REVIEW

Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: *JAK2*, *MPL*, *TET2*, *ASXL1*, *CBL*, *IDH* and *IKZF1*

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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 blastic transformation.

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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.¹ Among these, the first four were first assembled in 1951 by William Dameshek,² 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 MPN’.³

PV, ET and PMF are traditionally considered as stem cell-derived monoclonal hemopathies.^{4–6} Furthermore, family studies and Janus kinase 2 (*JAK2*) haplotype analysis have suggested a hereditary component for disease susceptibility.^{7–14} 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).^{15–21} In the past 5 years, a number of stem cell-derived^{19,22–26} mutations involving *JAK2* (exon 14^{27–30} and exon 12),³¹ Myeloproliferative Leukemia Virus (*MPL*) (exon 10),^{32,33} TET oncogene family member 2 (*TET2*) (across several exons),²⁵ Additional Sex Combs-Like 1 (*ASXL1*) (exon 12),²⁶ Casitas B-lineage lymphoma proto-oncogene (*CBL*) (exons 8 and 9),³⁴ Isocitrate dehydrogenase 1 (*IDH1*) (exon 4),^{35,36} *IDH2* (exon 4)^{35,37} 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).

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.^{38,39} 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).^{39–44}

JAK2V617F

Oncogenic *JAK1*, *JAK2* and *JAK3* mutations have been associated with both lymphoid and myeloid neoplasms.⁴⁵ Of particular relevance to MPN, *JAK2V617F* was discovered in 2004²⁷ and the first reports appeared in early 2005.^{27–30} *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),⁴⁵ but it is also seen in some patients with myelodysplastic syndrome (MDS)/MPN (for example, refractory anemia with ring sideroblasts and thrombocytosis)^{46–48} and, rarely, in primary acute myeloid leukemia (AML), MDS or CML.^{49–52} However, this should not undermine

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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 zinc finger 1.

its broad specificity to patients with myeloid neoplasms (including those with occult disease and splanchnic vein thrombosis)^{53,54} and the fact that the mutation is not seen in patients with lymphoid neoplasms, reactive myeloproliferation or in healthy volunteers.^{55–58}

JAK2V617F 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 617.⁵⁹ The mutation affects the noncatalytic ‘pseudo-kinase’ domain and is believed to derail its kinase-regulatory activity. JAK2V617F-mediated transformation is believed to require coexpression of type I cytokine receptor and leads to STAT5/3 activation,^{60–63} in addition, a recent study has suggested an epigenetic effect through nuclear translocation of the mutant molecule and direct phosphorylation of histone H3.⁶² Such a noncanonical mode of action has previously been reported to disrupt heterochromatin-mediated tumor suppression in *Drosophila*.⁶⁴ Some patients with MPN might carry multiple JAK2 mutations, sometimes occurring in the same exon and *in cis* configuration.⁶⁵ Such events might have functional relevance as they might alter specific signaling.

JAK2V617F induces PV-like phenotype in mouse transplantation models,²⁷ and this observation has been further confirmed by a recent report of an inducible JAK2V617F 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.⁶⁶ Such experimental data along with the fact that virtually all patients with PV carry a JAK2 mutation,⁶⁷ suggest a cause–effect relationship with erythrocytosis.^{31,68–71} Somewhat consistent with this contention, JAK2V617F homozygosity is infrequent in ET and its frequent occurrence in PV has been ascribed to mitotic recombination, possibly facilitated by JAK2V617F-induced

genetic instability.⁷² However, both ET- and PMF-like disease are also induced in mice by experimental manipulation of the JAK2V617F allele burden,^{73,74} and mutant allele burden in PMF is often as high as that seen in PV and its level increases further during fibrotic transformation.⁷⁵ These observations suggest the presence of additional phenotype determinants in primary and post-PV/ET MF.

Despite the above-described experimental and clinical observations, JAK2V617F does not appear to be the disease-initiating event and probably defines an MPN subclone, which does not always account for leukemic transformation.^{18,76,77} In the latter regard, JAK2V617F-positive, as opposed to JAK2V617F-negative, blast-phase MPN might require a fibrotic phase disease transition.¹⁸ On the other hand, JAK2 wild-type AML that develops in the setting of JAK2V617F-positive MPN does not necessarily arise from originally mutation-positive clones that have undergone mitotic recombination of wild-type JAK2.¹⁸ 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.^{16,36,78}

JAK2V617F-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).⁷⁵ 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).^{79–83} However, save for some contrary observations,^{80,84} the mere presence of JAK2V617F or increased mutant allele burden does not seem to affect survival or leukemic transformation.^{83,85–90} Instead, a lower mutant allele burden has been associated with

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
<i>JAK2</i> V617F 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
<i>JAK2</i> exon 12	9p24	PV ~ 3% ET ~ rare PMF ~ rare Blast-phase MPN ~ ?	Believed to contribute to primarily erythroid myeloproliferation	Not enough information
<i>MPL</i> 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
<i>TET2</i> 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
<i>ASXL1</i> 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
<i>CBL</i> 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
<i>IDH1/IDH2</i> exon 4/exon 4 (isocitrate dehydrogenase)	2q33.3/15q26.1	PV ~ rare ET ~ rare PMF ~ 4% Blast-phase MPN ~ 20%	Induces accumulation of 2-hydroxyglutarate, a possible oncoprotein	Not enough information
<i>IKZF1</i> (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.

