Oncogenes in myeloproliferative disorders

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Introduction

In 1951, William Dameshek (1900-1969) highlighted the phenotypic similarities among chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocytemia (ET), and primary myelofibrosis (PMF), and grouped them under the rubric of myeloproliferative disorders (MPDs).1 In 1960,2 CML became the first cancer to be associated with a specific cytogenetic marker, the Philadelphia chromosome (Ph1), which subsequently was shown to harbor a reciprocal chromosomal translocation, t(9;22)(q34;q11).3 These seminal observations ultimately led to the identification of the disease-causing mutation (BCR-ABL)4 in CML and development of molecularly targeted therapy.5 Accordingly, the “classic” MPDs are now sub-classified into CML (i.e. BCR-ABL+) and the BCR-ABL(-) classic MPDs (i.e. PV, ET, and PMF).6 The recently described association between all three BCR-ABL(-) classic MPDs and a novel gain-of-function (GOF) JAK2 mutation (JAK2V617F) validates their continued consideration as a distinct MPD category (Table 1).7-10 Both ABL and JAK2 encode for cytoplasmic tyrosine protein kinases (PTKs), that become constitutively activated as a result of these mutations.

Between 1967 and 1981, Fialkow and colleagues used polymorphisms in the X-linked glucose-6-phosphate dehydrogenase locus to establish all four classic MPDs as clonal stem cell disorders, with evidence for involvement of myeloid11-14 and B lymphoid15,16 lineages. The stem cell origin of the classic MPDs has been confirmed by more recent studies demonstrating clonal involvement of B,17-19 T,17-19 and natural killer (NK)20 lymphocytes. Furthermore, early reports21 on the occurrence of Ph1-negative clonal B lymphocytes in CML have suggested that preclinical clonal myelopoiesis might antedate disease-causing mutations in classic MPDs. More recent observations that support such a contention include the emergence of new cytogenetic clones during successful treatment of CML with imatinib,22 the imperfect association between overall clonal load and JAK2V617F mutant allele burden in PV,23 and the observation that heritable MPD syndromes have been observed in which the JAK2V617F allele is acquired with development of MPD, but is not present in the germline of affected family members, indicating the presence of an antecedent predisposition allele.24

In addition to the classic MPDs, myeloid malignancies also include acute myeloid leukemia (AML), the myelodysplastic syndrome (MDS), and other chronic myeloid neoplasms that are now classified either under one all-inclusive category (i.e. “non-classic” MPDs; Table 1)6 or into multiple subcategories.25 The distinction between MPD and MDS is based primarily on bone marrow histology; MDS is usually characterized by trilineage dys hematopoiesis in the absence of monocytosis.26 However, precise histological distinction between MPD and MDS is not always possible; we have arbitrarily grouped such cases with other “non-classic” MPDs (Table 1)6 whereas the World Health Organization classification system have assigned them to a separate category (i.e. mixed MDS/MPD).25 Regardless, both classic and non-classic MPDs share the common characteristics of stem cell-derived clonal myeloproliferation, in most instances, and activation of intracellular signal transduction pathways through mutations of PTKs or their effector molecules, in some instances.27-29

Examples of mutant PTK genes in MPDs include BCR-ABL (invariably associated with CML),30 JAK2V617F (seen in approximately 95% of patients with PV and 50% of those with either ET or PMF),31 JAK2 exon 12 mutations (seen in the majority of patients with JAK2V617F-negative PV),32 FIP1L1-PDGFRα (seen in a unique subset of patients with chronic eosinophilic leukemia associated with systemic mastocytosis; CEL-SM),33 ETV6-PDGFRα (a rare mutation seen in CEL that is sometimes associated with monocytosis),34,35 ZNF198-FGFR1 (seen in patients with stem cell leukemia-lymphoma...
syndrome; SCLL),36 and KITD816V (seen in virtually all adult patients with SM).37 Examples of mutations in PTK effector molecules include MPLW515L/K (seen in approximately 5% and 1% of patients with PMF and ET, respectively)38,39 and RAS, PTPN11, or NF1 mutations, each seen in 15% to 30% of patients with juvenile myelomonocytic leukemia (JMML).40-42 In the current review, we will discuss the aforementioned MPD-associated oncogenes and highlight the use of their corresponding oncoproteins as drug targets. For the sake of completeness, we have also included a section on GATA-1 mutations and Down syndrome-associated transient MPD.43

**ABL mutations**

ABL is a cytoplasmic PTK encoded by the ABL gene (Abelson murine leukemia viral (v-abl) oncogene homolog 1) located on chromosome 9q34.1. Physiologic functions of ABL include non-erythroid myelopoiesis44 and cytoskeletal rearrangement including regulation of small GTPases, inhibition of cell migration, and F-actin binding.45 Wild-type ABL exists in two isoforms that can localize to both the cytoplasm and nucleus, influencing cell proliferation/survival and apoptosis, respectively, through complex interactions with other cellular proteins.46-48 ABL contains both an SH2 and an SH3 (autoregulatory) domain in addition to the catalytic kinase domain and undergoes a treatment-relevant conformational change when activated by phosphorylation of the activation loop tyrosine residues.49

The most extensively studied ABL mutation is BCR-ABL; the disease causing mutation in CML.50,51 CML is the first leukemia to be described52 and also the first to be associated with a consistent cytogenetic abnormality, the Philadelphia chromosome (Ph1).53 Ph1 is a shortened chromosome 22 that is the consequence of a reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11).3 The chromosome 9 breakpoints involves a large, ~200 kb region within the ABL alternative first exons (1a and 1b), but invariably result in fusion genes that incorporate ABL exon 2.54 In contrast, the breakpoints on chromosome 22 are clustered within three much smaller regions of the BCR gene;55 the major breakpoint cluster region (M-bcr; a 5.8 kb region spanning exons 12-16 and resulting in a p210 fusion protein),56 the minor breakpoint cluster region (m-bcr; upstream of M-bcr and involving the first intron and resulting in a p190 fusion protein),57,58 and µ-bcr involving intron 19 that is downstream of M-bcr and resulting in a p230 fusion protein.59 By far the most frequent chromosome 22 breakpoint in CML is M-bcr and the other two, in the context of CML, are extremely rare. There are usually two junction variants of M-bcr; b2a2 and b3a2, without any documented clinical relevance.60

