Ham-Wasserman Lecture

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The Genetic Basis of Myeloproliferative Disorders

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For many decades, myeloproliferative disorders (MPD) were largely neglected orphan diseases. The conceptual work of William Dameshek in 1951 provided the basis for understanding MPD as a continuum of related syndromes, possibly with a common pathogenetic cause. Recognition of the clonal origin of peripheral blood cells in MPD in 1976 and the ability to grow erythroid colonies *in vitro* in the absence of added growth factors in 1974 initiated the search for genetic alterations that might be responsible for myeloproliferation. Mutations in the genes for the erythropoietin receptor, thrombopoietin and the von Hippel–Lindau protein were found to cause familial syndromes resembling MPD, but despite their phenotypic similarities, none of these mutations were later found in patients with the sporadic form of MPD. The discovery of activating mutations in the Janus kinase 2 (*JAK2*) in most patients with MPD has fully transformed and energized the MPD field. Sensitive assays for detecting the *JAK2*-V617F mutation have become an essential part of the diagnostic work-up, and *JAK2* now constitutes a prime target for developing specific inhibitors for the treatment of patients with MPD. Despite this progress, many questions remain unsolved, including how a single *JAK2* mutation causes three different MPD phenotypes, what other genes might be involved in the pathogenesis, and what are the factors determining the progression to acute leukemia.

Introduction

Myeloproliferative disorders (MPD) are a heterogeneous group of diseases characterized by increased proliferation of the erythroid, megakaryocytic or myeloid lineages. The entities subsumed into "MPD" have varied over time. Initially, chronic myelogenous leukemia (CML) was considered part of MPD,[1] but today is regarded as a separate entity characterized by the presence of *BCR/ABL*. Currently, the Philadelphia chromosome–negative MPDs comprise polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). The classification of MPD by the World Health Organization (WHO) also includes a number of rare disorders, that will not be considered in this review, such as chronic neutrophilic leukemia, chronic eosinophilic leukemia and hypereosinophilic syndrome.[2]

Early Work and MPD Milestones

PV was the first of the MPD syndromes to be recognized as a distinct clinical entity. Patients with plethora had been already noticed by Hippocrates, but it was Luis Henry Vaquez in 1892 who first published a detailed description of a patient with polycythemia, a case he called "cyanosis with persistent hyperglobulie."[3] Although he suspected that a congenital heart disease was causing the phenotype, he also considered the possibility that a "vital alteration of the hematopoietic organs" may be involved. In 1903, William Osler reported 4 additional cases, which he recognized as a new clinical entity.[4] He also discussed the report by Vaquez and concluded that these patients suffered from "true polycythemia" (i.e., an actual increase in the number of blood cells) as opposed to "relative polyglobulism" secondary to hemoconstriction.[4,5] At his time, the definition of "true polycythemia" did not yet exclude increased production of blood cells due to hypoxia or heart disease. Today, such cases would be classified as "secondary erythrocytosis." Epstein and Goeddel described the first case of ET in 1934, which they called "hemorrhagic thrombocythemia."[6] Myelofibrosis (sometimes also called myeloid metaplasia, i.e., extramedullary hematopoiesis) remains the most enigmatic of the MPD entities. In 1879, Heuck described the first two cases of myelofibrosis, which he called "leukemia with peculiar blood, respectively bone marrow findings."[7] The phenotypic variability and difficulty in delineating myelofibrosis/myeloid metaplasia from thrombocythemia, polycythemia and leukemia caused considerable confusion, which is illustrated by the fact that since

Heuck's first report more than a dozen different names for myelofibrosis have been used to describe it.[8] Very recently, another a new name, "primary myelofibrosis" (PMF), has been proposed.[9]

