Myeloproliferative neoplasms (MPNs) originate from genetically transformed hematopoietic stem cells that retain the capacity for multilineage differentiation and effective myelopoiesis. Beginning in early 2005, a number of novel mutations involving Janus kinase 2 (JAK2), Myeloproliferative Leukemia Virus (MPL), TET oncogene family member 2 (TET2), Additional Sex Combs-Like 1 (ASXL1), Casitas B-lineage lymphoma proto-oncogene (CBL), Isocitrate dehydrogenase (IDH) and IKAROS family zinc finger 1 (IKZF1) have been described in BCR-ABL1-negative MPNs. However, none of these mutations were MPN specific, displayed mutual exclusivity or could be traced back to a common ancestral clone. JAK2 and MPL mutations appear to exert a phenotype-modifying effect and are distinctly associated with polycythemia vera, essential thrombocythemia and primary myelofibrosis; the corresponding mutational frequencies are ~99, 55 and 65% for JAK2 and 0, 3 and 10% for MPL mutations. The incidence of TET2, ASXL1, CBL, IDH or IKZF1 mutations in these disorders ranges from 0 to 17%; these latter mutations are more common in chronic (TET2, ASXL1, CBL) or juvenile (CBL) myelomonocytic leukemias, mastocytosis (TET2), myelodysplastic syndromes (TET2, ASXL1) and secondary acute myeloid leukemia, including blast-phase MPN (IDH, ASXL1, IKZF1). The functional consequences of MPN-associated mutations include unregulated JAK-STAT (Janus kinase/signal transducer and activator of transcription) signaling, epigenetic modulation of transcription and abnormal accumulation of oncoproteins. However, it is not clear as to whether and how these abnormalities contribute to disease initiation, clonal evolution or blast transformation.

JAK2 mutations

JAK2 is located on chromosome 9p24 and includes 25 exons and its protein 1132 amino acids. JAK2 is one of the four Janus family nonreceptor protein tyrosine kinases; JAK1, JAK2 and TYK2 are ubiquitously expressed in mammalian cells, whereas JAK3 expression is limited to hematopoietic cells. Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling is important for a wide spectrum of cellular processes, including proliferation, survival or normal functioning of hematopoietic, immune, cardiac and other cells. JAKs transduce signals from their cognate type I and type II nonkinase cytokine receptors. Selective association of a JAK family member with specific cytokines or growth factors might explain some of the differences in therapeutic and side-effect profiles among drugs that primarily target JAK1, JAK2, JAK3 or multiple JAKs (Figure 2).

JAK2V617F

Oncogenic JAK1, JAK2 and JAK3 mutations have been associated with both lymphoid and myeloid neoplasms. Of particular relevance to MPN, JAK2V617F was discovered in 2004 and the first reports appeared in early 2005. JAK2V617F is by far the most prevalent mutation in BCR-ABL1-negative MPN (occurs in ~95% of patients with PV, in ~55% with ET and in ~65% with PMF), but it is also seen in some patients with myelodysplastic syndrome (MDS)/MPN (for example, refractory anemia with ring sideroblasts and thrombocytosis and rarely, in primary acute myeloid leukemia (AML), MDS or CML). However, this should not undermine

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**Introduction**

The WHO (World Health Organization) classification system for hematological malignancies includes eight clinicopathological entities under the category of myeloproliferative neoplasms (MPNs): chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic neutrophilic leukemia, chronic eosinophilic leukemia—not otherwise specified, mastocytosis and MPN-unclassifiable. Among these, the first four were first assembled in 1951 by William Dameshek, as ‘myeloproliferative disorders’; accordingly, they are now referred to as ‘classic’ MPNs. As CML is invariably and specifically associated with BCR-ABL1, the other three (that is, PV, ET and PMF) are operationally dubbed as ‘BCR-ABL1-negative MPNs.’

