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Review

Oncogenic mechanisms in myeloproliferative disorders

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Abstract. Myeloproliferative disorders (MPDs) are clonal haematopoietic malignancies involving the abnormal proliferation of myeloid lineages. The World Health Organisation (WHO) classification of haematopoietic malignancies distinguishes MPDs from myelodysplastic/myeloproliferative disorders and systemic mastocytosis. These malignancies frequently involve constitutive tyrosine kinase activity, resulting from either oncogenic fusion protein production or from point mutations. Chronic myelogenous leukaemia is the model used for studies of the consequences of such molecular defects. However, the heterogeneity of the clinical course of MPDs should be seen in a more rationale conceptual framework, including the many molecular events associated with these diseases. This review focuses on the various tyrosine kinase-related molecular mechanisms underlying both MPDs and rare diseases with myeloproliferative features. We pay particular attention to the newly identified JAK2 V617F mutation in polycythaemia vera, essential thrombocythaemia and idiopathic myelofibrosis and deal with disease heterogeneity and putative additional molecular mechanisms.

Keywords. Myeloproliferative disorders, tyrosine kinase, BCR/ABL, signal transduction, JAK2, leukaemogenesis.

Introduction

Myeloproliferative disorders (MPDs) are clonal haematopoietic malignancies in which one or several myeloid lineages (*i.e.* granulocytic, erythroid and megakaryocytic) are abnormally amplified. This amplification is thought to result from the deregulation of haematopoietic stem cells with a downstream selective proliferative advantage in late myeloid differentiation. These disorders can be divided into two groups: (i) classic MPDs, comprising chronic myelogenous leukaemia (CML), polycythaemia vera (PV), essential thrombocythaemia (ET) and idiopathic myelofibrosis (IMF) and (ii) a group of less frequent MPDs including chronic neutrophilic leukaemia (CNL), hypereosinophilic syndrome/chronic eosinophilic leukaemia (HEL/CEL) and other unclassifiable myeloproliferations. Systemic mast cell proliferations are also considered to be a type of MPD and some intermediate entities, such as chronic myelo-monocytic leukaemia (CMML) and atypical chronic myelogenous leukaemia are classified as myelodysplastic/myeloproliferative diseases (MDS/MPDs) (Fig. 1a).

MPDs frequently involve the deregulation of a tyrosine kinase, the archetype of which is BCR/ABL in CML. However, MPDs are heterogeneous in terms of their molecular origin, lineage involvement and natural course. The recent discovery of a recurrent mutation in the tyrosine kinase Janus kinase 2 (*JAK2*) gene in most cases

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of PV and in about half of the patients with ET and IMF [1–5] has led to a radical reassessment of MPD classification, the criteria for diagnosis and perspectives for treatment. Gene fusion rearrangements involving the platelet-derived growth factor receptors α (*PDGFRA*) and β (*PDGFRB*), fibroblast growth factor receptor-1 (*FGFR1*) and *JAK2* genes have been identified in rare MPDs [6–12]. Mutations have also been identified in the *KIT* gene, which encodes the stem cell factor (SCF) receptor, in systemic mastocytosis (SM) [13–15]. These advances, together with the discovery of the JAK2 V617F mutation, constitute a major breakthrough, greatly increasing our knowledge of the molecular aetiology of MPDs and related diseases. Indeed, thanks to this work, the molecular origin of more than half of these haematopoietic malignancies has now been determined (Fig. 1b). In line with World Health Organisation (WHO) aims to develop a rational classification of tumours of haematopoietic and lymphoid tissues [16], genetic features have been introduced as essential diagnosis criteria for various haematopoietic malignancies. The identification of new acquired genetic abnormalities in haematological malignancies increases our understanding of the pathogenesis of these diseases and provides new specific diagnostic, prognostic and therapeutic tools for the management of patients.

In this review, we describe the various molecular mechanisms underlying classic MPDs with tyrosine kinase dysregulation, focusing particularly on the recently identified JAK2 V617F mutation. We then discuss the hypothetical mechanisms proposed for the remaining BCR/ABL- and JAK2 V617F-negative MPDs. In the next section, we focus on the molecular mechanisms underlying other rare and difficult-to-classify MPDs. Finally, we discuss the consequences of tyrosine kinase involvement in MPDs, in terms of advances in treatment and disease classification.

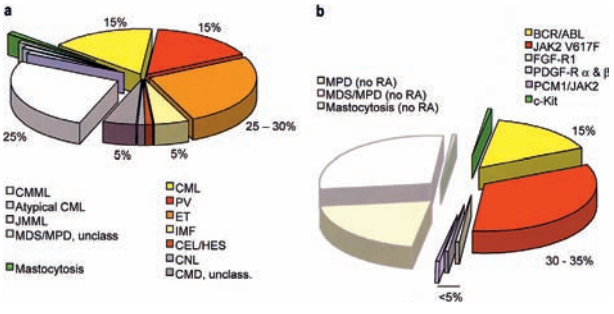

Figure 1. Distribution of MPD, MDS/MPD and SM, according to the WHO classification or a molecular classification. (*a*) The estimated frequency of each WHO entity is shown [16, 128–131]. The right part of the diagram represents MPDs and the left part MDS/MPD and SM. The overall incidence of MPDs, MDS/MPDs and mastocytosis is estimated to be approximately 10 per 100 000 individuals annually. The estimated percentages are given for the most frequent MPDs and MDS/MPDs. Smallest parts of the pie graph represent rare diseases with an estimated incidence below 1 case per million individuals annually. (*b*) The estimated frequency of each molecular entity is shown, with respect to the overall incidence of each disease as shown in *a*). The right part of the diagram shows the frequencies of the known molecular entities listed in the legend to the right-hand column (more than 50%) [16, 19, 93, 115, 128]. The left part corresponds to diseases with myeloproliferative features but with no known recurrent abnormality (no RA: no recurrent abnormality).

