REVIEW ARTICLE

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Myeloproliferative Neoplasms

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THE MYELOPROLIFERATIVE NEOPLASMS — POLYCYTHEMIA VERA, ESSENTIAL thrombocytosis, and primary myelofibrosis — are unique hematopoietic stem-cell disorders that share mutations that constitutively activate the physiologic signal-transduction pathways responsible for hematopoiesis (Table 1). Consequently, these disorders engage in phenotypic mimicry among themselves, as well as with myeloid neoplasms and even benign hematopoietic disorders. In contrast to the myeloid neoplasms, the myeloproliferative neoplasms have a natural history, with supportive care alone, that is usually measured in decades rather than years.[1] However, a facade of benign myeloproliferation masks a clone of transformed hematopoietic stem cells capable of expansion and transformation to an aggressive form of bone marrow failure or acute leukemia, albeit at varying frequencies in each of these disorders. In addition to phenotypic mimicry, each type of myeloproliferative neoplasm is capable of evolving into another type, making diagnosis, risk assessment, and therapeutic choices difficult. Furthermore, despite more than a century of scrutiny, the pathogenesis of myeloproliferative neoplasms has been enigmatic, and therapy largely supportive. Recently, however, driver mutations have been identified in more than 90% of patients with myeloproliferative neoplasms, providing substantial insight into their pathogenesis. The current challenge is to integrate this new knowledge with the accumulated decades of clinical knowledge to improve diagnosis, risk assessment, and therapy.

MUTATIONAL LANDSCAPE

HOST GENETIC VARIATION

Host genetic variation, including sex and age, has an essential role in the mutational landscape of myeloproliferative neoplasms.[2] Genomewide association studies have identified single-nucleotide variants that increase the probability that such neoplasms will develop. For example, in one study, a single-nucleotide variant haplotype, designated 46/1 (*GGCC*) and located in *cis* on the Janus kinase 2 (*JAK2*) allele, was associated with an increase by a factor of 3 in the risk of a JAK2-activating mutation[3]; in other studies, other single-nucleotide variants were associated with mutations in the genes encoding calreticulin (*CALR*) and the thrombopoietin receptor (*MPL*),[4] or the individual myeloproliferative neoplasms.[4,5] These genetic predispositions may explain the co-occurrence of stem-cell clones harboring *JAK2*, *MPL*, or *CALR* mutations[6,7] in the same person.

It is important to note that a single-nucleotide variant in *TERT*, which is linked to activated myeloid hematopoiesis, is associated with all three myeloproliferative neoplasms[8] but most significantly with their familial forms,[9] and this variant, in combination with 46/1 and other single-nucleotide variants, has an additive effect on susceptibility to myeloproliferative neoplasms.[4,5] The *TERT* variant also confers a predisposition to the co-occurrence of solid tumors in patients with myeloproliferative neoplasms.[10]

HEREDITARY AND FAMILIAL MYELOPROLIFERATIVE DISORDERS

In addition to these common but weakly penetrant single-nucleotide variants that increase disease susceptibility, rare but highly penetrant germline mutations in the *JAK2* JH1 and JH2 domains[11] (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org) and the *MPL* transmembrane domain[12] (Fig. S3 in the Supplementary Appendix) cause hereditary thrombocytosis, mimicking sporadic, clonal essential thrombocytosis, including myelofibrotic transformation.[13] Conversely, germline single-nucleotide variants in the *MPL* extracellular domain, which occur in 7% of African-American populations[14] and 6% of Arabic populations,[15] have a benign thrombocytosis phenotype.

In adults, myeloproliferative neoplasms due to somatic *JAK2*, *MPL*, or *CALR* mutations are usually sporadic. However, 7% of cases involve a familial predisposition (a feature unique to these disorders), with first-degree relatives of an affected patient at increased risk, by a factor of 5 to 7, for the same myeloproliferative neoplasm (in some families) or for different myeloproliferative neoplasms (in other families), involving *JAK2*, *MPL*, and *CALR* mutations or no identifiable mutation.[16,17] Predisposing genetic risk factors include female sex and the *TERT* single-nucleotide variant. Penetrance is incomplete, and generational skipping occurs.