The Philadelphia translocation is an acquired somatic mutation involving the hematopoietic stem cell11 and results in fusion of the ABL gene (225 kb total gene size) from chromosome 9 to the BCR gene (135 kb total gene size) on chromosome 22.36,61,62 A chimeric mRNA (8.5-kb) is thus transcribed instead of the normal c-Abl mRNA (a 6- or 7-kb)63 and subsequently translated to an activated BCR-ABL gene product (most commonly 210-kD) instead of the normal ABL gene product (145-kD).64 BCR-ABL localizes to the cytoskeleton and displays an up-regulated tyrosine kinase activity65 that leads to the recruitment of downstream effectors of cell proliferation and cell survival and consequently leukemogenesis, as has been demonstrated in cell lines, primary cells, and mouse transplant or transgenic models.66-71 BCR-ABL signal transduction involves several adapter molecules (e.g. GRB2, GAB2, CRKL, etc.) and signaling pathways (e.g. Ras, PI3K, JAK-STAT, etc.) that are all thought to contribute to the pathogenesis of CML (Figure 1).49,52,73

CML is currently considered the “poster child” for molecularly targeted therapy. In 1996, Brian Druker and his colleagues described the BCR-ABL selective in vitro activity of imatinib mesylate (imatinib), a 2-phenylaminopyrimidine class PTK inhibitor that is a selective inhibitor of ABL, ARG, PDGFRα, PDGFRβ, and KIT.74 Imatinib targets the ATP binding site within the BCR-ABL tyrosine kinase and competitively inhibits ATP binding with subsequent disruption of the oncogenic signal.74-76 The co-crystal structure of imatinib bound to the ABL kinase indicates that the drug stabilizes the oncoprotein in an enzymatically inactive conformation.76 This in vitro activity of imatinib has been clinically validated in chronic phase CML; in newly diagnosed patients, the drug produced 95% and 74% complete hematologic and cytogenetic responses, respectively.77-80 However, the drug is not as effective in advanced phases of CML7 and imatinib-resistant BCR-ABL oncoproteins are emerging as an important
clinical challenge. The latter are most often due to acquired ABL point mutations that either directly hinder drug binding or prevent the formation of the inactive structural conformation that is required for imatinib inhibitory activity. Examples of such kinase domain mutations include M351T, E255K, Q252H, T315I, E355G, F359V, F317Leu, and G250E. The development of second generation kinase inhibitors and better understanding of mutant ABL conformation dynamics have effectively addressed these new challenges, although some mutations such as T315I have so far remained resistant to even second generation kinase inhibitors.

To date, ETV6 is the only known non-BCR fusion partner for ABL. ETV6-ABL (t(9;12)(q34;p13) has rarely been associated with ALL, AML (transient response to imatinib), and atypical CML-like MPD (minor response to imatinib). Like BCR-ABL, ETV6-ABL encodes for an activated and transforming ABL. The PNT oligomerization motif of ETV6 is thought to activate the ABL kinase by a similar mechanism to the BCR coiled coil oligomerization domain in the context of the BCR-ABL fusion protein. The same ETV6 activation domain is involved in CEL-associated ETV6-PDGFRB, (t(5;12)(q33;p13), AML-associated ETV6-ARG, (t(1;12)(q25;p13), ALL/atypical MPD-associated ETV6-JAK2, (t(9;12)(p24;p13), and peripheral T cell lymphoma-associated ETV6-FGFR3, (t(4;12)(p16;p13).

**JAK mutations in MPD**

The Janus family of tyrosine kinases (JAK1, JAK2, JAK3, TYK2) are cytoplasmic PTKs that mediate signaling downstream of cytokine receptors. Janus kinases are named after the Roman god with two faces, and are unique among tyrosine kinases in that they contain two homologous kinase domains: a catalytically active JH1 domain and JH2 domain that is nearly identical in amino acid sequence to JH1, but lacks catalytic activity. The JH2, or pseudo-kinase, domain is thought to play a negative autoregulatory role in that deletion of JH2 results in constitutive activation of the JAK2 kinase. Activation of the JAK-cytokine receptor complex results in recruitment and JAK-mediated phosphorylation of substrate molecules including STAT proteins whose subsequent nuclear translocation induces target gene transcription. JAK-STAT signaling has pleiotropic effects on cellular proliferation, cell survival, and immune responses. JAK1 and JAK2 knockouts are not viable and are devoid of either definitive erythropoiesis (JAK2) or normal lymphoid development (JAK1). JAK3 and TYK2 knockout mice are viable and manifest either severe combined immunodeficiency (SCID; JAK3) or impaired interferon signaling (TYK2).