Classic myeloproliferative disorders

Classic MPDs were first recognised as a relatively homogeneous group of diseases, based on their similar clinical features and biological findings, by William Dameshek in 1951 [17]. CML, PV, ET and IMF are all characterised by primitive enhanced myeloid proliferation with various degrees of extramedullary haematopoiesis, leading to splenomegaly, spontaneous transformation into acute leukaemia and the development of marrow fibrosis and a secondary high risk of thrombotic and haemorrhagic events [18, 19]. CML, which differs from other classic MPDs in terms of the principal lineage involved, prognosis and natural course, was first separated from the other classic MPDs following the discovery of the recurrent t(9;22)(q34;q11) balanced translocation, revealed by the presence of the Philadelphia (Ph) chromosome [20]. This breakthrough in oncology was the first of a series of 'firsts' in the second half of the Twentieth Century. The discovery of this recurrent chromosomal abnormality [20, 21] led to the identification of the first oncogene produced by a fusion gene (*BCR/ABL*) [22], and to the development of the first anti-cancer therapy specifically targeting a molecular defect, by inhibiting the tyrosine kinase activity of BCR/ABL [23]. It also provided an impetus to research trying to identify recurrent cytogenetic abnormalities and to determine the role of other tyrosine kinases in Ph-negative MPDs. This led to the discovery of the recurrent *PDGFRB*, *PDGFRA* and *FGFR1* rearrangements [6–10, 12] in rare MPDs and, more recently, to the identification of the *JAK2* G1849T (V617F) mutation in PV, ET and IMF [2–5, 24].

Chronic myelogenous leukaemia

CML is now defined as a molecular entity with various myeloproliferative features. The natural course of CML differs little between patients and can be divided into three successive phases: the myeloproliferative phase, also known as the 'chronic phase', progresses towards an accelerating phase, characterised by the progressive cessation of normal haematopoietic maturation, and then towards acute leukaemia. Immediate transformation into acute leukaemia may occur in some cases, or isolated

thrombocytosis may lead to detection of the disease at a very early stage [25, 26]. As CML was one of the first MPDs to be discovered, a large number of studies of the molecular defects underlying this disease have been carried out. The results of these studies have improved patient management in terms of diagnosis, treatment and follow-up. The t(9;22)(q34;q11) translocation generates a fusion gene, which gives rise to a fused transcript that is translated into the BCR/ABL oncoprotein [27]. There are various possible breakpoints in this translocation, resulting in the generation of different transcripts, with different frequencies; some of these transcripts result in particular phenotypes [27]. The most frequent transcripts, b2a2 and b3a2, generating the $p210^{BCR/ABL}$ protein, are responsible for typical cases of CML (Fig. 2a). In contrast, rare transcripts, such as e1a2 and e19a2, are associated with particular cases with monocytosis or prominent mature granulocyte proliferation (Fig. 2a). Other transcripts, such as e1a3, e6a2, e13a3 and e14a3, occur only very rarely [27]. It should be noted that e1a2 ($p190^{BCR/ABL}$) is also found in *de novo* B cell progenitor acute lymphoblastic leukaemia [27].

What are the oncogenic mechanisms involved in CML? The molecular processes leading to leukaemogenesis are mostly dependent on the constitutive tyrosine kinase activity of the BCR/ABL oncoprotein. ABL is a widely expressed non-receptor tyrosine kinase present in both the nucleus and the cytoplasm of the cell. When functioning normally, it regulates the cytoskeleton structure *via* cell surface signalling pathways [28, 29]. BCR is also a signalling protein. It provides ABL with new regulatory and oligomerisation domains in the fusion protein and directs the ABL tyrosine kinase to the cytoplasm [30]. Whatever the BCR/ABL transcript produced, the ABL tyrosine kinase in the fusion protein is activated by oligomerisation *via* the BCR coiled-coil fused domain, resulting in the dysregulation of many signalling pathways (summarised in Fig. 2b) [30, 31]. BCR/ABL-dependent interactions and/or phosphorylation of signalling proteins leads to the activation of many targets, such as RAS, phosphatidylinositol 3-kinase (PI3K), protein kinase B (AKT), c-Jun kinase (JNK) and SRC kinases, protein and lipid phosphatases and transcription factors, such as signal transducers and activators of transcription

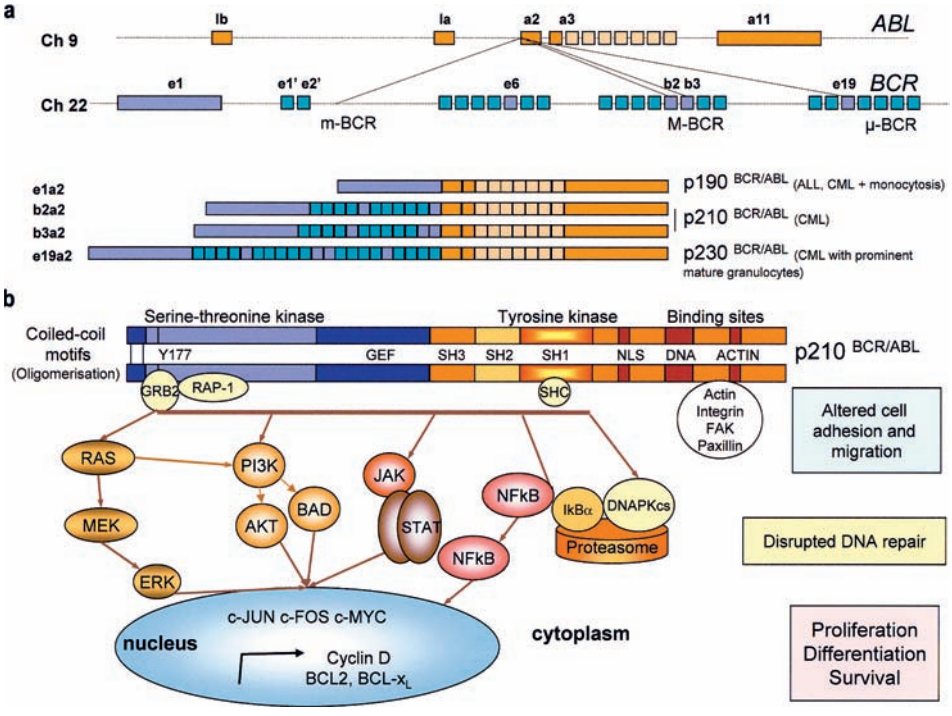

Figure 2. BCR/ABL breakpoints, transcripts and oncogenic mechanisms in CML. (*a*) The organisation of *ABL* and *BCR* genes on chromosomes 9 (Ch 9) and 22 (Ch 22) is shown: exons (squares) and introns (dashed lines) are represented, together with the most frequent breakpoints involved in CML (M: major, m: minor, µ: micro). The transcripts generated by the various fusions are shown: left legend: names of the transcripts; right legend: BCR/ABL proteins (p190, p210 and p230) and their associated diseases. (*b*) The $p210^{BCR/ABL}$ is oligomerised via its coiled-coil motif. The constitutive activation of the BCR/ABL tyrosine kinase activates multiple signalling pathways. Trans- and auto-phosphorylation at Y177 promotes growth factor receptor-bound protein 2 (GRB2) binding and leads to RAS/MAPK signalling. BCR/ABL also activates PI3K/AKT and JAK/STAT signalling pathways. The consequences of these effects in the transcriptional up-regulation and control of critical genes are indicated. Through the proteasome machinery, BCR/ABL also increases NFκB by IkB alpha degradation and modulates DNA repair proteins (DNA-PKcs). ABL binding site domains bind actin, integrins, FAK and paxillin, leading to the dysregulation of cell adhesion and/or migration. The multiple oncogenic effects induced by BCR/ABL are summarised in the boxes in the right part of the diagram.