In his now-famous 1951 editorial entitled "Some Speculations on the Myeloproliferative Syndromes," William Dameshek suggested these conflicts be resolved by considering that the various diseases, including "chronic granulocytic leukemia, polycythemia vera, idiopathic myeloid metaplasia, thrombocythemia, megakaryocytic leukemia and erythroleukemia," are closely interrelated. Dameshek proposed "that these various conditions—"myeloproliferative disorders"—are all somewhat variable manifestations of proliferative activity of bone marrow cells, perhaps due to a hitherto undiscovered stimulus."[1] He was attracted by the concept of a "myelostimulatory principle, perhaps hormonal or steroid in type,"[1] for which some experimental evidence had accumulated at that time.[10] More than 20 years would pass before Jaroslav F. Prchal and Arthur A. Axelrad in 1974 proved that the expansion of hematopoietic progenitors in patients with PV is not driven by elevated levels of exogenous growth factors, but rather that the hematopoietic progenitors exhibited an endogenous growth potential, i.e., these progenitors are capable of forming endogenous erythroid colonies (EEC) *in vitro*.[11] EECs were later shown to be present also in a proportion of patients with ET and IMF. The concept of MPD as a disease of the hematopoietic stem cells was further supported by the work of Adamson, Fialkow, and colleagues, who in 1976 demonstrated that peripheral blood cells of PV patients are of clonal origin, suggesting that the defect in PV originates at the stem cell level.[12] By using restriction-fragment length polymorphisms in the X-chromosomal gene glucose-6-phosphate dehydrogenase (*G6PDH*),[13] they demonstrated that peripheral blood cells in a female patient expressed the *G6PDH* derived solely from one of the two parental X chromosomes. These reports set the stage for investigating the molecular and genetic basis of MPD.

Familial MPD

Familial syndromes resembling MPD were the first to be successfully examined by using molecular genetic approaches. The phenotypes can be grouped into two classes: (1) inherited disorders with Mendelian transmission, high penetrance, and polyclonal hematopoiesis with increased proliferation of a single hematopoietic lineage, e.g., erythropoiesis or megakaryopoiesis; and (2) hereditary predisposition to true MPD, characterized by low penetrance, clonal hematopoiesis and frequent occurrence of somatic mutations, e.g., in *JAK2*.

The prototypes for the first category of inherited disorders are the congenital gain-of-function mutations of the erythropoietin receptor gene (*EPOR)*, leading to primary familial and congenital polycythemia (PFCP).[14,15] Typically, multiple family members are affected, and the polycythemia is apparent at an early age with high penetrance. Affected PFCP family members display elevated erythrocyte mass, low erythropoietin (Epo) serum levels, polyclonal hematopoiesis, and normal platelet and leukocyte counts. To date, 10 different mutant alleles of the *EPOR* alleles have been described, all causing truncations of the cytoplasmic domain of the EpoR protein (**Table 1**).[16-26] These mutations result in a loss of a negative regulatory domain located at the C-terminus of EpoR. As a consequence, the erythroid colonies in these patients are hypersensitive to low concentrations of Epo but usually do not grow in the absence of Epo.[19,24,27,28] Truncations of the EpoR have so far not been detected in patients with sporadic MPD.[17,29] *EPOR* mutations account for only 10% to 20% of all PFCP.[24] In the remaining families, *EPOR* can be excluded by genetic analysis, and the gene mutation remains unknown. Recently, a number of familial polycythemia syndromes with high Epo serum levels have been described. No mutations in the *EPO* gene have detected to date. Instead, alterations in the oxygen-dependent regulation of *EPO* expression can result in overproduction of Epo protein. The best studied are autosomal recessive mutations in the von Hippel–Lindau (*VHL*) gene that were first discovered in patients suffering from polycythemia in the Chuvash Republic in central Asia, where this mutation is endemic.[30-32] Chuvash polycythemia is caused by a homozygous germline mutation in *VHL* (598C>T), re-

Table 1. Mutations in causing primary familial and congenital polycythemia.