**REFERENCES**

PV, ET and PMF are traditionally considered as stem cell-derived monoclonal hemopathies. Furthermore, family studies and Janus kinase 2 (JAK2) haplotype analysis have suggested a hereditary component for disease susceptibility. The possibility of independently emerging multiple abnormal clones (that is, leading to oligoclonal rather than monoclonal myeloproliferation) has recently been raised and challenges the prevailing concept that considers an ancestral abnormal clone that gives rise to mutually exclusive subclones (Figure 1). In the past 5 years, a number of stem cell-derived mutations involving JAK2 (exon 14–27 and exon 12), Myeloproliferative Leukemia Virus (MPL) (exon 10), TET oncogene family member 2 (TET2) (across several exons), Additional Sex Combs-Like 1 (ASXL1) (exon 12), Casitas B-lineage lymphoma proto-oncogene (CBL) (exons 8 and 9), Isocitrate dehydrogenase (IDH1) (exon 4), IDH2 (exon 4) and IKAROS family zinc finger 1 (IKZF1) (deletion of several exons) have been described in chronic- or blast-phase MPN and are discussed in this review (Table 1).
its broad specificity to patients with myeloid neoplasms (including those with occult disease and splanchic vein thrombosis)\(^5\),\(^6\) and the fact that the mutation is not seen in patients with lymphoid neoplasms, reactive myeloproliferation or in healthy volunteers.\(^5\)–\(^8\)

JAK2\(V617F\) results from a somatic G to T mutation involving JAK2 exon 14, which leads to nucleotide change at position 1849 and the substitution of valine to phenylalanine at codon 617.\(^9\) The mutation affects the noncatalytic ‘pseudo-kinase’ domain and is believed to derail its kinase-regulatory activity. JAK2\(V617F\)-mediated transformation is believed to require coexpression of type I cytokine receptor and leads to STAT5/3 activation;\(^6\)–\(^8\) in addition, a recent study has suggested an epigenetic effect through nuclear translocation of the mutant molecule and direct phosphorylation of histone H3.\(^6\) Such a noncanonical mode of action has previously been reported to disrupt heterochromatin-mediated tumor suppression in Drosophila.\(^6\) Some patients with MPN might carry multiple JAK2 mutations, sometimes occurring in the same exon and in cis configuration.\(^6\) Such events might have functional relevance as they might alter specific signaling.

JAK2\(V617F\) induces PV-like phenotype in mouse transplantation models,\(^6\) and this observation has been further confirmed by a recent report of an inducible JAK2\(V617F\) knock-in mouse model, in which both heterozygous and homozygous mutation expressions induced PV-like disease, with the latter causing a more aggressive phenotype with myelofibrosis.\(^6\) Such experimental data along with the fact that virtually all patients with PV carry a JAK2 mutation,\(^6\) suggest a cause–effect relationship with erythrocytosis.\(^3\)\(^1\),\(^6\)–\(^7\) Somewhat consistent with this contention, JAK2\(V617F\) homozygosity is infrequent in ET and its frequent occurrence in PV has been ascribed to mitotic recombination, possibly facilitated by JAK2\(V617F\)-induced genetic instability.\(^7\) However, both ET- and PMF-like disease are also induced in mice by experimental manipulation of the JAK2\(V617F\) allele burden,\(^7\)\(^3\),\(^7\) and mutant allele burden in PMF is often as high as that seen in PV and its level increases further during fibrotic transformation.\(^7\) These observations suggest the presence of additional phenotype determinants in primary and post-PV/ET MF.

Despite the above-described experimental and clinical observations, JAK2\(V617F\) does not appear to be the disease-initiating event and probably defines an MPN subclone, which does not always account for leukemic transformation.\(^7\),\(^6\)\(^7\) In the latter regard, JAK2\(V617F\)-positive, as opposed to JAK2\(V617F\)-negative, blast-phase MPN might require a fibrotic phase disease transition.\(^8\) On the other hand, JAK2 wild-type AML that develops in the setting of JAK2\(V617F\)-positive MPN does not necessarily arise from originally mutation-positive clones that have undergone mitotic recombination of wild-type JAK2.\(^8\) The complexity of clonal hierarchy and structure in MPN has become more evident with recent demonstrations of multiple mutations occurring in the same patient and the fact that such mutations are neither necessarily mutually exclusive nor follow a predictable sequence of occurrence.\(^6\),\(^6\)\(^7\),\(^8\)

