferative neoplasms shows recurrent SRSF2 mutations that are associated with adverse outcome. *Blood*. 2012;119(19):4480-5. DOI: 10.1182/blood-2011-11-390252.
 142. Hobbs GS, Rampal RK. Clinical and molecular genetic characterization of myelofibrosis. *Curr Opin Hematol*. 2015;22(2):177-83. DOI: 10.1097/MOH.000000000000122.
 143. Tefferi A, Lasho TL, Finke CM, Knudson RA, Ketterling R, Hanson CH, Maffioli M, Caramazza D, Passamonti F, Pardanani A. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. *Leukemia*. 2014;28(7):1472-7. DOI: 10.1038/leu.2014.3.
 144. Tefferi A, Guglielmelli P, Lasho TL, Rotunno G, Finke C, Mannarelli C, Belachew AA, Pancrazzi A, Wassie EA,

- Ketterling RP, Hanson CA, Pardanani A, Vannucchi AM. CALR and ASXL1 mutations-based molecular prognostication in primary myelofibrosis: an international study of 570 patients. *Leukemia*. 2014;28(7):1494-500.
DOI: 10.1038/leu.2014.57.
145. Guglielmelli P, Biamonte F, Rotunno G, Artusi V, Artuso L, Bernardis I, Tenedini E, Pieri L, Paoli C, Mannarelli C, Fjerza R, Rumi E, Stalbovskaya V, Squires M, Cazzola M, Manfredini R, Harrison C, Tagliafico E, Vannucchi AM; COMFORT-II Investigators; Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative (AGIMM) Investigators. Impact of mutational status on outcomes in myelofibrosis patients treated with ruxolitinib in the COMFORT-II study. *Blood*. 2014;123(14):2157-60.
DOI: 10.1182/blood-2013-11-536557.

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