Report	EPOR mutation	Consequences of the mutation
Arcasoy[25]	5881G>T	Glu399 > ter; truncation (loss of 110AA)
Kralovics[24]	5959G>T	Glu425 > ter; truncation (loss of 84AA)
Kralovics[21,26]	5964C>G	Tyr426 > ter; truncation (loss of 83AA)
Furukawa[18]	5986C>T	Gln435 > ter; truncation (loss of 75AA)
De la Chapelle,[16] Percy[22]	6002G>A	Trp439 > ter; truncation (loss of 70AA)
Rives[26]	6003 G>A	Trp439 > ter; truncation (loss of 70AA)
Sokol[133]	5974insG	Frameshift > ter; truncation (loss of 65AA)
Kralovics,[19] Arcasoy[20]	del5985-5991	Deletion (7 bp), frameshift > ter (loss of 65AA)
Kralovics[19]	5967insT	Frameshift > ter; truncation (loss of 59AA)
Watowich[23]	Duplication 5968-5975	Duplication (8 bp), frameshift > ter; truncation

The mutations are listed in the order of decreasing length of the deleted cytoplasmic regions.
Abbreviations: EPOR, erythropoietin receptor; ins, insertion; del, deletion; ter, terminator codon; bp, base pair

sulting in impaired rate of ubiquitin-mediated degradation of the transcription factor hypoxia induced factor (*HIF*)–1α. As a result, the level of the *HIF1* heterodimers increases and leads to increased expression of *EPO* and other target genes.[31,32] The patients suffer from an increased incidence of thrombosis, bleeding, and cerebrovascular events. Additional alleles of *VHL* have been described that also occur in other ethnic groups.[33-35] In contrast to the classic autosomal dominant *VHL* syndrome characterized by variety of malignant and benign neoplasms of the central nervous system and the kidneys,[36] patients with homozygous *VHL* mutations and polycythemia do not develop tumors.[37] Recently, a mutation in the prolyl hydroxylase domain protein 2 (*PHD2*) has been described that results in a proline-to-arginine substitution at amino acid position 317 (P317R) in a family with polycythemia and elevated Epo serum levels. *PHD2* is part of the oxygen sensor and mediates O_2-dependent hydroxylation of *HIF1*α on proline residues, which improves VHL binding to Hif1α and results in proteasome-mediated degradation. A second allele of *PHD2* (R371H) that causes a similar phenotype has been reported.[38] In both families, an autosomal-dominant inheritance was found. These studies continue to advance our understanding of the molecular mechanisms regulating erythropoiesis by controlling Epo production and EpoR signaling. Despite this progress, in the majority of familial polycythemias, the gene mutations still remain to be determined.

Hereditary thrombocythemia (also called familial thrombocytosis) can be caused by mutations in thrombopoietin (Tpo protein; official gene symbol *THPO*) or its receptor "myeloproliferative leukemia" (*MPL*). Activating mutations in the *THPO* that cause overproduction of Tpo protein by a mechanism of increased translational efficiency for the mutant *THPO* mRNA have been found in 4 families (**Table 2**).[39-44] In my laboratory, Adrian Wiestner initially found a point mutation that inactivated the splice donor of intron 3 of *THPO;* we were puzzled, since at first sight the mutation looked like a loss of function of the *THPO* gene.[40] However, the affected family members had thrombocytosis with elevated serum Tpo levels, which implied that a gain-of-function mutation was present. The dilemma was resolved as we found that under physiologic conditions, translation of *THPO* mRNA is strongly inhibited by the presence of several AUG codons located in the 5′-untranslated region (5′-UTR) of the *THPO* mRNA.[40,45] The upstream open reading frame defined by the 7th uAUG (uORF7) had a very strong negative effect on translation. The splice donor mutation caused alternative splicing of the *THPO* mRNA, resulting in loss of this inhibitory uORF7 and increased translational efficiency (**Figure 1**; see Color Figures, page 518). Similarly, the 3 other known mutations in *THPO* also lead to the loss the inhibitory uORF7 (**Figure 1**; see Color Figures, page 518). The clinical course in affected family members is mild, with occasional thrombotic or bleeding complications, but without leukemic transformation. *THPO* mutations have not been found in patients with sporadic ET.[46] A mutation in *MPL,* exchanging a serine in position 505 with an asparagine (S505N) in the transmembrane domain of the Mpl protein, was reported in a family with autosomal-dominant thrombocytosis.[47] Interestingly, the same mutation was also found in a mutational screening using retroviruses in mice.[48] The S505N mutation has also been detected in 3 other families, but not in sporadic ET.[49] In contrast, mutations in position 515 of Mpl that exchange a tryptophane with leucine (W515L) or lysine (W515K) have been found in 1% of patients with ET and 5% of patients with idiopathic myelofibrosis (IMF).[50,51] Both the familial mutation in position 505 and the sporadic mutations in position 515 appear to activate the Mpl protein independent of ligand binding. In other families with familial thrombocythemias, *THPO* and *MPL* were excluded as the disease-causing gene through absence of linkage and/or sequencing.[52-55] Thus, in most pedigrees with thrombocythemia, the disease-causing gene still remains unknown.