(STATs), nuclear factor-kappa B (NF-kB) and MYC [30, 32]). The production of cytokines such as interleukin-3 (IL-3), granulocyte colony-stimulating factor (G-CSF) and granulocyte/macrophage colony-stimulating factor (GM-CSF) is also enhanced by BCR/ABL [33, 34]. In the murine haematopoietic FDCP-Mix cell line ectopically overexpressing $p210^{BCR/ABL}$, expression of the nephroblastoma overexpressed gene (*NOV* also called *CCN3*), a proto-oncogene involved in multiple internal and external signalling pathways influencing cell proliferation and differentiation, is down-regulated whereas secretion of the product of this gene is stimulated [35]. Studies of intracellular CCN3 depletion are currently underway, to determine the contribution of this protein to CML pathogenesis [35]. Another recent report also demonstrated the activation of lipocalin 24p3 expression by BCR/ABL in transformed murine cells, with the concomitant repression of its receptor, rendering BCR/ABL cells refractory to lipocalin 24p3 [36]. Lipocalin 24p3 regulates intracellular levels of iron, the depletion of which induces apoptosis *via* the pro-apoptotic protein BIM. It was therefore suggested that the regulation of apoptosis by intracellular iron level in BCR/ABL-positive cells would provide transformed cells with a selective advantage over normal cells [36]. These multiple activation pathways may account for the extraordinary ability of BCR/ABL to promote leukaemogenesis through proliferative advantage, anti-apoptotic properties, and changes in cytokine secretion, cell adhesion and migration (Fig. 2b) [30]. These properties may account for the onset of an MPD originating from a deregulated haematopoietic stem cell producing a larger than usual number of myeloid cells able to undergo terminal differentiation, but they leave several questions concerning the progression of CML to acute leukaemia unanswered. Are additional events absolutely necessary for blastic transformation? If so, why do these events occur in virtually all cases? BCR/ABL tyrosine kinase activity increases during disease progression from the chronic phase to blast crisis. This increase may result from amplification of the translocated *BCR/ABL* gene [37, 38] and may be directly involved in the blast crisis. BCR/ABL directly regulates the levels of certain transcription factors, including BACH2 [39], C/EBPα and C/EBPβ [40–42] and Ikaros splicing [43], blocking differentiation. Cytogenetic and/or genetic changes are frequently detected during the accelerated and blast phases, suggesting that additional events co-operate with BCR/ABL to produce the blast crisis. The genetic changes detected include mutations in *TP53*, *RB* and $p16^{INK4A}$, chromosome translocations including t(8;21)(q22;q22) (*AML1-ETO*), t(3;21)(q26;q22) (*AML1-EVI1*), inv16(p13;q22) (*CBFβ-SMMHC*), t(7;11)(p15;p15) (*NUP98-HOXA9*) and other abnormalities [30, 44–47]. Analyses of the consequences of these mutations and translocations provide clues to the way in which these additional genetic events modifying cell cycle or transcription factor activity co-operate with BCR/ABL to block myeloid differentiation and provoke a blast crisis. The acquisition of stem cell properties such as self-renewal by CML progenitor cells may also be an essential event in CML progression. In this regard, the activation of β-catenin in CML granulocyte-macrophage progenitors appears to enhance the self renewal of these cells, promoting blast crisis [48]. As a degree of genetic instability is required for the occurrence of such additional events, it seems likely that BCR/ABL disrupts DNA damage repair sufficiently to cause acute leukaemia. Indeed, BCR/ABL interferes with DNA repair mechanisms by increasing the rate of homologous recombination repair [49]. BCR/ABL increases the proteasome-dependent degradation of DNA-PKcs, which plays a major role in non-homologous end-joining repair [50] and down-regulates BRCA1 [51], thereby promoting the unfaithful repair of DNA double-strand breaks [52, 53]. BCR/ABL also disrupts cell cycle checkpoints and acts on BCL-2 members, thereby increasing the time available for the potentially deleterious repair of DNA lesions [49]. In addition, BCR/ABL-transformed cells produce reactive oxygen species in excess, resulting in oxidative damage and DNA lesions [54]. These mechanisms may account for the progression of BCR/ABL-positive MPD to acute leukaemia.

JAK2V617F-positive myeloproliferative disorders

The molecular defects underlying ET, PV and IMF have only recently been identified. These MPDs were considered to be promiscuous diseases because they have a number of biological, clinical and progression features in common. In PV, ET and IMF, haematopoietic progenitors are hypersensitive to various cytokines, including erythropoietin (Epo), IL-3, SCF, insulin-like growth factor-1 (IGF-1), GM-CSF and thrombopoietin (Tpo) [1, 55–58]. The biological features common to these diseases include the presence of endogenous erythroid colonies (EEC) [59], overproduction of polycythaemia rubra vera 1 (PRV-1) mRNA in granulocytes [60] and the low level of cell-surface expression of the Tpo receptor (Tpo-R or Mpl) on the platelets and megakaryocytes of most patients with PV, and of some patients with ET or IMF [61]. Finally, clinical progression is frequently observed from ET to PV or IMF and from PV to IMF. It was, therefore, thought likely that the molecular defects responsible for these MPDs would be very similar, if not identical.

Several groups, using different approaches, simultaneously identified the major molecular aetiology of Ph-negative classic MPDs. We used a functional strategy to investigate the mechanisms underlying EEC formation in PV. The involvement of several Epo receptor (Epo-R)-dependent signalling pathways was shown, with JAK2

the key upstream molecule [62]. Studies focusing on the JAK2 signalling pathway then led to identification of the V617F mutation [1]. Kralovics *et al*. used a different approach, delimiting the smallest interval common to PV patients with a loss of heterozygozity of the short arm of chromosome 9 (9pLOH). These authors identified *JAK2* as a main candidate gene and subsequently sequenced it [3]. Based on the high probability of tyrosine kinase involvement in these MPDs [63], other groups undertook the systematic sequencing of all the genes encoding tyrosine kinase proteins [2, 4, 5]. As a result of all these approaches, a recurrent point mutation in the *JAK2* gene was identified that is found in around 90% of patients with PV and 50% of patients with ET and IMF [19].