Figure 2 The spectrum of cytokines and growth factors that use Janus kinases (JAKs) for signal transduction.

inferior survival in PMF.^{80,84} This particular finding illustrates prognostically relevant clonal complexity in PMF. *JAK2V617F* allele burden increases with time in PV and PMF,^{80,82,91} but not in ET.⁸³ This phenomenon in PV and PMF coincides with the development of post-PV myelofibrosis, marked splenomegaly and requirement for chemotherapy.^{79,90,92,93} Current evidence is not conclusive with regard to the relationship between *JAK2V617F* and thrombosis.^{82,83,85,86,93–95}

JAK2 exon 12 mutations

JAK2 exon 12 mutations are relatively specific to *JAK2V617F*-negative PV and were first described in 2007.³¹ Subsequent studies have identified N542-E543del as the most frequent among the >10 *JAK2* exon 12 mutations described so far.^{31,68,69,96} *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 erythrocytosis in mice.³¹ *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.^{31,97,98} The clinical course of these patients seems to be similar to that of patients with *JAK2V617F*-positive PV.^{68,98,99}

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 thrombocytosis (S505N) that is, interestingly, associated with an MPN phenotype, including splenomegaly, myelofibrosis and an increased risk of thrombosis.¹⁰⁰ 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 ~7% of African Americans and is associated with higher platelet counts.¹⁰¹

Somatic *MPL* mutations are rare and their occurrence is largely limited to patients with MPN, although their occurrence in acute megakaryocytic leukemic patients has also been reported.¹⁰² *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 thrombocytosis in mice.³² Subsequently, *MPLW515K* and other exon 10 *MPL* mutations (such as *MPLW515S* and *MPLS505N*) were described in ET and PMF with mutational frequencies that range from 3 to 15%.^{32,33,103–106} *MPLW515L* is the most frequent MPN-associated *MPL* mutation, whereas *MPLS505N* also occurs in the setting of hereditary thrombocythemia, as mentioned above.¹⁰⁰

As is the case with *JAK2* mutations, *MPLS515* mutations are stem cell-derived events that involve both myeloid and lymphoid progenitors.^{24,33,107} *MPL* mutant-induced oncogenesis also results in constitutive JAK-STAT activation and might require specific *MPL* mutant variants (such as *MPLW515*)¹⁰⁸ and receptor residues (such as Y112).¹⁰⁹ 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.^{104,110} Homozygosity for *MPL* mutations is also

ascribed to acquired uniparental disomy, as is the case with *JAK2V617F*.¹¹¹

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.^{106,112} The presence of *MPL* mutation did not appear to affect survival, fibrotic or leukemic transformation.¹⁰⁶ *MPL*-mutated PMF has been associated with the female gender, older age, lower hemoglobin level and a higher likelihood of becoming transfusion dependent.¹⁰⁵ 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*,¹¹³ 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 *MLL* during an AML-associated chromosomal translocation, t(10;11)(q22;q23).¹¹⁴ *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).¹¹⁵ *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 2008,²⁵ 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.¹¹⁶ Higher incidences of *TET2* mutations have been reported in systemic mastocytosis, MPN-unclassifiable, chronic myelomonocytic leukemia (CMML), MDS, MDS/MPN, AML and idic(X)(q13)-positive myeloid malignancies;^{117–124} in addition, a germline *TET2* mutation was recently described in a patient with PV.¹⁶ Furthermore, *TET2* mutations have been shown to coexist with other pathogenetically relevant mutations involving *RARA*, *MPL*, *KIT*, *FLT3*, *RAS*, *MLL*, *CEBPA* or *NPM1*.^{117–120} *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 biclonal pattern.^{16,18,25}

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.^{116,125–127} By contrast, the presence of *TET2* mutations was associated with superior survival in MDS¹²¹ and inferior survival in AML¹²⁰ and CMML.¹²⁸ 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;³⁶ 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.^{126,129} 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.¹²⁹

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.¹³⁰ *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.¹³¹ *PAX5-ASXL1* has been associated with the B precursor acute lymphoblastic leukemia.¹³² 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.^{133,134} 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 $-7/7q-$ and infrequent in the presence of $-5/5q-$. In AML with normal karyotype, *ASXL1* mutations were often absent in patients with *NPM1* or *FLT3* mutations; mutational frequency was 34% in non-*NPM1* cases.¹³⁴