The second category of familial MPD, hereditary predisposition to true MPD, is only now beginning to be appreciated as a relatively frequent phenomenon. This is most likely due to the fact that penetrance in these families is low, and frequently only two family members are affected (pairs of parent-child, two siblings, or more distant relatives). If we assume an incidence of sporadic MPD in the general population in the range of 1 to 2 per 100,000 per year, the likelihood of finding 2 patients affected by MPD in the same family without genetic predisposition is small.

Table 2. Mutations causing hereditary thrombocythemia.

Report	Gene mutation	Consequence
Wiestner et al[40]	*THPO*, G>C in intron3 position +1	Loss of uORF-mediated repression
Kondo et al,[41] Ghilardi and Skoda[43]	*THPO*, deletion of G in 5′-UTR	Loss of uORF-mediated repression
Ghilardi et al[42]	*THPO*, G>T in 5′-UTR	Loss of uORF-mediated repression
Jorgensen et al[44]	*THPO*, A>G in intron 3 position +5	Not studied
Ding et al[134]	*MPL,* G>A in exon 10 resulting in S505N in Mpl protein	Constitutively active Mpl protein

Abbreviations: *THPO*, thrombopoietin gene; uORF, upstream open reading frame; IVS, intron; UTR, untranslated region; *MPL*, thrombopoietin receptor (myeloproliferative leukemia)

Only a small number of kindreds have been described with 4 or more affected family members.[56-58] A more systematic search for such families has revealed a number of additional kindreds with MPD,[54] and new studies have been initiated to estimate the frequency of MPD affecting 2 or more relatives. Affected family members have clonal hematopoiesis and display growth of EECs in methylcellulose,[59] and interestingly, most but not all affected family members carry an acquired somatic mutation in the *JAK2* gene.[54,60] This strongly suggests that only the predisposition to acquiring additional somatic mutations, such as *JAK2*-V617F, is inherited in these families, thus explaining the late onset and low penetrance of the MPD phenotype and the clonal hematopoiesis.