A limited number of JAK mutations have been linked to human disease; these have so far involved only JAK3 and JAK2 (Table 2). Germline JAK3 loss-of-function (LOF) mutations have been associated with autosomal recessive SCID whereas somatic GOF JAK3 mutations (JAK3A572V, JAK3V722I, and JAK3P132T) were recently detected in acute megakaryocytic leukemia cell lines or primary cells. The oncogenic potential of JAK2 mutations in man was first suggested by a set of observations made in the 1990s; a dominant mutation in either the JH4 (HOP humming) or JH2 (HOP742) domain of HOP, a JAK homolog in Drosophila, resulted in leukemia-like defects whereas a similar point mutation in murine JAK2 (JAK2E661F) resulted in an activated protein. Similarly, others demonstrated inhibition of ALL cell growth by a JAK2 kinase inhibitor (AG-490) and the association of ETV6-JAK2, t(9;12)(p24;p13), with both T and pre-B ALL as well as atypical MPD. Most recently, two other JAK2 fusion mutants were described; PCM1-JAK2, t(8;9)(p22;p24), in eosinophilia-associated atypical MPD, ALL/AML, or T cell lymphoma and BCR-JAK2, t(9;22)(p24;q11.2), in atypical MPD that was not responsive to imatinib. However, JAK2V617F constitutes the most MPD-relevant JAK mutation because it is found in virtually all patients with PV and about half of those with ET or PMF. JAK2V617F is a G to T somatic mutation of JAK2, at nucleotide 1849, in exon 14, resulting in the substitution of valine to phenylalanine at codon 617. The association of JAK2V617F with MPDs, including PV, ET, and PMF, was first reported in 2005. Since then, however, the mutation has been described, at a lower frequency, in a spectrum of other myeloid disorders including non-classic MPDs and MDS but not in lymphoid disorders, solid tumor, or secondary myeloproliferation. Furthermore, whereas mutational frequency exceeds 50% in BCR-ABL classic MPD, it is less than...
JAK2V617F occurs in certain acute leukemia cell lines including the human erythroleukemia (HEL) cell line. HEL cells carry the JAK2V617F mutation in a homozygous state; display constitutive activation of JAK2, STAT5, and ERK; and their growth is inhibited by a small molecule JAK2 inhibitor. Similarly, when JAK2V617F is transfected in various cell lines, it promotes cytokine-independent and erythropoietin (Epo)- or interleukin-3 (IL-3)-hypersensitive growth accompanied by constitutive activation of JAK2, STAT5, and PI3K, ERK, and Akt pathways. Similarly, primary blood cells from mice with JAK2V617F-induced MPD display constitutive STAT5 activation and endogenous and Epo-hypersensitive erythroid colony formation. In vivo, such mice manifest a PV-like disease with erythrocytosis but not thrombocytosis; also seen are low serum Epo level, splenomegaly, extramedullary hematopoiesis, megakaryocytic hyperplasia, bone marrow fibrosis, and anemia. The STAT5/Bcl-xL pathway might be the key mediator of JAK2V617F effect since Epo-independent terminal differentiation of erythroid precursors as well as endogenous erythroid colony formation have been demonstrated through STAT5 activation or Bcl-xL overexpression. Additional laboratory studies have also suggested that there may be aberrant cytokine receptor interactions and the need for the expression of homodimeric cytokine receptors for JAK2V617F functionality.

The role of JAK2V617F in human disease is currently under intense investigation. The mutation occurs at a primitive stem cell level and is chronologically an early event. In this regard, some studies have suggested clonal involvement of NK, T, and B lymphocytes while others did not. Familial MPD studies have confirmed the somatic nature of JAK2V617F and did not reveal differences in mutation distribution compared to that seen in sporadic MPD. These findings suggest that there is an as yet undefined allele in familial MPD that in some way predisposes to the acquisition of the JAK2V617F allele. The same might be true in sporadic MPD; there is evidence to suggest that JAK2V617F may not be the initial clonogenic event in either PV or other MPDs and its presence might not be mandatory for endogenous colony formation. The recent demonstration of JAK2V617F-negative leukemia clones arising from JAK2V617F-positive MPD lends further support in this regard.

At the same time, however, JAK2V617F or other JAK2 mutations might be an essential component of PV because of their variable association with the disease whereas germline genetic variation/host modifiers and/or the occurrence of other concomitant mutations might play a part in diverting the disease phenotype from PV toward ET or PMF, in the presence of the JAK2 mutation. Figure 2 presents a pathogenetic scenario that takes these observations into account. Furthermore, a high mutant allele burden, rather than the mere presence of JAK2V617F, might be essential for erythroid-weighted myeloproliferation and the acquisition of the PV phenotype in vivo; this is accomplished by mitotic recombination that leads to homozygosity for JAK2V617F. In other words, homozygosity for JAK2V617F is believed to contribute towards full phenotypic penetrance in PV. Accordingly, JAK2V617F clonal load is significantly higher in PV compared to ET, where a pure mutant allele state is rarely encountered even at a single colony level. Most recently, other JAK2 mutations were described in JAK2V617F-negative patients with PV and in “idiopathic” erythrocytosis. The majority of such cases (10 of 11 in one study) were found to harbor one of four exon 12 JAK2 mutant alleles with functional relevance that is similar to that of JAK2V617F; they induce cytokine-independent/hypersensitive proliferation in erythropoietin receptor-expressing cell lines and a PV-like phenotype in mice. The four newly described exon 12 mutations, which include both in-frame deletions and tandem point mutations, appear to be specific to either PV or idiopathic erythrocytosis. The latter refers to a phenotype with increased red cell mass that fails to fulfill conventional diagnostic criteria for either PV or secondary polycythemia. Unlike the case with PV-associated JAK2V617F, exon 12 JAK2 mutations are heterozygous but associated with stronger abnormal JAK2 activation.

Although the precise role of JAK2 mutations in MPD pathogenesis remains under investigation, the currently available information warrants consideration of JAK2 and other JAK-STAT pathway molecules as potential drug targets in the treatment of MPD. Endogenous regulation of JAK2 signaling
occurs at multiple levels including direct dephosphorylation by specific protein tyrosine phosphatases, proteolytic degradation of JAK2 through binding with a family of suppressors of cytokine signaling, and inhibition of DNA binding of STAT by protein inhibitors of activated STAT.\textsuperscript{173-176} Exogenous negative regulation is usually accomplished by variably specific JAK kinase inhibitors.\textsuperscript{177} One such compound is the tyrphostin AG-490, which \textit{in vitro} induces death to pre-B ALL cells with constitutive JAK2 expression.\textsuperscript{178} Similarly, both AG-490 and other small molecule pan-JAK inhibitors\textsuperscript{8} inhibit JAK2V617F-associated signaling and/or proliferation in the context of both HEL cells\textsuperscript{8,152} and primary cells.\textsuperscript{158} Furthermore, such JAK-STAT inhibition is not restricted to mutant JAK but is also seen with MPD-associated mutant MPL.\textsuperscript{38} However, none of these compounds are candidate drugs and their activity is relatively non-specific.\textsuperscript{179,180} There is currently intense activity in the development and testing of both specific and non-specific (e.g. aurora kinase inhibitors)\textsuperscript{81} JAK2 inhibitors and the first clinical trials have already been initiated.