JAK2\(V617F\)-positive MPN has been associated with older age at diagnosis (ET and PMF), higher hemoglobin level (ET and PMF), leukocytosis (ET and PMF) and lower platelet count (ET).\(^7\) A higher mutant allele burden has been associated with pruritus (PV and PMF), higher hemoglobin level (PV), leukocytosis (PV, ET and PMF) and larger spleen size (PV, ET and PMF).\(^7\),\(^8\) However, save for some contrary observations,\(^8\),\(^8\) the mere presence of JAK2\(V617F\) or increased mutant allele burden does not seem to affect survival or leukemic transformation.\(^8\) Instead, a lower mutant allele burden has been associated with

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**Figure 1** Clonal origination and evolution in myeloproliferative neoplasms (MPNs). PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; CML, chronic myelogenous leukemia; AML, acute myeloid leukemia; JAK2, Janus kinase 2; MPL, thrombopoietin receptor; TET2, TET oncogene family member 2; ASXL1, Additional Sex Combs-Like 1; CBL, Casitas B-lineage Lymphoma proto-oncogene; IDH, isocitrate dehydrogenase; IKZF1, IKAROS family zink finger 1.
### Table 1  Novel mutations in polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF) and blast-phase myeloproliferative neoplasm (MPN)

| Mutations | Chromosome location | Mutational frequency | Pathogenetic relevance | Prognostic relevance |
|-----------|---------------------|----------------------|------------------------|----------------------|
| JAK2V617F exon 14 (Janus kinase 2) | 9p24 | PV ~ 96% ET ~ 55% PMF ~ 65% Blast-phase MPN ~ 50% | Believed to contribute to myeloproliferation and progenitor cell growth factor hypersensitivity | Limited |
| JAK2 exon 12 | 9p24 | PV ~ 3% ET ~ rare PMF ~ rare Blast-phase MPN ~ ? | Believed to contribute to primarily erythroid myeloproliferation | Not enough information |
| MPL exon 10 (Myeloproliferative Leukemia Virus oncogene) (encodes for thrombopoietin receptor) | 1p34 | PV ~ rare ET ~ 3% PMF ~ 10% Blast-phase MPN ~ ? | Believed to contribute to primarily megakaryocytic myeloproliferation | Not enough information |
| TET2 mutations occur across several of the gene's 12 exons (TET oncogene family member 2) | 4q24 | PV ~ 16% ET ~ 5% PMF ~ 17% Blast-phase MPN ~ 17% | Might contribute to epigenetic modulation of transcription (TET1 catalyzes conversion of 5-methylcytosine to 5-hydroxymethylcytosine) | Not enough information |
| ASXL1 exon 12 (Additional Sex Combs-Like 1) | 20q11.1 | PV ~ ? ET ~ ? PMF ~ ? Blast-phase MPN ~ 19% | Believed to affect regulation of transcription and RAR-mediated signaling | Not enough information |
| CBL exons 8 and 9 (Casitas B-lineage lymphoma proto-oncogene) | 11q23.3 | PV ~ rare ET ~ rare PMF ~ 6% Blast-phase MPN ~ ? | Believed to alter the regulatory function of wild-type CBL against kinase signaling because of defective ubiquitylation of oncoproteins | Not enough information |
| IDH1/IDH2 exon 4/exon 4 (Isocitrate dehydrogenase) | 2q33.3/15q26.1 | PV ~ rare ET ~ rare PMF ~ 4% Blast-phase MPN ~ 20% | Induces accumulation of 2-hydroxylglutarate, a possible oncoprotein | Not enough information |
| IKZF1 (IKAROS family zinc finger 1) | 7p12 | PV ~ rare ET ~ rare PMF ~ rare Blast-phase MPN ~ 19% | Believed to alter tumor suppressor activity of the wild-type protein | Not enough information |

Abbreviation: RAR, retinoic acid receptor.

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**Figure 2**  The spectrum of cytokines and growth factors that use Janus kinases (JAKs) for signal transduction.
inferior survival in PMF. This particular finding illustrates prognostically relevant clonal complexity in PMF. JAK2V617F allele burden increases with time in PV and PMF, but not in ET. This phenomenon in PV and PMF coincides with the development of post-PV myelofibrosis, marked splenomegaly and requirement for chemotherapy. Current evidence is not conclusive with regard to the relationship between JAK2V617F and thrombosis.