Clonality and Chromosomal Alterations in MPD

Clonal origin of hematopoiesis has been recognized as a key feature of the MPD pathophysiology.[12] Since the myeloid and often also lymphoid lineages are part of the clone, MPD is considered to be a hematopoietic stem cell disorder.[12,61-64] Clonal hematopoiesis arises when a progenitor or stem cell acquires a somatic mutation that provides a competitive advantage. In the course of the disease, additional clonal alterations are often acquired and are usually linked to the progression of chronic MPD into myelofibrosis and acute leukemia.[65-67] Although PV and IMF are invariantly clonal, a subgroup of patients with ET has been reported to display polyclonal hematopoiesis.[28,68] Clonality can be detected either indirectly, e.g., using markers that allow detection of X-chromosome inactivation pattern (XCIP) in female patients, or directly by detecting chromosomal alterations or mutations at the DNA level as molecular genetic markers linked to the presence of the disease (e.g., *BCR/ABL*, *JAK2-V617F,* or rearranged immunoglobulin gene). Considerable confusion exists as to what "clonal disease" means. This is in part due to the different sensibilities of the methodologies used for assessing clonality. To detect clonality by XCIP analysis, the clone must have expanded over an arbitrary limit of 70% to 80% of the cell population.[69] In contrast, the presence of small number of clonal cells can be detected using molecular genetic markers, e.g., the presence of *BCR/ABL* or *JAK2*-V617F. Thus, the statement that some patients with ET display polyclonal hematopoiesis may be true from the perspective of using XCIP to detect clonality, but patients with "polyclonal ET" can nevertheless have a subset of up to 50% of cells that are clonal, when a molecular marker is available to detect them, e.g. the *JAK2*-V617F mutation.[70,71] Therefore, XCIP studies that fail to demonstrate clonality cannot be taken as a proof that the disease condition is not caused by a clone of cells. True polyclonal MPD would imply that increased hematopoiesis is secondary to stimulation by a growth factor or an infectious agent, or is inherited through the germline.

The genetic basis for clonal hematopoiesis has been extensively studied in MPD by using cytogenetic analysis. However, only 10% to 15% of patients with PV have abnormal karyotype at diagnosis, and the most common abnormalities include trisomies (+8, +9, +1) and 20q deletions (del(20q)).[72-74] Using more sensitive methods of detection, e.g., interphase fluorescence *in situ* hybridization (FISH), the most frequent aberrations were del(20q), and trisomies 8 and 9.[75,76] In IMF, about 35% of patients have an aberrant karyotype, and the most frequent aberrations included del(13q), del(20q), and partial trisomy 1q.[77] The functional relevance of some of these alterations remains questionable, since they are often present only in a small proportion of cells. Appearance of such subclones has been demonstrated in several studies: karyotypic analysis of single erythroid colonies grown in the absence of Epo in a patient with PV revealed that trisomy 8 was present in only a subset of colonies.[78] Similar results have been obtained for del(20q), in which case the del(20q) clone was present in the bone marrow, but was absent in clonal peripheral blood granulocytes.[79] The common deleted region on chromosome 20q has been studied extensively. This abnormality is not unique to MPD, but can be also found in other myeloid malignancies such as myelodysplastic syndrome or acute myeloid leukemia. A minimal deleted region of 2.7 Mb has been defined for MPD and 2.6 Mb for other myeloid malignancies, and the overlap between these regions is 1.6 Mb.[79-82] A detailed expression map of this region was assembled, and analyses of candidate tumor suppressor genes within the common deleted region have been initiated, but the molecular defect has not yet been identified.[83,84]

Loss of Heterozygosity on Chromosome 9p

To search for chromosomal alterations that are hidden to cytogenetic and FISH analysis, Kralovics, Guan and Prchal initiated a genome wide screening for loss of heterozygosity (LOH) using microsatellite markers.[85] DNA from granulocytes was compared with DNA from nonhematopoietic tissues or T cells, and differences between in the pattern were scored. Initially, 6 of 20 (33%) of patients with PV showed LOH on chromosome 9p, 1 showed LOH on chromosome 10q, and 1 showed LOH on chromosome 11q.[85] The mechanism was found to involve mitotic recombination of homologous chromosome 9 and was present also in cells of the erythroid lineage and in $CD34^+$ cells.[85] The *JAK2* gene was partially sequenced, but no mutation was found. In retrospect, the failure to detect the *JAK2*-V617F mutation was due to the fact that the primers for amplifying the DNA were covering the mutated base. Instead, expression of the transcription factor *NFIB* was found to be elevated. After joining my laboratory, Robert Kralovics extended the 9pLOH studies. First he excluded *NFIB* as a candidate gene, since expression levels did not correlate with 9pLOH in a second independent cohort of patients with MPD.[86] We then further increased the number of patients and determined the frequency of 9pLOH in a total of 244 patients with MPD (128 with PV, 93 with ET, and 23 with IMF) from Switzerland and Italy.[87] The 9pLOH was predominantly present in patients with PV (36%) and IMF