\textbf{MPL mutations}

Thrombopoietin (Tpo) is the key growth factor for megakaryopoiesis and is essential for platelet production.\textsuperscript{182} Tpo acts through its receptor, Mpl, and the JAK-STAT signal transduction pathway.\textsuperscript{183} The human \textit{TPO} gene is located at chromosome 3q27\textsuperscript{184} and that of \textit{MPL} at chromosome 1p34.\textsuperscript{185} Mutations involving \textit{TPO} in man have been described in autosomal-dominant familial thrombocytosis\textsuperscript{186,187} and those of MPL in either familial thrombocytosis\textsuperscript{188} or congenital amegakaryocytic thrombocytopenia.\textsuperscript{189-191} Such mutations, however, have not been found in sporadic cases of ET.\textsuperscript{192,193} Nonetheless, the potential pathogenetic contribution of the Tpo-Mpl axis to human MPD has been suggested by two important observations; systemic over-expression of Tpo causes myelofibrosis in mice\textsuperscript{194-197} and Mpl expression is markedly decreased in megakaryocytes and platelets of patients with PV and other MPDs.\textsuperscript{198,199} In ET, the latter phenomenon has been attributed to reduced \textit{MPL} transcription\textsuperscript{198,200} and in PV to post-translational hypo-glycosylation and defective membrane localization.\textsuperscript{201}

Most recently, a somatic GOF \textit{MPLW515L} mutation (a G to T transition at nucleotide 1544 resulting in a tryptophan to leucine substitution at codon 515 of the transmembrane region) was described in \textit{JAK2V617F}-negative PMF.\textsuperscript{38} Subsequently, an additional \textit{MPL} mutation involving the same 515 codon (\textit{MPLW515K}) was incidentally discovered during screening for \textit{MPLW515L} and the prevalence of both mutations was determined at approximately 5\% in PMF and 1\% in ET.\textsuperscript{39} Interestingly, some patients with \textit{MPL} mutations also displayed a minor \textit{JAK2V617F} clone, an observation that is not easily explained and underscores the complexity of pathogenetic mechanisms in MPD.

As is the case with \textit{JAK2V617F}, \textit{MPL515} mutations are early, stem cell-derived events\textsuperscript{163} and \textit{MPLW515L} has been shown to transform cell lines in terms of both cytokine-independent growth and Tpo hypersensitivity, activate JAK-STAT/ERK/Akt, and induce PMF-like disease in mice that is characterized by a rapid fatal course, marked thrombocytosis, leukocytosis, hepatosplenomegaly, and bone marrow fibrosis.\textsuperscript{38} Furthermore, \textit{MPLW515L}-induced cell growth was effectively inhibited by a small molecule JAK inhibitor raising the prospect of a similar treatment strategy for both \textit{MPL} and \textit{JAK2} mutation-associated MPD.\textsuperscript{38} Finally, it should be noted that one of the aforementioned germline \textit{MPL} mutations associated with familial thrombocytosis also occurred in the transmembrane region and was activating.\textsuperscript{188}

\textbf{PDGFR mutations}

Both platelet-derived growth factor receptors \textit{a} (\textit{PDGFRA} located on chromosome 4q12) and \textit{b} (\textit{PDGFRB} located on chromosome 5q31-q32) are involved in MPD-relevant activating mutations (Table 2). Clinical phenotype in both instances includes prominent blood eosinophilia and excellent response to imatinib therapy.

In regards to \textit{PDGFRA} mutations, the most intensively studied has been the \textit{FIP1L1-PDGFR}, a karyotypically occult del(4)(q12), that was described in the year 2003 as an imatinib-sensitive activating mutation.\textsuperscript{202} Subsequent studies have demonstrated the stem cell origin of the particular mutation\textsuperscript{203} and functional studies have demonstrated transforming properties in cell lines and the induction of MPD in
mice. Cloning of the FIP1L1-PDGFRA fusion gene identified a novel molecular mechanism for generating this constitutively active fusion tyrosine kinase, wherein a ~800kb interstitial deletion within 4q12 fuses the 5’ portion of FIP1L1 to the 3’ portion of PDGFRA. Molecular studies have shown that the breakpoint in FIP1L1 is relatively promiscuous, while the PDGFRA breakpoint is restricted to exon 12 that encodes part of the protein-protein interaction module with two fully conserved tryptophans (WW domain)-containing the JM region with resultant disruption of its autoinhibitory activity. Further biochemical analysis has shown that, in contrast with most tyrosine kinase fusions associated with human cancers, the FIP1L1 encoded sequences are dispensable for transformation, and there is no requirement for a dimerization motif; disruption of the autoinhibitory juxtamembrane motif as an invariant consequence of disruption of exon 12 is the basis for constitutive activation of PDGFRA kinase activity.

FIP1L1-PDGFRA occurs in a very small subset of patients who present with the phenotypic features of both CEL and SM (i.e. CEL-SM) but the presence of the mutation reliably predicts complete hematologic and molecular response to imatinib therapy. The breakpoint in FIP1L1 is relatively promiscuous, while the PDGFRA breakpoint is restricted to exon 12 that encodes part of the protein-protein interaction module with two fully conserved tryptophans (WW domain)-containing the JM region with resultant disruption of its autoinhibitory activity. Further biochemical analysis has shown that, in contrast with most tyrosine kinase fusions associated with human cancers, the FIP1L1 encoded sequences are dispensable for transformation, and there is no requirement for a dimerization motif; disruption of the autoinhibitory juxtamembrane motif as an invariant consequence of disruption of exon 12 is the basis for constitutive activation of PDGFRA kinase activity.

PDGFRA activation associated with CEL has also been described with karyotypically apparent fusion mutations including KIF5B-PDGFRA, t(4;10)(q12;p11), BCR-PDGFRA, t(4;22)(q12;q11), and CDK5RAP2-PDGFRA, ins(9;4)(q33;q12q25). In the former instance, the breakpoints involved exon 3 of the kinesin family member 5B and exon 12 of PDGFRA resulting in an in-frame fusion. The patient achieved complete hematological and molecular remission with imatinib therapy. BCR-PDGFRA represents an in-frame fusion with BCR breakpoints in intron 7/exon 12/exon 1/exon 17 and PDGFRA breakpoint in exon 12/exon 13 and is also sensitive to imatinib therapy. CDK5RAP2-PDGFRA also represents an imatinib-sensitive in-frame fusion involving exon 13 of CDK5RAP2 and intron 9/exon 12 of PDGFRA. As is the case with FIP1L1-PDGFRA, currently known PDGFRA breakpoints are noted to be tightly clustered in the JM region, which once again highlights a key regulatory role for this domain.