GENOTYPE AND PHENOTYPE RELATIONSHIPS

Table 2, and Table S1 in the Supplementary Appendix, list the most common gene mutations associated with clinical phenotypes of myeloproliferative neoplasms. Myeloproliferative neoplasms have a low mutation frequency (0.2 per megabase), as do the myeloid neoplasms, and likewise, the median number of mutations (6.5 in essential thrombocytosis and polycythemia vera and 13.0 in myelofibrosis) is similar.[18] Mutation number is a function of host age, not disease duration or a particular driver mutation. Pathogenic mutations have been identified in more than 90% of patients with myeloproliferative neoplasms; 50 to 60% of patients have only a driver mutation: *JAK2* V617F, *CALR*, *MPL*, or in rare cases, *LNK*[7]; the remainder have additional mutations, most often affecting genes coding for signal transduction or for epigenetic regulatory, tumor-suppressor, or splicing proteins. Such grouping belies the fact that mutated genes in one category frequently influence function in another category[19] and rarely operate alone; remarkably, as few as two mutations can shorten the life span.[7,20]

In "triple-negative" patients (i.e., those who have a myeloproliferative neoplasm phenotype [Table 2] but do not have canonical *JAK2*, *MPL*, or *CALR* driver mutations), novel somatic or germline *JAK2* or *MPL* mutations have been identified by means of deep sequencing. Other patients have been found to have a clonal disorder without a defined driver mutation, and in some patients, hematopoiesis is polyclonal, a feature that is consistent with a hereditary disorder.[21,22]

AGE, SEX, AND PHENOTYPE

In adults, sporadic acquisition of a myeloproliferative neoplasm driver mutation by a hematopoietic stem cell does not guarantee its clonal expansion at the expense of unaffected stem cells[8]; clonal expansion appears to be dictated in part by the patient's sex and age.[23,24] For example, *JAK2* V617F acquisition can occur at any age, but myeloproliferative neoplasms are uncommon before the age of 50 years, and two of the three types — polycythemia vera and essential thrombocytosis — occur mainly in women. After the age of 60 years, the incidence of myeloid and myeloproliferative neoplasms increases exponentially[25] in association with an increased incidence of *DNMT3A*, *TET2*, *ASXL1*, *JAK2* V617F, and *TP53* mutations.[26] In this age group, myeloproliferative neoplasms are more common in men than in women and are associated with primary myelofibrosis and acute leukemia. The order of mutation acquisition does not affect the clinical phenotype.[27]

EPIGENETIC AND CYTOGENETIC ABNORMALITIES

Aberrant DNA methylation is a feature of the myeloproliferative neoplasms that is independent of driver mutations and is associated with disease transformation[28]; the mechanisms are undefined. Most patients do not have *DNMT3a* or *TET2* mutations, which regulate DNA methylation and, by extension, the size of the hematopoietic stem-cell pool. Patients do, however, have age-associated changes in stem-cell DNA methylation that mimic cancer-associated DNA methylation abnormalities[29] and promote stem-cell monoclonality.[30]

Altered DNA methylation, associated with

both age and mutations, also causes DNA breakage,[31] leading to gene deletions (del5q, del7q, and del17p) and duplications (8q and *MYC*). These are as important prognostically with respect to leukemic transformation as are acquired point mutations.[32,33] Telomere shortening occurs in myeloproliferative neoplasms,[34] but whether it is associated with aneuploidy is unknown.

GENE EXPRESSION

Gene-expression profiling integrates the consequences of genetic abnormalities for cellular processes. Neutrophil gene expression in patients with myeloproliferative neoplasms differs from expression in persons without such neoplasms but does not differ among the three diseases[35]; notably, there is activation of genes involved in inflammatory signaling pathways, including interleukin-6, interleukin-8, interleukin-10, granulocyte–macrophage colony-stimulating factor, and transforming growth factor (TGF) β.[36] By contrast, hematopoietic stem-cell gene expression in patients with the three types of myeloproliferative neoplasms not only differs from expression in persons without such neoplasms

Table 1. Types of Myeloproliferative Neoplasms and Associated Driver Mutations.