JAK2 tyrosine kinase. JAK2 is a member of a group of proteins with tyrosine kinase activity, the Janus kinases (JAK). These proteins bind the intracytoplasmic sequences of multiple cytokine receptors *via* their FERM (4.1 Ezrin Radixin Moesin)-like domain, promoting downstream cell signalling [64, 65]. JAK2 mediates signalling by various subfamilies of haematopoietic receptors without endogenous tyrosine kinase activity, such as type 1 homodimeric receptors (Epo-R; Tpo-R or Mpl; and G-CSF receptor) and type 1 and type 2 heteromeric receptors (gp130 and IL-3 receptor family, IFN-γ receptor) [66]. JAK2 is also involved, to a lesser extent, in signalling by receptors with tyrosine kinase activity, such as KIT [67]. Following cytokine binding, the two JAK2 proteins appended to the cytosolic domain of the receptor are activated by transphosphorylation. They then phosphorylate the tyrosine residues of the receptor. This induces the recruitment and phosphorylation of kinases involved in signal transduction pathways, such as PI3K, RAS complex and STAT5a/b [65]. JAK2 directly phosphorylates STAT5 docked to the receptor, leading to STAT5 dimerisation, nuclear translocation and transcriptional activity. Thus, JAK2 mediates cytokine signalling to regulate cell proliferation, differentiation and anti-apoptotic events. JAK2 also promotes maturation and is required for the efficient trafficking of homodimeric type 1 receptors, such as EpoR [68] and Mpl [69]. JAK2 stabilises the mature form of Mpl and promotes its recycling [69]. These two processes are defective in MPDs [61].

The JAK2 V617F mutation. This point mutation at position 1849 (G to T), resulting in a valine-to-phenylalanine substitution at codon 617, is located in the JH2 (JAK ho-

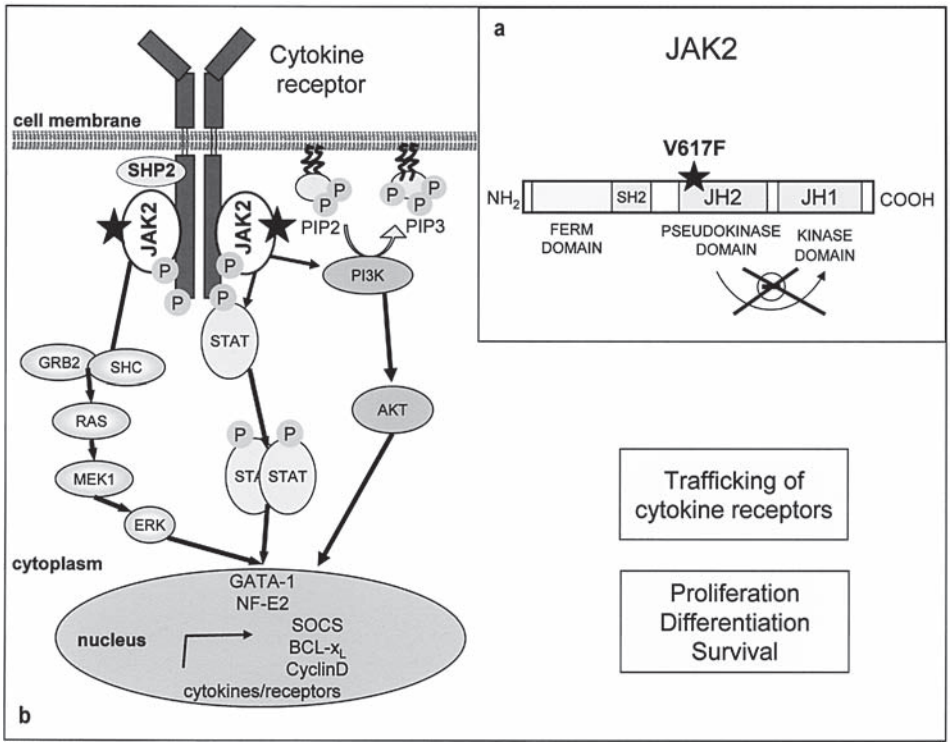

Figure 3. JAK2 V617F signalling in myeloproliferative disorders. (*a*) Structure of JAK2 V617F: the mutation is located in pseudokinase JAK homology domain 2 (JH2) and disrupts the auto-inhibition of this regulatory domain. Consequently, the tyrosine kinase corresponding to the JH1 domain is constitutively activated. (*b*) In the presence of a homodimeric cytokine receptor (for example Epo-R), the two JAK2 V617F proteins bound to the intracellular domain of the receptor transphosphorylate its tyrosine residues. In turn, STAT5, PI3K and RAS signalling pathways are activated, leading to the downstream modulation of transcription and protein levels for cell cycle, proliferation and apoptosis-related factors. P: phosphate, PIP2 and PIP3: phosphatidyl inositol bi- and tri-phosphate.

mology 2) domain of JAK2 [70, 71]. The crystal structure of the FGF tyrosine kinase receptor suggests that the JH2 domain would block the JAK2 kinase domain (JH1) [72]. The mutation may therefore disrupt an auto-inhibitory effect of the JH2 domain on JH1. In fact, JAK2 V617F is constitutively phosphorylated at the activation loop Y1007 and activates downstream kinase signalling pathways (JAK2-STAT5, ERK1/2 MAPK and PI3K/AKT) in a cytokine-independent or hypersensitive manner [1, 73, 74] (Fig. 3). This cytokine-independent or hypersensitive behaviour may be the result from partial constitutive activation associated with the ability of the mutant protein to be activated by cytokine receptor stimulation [1, 73, 74]. As reported *in vitro*, JAK2 V617F activity seems to be partly dependent on homodimeric type 1 cytokine receptor binding [73], particularly when the ratio of mutant to wild-type protein is low. This may account for the restriction to the myeloid lineage of JAK2 V617F pathogenesis, as the lymphoid lineage does not express type 1 receptors. However, if mutant JAK2 is expressed in large amounts (mutant to wild-type ratio > 2), low endogenous levels of IL3-R β chain or other cytokine receptors are sufficient for the transformation of Ba/F3 cells [74, 75]. As Mpl and gp130 are expressed in haematopoietic stem cells, it would be interesting to determine whether JAK2 V617F modifies the biological properties of these receptors through their activation or through other receptors, such as KIT or the IGF-1 receptor. PV erythroid progenitors are hypersensitive to cytokines and growth factors, including IGF-1 [58]. JAK2 V617F was expressed in IL-3-dependent Ba/F3 cells, and cells not selected for JAK2 V617F-induced autonomous growth were examined for sensitivity to IGF-1. These cells were found to be 10 times more sensitive to IGF-1 than the parental cells [74]. Moreover, in cells that have acquired autonomous growth after JAK2 V617F expression, IGF-1 induces the tyrosine phosphorylation of STAT5 and STAT3 and additional phosphorylation of JAK2 [74]. These data suggest that JAK2 V617F expression may connect cytokine receptor and tyrosine kinase receptor signalling. Such cross talk has been reported between EpoR and KIT, involving direct association and phosphorylation of the cytosolic EpoR tyrosine residues by the KIT tyrosine kinase [76]. Haematopoietic stem cell renewal is thought to be induced by IGF-2 [77]. Thus, the expression of JAK2 V617F in haematopoietic stem cells may modify the self-renewal rate of these cells, conferring an advantage on mutated cells. It remains to be determined whether JAK2 V617F can increase the very low affinity or reversible association between IGF-1-R and cytokine receptors.