The association between eosinophilic myeloid malignancies and PDGFRB rearrangement was first characterized and published in 1994 where fusion of the tyrosine kinase encoding region of PDGFRB to the ets- like gene, ETV6 (ETV6-PDGFRB, t(5;12)(q33;p13) was demonstrated. The fusion protein was transforming to cell lines and resulted in constitutive activation of PDGFRB signaling. Since then, several other PDGFRB fusion transcripts with similar disease phenotypes have been described (Table 2), cell line transformation and MPD-induction in mice has been demonstrated, and imatinib therapy was effective when employed. Additional evidence regarding the oncogenicity of activated PDGFRB comes from experiments with mice where either ETV6-PDGFRB or H4-PDGFRB induced lymphoblastic lymphoma. In most of these mutations, PDGFRB is fused to the N-terminal segment of a partner protein that encodes for one or more oligomerization domains.

FGFR1 mutations

Human stem cell leukemic/lymphoma syndrome (SCLL), also known as the 8p11 myeloproliferative syndrome, constitutes a clinical phenotype with features of both lymphoma and eosinophilic MPD and characterized by a fusion mutation that involves the gene for fibroblast growth factor receptor-1 (FGFR1), which is located on chromosome 8p11. In SCLL, both myeloid and lymphoid lineage cells exhibit the 8p11 translocation, thus demonstrating the stem cell origin of the disease. The disease features several 8p11-linked chromosome translocations as outlined in Table 2, and MPD-induction in mice has been demonstrated, and imatinib therapy was effective when employed. Additional evidence regarding the oncogenicity of activated PDGFRB comes from experiments with mice where either ETV6-PDGFRB or H4-PDGFRB induced lymphoblastic lymphoma. In most of these mutations, PDGFRB is fused to the N-terminal segment of a partner protein that encodes for one or more oligomerization domains.
to that seen with PDGFRB-associated CEL-UMP; the tyrosine kinase domain of FGFR1 is juxtaposed to a dimerization domain from the partner gene. SCLL is refractory to usual chemotherapy and some (e.g. PKC412) but not other (e.g. imatinib) kinase inhibitors have been shown to inhibit in vitro kinase activity as well as cell proliferation induced by ZNF198-FGFR1.

**KIT mutations**

Kit, encoded by KIT (located at chromosome 4q12), is a receptor tyrosine kinase, and along with its ligand, stem cell factor (SCF), is important in hematopoiesis, gametogenesis, and melanogenesis. Activating Kit mutations have been described in a spectrum of hematologic (e.g. mastocytosis, acute leukemia) and non-hematologic (e.g. gastrointestinal stromal cell tumor (GIST), germ cell tumor) malignancies. Among these, SM is considered a MPD by virtue of its clonal derivation from the hematopoietic stem cell and the SCF-Kit axis is considered key for human mast cell growth and survival.

In mast cells from normal mice, SCF induces wild-type Kit tyrosine phosphorylation whereas such phosphorylation is constitutive in the murine mastocytoma cell line, P-815; receptor association with PI3K but not GAP-Ras occurs in both instances. The constitutive Kit activation in P-815 was later traced to a dominant-positive, catalytic domain point mutation at nucleotide 2468 (GC to TA transversion) resulting in an amino acid substitution at codon 814 (KITD814Y). A similar scenario occurs with a human mast cell leukemia cell line (HMC-1) where a homologous KITD816V mutation (A to T transition at nucleotide 2468) as well as another juxtamembrane domain activating mutation, KITV560G (mouse homolog KITD559G), have been demonstrated.

KITD816V was initially detected from peripheral blood mononuclear cells of patients with MDS/MPD-associated SM but not from those with indolent or aggressive SM or solitary mastocytoma. However, although in an inconsistent manner, a series of subsequent studies demonstrated the presence of KITD816V and other KIT mutations (KITD820G, KITV560G, KITD816Y, KITE839K, KITD816F, KITD559G) in all SM variants including indolent SM in children (skin) or adults (skin), aggressive SM (mast cells), and a rare variant with morphologically and immunophenotypically mature-appearing mastocytosis (Figure 7). The discrepancy in the literature regarding the prevalence of KIT mutations in SM is partly explained by differences among studies in terms of the type of tissue screened for the mutation and it is possible that mast cell-derived DNA carries the mutation in all instances of SM in either adults or children.

KITD814Y induces growth factor independent growth, mast cell differentiation, and oncogenic transformation in mast cell lines. Similarly, retroviral infection of hematopoietic progenitors with mouse KITD814Y and KITV559G mutants induces autonomous myeloid and mast cell colony formation as well acute leukemia in murine transplant models. Furthermore, transgenic mice expressing KITD816V restricted to their mast cells display an SM phenotype that closely resembles the clinically heterogeneous disease in man. This is in contrast to another report that suggested a differentiating but not transforming potential for the mutation, which nevertheless probably participates in enhancing mast cell chemotaxis and clustering. Mutant Kit signaling might involve both PI3K and Src participation although utilization of pathway molecules might be different between the wild-type and mutant protein.