JAK2 exon 12 mutations

JAK2 exon 12 mutations are relatively specific to JAK2V617F-negative PV and were first described in 2007. Subsequent studies have identified N542-E543del as the most frequent among the >10 JAK2 exon 12 mutations described so far. JAK2 exon 12 mutations include in-frame deletions, point mutations and duplications, mostly affecting seven highly conserved amino-acid residues (F537–E543). As is the case with its exon 14 counterpart (that is, JAK2V617F), the JAK2K539L exon 12 mutation has also been shown to induce erythroid crisis in mice. JAK2 exon 12 mutation-positive PV patients are often heterozygous for the mutation and are characterized by predominantly erythroid myelopoiesis, subnormal serum erythropoietin level and younger age at diagnosis. The clinical course of these patients seems to be similar to that of patients with JAK2V617F-positive PV.

**MPL mutations**

MPL, located on chromosome 1p34, includes 12 exons and encodes for the thrombopoietin receptor (635–680 amino acids). MPL is the key growth and survival factor for megakaryocytes. Gain-of-function germline MPL mutations have been associated with familial thrombocytopathy (SS05N) that is, interestingly, associated with an MPN phenotype, including splenomegaly, myelofibrosis and an increased risk of thrombosis. The particular observation further attests to the phenotype-modifying effect of somatic MPL mutations in MPN. An MPL single-nucleotide polymorphism (G1238T) that results in a K39N substitution is found in single-nucleotide polymorphism (G1238T) that results in a K39N substitution is found in approximately 7% of African Americans and is associated with higher platelet counts.

Somatic MPL mutations are rare and their occurrence is largely limited to patients with MPN, although their occurrence in acute megakaryocytic leukemia patients has also been reported. MPLW515L results from a G to T transition at nucleotide 1544 (exon 10), resulting in a tryptophan to leucine substitution at codon 515. MPLW515L was first described in 2006 among patients with JAK2V617F-negative PMF and induces a PMF-like disease with thrombocytopathy in mice. Subsequently, MPLW515K and other exon 10 MPL mutations (such as MPLW515S and MPLS05N) were described in ET and PMF with mutational frequencies that range from 3 to 15%. MPLW515L is the most frequent MPL-associated mutation, whereas MPLS05N also occurs in the setting of hereditary thrombocythemia, as mentioned above.

As is the case with JAK2 mutations, MPLS15 mutations are stem cell-derived events that involve both myeloid and lymphoid progenitors. MPL mutant-induced oncogenesis also results in constitutive JAK-STAT activation and might require specific MPL mutant variants (such as MPLW515L) and receptor residues (such as Y112). Some patients with ET or PMF display multiple MPL mutations and others a low allele burden JAK2V617F clone together with a higher allele burden MPL mutation. Homozygosity for MPL mutations is also ascribed to acquired uniparental disomy, as is the case with JAK2V617F.

MPL-mutated ET has been associated with older age, lower hemoglobin level, higher platelet count, microvascular symptoms and a higher risk of post-diagnosis arterial thrombosis. The presence of MPL mutation did not appear to affect survival, fibrotic or leukemic transformation. MPL-mutated PMF has been associated with the female gender, older age, lower hemoglobin level and a higher likelihood of becoming transfusion dependent. This set of findings suggests a phenotype-modifying effect that is different from that seen with a JAK2 mutation.

**TET2 mutations**

TET2 is one of three homologous human proteins (that is, TET1, TET2 and TET3) the function of which, based on a recent report on TET1, might include conversion of 5-methylcytosine to 5-hydroxymethylcytosine, and thus possibly affect the epigenetic regulation of transcription. TET1 was the first of the three TET genes to be described and the name is derived from ‘ten-eleven translocation 1’—a name given to a novel gene located at chromosome 10q22 and was identified as the fusion partner of TET1 during an AML-associated chromosomal translocation, t(10;11)(q22;q31). TET2 is located on chromosome 4q24, which is a breakpoint that is also involved in other AML-associated translocations, including t(3;4)(q26;q24), t(4;5)(q24;p16), t(4;7)(q24;q21) and del(4)(q23q24). TET2 has multiple isoforms and isoform A, which is affected by most of the TET2 mutations described so far, and includes 12 exons. TET3 is located at 2p13.1.