The absence of lymphoid proliferation in JAK2 V617F diseases may be accounted for by the absence of homodimeric receptors in these lineages. However, the possibility that the signal mediated by JAK2 V617F may not be strong enough to induce proliferation in T cells cannot be excluded. Indeed, in mouse CTLL-2 cells, EpoR expression and JAK2 overexpression are not sufficient to trigger cell proliferation, due to a defect in MAP kinase activation [78]. Mutant JAK2 also alters Mpl trafficking (Staerk *et al*., manuscript in preparation), which normally requires JAK2 [69]. However, it remains unclear whether the defective Mpl processing reported in MPDs [61] is correlated with the presence of JAK2 V617F.

It has also been shown that wild-type JAK2 competes with JAK2 V617F, abolishing its effects, in Ba/F3 cells [1]. This observation may have implications for our understanding of the disease heterogeneity associated with JAK2 V617F zygosity, as discussed below. Moreover, the dominant negative effect of the wild-type JAK2 on the mutated protein may be involved in the selection of rare 9pLOH-positive subclones, which eliminate the wild-type gene by mitotic recombination, as observed in PV patients [1, 3, 75]. As competition by the wild-type JAK2 does not appear to decrease the intrinsic tyrosine phosphorylation of JAK2 V617F [4], competition probably involves binding to either a limiting number of cytokine receptors or to downstream signalling molecules.

Involvement of the JAK2 V617F mutation in MPDs. JAK2 V617F is detected in most cases of PV, in up to 70% of ET cases and 50% of IMF cases. This mutation has also been detected in rare MPDs such as CNL, some CMML and a few *de novo* acute leukaemias [19]. These differences in the frequency of this mutation in myeloid malignancies raise two questions: is the JAK2 mutation the direct primary cause of these diseases and how can we explain their clinical and biological heterogeneity?

Recent studies have shown that JAK2 V617F is not a germline mutation in familial MPDs [79, 80]. The coexistence of JAK2 mutated and non mutated MPDs in the same families suggests that alternative events, considered as predisposing factors, may promote the onset of MPDs. In these families, the occurrence of the JAK2 mutation and/or other somatic events may participate to a multiple step process leading to MPDs. There is strong evidence that JAK2 V617F plays a major role in the pathogenesis of PV. Indeed, in addition to the high frequency of the mutation, mice transplanted with bone marrow cells transduced by a JAK2 V617F retrovirus develop rapid erythrocytosis, progressing within a few months to a myelofibrotic state, thus mimicking the natural progression of PV [1, 81, 82]. However, there is still a lack of conclusive evidence for ET and IMF. Mutated JAK2 levels in granulocytes and bone marrow samples have been found to be markedly lower in ET patients than in PV and IMF patients. In addition, low levels of JAK2 V617F expression have been associated with the onset of thrombocytosis in the mouse model [82], suggesting that low levels of JAK2 V617F are associated with a thrombocythaemic state, whereas higher levels may trigger polycythaemic

and myelofibrotic diseases. This 'dosage' hypothesis is one of the explanations proposed to account for the heterogeneity of JAK2 V617F-positive MPDs.

It is indeed surprising that one single-point mutation can cause such different diseases [83], although ET, PV and IMF have phenotypic features and a characteristic natural clinical course in common [18, 84, 85]. The frequent progression of PV towards a myelofibrotic state and the progression of ET towards PV and myelofibrosis have been extensively described. However, it has been suggested that some cases of ET may actually correspond to the early stages of IMF [86, 87]. Three hypotheses have been proposed to explain why MPDs caused by the same mutation have different phenotypes: (i) according to the 'dosage' hypothesis, differences in the level of JAK2 V617F kinase activity determine the phenotype; (ii) according to the 'additional events' hypothesis, alternative and/or additional molecular abnormalities may modify, or even precede [88] a homogeneous state conferred by JAK2 V617F alone; and (iii) according to the 'stem cell fate' hypothesis, the identity of the progenitor/stem cell in which the mutation occurs dictates the nature of the lineage expanded. This third hypothesis is quite unlikely because there is growing evidence to suggest the involvement of a pluripotent haematopoietic stem cell with myeloid [1, 2, 89], B, NK and T potential in all these classic MPDs, based on clonality assays [90–92], 9pLOH [75] or direct JAK2 V617F detection in lymphoid lineages ([79] and Delhommeau, unpublished observations). Consequently, only additional specific molecular events or polymorphisms are likely to modify the consequences of the mutation in a lineage-dependent manner, in line with the 'additional events' hypothesis. The phenotypic diversity of JAK2 V617F-positive diseases is better explained by the 'dosage' hypothesis. Low levels of kinase activity would favour a thrombocytic phenotype, whereas higher levels would lead to an erythrocytic phenotype or a myelofibrotic state. As discussed above, this hypothesis is supported by animal models [1, 81, 82]. It is also supported by studies quantifying the mutated allele in ET, PV and IMF [1–5, 93]. Indeed, the vast majority (93–100%) of ET patients have low levels of mutated JAK2 and are described as 'heterozygous' for the mutation. In contrast, one third of PV and some IMF patients positive for V617F are described as 'homozygous', meaning that their granulocytes or bone marrow mononuclear cells harbour more mutated than wild-type JAK2 alleles [94, 95]. As these analyses were performed with cell populations rather than by clonal assays, the frequency of mitotic recombination leading to homozygous subclones may have been underestimated. Indeed, it has been demonstrated that most PV patients with less than 50% of mutated allele in their granulocytes have homozygous erythroid or granulocyte-macrophage progenitors in contrast to ET patient who had no homozygous progenitors [96]. It is now thought that the progression from a mixed heterozygous/homozygous population to a prominent homozygous clone observed in some patients [97] is the result of a growth advantage conferred by the higher level of kinase activity due to the presence of two mutated JAK2 alleles. Mechanisms other than mitotic recombination may increase kinase activity, including duplication of the mutated allele, which is observed in a proportion of PV and IMF patients displaying a gain of 9p, mostly due to trisomy 9 [98–100]. JAK2 V617F kinase activity may be dependent not only on the amounts of the mutant protein and its normal counterpart, but also on the various pathways regulating JAK2 activity. There may, therefore, be an overlap between the 'dosage' and 'additional molecular events' hypotheses. Indeed, some of these putative events may have an intrinsic ability to interfere with JAK2 kinase activity. These events include the deregulation of phosphatases [101] and Suppressors of cytokine signalling (SOCS) proteins [102], polymorphisms or mutations in cytokine receptors [103]. Other features suggesting the occurrence of additional molecular events in MPDs not directly related to JAK2 kinase activity also provide support for this alternative hypothesis. Sex appears to be a powerful 'genetic' modifier in V617F-positive MPDs, as ET is more common in women and PV is more common in men [104]. Mouse models have also shown that different diseases occur in different genetic backgrounds [81]. It would therefore seem necessary to analyse putative cytokine receptor polymorphisms [105, 106] that might influence white blood cell and platelet counts or haemoglobin levels in the presence of a JAK2 V617F mutation carefully, taking the sex of the patient into account. Finally, the frequencies of various chromosomal abnormalities are high in IMF patients [98, 107]. These molecular events clearly co-operate with the JAK2 V617F mutation in the determination of disease phenotype, but it remains unclear whether these events are primary or secondary. Clonality assays [88, 108, 109] and long-term studies are therefore required to delineate clearly the chronology and impact of the occurrence of putative additional molecular abnormalities, especially in cases of IMF and ET, in which the JAK2 V617F mutation may not be the primary event. Figure 4 summarises the heterogeneity of Ph-negative classic MPDs.