Wild-type Kit is inhibited by imatinib whereas such drug sensitivity is displayed by some (e.g. V560G, F522C) but not other (e.g. D816V) mutant Kit both in vitro and in vivo. Other kinase inhibitors, such as PKC412, nilotinib, dasatinib, indoliones (e.g. SU11652, SU11654), and Src-selective kinase inhibitors might or might not overcome drug resistance by KITD816V. Accordingly, the first three agents are currently undergoing clinical trials in patients with SM. An alternative approach to the treatment of SM considers the use of drugs that target downstream molecules of mutant Kit such as m-TOR. Also, recent studies have suggested the therapeutic utility of the heat shock protein binding antibiotic, 17-Allylamino-17-demethoxygeldanamycin, and specific NF-κB inhibitors. Finally, it is worth mentioning that KIT mutations are also identified as a treatment-relevant disease markers in both core binding factor (CBF) AML and GIST (Figure 7).
Mutations involving Ras signaling pathway molecules

Mutations involving the Ras signal transduction pathway are most prevalent in the childhood MPD, JMML, a disease characterized by monocytosis, hepatosplenomegaly, thrombocytopenia, and elevated fetal hemoglobin.291 JMML is often associated with monosomy 7292-294 and arises at the level of the pluripotent stem cell.295-297 Myeloid progenitors in JMML display selective granulocyte-macrophage colony-stimulating factor (GM-CSF) hypersensitivity298 and the use of GM-CSF antagonists either in vitro against primary cells299 or in mouse model of the disease300 profoundly inhibits JMML cell growth. This phenomenon has been attributed to altered Ras signaling as a result of mutually exclusive mutations affecting one of the pathway regulatory molecules including the genes for RAS, PTPN11, and NF1.293,301 A third of patients with JMML display PTPN11 mutations40,301 whereas the incidence of NF1293 and RAS29 mutations are approximately 15-20% each. These mutations do not appear to segregate with specific clinical or cytogenetic phenotypes.301 Other MPD-characteristic mutations occur rarely in JMML and include PDGFRB-HCMOGT-1224 and JAK2V617F.302

Ras is a GTP-dependent protein (G-protein) and is localized at the inner side of the cell membrane. This 21 kD protein is encoded by the RAS gene family (KRAS (12p12.1), HRAS (11p15.5), and NRAS (1p13.2)), binds guanine nucleotides, displays intrinsic GTPase activity, and transduces signal from growth factor receptors to downstream effectors (Figure 8). The GTPase-mediated cycling between Ras bound GTP (active conformation) and GDP (inactive conformation) serves as a regulatory switch for signal transduction. Ras point mutations, often involving codons 12, 13, and 61, disrupt this function by diminishing Ras-associated GTPase activity and locking Ras in the active state (Ras-GTP). Such mutations, especially those involving NRAS, are ubiquitous (5-15% incidence) among myeloid malignancies including AML,303 MDS,304 and atypical BCR-ABL(-) MPDs,305 especially CMML.306 In JMML, RAS mutations often represent single nucleotide substitutions, involve the P loop domain of either KRAS (~15%)293,307 or NRAS (~30%),29,308 and have been shown to cause the disease phenotype in mice.309 In cell lines, RAS mutations are transforming310,311 and have been shown to activate Ras signaling311 and cause accumulation of GTP-bound Ras.311,312 Similarly, in murine-derived primary hematopoietic cells, KRAS mutations have been shown to induce cytokine independent growth of CFU-GM and GM-CSF hypersensitivity.311

Neurofibromatosis, type 1 (NF1), located on chromosome 17q11.2, is a tumor suppressor gene that encodes for the 327-kD neurofibromin protein that inactivates Ras through acceleration of Ras-associated GTP hydrolysis (i.e. NF1 is a GTPase activating protein (GAP) for Ras).313 Germline NF1 mutations cause Von Recklinghausen’s disease (a disease characterized by café au lait spots and peripheral nerve tumors such as neurofibromas), NF-1, whereas somatic mutations of the same cause cancer.314 Children with NF-1 are predisposed to JMML315,316 and display NF1 allelic losses (i.e. loss-of-heterozygosity) in myeloid progenitor cells.41,317 Primary leukemic cells from such patients accumulate Ras in the active GTP-bound state.318 NF1 allelic losses are also seen in isolated JMML317,319 where the incidence of NF1 mutations is estimated at 15%.293 NF1 knockout mice develop an MPD-like disease and display GM-CSF hypersensitivity, which appears to be central to the disease phenotype.318,320

PTPN11 (Protein-Tyrosine Phosphatase, Nonreceptor-type, 11), located on chromosome 12q24.1, encodes for the non-receptor protein tyrosine phosphatase, SHP-2, that transmits signals from growth factor receptors to Ras. Germline, gain-of-function PTPN11 mutations are found in approximately 45% of patients with Noonan syndrome (others may carry germline KRAS mutations),312 a developmental disorder with cardiac and skeletal defects.321 Such patients are at risk of developing JMML.322 Conversely, several large studies of isolated JMML suggest a greater than 30% incidence of PTPN11 mutations.40,301,323 JMML-associated PTPN11 mutations are somatic, usually distinct and more potent than those associated with Noonan syndrome,321 and often involve exon 3 in the SH2 domain and codon 76 (E76K is the most common).324 In cell lines, PTPN11 mutations induce increased enzymatic activity and cell proliferation.40,301,321,325 In mice, transduction of PTPN11 mutant alleles in bone marrow cells induces GM-CSF hypersensitivity,313,324,326,327 constitutive Erk and Akt activation,52 and an MPD phenotype.52 PTPN11 mutations are also found in other hematological and other malignancies328-331 and the mechanism of leukemogenesis might involve
enhanced interaction between the SH2 domain of the mutant molecule and Gab2/Grb2/p85 that leads to activation of Erk and PI3K pathways.326

The above discussed pathogenetic disclosure in JMML has ignited interest in testing the therapeutic potential of molecules that interfere with the RAS signaling pathway.332 Accordingly, a recent multi-center study evaluated the farnesyltransferase inhibitor R115777 (Zarnestra) in JMML although the results were not impressive.333 The particular study was based on previous laboratory studies of farnesyltransferase inhibitors that showed suppression of JMML colony growth.332 The mechanism of such action is linked to drug-induced blockage of Ras prenylation that is required for membrane localization of Ras, which is functionally essential. Similarly, in vitro cell transformation by mutant PTPN11 is suppressed by inhibitors of individual components of the Ras signal transduction pathway including PTP (sodium orthovanadate),321 Erk (UO126),321 mTOR (rapamycin),321 and Raf-1 (BAY 43-9006).334

**GATA1 mutations**

GATA-1 is a zinc-finger transcription factor for erythroid, megakaryocyte, mast, eosinophil, and Sertoli cell-specific genes and is essential for cell differentiation and survival.335-343 Friend of GATA-1 (FOG) is a cofactor in this process.344 The genes encoding for these two factors are located on chromosomes Xp11.23 and 16q24.3, respectively. GATA-1 and/or FOG knockout mice die in utero and are deficient in definitive erythropoiesis and display retarded maturation of megakaryocytes with thrombocytopenia and hyperproliferation of immature megakaryocytes.345-347 In this regard, recent information suggests that GATA-1 might exert its influence at the level of megakaryocyte-erythroid bifunctional progenitors.348