Types of Myeloproliferative Neoplasms

Polycythemia vera, the most common myeloproliferative neoplasm, is a panmyelopathy and the ultimate phenotypic consequence of *JAK2* gain-of-function gene mutations and, in rare cases, *CALR* or *LNK* mutations. Unlike the two other types of myeloproliferative neoplasms, polycythemia vera is characterized by erythrocytosis, with a progressive increase over time in erythropoiesis, granulopoiesis, and thrombopoiesis. The most common complications are arterial and venous thrombosis due to red-cell-mass–induced hyperviscosity; transient ischemic attacks, ocular migraine, or erythromelalgia due to activated platelets; aquagenic pruritus due to activated basophils; acquired von Willebrand's disease and pseudohyperkalemia due to extreme thrombocytosis; splenomegaly due to migration of the involved hematopoietic stem cells from the marrow (extramedullary hematopoiesis); and in some patients, transformation to bone marrow failure, myelofibrosis, and acute leukemia.

Essential thrombocytosis, the most indolent myeloproliferative neoplasm, is characterized by thrombocytosis alone and is caused by *JAK2* V617F, *CALR*, or *MPL* mutations and infrequently by germline single-nucleotide variants. Complications include transient ischemic attacks, ocular migraine, erythromelalgia, acquired von Willebrand's disease, and pseudohyperkalemia due to extreme thrombocytosis, and less commonly, arterial or venous thrombosis and transformation to bone marrow failure, myelofibrosis, and acute leukemia. Essential thrombocytosis is a diagnosis of exclusion because the thrombopoietin receptor, MPL, is the only hematopoietic growth factor receptor expressed by hematopoietic stem cells, and isolated thrombocytosis may be the first manifestation of polycythemia vera or primary myelofibrosis.

Primary myelofibrosis, the least common and most aggressive myeloproliferative neoplasm, is caused by *JAK2* V617F, *CALR*, or *MPL* mutations and is manifested as new bone marrow fibrosis, splenomegaly due to extramedullary hematopoiesis, an increase in circulating CD34+ cells, anemia, variable changes in the platelet and leukocyte counts, and constitutional symptoms due to inflammatory cytokine production. The disease has a progressive course characterized by bone marrow failure; organ failure due to extramedullary hematopoiesis, including pulmonary hypertension; and transformation to acute leukemia.

Driver mutations

JAK2 is the most common myeloproliferative neoplasm driver gene. A member of the Janus kinase family, JAK2 serves as the cognate tyrosine kinase for the erythropoietin and thrombopoietin receptors and can also be used by the granulocyte colony-stimulating factor receptor, all of which lack an intrinsic kinase domain. JAK2 has a dual kinase structure: a canonical tyrosine kinase domain (JH1) paired in tandem with a weakly active pseudokinase domain (JH2), which normally inhibits JH1 kinase activity in the absence of ligand binding (Fig. S1 in the Supplementary Appendix).

Whether the JH1–JH2 interaction occurs in *cis* or *trans* is unresolved, but current data favor an interaction in *trans*, in which the JAK2 JH2 pseudokinase domain on one receptor monomer inhibits the JH1 kinase domain of the JAK2 molecule on its partner receptor monomer and vice versa, an inhibition that is abrogated physiologically with receptor-ligand binding as a result of a change in the receptor dimer conformation. The most common myeloproliferative neoplasm mutation, *JAK2* V617F, an exon 14 point mutation in the *JAK2* JH2 pseudokinase domain, impairs its physiologic inhibitory influence on the JH1 kinase domain. How *JAK2* V617F and other JH2 domain mutations alleviate this inhibition is unknown, but the mechanism probably involves changes in the JAK2 Src homology 2 (SH2)–JH2 linker region, which alter the interface between the JH2 and JH1 domains. In the heterozygous state, JAK2 V617F–bearing receptors are still responsive to growth factors. Only with *JAK2* V617F homozygosity, usually due to 9p uniparental disomy, do these receptors become autonomous with respect to growth factor. Approximately 3% of patients with polycythemia vera have insertions or deletions in *JAK2* exon 12 at the interface of the *JAK2* SH2 and JH2 domains (Fig. S1 in the Supplementary Appendix), which enable constitutive kinase activation, possibly also by altering the interface between the JH2 and JH1 domains. The *JAK2* exon 12 phenotype is usually more benign than that of *JAK2* V617F, often causing erythrocytosis alone, though a complete polycythemia vera phenotype can develop, as can homozygosity or coexistence with *JAK2* V617F.