BCR/ABL- and JAK2 V617F-negative classic myeloproliferative disorders

A small subset of PV cases and approximately half of all ET and IMF cases do not harbour the JAK2 V617F mutation. However, it is thought that molecular defects involving similar signalling pathways would be able to induce these MPDs, based on the similar clinical and biological features, including the *in vitro* formation of EEC

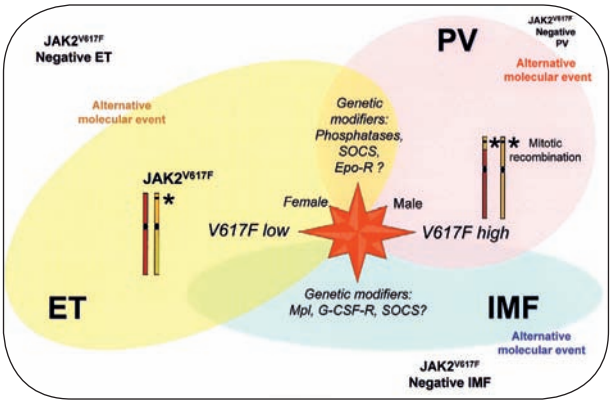

Figure 4. Heterogeneity of Ph-negative classic MPDs. ET, PV and IMF are classic Ph-negative MPDs. About 90% of PV cases, 50–70% of ET cases and 50% of IMF cases harbour the JAK2 V617F mutation and are characterised by common biological features and forms displaying similar courses. The parameters thought to be involved in disease heterogeneity are the level of mutated JAK2 tyrosine kinase activity (V617F low or V617F high, due to mitotic recombination or trisomy 9), sex (female, male) and various genetic modifiers such as polymorphisms or mutations in cytokine receptors or modifications in SOCS or phosphatase proteins. These parameters are represented around a compass rose indicating the direction towards which each modifier influences the phenotype. JAK2 V617F-negative ET, PV and IMF are also shown because the modifiers influencing the JAK2 V617F-positive MPD phenotype may overlap with the unknown molecular events underlying these MPDs.

or spontaneous megakaryocytic colonies in some cases of JAK2 V617F-negative ET and PV. Future studies should determine whether the molecular events underlying mutant JAK2-negative diseases are identical to the additional events in JAK2 V617F-positive malignancies. Defining this molecular overlap would also provide essential information about the primary event leading to the onset of MPDs. Thus, all the molecular hypotheses discussed above should be tested, in addition to functional and molecular approaches focusing on cytokine receptor signalling pathways. It remains unclear whether molecular defects not directly affecting tyrosine kinase proteins occur in these diseases. As familial forms of polycythaemia are associated with mutations in the Epo-R, the answer is probably 'yes', for at least a small subset of MPDs. Moreover, as demonstrated *in vitro*, mutant Mpl receptors with a disrupted motif at the junction between the transmembrane and cytoplasmic domains constitutively activate JAK2, TYK2, STAT5, and MAP kinase and induce haematopoietic myeloid differentiation in the absence of Tpo [110]. The careful screening of potential mutations in Epo-R, Mpl, G-CSF-R and other cytokine receptors was therefore logical. This strategy led to the recent identification of the MPL W515L and MPL W515K mutations [111, 112]. The MPL W515L mutation results in the constitutive activation of the Tpo-R signalling [111]. These MPL 515 mutations are detected in 5% of IMF cases and 1% of ET cases, and can occur in JAK2 V617F-positive cases [112].

Other myeloproliferative disorders

In addition to the classic chronic MPDs, various haematopoietic malignancies fully or partially meet the basic criteria for myeloproliferative diseases. Some of these heterogeneous entities, such as CEL and CNL, are classified as MPDs by the WHO, whereas others, such as CMML and atypical CML are classified as MDS/MPD. Finally, SM is now also considered to be a MPD. Like classic MPDs, these entities can be divided into two groups, based on the presence or absence of recurrent molecular abnormalities. Tyrosine kinase deregulation accounts for almost all these abnormalities, as summarised in Fig. 5.

Rare MPDs with recurrent genetic abnormalities

This heterogeneous group of diseases contains SM, myeloproliferations with high eosinophil levels, such as CEL and CMML with eosinophilia, and rare disorders known as stem cell leukaemia-lymphoma syndromes. All these diseases are characterised by the involvement of a proven or putative constitutively active tyrosine kinase generated by gene fusions or point mutations affecting platelet-derived growth factor receptor α and β (*PDGFRA, PDGFRB*), fibroblast growth factor receptor-1 (*FGFR1*), *JAK2*, and *KIT*.