GATA-1 mutations have been identified in patients with Down syndrome who develop either transient myeloproliferative disorder (TMD) or acute megakaryoblastic leukemia (AMKL).43 TMD is a myeloproliferative reaction (immature megakaryoblast proliferation in the peripheral blood, bone marrow and liver) that occurs in approximately 10% of infants with Down syndrome (DS) but can occasionally occur in phenotypically normal infants who however display trisomy 21 in their hematopoietic cells (i.e. GATA-1 associated TMD or AMKL does not occur in the absence of trisomy 21).350 TMD is usually recognized at birth and often undergoes spontaneous regression but can progress into AMKL in approximately 25% of the cases by age 3 years. Almost all patients with both TMD and TMD-derived AMKL, but not those with other DS-associated leukemias, display GATA1 mutations that are somatic, often occur in utero, involve exon 2 (insertions, deletions, or missense mutations) and thus affect the N-terminal transactivation domain of GATA-1, and are characterized by loss of full-length (50-kD) GATA-1 and its replacement with a shorter isoform (40-kD) that retains FOG-1 binding.351-360 In contrast, inherited forms of exon 2 GATA1 mutations produce a phenotype with anemia but not thrombocytopenia whereas exon 4 mutations that affect the N-terminal, FOG-1-interactive domain, produce familial dyserythropoietic anemia with thrombocytopenia or X-linked macrothrombocytopenia.361

It is currently not clear why TMD is transient in some patients while it progresses to AMKL in others. It is possible that GATA1 mutations are not by themselves sufficient to cause either TMD or AMKL unless accompanied by trisomy 21 and other co-operating mutations, respectively. RUNX1 is located on chromosome 21q22.3, serves as an essential transcription factor for terminal differentiation of megakaryocytes, and is frequently mutated in acute leukemia. RUNX1 has been shown to functionally and physically interact with GATA-1 (through its zinc-finger domain) and its over-expression favors megakaryocytic diversion of cell differentiation whereas inhibition of erythroid differentiation is seen with its deacetylation-mediated repression by mutant RUNX1. Furthermore, transgenic mouse experiments have suggested myeloid leukemogenicity of increased RUNX1 dosage Therefore, the extra-copy of RUNX1 in DS could represent the missing link for DS-associated TMD and AMKL.

The pathogenetic role of GATA-1 in MPDs unrelated to Down syndrome has been considered partly because of the demonstration of a PMF-like phenotype in mice in the presence of megakaryocyte lineage restricted under-expression of GATA-1. The particular animal model of PMF also displayed
other features of human PMF including decreased megakaryocyte MPL expression and abnormal interaction between megakaryocytes and neutrophils or osteoblasts.\textsuperscript{370,371} Consistent with these observations, GATA-1 protein levels in megakaryocytes of some patients with PMF are decreased\textsuperscript{372} although alterations in either gene structure or gene expression of either GATA-1 or FOG-1 have not been detected.\textsuperscript{372-375} Thrombopoietin (Tpo) treatment of GATA-1\textsuperscript{low} animal models of PMF restores megakaryocyte GATA-1 expression and abrogates development of myelofibrosis whereas the opposite effect (i.e. reduction of GATA-1 content) is seen in wild-type mice.\textsuperscript{376} Taken together, these observations suggest a fibrosis-relevant interaction between Tpo and GATA-1, whose levels in human PMF might be indirectly affected by alterations in other molecules.

**Concluding remarks**

Current information on the molecular pathogenesis of MPDs suggests the existence of a preclinical stage of clonal myelopoiesis that antedates the emergence of “disease-causing” mutations (e.g. $BCR-ABL$) whose phenotypic effects are further modified by additional “disease-patterning” mutations (e.g. $JAK2V617F$). The discovery of functional mutations in MPDs identifies pathogenetically relevant signal pathways and marks their molecules as potential drug targets. This is best exemplified by the CML-$BCR/ABL$-imatinib paradigm and recently reaffirmed by the impressive activity of imatinib in $PDGFRA/\beta$-rearranged MPDs,\textsuperscript{202} an observation that demonstrated the role of novel drugs in providing insight into disease pathogenesis as well as the advantage of limited molecular “promiscuity” by kinase inhibitors. Similarly, the remarkable association between $JAK2V617F$ and the classic $BCR-ABL$\textsuperscript{(c)} MPDs validates the fundamental role of JAK-STAT signaling in disease pathogenesis and has aroused major interest in small molecule drug development. At the same time, the occurrence of cytogenetic remissions with immunomodulatory drugs in MDS\textsuperscript{377} and PMF\textsuperscript{378} have added another layer of complexity to disease pathogenesis that involves the host immune system and cytokine network (Figure 5).\textsuperscript{379,384} Understanding these mechanisms offers another dimension for treatment options. Finally, it is underscored that the failure to demonstrate “cure” and/or eradication of mutant clones with novel therapeutic approaches does not undermine their ultimate value in preventing disease-associated death and morbidity, although it implies the need for continuous therapy.\textsuperscript{385}

![Figure 1. BCR-ABL signaling.](image-url)
Figure 2. The contribution of JAK2V617F to the pathogenesis of myeloproliferative disorders.

Figure 3. KIT mutations in systemic mastocytosis (SM), gastrointestinal stromal cell tumors (GIST), and core-binding factor acute myeloid leukemias (CBF-AML).
Figure 4. Mutations (red asterix) across the RAS signal transduction pathway in juvenile myelomonocytic leukemia.

Figure 5. Pathogenetic mechanisms in primary myelofibrosis.
## Table 1. Semi-molecular classification of myeloproliferative disorders (MPD).