(30%), but found in only 2 of 93 patients with ET. A diploid copy number was observed in all patients with 9pLOH examined. To determine the minimal LOH interval, we analyzed the 74 patients with 9pLOH with microsatellite markers covering the telomeric part of chromosome 9p. The LOH region extended from the telomere to variable distances on 9p. This allowed us to localize the minimal LOH region to the tip of chromosome 9p. The size of the minimal 9pLOH region has a physical size of 6.2 million bp and contains 33 genes, of which only 23 were expressed in $CD34^+$ cells. At this stage, we decided to sequence all 23 genes, including *JAK2*, which led to the detection of the *JAK2*-V617F mutation.[87]

JAK2 Mutations in MPD

The *JAK2*-V617F mutation, caused by a G>T transversion in exon 14 of *JAK2*, has been discovered by several teams using different approaches.[87-90] The team of William Vainchenker and colleagues examined the sensitivity of progenitor cell cultures from patients with PV to kinase inhibitors. They found that inhibitors of Jak2 (AG490), phosphatidylinositol-3-OH kinase (LY294002) and Src kinase family (PP2) interfered with the spontaneous growth of factor-independent erythroid cells.[91] Furthermore, siRNA against *JAK2* inhibited the growth of EECs, and sequencing of *JAK2* revealed the *JAK2*-V617F mutation.[88] The observation that chromosomal translocations in CML and in rare forms of MPD invariably involved protein tyrosine kinases led to the hypothesis that kinase activation may be a common pathogenetic theme in MPD.[92] The teams of Gary Gilliland and Tony Green followed this hypothesis and used high-throughput sequencing of kinase genes in the genomic DNA from PV patients.[89,90] The team of Joe Zhao sequenced the coding regions of cDNAs for candidate protein tyrosine kinases and phosphatases,[93] and as discussed in the previous paragraph, my team mapped the minimal 9pLOH region, which contained the *JAK2* gene, and found the *JAK2*-V617F mutation by sequencing DNA from patients with 9pLOH.[87] The mechanism of mitotic recombination that causes 9pLOH in a high proportion of patients with PV and IMF, but rarely in those with ET,[86] provided a plausible explanation for the presence of homozygous *JAK2*-V617F in patients with PV and IMF and by its absence in patients with ET.[87] It is remarkable that the vast majority of patients with MPD carry the identical *JAK2*-V617F mutation.[94] In some patients, the *JAK2*-V617F mutation is present in such a small proportion of peripheral blood cells that it can be missed by standard DNA sequencing techniques.[90] Using more sensitive methodologies, e.g., allele-specific PCR, the frequency of *JAK2*-V617F is close to 95% in PV, 58% in IMF and 50% in ET.[90] In rare cases, JAK2 mutations in positions other than codon 617 have been reported in acute leukemia or in cell lines.[95-97] Furthermore, mutations in exon 12 of the *JAK2* gene have been found in patients with PV who are negative for *JAK2*-V617F.[98] These exon 12 mutations alter various nucleotide positions in the vicinity of codon 539 and frequently also involve deletions of 1 to 2 codons, suggesting that a different mechanism is involved than in *JAK2*-V617F. Interestingly, each of the patients studied had mutations at slightly different positions in exon 12, in sharp contrast to the uniform pattern of *JAK2*-V617F.