JAK2 also serves as an endoplasmic reticulum chaperone for the erythropoietin and thrombopoietin receptors, transporting them to the cell surface, and increases the total number of thrombopoietin receptors by stabilizing the mature form of the receptor, enhancing receptor recycling, and preventing receptor degradation. However, in contrast to its effect on the erythropoietin receptor, JAK2 V617F appears to increase the quantity of immature MPL while increasing MPL degradation through ubiquitination and reducing its cell-surface expression.

In addition to functioning as a tyrosine kinase and chaperone, mutated *JAK2* is sumoylated, permitting it to shuttle to the nucleus, where it deregulates gene transcription directly through histone phosphorylation and indirectly by phosphorylating and inhibiting PRMT5, a histone arginine methyltransferase.

Table 1. (Continued.)

CALR, the gene that encodes calreticulin, is the second most common myeloproliferative driver gene. Discovered by whole-exome sequencing (Fig. S2 in the Supplementary Appendix), it was an unlikely candidate. CALR is a multifunctional protein involved in glycoprotein folding and calcium homeostasis in the endoplasmic reticulum, as well as in cellular functions such as proliferation, phagocytosis, and apoptosis. *CALR* mutations consist of a wide variety of deletions or insertions in exon 9, all of which have the same consequence: a 1-bp frameshift, which removes KDEL, a canonical endoplasmic reticulum retrieval motif, together with a switch from a negatively charged to a positively charged peptide sequence in the CALR C terminal domain.

These mutations substantially alter CALR cellular distribution because mutant CALR is able to bind MPL through the receptor's extracellular domain and chaperone it to the plasma membrane. The mutant, positively charged CALR C terminal domain is obligatory for both MPL binding and cellular transformation, but how MPL JAK2 signaling is activated by mutant CALR is unknown. As is the case with *JAK2* and *MPL* mutations, the proportion of immature MPL in the cell is increased. Like receptors containing *JAK2* V617F, MPL bound by mutant CALR still requires growth factor stimulation for complete JAK2 activation in the heterozygous state.

To date, more than 50 different *CALR* mutations have been identified and classified according to their effect on DNA sequence: deletions have been designated as type 1 or type 1–like, of which L367fs*46, a 52-bp deletion, is the most common, and insertions as type 2 or type 2–like, of which K385fs*47, a 5-bp insertion, is the most common. Together, these account for 85% of the *CALR* mutations; type 1 mutations are more common in primary myelofibrosis, whereas type 1 and type 2 occur with similar frequency in essential thrombocytosis.

Like *JAK2* and *MPL* mutations, mutated *CALR* is expressed in hematopoietic stem cells. It is a driver mutation primarily in essential thrombocytosis and primary myelofibrosis; is occasionally homozygous as a result of 19 uniparental disomy, usually with type 2 mutations; is not mutually exclusive of *JAK2* V617F; and causes polycythemia vera in rare cases.

In some but not all studies, the *CALR* type 1 mutation appeared to be associated with a survival advantage as compared with *JAK2* V617F and *MPL* mutations, but the three mutations did not differ with respect to leukemic transformation. Among patients with essential thrombocytosis, those with the *CALR* type 2 mutation were younger and had higher platelet counts than their counterparts with type 1 mutations, and type 1 was more common in men than in women, but there was no difference in survival between the type 1 and 2 mutations or between them and *JAK2* V617F or *MPL*. Myelofibrotic transformation of essential thrombocytosis appeared to be more common with type *CALR* 1 mutations than with type 2 or *JAK2* V617F mutations. Finally, although thrombotic risk appeared to be greater in patients with *JAK2* V617F–positive essential thrombocytosis than in their *CALR* mutation–positive counterparts, this observation must be tempered by the fact that many of the patients with so-called *JAK2* V617F–positive essential thrombocytosis had hemoglobin levels that were compatible with polycythemia vera rather than essential thrombocytosis.