TET2 mutations, first described in 2008, include frameshift, nonsense and missense mutations, scattered across several of its 12 exons, and are seen in both JAK2V617F-positive (17%) and JAK2V617F-negative (7%) MPNs with approximate mutational frequencies of 16% in PV, 5% in ET, 17% in PMF, 14% in post-PV MF, 14% in post-ET MF and 17% in blast-phase MPN. Higher incidences of TET2 mutations have been reported in systemic mastocytosis, MPN-unclassifiable, chronic myelomonocytic leukemia (CMLML), MDS, MDS/MPN, AML and idic(X)(q13)-positive myeloid malignancies; in addition, a germline TET2 mutation was recently described in a patient with PV. Furthermore, TET2 mutations have been shown to coexist with other pathogenetically relevant mutations involving RARA, MPL, KIT, FLT3, RAS, MLL, CEBPA or NPM1. TET2 mutations in MPN can either antedate or follow the acquisition of a JAK2 mutation (exon 12 or 14), or occur in an independent manner leading to a bicalonal pattern.

Taken together, the ubiquitous nature of TET2 mutations undermines their specific pathogenetic contribution to MPN. Furthermore, the presence of the mutant TET2 did not seem to affect survival, leukemic transformation, thrombosis risk or cytogenetic profile in either PV or PMF. By contrast, the presence of TET2 mutations was associated with superior survival in MDS and inferior survival in AML and CMMML. A further twist in the TET2 story was recently reported by a study that suggested the possible acquisition of the mutation during leukemic transformation of MPN, a paired sample analysis in 14 patients disclosed the absence of TET2 mutations in chronic-phase disease but their presence in 5 cases during blast-phase disease, regardless of JAK2V617F mutational status. However, these results were not reproduced by two other
studies that looked for the presence of TET2 mutations in patient samples obtained during chronic- and blast-phase diseases. One of the latter studies also showed that post-MPN AML can develop in the presence or absence of TET2 or JAK2 mutations in a mutually exclusive manner or not.

**ASXL1 mutations**

ASXL1 (includes 12 or 13 exons) maps to chromosome 20q11.1 and belongs to the Enhancer of trithorax and Polycomb gene family. Gene function is believed to include dual activator/ suppressor activity toward transcription and includes repression of retinoic acid receptor-mediated transcription. ASXL1 is expressed in most hematopoietic cell types, and a knockout mouse model displayed mild defects in myelopoiesis but did not develop MDS or other hematological malignancy. PAX5-ASXL1 has been associated with the B precursor acute lymphoblastic leukemia. Truncation exon 12 mutations, which affect the C-terminal PHD (plant homeodomain), have recently (2009) been described in 11% of patients with MDS, 43% of those with CMML, 7% with primary and 47% with secondary AML. In a more recent study of 300 patients with a spectrum of non-MPN myeloid malignancies, ASXL1 mutations were found in 62 patients (~21%); ~7% in MDS without excess blasts, 11–17% in MDS with ring sideroblasts, 31% in MDS with excess blasts, 23% in post-MDS AML, 33% in CMML and 30% in primary AML. ASXL1 mutations might be more common in patients with normal karyotype or ~77q− and infrequent in the presence of ~5q−. In AML with normal karyotype, ASXL1 mutations were often absent in patients with NPM1 or FLT3 mutations; mutational frequency was 34% in non-MPN1 cases.

ASXL1 mutations occur in both chronic- and blast-phase MPNs; in a study of 64 patients with ET (n = 35), PMF (n = 11), PV (n = 10), blast-phase MPN (n = 5) and MPN-unclassifiable (n = 3), heterozygous mutations of ASXL1 were identified in 5 cases who were all JAK2V617F negative (~8%; 3 PMF, 1 ET and 1 blast-phase ET). In an even more recent study of 63 patients with post-MPN AML, ASXL1 mutations were seen in 12 (19%) cases and did not appear to be acquired during leukemic transformation. ASXL1 mutations in the latter study were shown to coexist with JAK2 or TET2 but not IDH1 mutations and, in some instances, appeared to predate the acquisition of both JAK2 and TET2 mutations. Obviously, larger studies are required to confirm these findings and determine prognostic impact, especially in terms of leukemic transformation risk. Similarly, additional laboratory studies are required to clarify ASXL1 mutation-mediated oncogenesis and whether it involves loss of tumor suppression or aberrant retinoic acid receptor signaling.