ASXL1 mutations occur in both chronic- and blast-phase MPNs;^{26,36} 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).²⁶ 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.³⁶ *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.³⁶ 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.¹³⁵ *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*,¹³⁶ *KIT*¹³⁷ and *FLT3*,¹³⁸ and ubiquitylation of the latter two proteins was shown to be defective in the presence of mutant *CBL*.^{137,138} Mutant *CBL* induces oncogenic phenotype in various cell lines and promotes growth factor independence.¹³⁹ *CBL* knockout mice display expanded hematopoietic stem cell pool, splenomegaly and enhanced growth factor sensitivity of hematopoietic progenitor cells.¹³⁹ Retroviral expression of mutant *CBL* in transplanted bone marrow induced extensive and diffuse multiorgan infiltration by mast cells accompanied by mast cell sarcoma, myeloproliferative phenotype and acute leukemia in some instances.¹³⁷ In contrast to this observation, *CBL* mutations were not detected in any of the 60 patients with systemic mastocytosis.³⁴

CBL mutations in myeloid malignancies are usually associated with 11q acquired uniparental disomy¹³⁹ and were first recognized in AML as an *MLL-CBL* fusion resulting from interstitial *CBL* deletion.¹⁴⁰ Subsequent studies have shown that *CBL* mutations were most frequent in juvenile monomyelocytic leukemia (JMML) and CMML. In one large study,¹⁴¹ 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.¹⁴¹ The respective mutational frequencies for JMML and CMML, from another group of investigators, were 10 and 5%.^{142,143} Others have also shown relatively high *CBL* mutation rates in CMML (13–15%)^{34,139} and one of the latter studies reported an 8% incidence in *BCR-ABL1*-negative atypical CML.³⁴ It is to be recalled that CMML, JMML and atypical CML are all subcategories of MDS/MPN.¹⁴⁴ By contrast, *CBL* mutations were infrequent in refractory anemia with ring sideroblasts and thrombocytosis (0 of 19 analyzed cases), a provisional MDS/MPN entity.¹⁴⁵

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.¹⁴¹ 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.^{141,143} By contrast, mutant *CBL* has been shown to coexist with mutations involving *RUNX1*, *FLT3*, *JAK2* and *TP53*.^{139,141}

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).³⁴ *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.^{34,139,142,146} Mutational frequency might be higher in post-MDS/MPN AML¹⁴² or in AML with core-binding factor or 11q aberrations.^{146,147} Acquisition of mutant *CBL* during disease progression from ET to post-ET MF was documented in one instance.³⁴ 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 *IDH3- α* , *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 AML^{149–151} and are infrequently seen in other tumors.^{152–155} These mutations were all heterozygous and affected three specific arginine residues: R132 (*IDH1*), R172 (the *IDH1* R132 analogous residue on *IDH2*) and R140 (*IDH2*). Functional characterization suggests a loss of activity toward isocitrate (that is, the mutant wild-type heterodimer has decreased affinity to isocitrate) and gain of function in catalyzing NADPH-dependent reduction of α -ketoglutarate to the (R) enantiomer of 2-hydroxyglutarate; the hypoxia-inducible factor-1 α pathway also appears to be activated.^{156,157} Excess accumulation of 2-hydroxyglutarate has been demonstrated in both glioma and AML with *IDH1* or *IDH2* mutations.^{150,151,156}

IDH1 (exon 4 affecting R132) and *IDH2* (exon 4 affecting R172) mutations are mutually exclusive and occur in >70% and <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.^{158,161,163} 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,¹⁵⁰ 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 *IDH* mutations.^{149,150,167}

IDH 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.³⁶ In this particular study, there was not 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.³⁶ In another study of AML occurring in the setting of *JAK2*-mutated MPN,³⁵ mutant *IDH* 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 *IDH* 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 *IDH2*R140Q, the mutation was detected in both *JAK2*V617F-positive erythroid colonies and leukemic blasts. The authors did not find *IDH* 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 *IDH* mutations including five *IDH1* and four *IDH2* were found and mutational frequencies were ~21% for blast-phase MPN and ~4% for PMF. No mutations were seen in PV or ET. Furthermore, *IDH* 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 *IDH*-mutated case. The specific *IDH1* mutations found in this study included R132C and R132S and the *IDH2* mutations R140Q and R140W. *IDH* mutations coexisted with *JAK2*V617F. The results of this and the aforementioned study suggest that *IDH* 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 MPN but were detected in approximately 19% of patients with blast-phase MPN.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 2005.² Unlike CML, pathogenetic mechanisms in these *BCR-ABL1*-negative MPN are turning out

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*, *IDH* 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,^{27,31,32,68,69,105,112} 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 INCB018424 (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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