Diagnostic Work-up and Clinical Relevance of *JAK2* Mutations

A proposal for a revision of the WHO diagnostic criteria for MPD have recently been published, and the presence of the *JAK2*-V617F mutation or other *JAK2* mutations, including exon 12, are now scored as major criteria for the diagnosis of MPD.[9] The *JAK2*-V617F is not limited to patients with MPD, as approximately 50% of patients with refractory anemia and ringed sideroblasts and thrombocytosis (RARS-T) and in rare cases also myelodysplastic syndromes, atypical MPD and acute leukemia can display *JAK2*-V617F. However, the presence of a JAK2 mutation is an important criterion for the exclusion of secondary erythrocytosis or thrombocytosis.[99] The clinical consequences of the JAK2 mutations have been analyzed mostly in retrospective studies. The presence of *JAK2*-V617F was found to be associated with growth of EECs, a higher likelihood of receiving cytoreductive treatment and with a higher rate of complications, including secondary fibrosis in PV and ET, and increased incidence of thrombosis and bleeding.[87] Subsequent studies confirmed the association with secondary fibrosis in PV,[100] but not in ET,[101] and the association with thrombosis remains controversial, with some studies confirming the initial results[102,103] and others rejecting it.[104] Presence of *JAK2*-V617F was found to correlate with poorer survival in IMF.[105] More recently, a correlation between the "allelic burden," i.e., the percentage of chromosome 9 that carry the *JAK2*-V617F mutation in a mixture of cells, and the rate of complications was found in a prospective study.[106]

JAK2 Mutations and Pathogenesis of MPD

What have we have learned and what are the remaining open questions? The *JAK2*-V617F mutation is located in the so called "pseudokinase domain" of the Jak2 protein. The pseudokinase domain has been shown to exert a negative effect on the tyrosine kinase activity of *JAK2*.[107,108] The position of the valine 617 is thought to be located at a hinge region, where the mutated phenylalanine 617 may interfere with the activation loop.[109,110] Depending on the expression levels and the cell type analyzed, the *JAK2*-V617F behaved either as a constitutively active kinase or as a kinase hypersensitive to incoming signals.[87-89] The presence of the *JAK2*-V617F correlated with EECs in patients,[87] and expression of *JAK2*-V617F in human progenitor cells induced Epo-independent growth *in vitro*.[111] The mechanism of how the V617F mutation alters the kinase activity appears to also involve altered responsiveness to the suppressors of cytokine signaling (Socs) proteins. Socs3 normally inhibits the action of Jak2 and the Epo receptor, but in the presence of Jak2-V617F, the Socs3 protein enhances

the effects of Epo-mediated activation of Jak2.[112] The expression of surrogate markers for MPD, e.g., *PRV1*, correlated with the presence and the allelic ratio of the *JAK2*-V617F mutation.[113,114] Retroviral transduction of bone marrow cells followed by transplantation into lethally irradiated mice demonstrated that the expression of Jak2-V617F is sufficient to induce a PV like phenotype.[88,115-118] The mice showed variable degree of myelofibrosis and normal platelet counts, except for a subgroup of secondary recipients of bone marrow transplantation in one report.[115] The phenotype was not diminished when donor mice deficient for the Scr family kinases Lyn, Hck and Fgr were used, but was dependent on the presence of Stat5 and inhibited by imatinib mesylate or the Jak2 inhibitor AG-490.[118,119] Using an inducible system for expressing *JAK2*-617F, we recently obtained transgenic mice with a PV phenotype that also exhibited thrombocytosis.[120]