<table>
<thead>
<tr>
<th>Main categories</th>
<th>Clinicopathologic subcategories</th>
<th>Molecular signatures</th>
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</thead>
<tbody>
<tr>
<td>I. Classic MPD</td>
<td>1. Chronic myeloid leukemia (CML)</td>
<td>100% BCR-ABL&lt;sup&gt;(+)&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
|                 | 2. Polycythemia vera (PV)      | ~95% JAK2V617F<sup>(+)</sup>  
|                 |                                 | ~5% JAK2 exon 12 mutations |
|                 | 3. Essential thrombocytopenia (ET) | ~50% JAK2V617F<sup>(+)</sup>  
|                 |                                 | ~1% MPLW515L/K<sup>(+)</sup> |
|                 | 4. Primary myelofibrosis (PMF)  | ~50% JAK2V617F<sup>(+)</sup>  
|                 |                                 | ~5% MPLW515L/K<sup>(+)</sup> |
| II. Non-classic MPD | 1. Chronic neutrophilic leukemia (CNL) | ~20% JAK2V617F<sup>(+)</sup>  |
|                 | 2. Chronic eosinophilic leukemia (CEL) | 100% FIP1L1-PDGFRα<sup>(+)</sup>  
|                 | i. FIP1L1-PDGFRα<sup>(+)</sup> CEL-SM | 100% PDGFRB translocations  
|                 | ii. PDGFRB-rearranged CEL-UMPD | 100% PDGFRB translocations  
|                 | iii. Molecularly not characterized CEL | |
|                 | 3. Hypereosinophilic syndrome (HES) |  |
|                 | 4. Chronic basophilic leukemia (CBL) |  |
|                 | 5. Chronic myelomonocytic leukemia (CMML) | ~3% JAK2V617F<sup>(+)</sup>  |
|                 | 6. Juvenile myelomonocytic leukemia (JMML) | ~30% PTPN11 mutation<sup>(+)</sup>  
|                 |                                 | ~15% NF1 mutation<sup>(+)</sup>  
|                 |                                 | ~15% RAS mutation<sup>(+)</sup>  |
|                 | 7. Systemic mastocytosis (SM)     | ~100% KIT-mutation<sup>(+)</sup>  |
|                 | 8. Stem cell leukemia-lymphoma syndrome (SCLL) | 100% FGFR1 translocations  |
|                 | 9. Otherwise unclassified MPD (UMPD) |  |
|                 | i. BCR-ABL<sup>(+)</sup> CML-like MPD (a.k.a. atypical CML) | ~20% JAK2V617F<sup>(+)</sup>  
|                 | ii. Unclassified MDS/MPD including RARS-T | ~50% JAK2V617F<sup>(+)</sup>  |

Key: MDS, myelodysplastic syndrome; RARS-T, refractory anemia with ringed sideroblasts associated with thrombocytosis and megakaryocyte abnormalities similar to those seen in MPDs.
Table 2. Mutations of putative pathogenetic relevance in myeloproliferative disorders (MPD).

<table>
<thead>
<tr>
<th>JAK2 (phenotype)</th>
<th>MPL (phenotype)</th>
<th>PDGFR A (phenotype)</th>
<th>PDGFR B (phenotype)</th>
<th>FGFR1 (phenotype)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2V617F (PV, ET, PMF)</td>
<td>MPLW515L/K (PMF, ET)</td>
<td>FIP1L1-PDGFR A del(14q12) (CEL with mastocytosis)</td>
<td>ETV6-PDGFR B t(5;12)(q33;p13) (CMML with eosinophilia)</td>
<td>ZNF 198-FGFR1 t(8;13)(p11;q12) (SCLL)</td>
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<tr>
<td>JAK2 Exon 12 mutations</td>
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<tr>
<td>F537-K539delinsL</td>
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<tr>
<td>H538K</td>
<td>K539L</td>
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<tr>
<td>N542-E543del (PV)</td>
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<tr>
<td>BCR-PDGFR A</td>
<td>t(4;22)(q12;q11) (CEL, UMPD)</td>
<td></td>
<td></td>
<td>FOP-FGFR1 t(6;8)(p27;p11) (SCLL)</td>
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<tr>
<td>ETV6-JAK2</td>
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<tr>
<td>t(9;12)(p24;p13) (AML, UMPD)</td>
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<tr>
<td>KIF5B-PDGFR A</td>
<td>t(4;10)(q12;p11) (CEL)</td>
<td>CEV14-PDGFR B t(5;14)(q33;q32) (AML with eosinophilia)</td>
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<tr>
<td>NIN-PDGFR B</td>
<td>t(5;14)(q33;q24) (UMPD with eosinophilia)</td>
<td>KIAA1509-PDGFR B t(5;14)(q31;q32) (CMML with eosinophilia)</td>
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<tr>
<td>BCR-JAK2</td>
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<tr>
<td>t(9;22)(p24;q11.2) (UMPD)</td>
<td></td>
<td></td>
<td>TIF1-FGFR1 t(7;8)(q34;p11) (SCLL)</td>
<td></td>
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<tr>
<td>PDE4DIP-PDGFR B</td>
<td>t(1;5)(q23;q33) (UMPD with eosinophilia)</td>
<td>MYO18A-FGFR1 t(8;17)(p11;q23) (SCLL)</td>
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<tr>
<td>PCMI-JAK2</td>
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<tr>
<td>t(8;9)(p22;p24) (AML, UMPD)</td>
<td></td>
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<td>HERV-K-FGFR1 t(8;19)(p12;q13.3) (SCLL)</td>
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<tr>
<td>HIP1-PDGFR B</td>
<td>t(5;7)(q33;q11.2) (CMML with eosinophilia)</td>
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<tr>
<td>H4-PDGFR B</td>
<td>t(5;10)(q33;q22) (UMPD)</td>
<td></td>
<td>BCR-FGFR1 t(8;22)(p11;q11) (CML-like MPD)</td>
<td></td>
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<tr>
<td>CEP110-FGFR1</td>
<td>t(8;9)(p12;q33) (SCLL)</td>
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Key: PV, polycythemia vera; ET, essential thrombocytopenia; PMF, primary myelofibrosis; AML, acute myeloid leukemia; UMPD, unclassified MPD; CEL-SM, chronic eosinophilic leukemia associated with systemic mastocytosis; CMML, chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia; SCLL, stem cell leukemia-lymphoma syndrome.
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