MPL, a truncated form of the thrombopoietin receptor gene, is the oncogene of the *MPLV* retrovirus, which causes murine polycythemia vera. *MPL* mutations are the least common myeloproliferative neoplasm driver mutations, occurring in primary myelofibrosis and essential thrombocytosis, but overall, compromised MPL function due to incomplete glycosylation and impaired MPL cell-surface expression may have a more important role in the pathophysiology of myeloproliferative neoplasms than any *MPL* mutation.

MPL is a unique type I hematopoietic cytokine receptor because it is the only one expressed in hematopoietic stem cells; it also has a reduplicated extracellular cytokine-binding domain. Somatic *MPL* mutations occur most often in exon 10 (Fig. S3 in the Supplementary Appendix) and result in a switch from tryptophan to leucine or lysine or, less frequently, to arginine or alanine at amino acid 515 (*MPL* W515L/K or W515R/A) in the MPL juxtamembrane domain.

A less common mutation, S505N, in the MPL transmembrane domain, in which serine is switched to asparagine, can be inherited or acquired and causes essential thrombocytosis. *MPL* mutations force a change in receptor conformation, activating JAK2 in the absence of thrombopoietin binding. Like *JAK2* and *CALR* mutations, however, *MPL* mutations require a hematopoietic growth factor, in this case thrombopoietin, for complete kinase activation in the heterozygous state.

Myeloproliferative neoplasm driver mutations also occur in the *MPL* extracellular distal cytokine domain (Fig. S3 in the Supplementary Appendix). For example, *MPL* S204P/F are acquired mutations causing essential thrombocytosis or primary myelofibrosis, whereas the germline *MPL* variants, K39N (*MPL* Baltimore) and P106L, cause a benign form of thrombocytosis in African-American and Arabic populations, respectively, which is most marked in the homozygous state.

Somatic *MPL* mutations and germline single-nucleotide variants are not mutually exclusive of *JAK2* V617F, though they are not in the same clone. In patients with essential thrombocytosis, *MPL* mutations are associated with greater myelofibrotic transformation, but there is no difference in overall or leukemia-free survival between patients with *MPL* mutations and those with *JAK2* V617F, and there appears to be no survival difference between patients with primary myelofibrosis who have *MPL* mutations and those who have *JAK2* V617F mutations.

JAK2 V617F impairs MPL maturation, increasing the proportion of immature receptors in the plasma membrane; reduces MPL recycling; and increases its degradation. Impaired MPL cell-surface expression, which is also a feature of *CALR* and *MPL* mutations, results in elevated plasma thrombopoietin levels, as a result of reduced clearance of thrombopoietin from the plasma by megakaryocytes and platelets, and may also be involved in the emigration of involved hematopoietic stem cells from their marrow niches (Fig. S4 in the Supplementary Appendix). In this regard, congenital amegakaryocytic thrombocytopenia, an autosomal recessive disorder caused by compound heterozygous or homozygous mutations in the *MPL* distal cytokine homology domain, which is manifested initially as thrombocytopenia but eventually evolves to aplastic anemia, underscores the central role of MPL in hematopoietic stem-cell physiology.

but also, and more important, differs among the three types of neoplasms, indicating that they are genetically distinct diseases.[1,23,37-39]

In polycythemia vera, hematopoietic stem-cell gene expression differs between men and women. However, men and women have in common *JAK2* V617F–independent expression of 102 genes, which are differentially expressed in patients with aggressive disease and those with indolent disease. Included are genes involved in stem-cell expansion, myelofibrosis, inflammation, coagulation, and leukemic transformation.[23] A total of 55 of these genes are differentially regulated in chronic and blast-phase chronic myeloid leukemia,[23] suggesting that the two diseases have the same molecular pathways for leukemic transformation.