PDGFRB, FGFR and JAK2 rearrangements. *PDGFRB* rearrangements are mostly observed in diseases with both myelodysplastic and myeloproliferative features, which are therefore classified as MDS/MPD in the WHO classification. The underlying molecular abnormalities affecting *PDGFRB* were first identified with the implication of t(5;12)(q31; p12). This translocation fuses the *ETV6* (*TEL*) gene to the *PDGFB* gene and generates an activated form of the tyrosine kinase. Like BCR/ABL, the fusion protein (PDGFRβ-TEL) is activated by oligomerisation due to the presence of the non-tyrosine kinase partner gene. CMML with eosinophilia accounts for no more than 2% of all cases of CMML, and is considered a separate entity by some authors [6, 113]. In CMML with and without eosinophilia, numerous translocations leading to the activation of the PBGFRβ tyrosine kinase have been described, involving the partners listed in Fig. 5 [63].

FGFR1 rearrangements have been observed in rare MPDs coupled with lymphoid proliferations – so-called stem cell leukaemia-lymphoma syndromes. The first recurring translocation described in this disorder was t(8;13)(p11;q12), fusing a zinc finger gene, *ZNF198*, to *FGFR1* [7–9]. This translocation leads to the constitutive

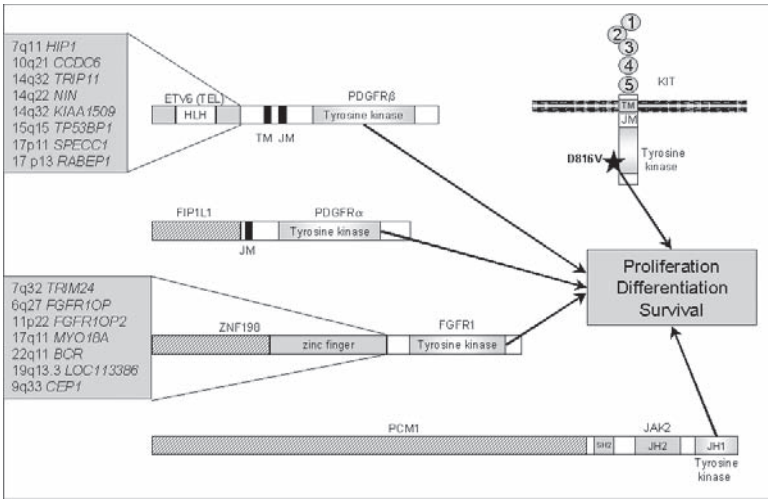

Figure 5. Recurrent tyrosine kinase abnormalities in Ph- and JAK2 V617F-negative MPDs. Protein structure of the tyrosine kinases involved in the pathogenesis of SM (KIT), CMML with eosinophilia (ETV6/PDGFRβ), CEL (FIP1L1/PDGFRα) and other rare haematopoietic malignancies with myeloproliferative features (ZNF198/FGFR1, PCM1/JAK2). The boxes in the left part of the diagram list the various alternative partner genes fused to *PDGFRB* in CMML or to *FGFR1* in stem cell leukaemia-lymphoma syndrome. The constitutive activation of these tyrosine kinases leads to proven or putative downstream signalling pathways similar to those shown in Figures 2 and 3, the deregulation of proliferation, survival and differentiation of the malignant cells. The main functional domains of each protein are indicated: HLH, helix loop helix; TM, transmembrane domain; JM, juxtamembrane domain; JH, Jak homology domain; SH2, Src homology domain 2; circles 1–5, immunoglobulin-like domains of the SCF receptor, KIT.

activation of a tyrosine kinase, the partner gene providing the tyrosine kinase gene with sequences encoding oligomerisation domains. Like *PDGFRB* rearrangements, *FGFR1* rearrangements involve a large number of partners, listed in Fig. 5 [63].

Various rare MPDs, acute myeloid leukaemia and T cell lymphoma have been shown to be related to t(8;9)(p22;p24), which results in fusion of the *PCM1* and *JAK2* genes [11]. According to the *ABL*, *FGFR1* and *PDGFRB* models, this *PCM1/JAK2* rearrangement is thought to induce constitutive activation of the JAK2 tyrosine kinase. In addition, marginal cases of *BCR/JAK2* rearrangement due to t(9;22)(p24;q11), with a typical CML phenotype, have been described [12].

Chronic eosinophilic leukaemia with *FIP1L1/PDGFRA* rearrangement. Chronic eosinophilic leukaemia (CEL) and hypereosinophilic syndrome (HES) are defined as proliferations of the eosinophilic lineage in the WHO classification. The diagnostic criteria for these disorders include persistently high peripheral eosinophil levels, the absence of parasitic, allergic or other recognised causes of eosinophilia and the existence of signs of organ system involvement, such as cardiac, lung, central nervous system or gastrointestinal tract lesions [114]. When there is evidence for eosinophilic clonality or an increase in blasts in the blood or bone marrow, the term preferred by the WHO is CEL. The molecular defect underlying a proportion (12–47%) of CEL cases [10, 115, 116] is an 800-kb deletion on chromosome 4, resulting in fusion of the *FIP1L1* and *PDGFRA* genes. This fusion generates a constitutive tyrosine kinase activity due to the disruption of the autoinhibitory domain of PDGFR-α, which does not involve dimerisation and is independent of FIP1L1 [117]. Interestingly, the features of CEL overlap with those of SM. No evidence implicating KIT mutations has been obtained, but a proportion of CEL cases with FIP1L1/PDGFRA rearrangement present both an increase in the number of pathological mast cells in the bone marrow and some typical symptoms of SM [115, 118].

***KIT* mutations in systemic mastocytosis.** Mastocytosis is a group of diseases divided into seven subtypes by the WHO classification, some of which are considered to be SM. Systemic mastocytosis comprises indolent SM, SM with associated clonal, non-mast cell lineage disease, aggressive SM and mast cell leukaemia. These diseases are characterised by the abnormal growth and accumulation of mast cells in one or more organ systems, including the bone marrow. As mast cells arise from haematopoietic stem cells and have several characteristic features in common with basophils, SM is often considered a true MPD with a specific clinical presentation. This point of view is supported by the involvement of a tyrosine kinase receptor, KIT, and by the promiscuity between SM and some cases of CEL with FIP1L1/PDGFRα, as discussed above. In most cases of adult SM, the *KIT* gene is mutated. KIT is the SCF receptor, a transmembrane protein harbouring an intracytoplasmic domain with intrinsic tyrosine kinase activity, requiring the binding of its ligand for activation.