Analysis of the hematopoietic lineage distribution of *JAK2*-V617F in patients with MPD revealed that *JAK2*-V617F is present in myeloid and sometimes lymphoid lineages and can be detected in purified human hematopoietic stem cells.[121-123] No evidence for differences in lineage distribution have been found between patients with PV, ET and IMF.[121,122] The question why the same mutation causes three apparently different phenotypes in patients remains unclear. It has been noted that patients with ET with *JAK2*-V617F have slightly higher hematocrit and hemoglobin levels and lower platelet counts than patients with ET negative for *JAK2*-V617F.[101] A continuum of phenotypic variation between ET and PV has been proposed, with *JAK2*-V617F$^+$ ET representing a *forme fruste* of PV that later develops into PV.[101] Although transitions between ET and PV do occur, their frequency is far lower than predicted by this model. If *JAK2*-V617F$^+$ ET is *forme fruste* of PV, it is not clear why these patients with ET display biologically different features from PV in that they almost never display homozygous progenitor colonies in methylcellulose assays, whereas practically all patients with PV do, irrespective of the allelic ratio of *JAK2*-V617F in peripheral blood granulocytes.[124]

Another puzzling feature in patients with MPD is that many patients with ET, but also some with PV and IMF, display low allelic ratios of *JAK2*-V617F. This is surprising if we assume that *JAK2*-V617F is the driving mutation and acts as a single-step oncogene. Analysis of granulocytes from female patients with ET or PV with allelic ratio of the mutant allele below 25% revealed that the granulocytes negative for the mutation in most cases were clonal by XCIP.[71] Furthermore, in 2 patients with del(20q)$^+$, the *JAK2*-V617F clone was considerably smaller than the del(20q)$^+$ clone, suggesting that in these patients, del(20q) preceded the acquisition of *JAK2*-V617F.[71] Additional indirect evidence for the presence of clonal events that precede the acquisition of the *JAK2*-V617F mutation come from studies of erythroid progenitors in methylcellulose. Analysis of single colonies revealed that some EECs in patients with PV can grow in the absence of *JAK2*-V617F,[125] and expansion of erythroid cells in liquid culture appears to favor *JAK2*-V617F$^-$ cells.[126] Another unexpected finding is that leukemic transformation in patients carrying *JAK2*-V617F at the diagnosis of MPD frequently results in an absence of *JAK2*-V617F in leukemic blasts.[127,128] Although the present studies were unable to rule out the possibility that the acute leukemias arose *de novo*, the results are compatible with a common clonal origin of the MPD and AML clone. Such a pre-*JAK2* stage is also suggested by the data from familial clonal MPD.[54] Thus, in contrast to the work in mouse models, analysis of human MPD reveals a more complex picture summarized in a model, in which a dormant stem cell clone with a predisposition to acquiring a *JAK2* mutation or other mutations that can lead to AML exists before the patients show signs of hematologic disease (**Figure 2**; see Color Figures, page 518).

Outlook

Physicians and patients with MPD are eagerly awaiting the results of the first clinical trials with *JAK2* inhibitors. Several kinase inhibitors with a relatively broad specificity and favorable "secondary-target" effects have been found to also inhibit Jak2, and some of these have been already studied for other applications, e.g., lestaurtinib (CEP701) for activated *FLT3* in AML or erlotinib for mutated *EGFR*.[129-132] A number of compounds more specific for Jak2 are under development. Since all of these compounds also inhibit the wild-type Jak2 protein, it will be interesting to see whether the MPD cells expressing Jak2-V617F are more sensitive to the inhibition than the wild-type cells. Strong inhibition of the wild-type Jak2 is expected to cause serious adverse effects, including anemia, neutropenia, and thrombocytopenia. Although considered unlikely by most experts, a slight chance remains that inhibitors specific for the mutant Jak2-V617F protein can be found. The search for additional mutations in MPD patients negative for *JAK2*-V617F, *JAK2*-exon12, and MPLW515 mutations and for predisposing mutations in familial MPD remains a high priority. More data on the putative pre-*JAK2* stem cell pool in MPD are needed, in particular with respect to genomic instability and leukemic transformation. Our understanding of the genetic basis of MPD has increased enormously in the first two years since the discovery of the *JAK2*-V617F mutation, and the current pace of progress raises hopes that many of the open questions will be answered soon.

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