Table 2. Gene Mutations in the Chronic Phase of Myeloproliferative Neoplasms, According to Phenotype and Driver Mutation.*

Gene Mutation	Polycythemia Vera			Essential Thrombocytosis				Primary Myelofibrosis			
	JAK2 V617F	*JAK2* Exon 12	*CALR*	*JAK2* V617F	*CALR*	*MPL*	Triple Negative	*JAK2* V617F	*CALR*	*MPL*	Triple Negative
	percent of patients†										
Tyrosine kinases											
JAK2 V617F	92	0	Frequency unknown	55	0	0	Frequency unknown	50	0	0	0
JAK2 exon 12	Frequency unknown	5	NA	0	0	0	0	0	0	0	0
Receptors											
CALR	Frequency unknown	0	Frequency unknown	0	36	0	0	0	30	0	0
MPL	Frequency unknown	0	0	0	0	4	Frequency unknown	Frequency unknown	0	8	0
NTRK1	14	NA	NA	NA	NA	NA	Frequency unknown	10	NA	NA	NA
DNA methylation											
TET2	12	NA	NA	6	NA	NA	NA	12	Frequency unknown	NA	Frequency unknown
DNMT3A	8	NA	NA	9	NA	NA	NA	6	NA	NA	0
IDH1/2	3	NA	NA	0	NA	NA	NA	4	7	0	9
Histone methylation											
ASXL1	7	NA	NA	2	NA	NA	NA	30	32	30	32
EZH2	2	NA	NA	1	NA	NA	NA	7	7	10	5
SUZ12	3	NA	NA	0	NA	NA	NA	3	NA	NA	0
Spliceosome											
U2AF1	0	NA	NA	0	NA	NA	NA	22	2	21	5
SRSF2	0	NA	NA	0	NA	NA	NA	13	3	15	23
SF3B1	10	NA	NA	0	NA	NA	NA	9	2	5	14
ZRSR2	0	NA	NA	0	NA	NA	NA	3	NA	NA	0

Tumor suppressors: *TP53*	5	NA	NA	6	NA	NA	NA	5	NA	NA	0
Transcription factors: *NF-E2*	2	NA	NA	Frequency unknown	NA	NA	NA	Frequency unknown	Frequency unknown	Frequency unknown	NA
Activated signaling											
NRAS/KRAS	3	NA	NA	0	NA	NA	NA	5	NA	NA	0
CBL	2	NA	NA	2	NA	NA	NA	6	NA	NA	Frequency unknown
NF1	3	NA	NA	0	NA	NA	NA	3	NA	NA	0
SH2B3 (LNK)‡	Frequency unknown	NA	NA	Frequency unknown	NA	NA	Frequency unknown	Frequency unknown	NA	NA	NA

* "Triple negative" refers to patients with a myeloproliferative neoplasm phenotype who do not have a canonical driver mutation. Some of these patients have germline mutations, suggesting that their disorder is hereditary. NA denotes not available, whereas "frequency unknown" indicates that there are too few reports to allow calculation of a frequency.
† The percentages represent the frequency of a particular mutation among patients with the same myeloproliferative neoplasm.
‡ Germline *LNK* single-nucleotide variants appear to influence LNK behavior.

HEMATOPOIETIC STEM-CELL BONE MARROW NICHES

Hematopoietic stem cells reside in two specialized bone marrow niches[40] (Fig. S4 in the Supplementary Appendix). The proliferative niche is sinusoidal. Here, thrombopoietin promotes DNA synthesis[41] and macrophages nurture developing erythroblasts.[42] The quiescent niche is endosteal, perfused by arterioles and innervated by sympathetic nerves. Here, stem cells are tethered to osteoblasts through their adhesion and thrombopoietin receptors.[41] Stem-cell quiescence is maintained by CXCL4 and TGF-β1 secretion from closely apposed megakaryocytes.[43]

Polycythemia vera stem cells up-regulate inflammatory cytokine genes (as do chronic myeloid leukemia stem cells[44]), including *CCL3*, tumor necrosis factor, *LCN2*, and *LGALS3*,[23,45] that inhibit normal stem-cell proliferation, promote osteomyelofibrosis, and damage niche sympathetic nerves, enhancing myeloproliferation.[46] Normal marrow stromal cells can be appropriated by the neoplastic clone to secrete inflammatory cytokines.[47] These abnormalities are augmented by age-associated microenvironmental changes that promote stem-cell monoclonality and myeloid predominance.[30]