The most frequent mutation in SM is the D816V mutation, affecting the activation loop of the tyrosine kinase domain [13, 14]. This mutation results in constitutive kinase activation, a mechanism thought to be involved in the proliferation and survival of pathological mast cells.

Rare and frontier forms with no known aetiology

These haematopoietic malignancies with myeloproliferative features but without recurrent molecular defects belong to three categories of diseases in the WHO classification: MPD, MDS/MPD and mastocytosis. Mastocytosis without *KIT* mutation is mostly a paediatric form of unknown aetiology. Rare MPDs include CNL and CEL without *JAK2* mutation or *FIP1L1/PDGFRA* rearrangement. Among MDS/MPDs, most cases of CMML do not involve eosinophilia and have no *PDGFRB* rearrangement. Patients with these disorders have an abnormal karyotype in 20–40% of the cases. Trisomy 8 or deletions of chromosome 7 are frequently observed. Another MDS/MPD displaying a degree of dysplasia is atypical CML. It is characterised by frequent trisomy 8, trisomy 13 and some 20q deletions, with *PDGFRB* rearrangement observed only in exceptional cases [119, 120]. These characteristics are not far from those of myelodysplastic syndromes. The identification of other underlying defects in CMML and atypical CML is, therefore, warranted to determine whether some cases would be better classified as MDS or MPD, on the basis of a molecular classification.

Detection of molecular hallmarks in clinical practice

In addition to increasing our understanding of the pathogenesis of MPDs, the assessment of a molecular abnormality in a disease makes it possible to improve the management of patients, particularly as concerns diagnosis and response to treatment.

The diagnosis of CML is now essentially based on the presence of BCR/ABL. This example clearly shows how the presence of a molecular defect has changed our attitudes to diagnosis and treatment. The detection of BCR/ABL leads to the administration of imatinib, directly targeting the molecular abnormality [23]. Molecular diagnosis has therefore now superseded clinical diagnosis, at least for CML. Other examples include the detection of *FIP1L1/PDGFRA* in the context of chronic hypereosinophilia, and the detection of *TEL/PDGFRB* in a case of CMML, both leading to a clear diagnosis and the prescription of imatinib, often improving the phenotype considerably. By analogy with these diseases, in which diagnosis is now essentially based on the presence of a molecular abnormality, we now need to consider whether the detection of JAK2 V617F constitutes a molecular marker of BCR-ABL-negative MPDs. Indeed, could the detection of molecular abnormalities form the basis of a new MPD classification, by analogy with recent changes in the WHO classification of acute leukaemias, in which molecular rearrangements delimit different subgroups of diseases [16]? Such a classification would distinguish between the *BCR/ABL*, *JAK2*, *PDGFRB*, *FGFR1*, *PDGFRA* and *KIT* groups of MPDs, but the molecular abnormalities underlying a proportion of MPDs would remain unknown. Further work is therefore required to identify these molecular defects and then to develop effective targeted therapy.

Deregulated protein kinases are a major therapeutic target. CML remains a model for demonstrations of the efficacy of kinase inhibitors. Indeed, determination of the molecular characteristics and biological consequences of constitutive activation of the BCR/ABL oncoprotein led to the development of imatinib, a tyrosine kinase inhibitor that targets ABL tyrosine kinase and certain other kinases, including c-KIT and PDGFR. Since its discovery [121], this treatment has been shown to be efficient, despite the occurrence of resistance, due in most cases to mutations in the ABL kinase domain. New tyrosine kinase inhibitors, such as AMN107 and dasatinib, have therefore been developed to overcome this drug resistance [122, 123]. As most MPDs involve tyrosine kinase deregulation, it was logical to apply this targeted strategy to Ph-negative MPDs. Imatinib has therefore been tested in various MPDs and other cancers [124]. However, the most striking results have been obtained in cases of CEL with *FIP1L1/PDGFRA,* in which the response to low-dose imatinib is spectacular [122]. In contrast, imatinib did not induce any response in SM with KIT D816V mutation, whereas dasatinib may be effective based on recent *in vitro* studies [125].

Finally, the detection of a molecular abnormality in a patient with an MPD makes it possible to assess the response to treatment precisely. CML has also served as a model disease in this respect. With the sequential measurement of BCR/ABL levels, it is now easy to assess the efficacy of treatment by imatinib or allogeneic stem cell transplantation, for example. Many publications dealing with minimal residual disease assessment have proposed treatment guidelines, all stressing the importance of BCR/ABL quantification during treatment [126].

One study recently stressed the value of monitoring minimal residual disease in patients with PV, by evaluating the percentage of JAK2 V617F by real-time PCR, in patients receiving IFN-α [127]. Kiladjian *et al.* have also shown that this drug preferentially targets the malignant clone, with a decrease in JAK2 V617F percentage observed following treatment in 24 of 27 patients (89%). This opens up new possibilities for the testing of drugs specifically designed to target JAK2 V617F, which will soon become available.

Conclusion

Where do the major advances towards understanding the precise molecular bases of MPDs lead us? One striking observation is the key role played by various tyrosine kinases, constitutively activated either by balanced translocations or deletions generating fusion oncoproteins, or by activating point mutations. These mechanisms seem to be the molecular hallmark of MPDs, although there are probably alternative mechanisms directly involving cytokine receptors, adaptor proteins or transcription factors. All the molecular defects identified in MPDs to date confer proliferation and survival advantages on transformed cells, which retain the capacity to differentiate into mature cells. Differentiation may be disrupted by additional events, such as transcription factor deregulation, as frequently observed in acute myeloid leukaemia. *KIT* mutations and *FLT3* abnormalities are frequently found in cases of acute myeloid leukaemia. However, these molecular defects are now considered to be secondary events. Strikingly, the diverse mutants and fusion proteins with constitutive tyrosine kinase activity each appear to stimulate a specific lineage. For example, PDGFR fusion proteins induce eosinophil differentiation, FGFR fusion proteins induce lymphoid malignancies, and JAK2 V617F mostly expands the erythroid compartment, whereas translocations involving the JAK2 kinase domain promote lymphoid proliferation. Thus, constitutive signalling via these different kinases is likely to result in effects on specific differentiation programs. Uncovering the molecular details of this specificity remains a major challenge, particularly as similar signalling molecules (*i.e.* STAT5, STAT3, RAS/MAPK, PI3K/AKT and others) are constitutively activated by all oncogenic fusion proteins.

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