**CBL mutations**

CBL (includes 16 exons) is located at 11q23.3, telomeric to MLL and encodes for a cytosolic protein capable of dual function: negative regulation of kinase signaling mediated by E3 ubiquitin ligase activity and an adaptor protein function with a positive effect on downstream signaling. CBL (906 amino acids) is one of three cytosolic CBL family of proteins (CBL, CBL-B and CBL-C/3) and its N terminal features tyrosine kinase-binding and zinc-binding RING-finger domains with a linker domain between them, and its C terminal is composed of a proline-rich region. E3 ubiquitin ligase activity is central to the primary function of CBL, which is the downregulation of activated receptor and nonreceptor protein-tyrosine kinases by ubiquitination, internalization and lysosomal/proteosomal degradation.

Of relevance to myeloid neoplasms, wild-type CBL has been shown to participate in the ubiquitination of MPL, KIT and FLT3 and ubiquitylation of the latter two proteins was shown to be defective in the presence of mutant CBL. Mutant CBL induces oncogenic phenotype in various cell lines and promotes growth factor independence. CBL knockout mouse display expanded hematopoietic stem cell pool, splenomegaly and enhanced growth factor sensitivity of hematopoietic progenitor cells. Retroviral expression of mutant CBL in transplanted bone marrow induced extensive and diffuse multigran infiltration by mast cells accompanied by mast cell sarcoma, myeloproliferative phenotype and acute leukemia in some instances. In contrast to this observation, CBL mutations were not detected in any of the 60 patients with systemic mastocytosis.

CBL mutations in myeloid malignancies are usually associated with 11q acquired uniparental disomy and were first recognized in AML as an MLL-CBL fusion resulting from interstitial CBL deletion. Subsequent studies have shown that CBL mutations were most frequent in juvenile mononucentic leukemia (JMML) and CMML. In one large study, mostly exon 8 CBL mutations were detected in 27 (17%) of 159 cases with JMML (40% mutational frequency among patients without known RAS pathway mutations) and 5 (11%) of 44 patients with CMML. The respective mutational frequencies for JMML and CMML, from another group of investigators, were 10 and 5%, respectively. Others have also shown relatively high CBL mutation rates in CMML (13–15%) and one of the latter studies reported an 8% incidence in BCR-ABL1-negative atypical CML. It is to be recalled that CMLM and atypical CML are all subcategories of MDS/MPN. By contrast, CBL mutations were infrequent in refractory anemia with ring sideroblasts and thrombocytosis (0 of 19 analyzed cases), a provisional MDS/MPN entity.

Most CBL mutations in JMML are homozygous, which suggests a tumor-suppressor function for the normal protein. This conjecture is supported by the observation that two patients with homozygous mutations in their hematopoietic cells displayed germline heterozygous mutations in their buccal or cord blood cells. In general, CBL mutations associated with JMML and CMML consist of missense substitutions or in-frame deletions and are located throughout the linker and RING-finger domain. JMML patients with mutant CBL do not express RAS or PTPN11 mutations but display similar biochemical (for example, cellular granulocyte macrophage-colony-stimulating factor hypersensitivity) and clinical features. By contrast, mutant CBL has been shown to coexist with mutations involving RUNX1, FLT3, JAK2 and TP53.

CBL mutations are infrequent in myeloid malignancies other than JMML or CMML. In a recent study of 577 patients with MPN or MDS/MPN, including 74 patients with PV, 24 with ET and 53 with PMF, CBL mutations in either exon 8 or 9 were identified in 3 (6%) patients with PMF and 1 of 96 patients with CEL/HES (chronic eosinophilic leukemia/hypereosinophilic syndrome). CBL mutations were found in <1% of patients with primary AML, MDS, systemic mastocytosis, CNL (chronic neutrophilic leukemia), blast-phase CML and T-acute lymphoblastic leukemia. Mutation frequency might be higher in post-MDS/MPN AML or in AML with core-binding factor or 11q aberrations. Acquisition of mutant CBL during disease progression from ET to post-ET MF was documented in one instance. Additional studies are required to clarify the pathogenetic contribution of altered CBL to PMF or
post-ET/PV MF and its potential role in fibrotic or leukemic disease transformation.

**IDH mutations**

**IDH1** (located on chromosome 2q33.3; includes 10 exons) and **IDH2** (located on chromosome 15q26.1; includes 11 exons) encode for isocitrate dehydrogenase 1 and 2, respectively, which are homodimeric NADP⁺-dependent enzymes that catalyze oxidative decarboxylation of isocitrate to α-ketoglutarate, generating NADPH from NADP⁺. **IDH1** and **IDH2** are different from the mitochondrial NAD⁺-dependent isocitrate-IDH3-β (IDH3-β) and IDH3-γ. **IDH1** (414 amino acids) is localized in the cytoplasm and peroxisomes, whereas **IDH2** (452 amino acids) is localized in the mitochondria. **IDH1** and **IDH2** mutations were first described in gliomas148 and subsequently in AML149–151 localized in the mitochondria. 

**IDH1** mutations are younger and display better survival and often express **EGFR** mutations.152,153 These mutations have also been described in post-MPN AML.35–37 In one such study, 18 (23%) of 78 IDH1/R132H, 19% R132S, 15% R132G and 4% R132L). In both IDH1 and IDH2 mutations clustered with normal karyotype, or 1%, respectively, of patients with WHO grade II or III histology and secondary glioblastomas but are infrequently (<5% incidence) seen in primary glioblastoma.158–161 In one study of 496 gliomas, >90% of the IDH1 mutations were IDH1/R132H.162 Paired sample analysis in glioma patients transforming from low- to high-grade histology showed that IDH mutations were early events. IDH-mutated glioma patients are younger and display better survival and often express TP53 but not PTEN, EGFR, CDKN2 or CDKN2B mutations.158,163 The superior survival associated with IDH mutations has been attributed to increased sensitivity to treatment, as a result of decreased NADPH production, and, therefore, reduced risk of progression.164–166

The first study on IDH mutations in AML included 188 patients with primary AML and reported IDH1 but not IDH2 mutations in 16 (∼9%) cases: R132C in 8, R132H in 7 and R132S in 1.149 In a subsequent AML study of 493 adult Chinese patients, 167 27 (∼6%) expressed IDH1 mutations (37% R132C, 26% R132H, 19% R132S, 15% R132G and 4% R132L). In both studies, IDH1 mutations clustered with normal karyotype, NPM1 mutations and trisomy 8. More recently, IDH2 exon 4 mutations, affecting R172 or R140, were also shown to occur in primary AML.150,151 In one of these studies, 18 (23%) of 78 patients displayed either IDH1 (n = 6; ∼8%) or IDH2 (n = 12; ∼15%; 7 R140Q and 5 R172K) mutations.150 AML patients with IDH2 mutations were also less likely to carry FLT3, NPM1 or ASXL1 mutations, whereas the above-mentioned study from China167 reported the coexistence of IDH1 mutations and RUNX1, PTPN11, NRAS, FLT3-ITD, FLT3-TKD or MLL-PTD mutations.150,167 In general, survival in primary AML did not seem to be affected by the presence of IDH1 mutations.149,150,167

**IDH1** mutations have also been described in post-MPN AML.35–37 In one such study, IDH1 mutations were seen in ∼8% (5 of 63) of patients, mostly occurring in the absence of TET2 and ASXL1 mutations.16 In this particular study, there was no significant difference in **IDH1** mutational frequency between post-MPN AML, post-MDS AML and primary AML. Furthermore, paired sample analysis did not suggest acquisition of **IDH1** mutation during leukemic transformation.16 In another study of AML occurring in the setting of JAK2-mutated MPN,35 mutant IDH1 was seen in 5 (31%) of 16 patients; 3 R132C (1 post-PMF, 1 post-ET and 1 post-PV with exon 12 mutation) and 2 R140Q (1 post-PMF with trisomy 8 and 7q– and 1 post-PV with complex karyotype). Three patients lost their mutant JAK2 at the time leukemic transformation; in two of these three patients, the **IDH1** mutation was present in leukemic blasts with wild-type JAK2 but absent from JAK2 mutation-positive progenitor colonies. By contrast, in the PMF patient with JAK2R140Q, the mutation was detected in both JAK2V617F-positive erythroid colonies and leukemic blasts. The authors did not find **IDH1** mutations in 180 patients with either PV or ET.35 Most recently, 200 patients with either chronic- or blast-phase MPN were screened for **IDH1** and **IDH2** mutations.37 A total of nine **IDH1** mutations including five **IDH1** and four **IDH2** were found and mutational frequencies were ∼21% for blast-phase AML and ∼4% for PMF. No mutations were seen in PV or ET. Furthermore, **IDH1** mutations were found in only 1 of 12 paired chronic- and blast-phase samples and the mutation was detected in both chronic- and blast-phase disease samples in the single **IDH1**-mutated case. The specific **IDH1** mutations found in this study included R132C and R132S and the **IDH2** mutations R140Q and R140W. **IDH1** mutations coexisted with JAK2V617F. The results of this and the aforementioned study suggest that **IDH1** mutations are relatively frequent in blast- but not chronic-phase MPN, but more studies are required to find out whether they represent early genetic events or are acquired during leukemic transformation.

**IKZF1 mutations**

IKAROS family zinc finger 1 (IKZF1; 7p12) encodes for Ikaros transcription factors, which are important regulators of lymphoid differentiation. **IKZF1** gene (seven translated exons) transcription is characterized by multiple alternatively spliced transcripts with common C- (inter-Ikaros protein dimerization) and N-terminal (DNA-binding) domains. **IKZF1** is believed to modulate expression of lineage-specific genes through a mechanism that involves chromatin remodeling and results in effective lymphoid development and tumor suppression. Loss-of-function animal models develop severe B, T and NK cell defects (homozygous gene deletions) or lymphoblastic leukemia (heterozygous for a dominant-negative allele).169 **IKZF1** mutations and overexpression of dominant-negative isoforms are prevalent in ALL, including blast-phase CML or **BCR-ABL1**-positive ALL, suggesting a pathogenetic contribution to leukemic transformation.170 A recent study demonstrated that **IKZF1** deletions were rare in chronic-phase CML but were detected in approximately 19% of patients with blast-phase CML.171 The occurrence of **IKZF1** mutations in MPN is particularly relevant, as part of their functional consequence might include JAK–STAT activation.

**Concluding remarks**

PMF–PV–ET were first described in 1879–1892–1934 and their close relationship was formally recognized in 1951 and molecularly validated in 2005. Unlike CML, pathogenetic mechanisms in these **BCR-ABL1**-negative MPN are turning out

Novel mutations in MPNs

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to be more complex than originally believed, and their trademark JAK2 and MPL mutations do not appear to be analogous to BCR-ABL1 in terms of their importance as therapeutic targets.41,78 The repertoire of other mutations (such as TET2, ASXL1, CBL, IDH1 mutations) in MPN is growing but their specific pathogenetic relevance is undermined by their omnipresence in other myeloid malignancies. Conversely, the particular scenario might reflect our collective oversight regarding the molecular inter-relationship among phenotypically disparate myeloid malignancies. Regardless, on the basis of the assumption that JAK-STAT is central to the pathogenesis of BCR-ABL1-negative MPN, a number of orally administered anti-JAK2 ATP mimetics have been developed and are undergoing clinical trials.42–44 So far, the two that have shown the most promising clinical activity are TG101348 (JAK2 inhibitor) and INCBO18424 (JAK1/2 inhibitor).41 Other drugs that are currently in clinical trials for PMF, PV or ET include other kinase inhibitors (such as CYT387, CEP-701, AZD1480, SB1518, erlotinib), histone deacetylase inhibitors (such as ITF2357, MK-0683, panobinostat) and the anti-vascular endothelial growth factor monoclonal antibody bevacizumab (http://ClinicalTrials.gov).42,168

Conflict of interest

The author declares no conflict of interest.

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