Myelofibrosis in the myeloproliferative neoplasms is fostered by elevated plasma thrombopoietin levels, possibly as a result of impaired thrombopoietin receptor expression,[48] that are unrelated to the driver mutation.[49] Myelofibrosis is a reactive and reversible process that does not impair marrow function.[20,50] Impaired marrow function is due to the transformed hematopoietic stem cells and occurs in approximately 15 to 20% of patients with polycythemia vera[50]; in some patients, a decline in the phlebotomy rate is an artifact of plasma-volume expansion and is not indicative of a bone marrow "spent phase."[51]

STEM-CELL CLONAL ARCHITECTURE

Driver mutations for myeloproliferative neoplasms are present in the long-term repopulating stem cells that are responsible for maintaining hematopoiesis[52,53] (Fig. 1) but do not alter the hematopoietic stem-cell hierarchy; instead, they expand the pool of JAK2-sensitive, committed myeloid progenitor cells.[54] Studies indicate that long-term repopulating stem cells can also differentiate

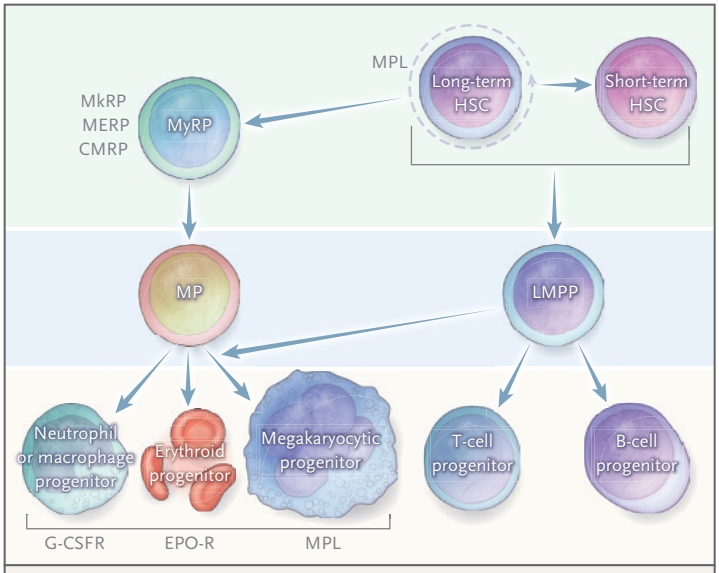


Figure 1. Hierarchy of Hematopoietic Stem and Progenitor Cells.

Hematopoietic stem cells (HSCs) are organized hierarchically into long-term and short-term HSCs according to their capacity for self-renewal and marrow repopulation. During homeostasis, long-term HSCs maintain the pool of short-term HSCs, which are responsible for daily replenishment of the lymphoid multipotent progenitor-cell (LMPP) and myeloid progenitor-cell (MP) pools. These pools, in turn, give rise to lineage-restricted neutrophil or macrophage, erythroid, megakaryocytic, B-cell, and T-cell progenitors. Lineage-restricted myeloid repopulating (MyRP) HSCs — specifically, megakaryocytic repopulating (MkRP), megakaryocytic–erythroid repopulating (MERP), and common myeloid long-term repopulating (CMRP) stem cells — can arise directly from long-term HSCs. Although *JAK2*, *CALR*, and *MPL* driver mutations arise in long-term HSCs, phenotypic mimicry among the myeloproliferative neoplasms may be due to the differential or changing involvement of specific lineage-restricted HSCs. The thrombopoietin receptor (MPL), in contrast to the granulocyte colony-stimulating factor receptor (G-CSFR) and the erythropoietin receptor (EPO-R), is the only hematopoietic growth factor receptor expressed in long-term HSCs and is essential for HSC osteoblastic niche residence in marrow, maintenance of HSC quiescence, DNA damage repair, and cell-cycle activation.

directly into megakaryocytic, megakaryocytic–erythroid, or myeloid progenitor cells,[55] which could explain platelet-restricted *JAK2* V617F expression in women with essential thrombocytosis,[56] the variable phenotypic presentations of polycythemia vera, and the evolution from one myeloproliferative neoplasm to another.

Figure 2 shows the relationship among sex, disease phenotype, disease duration,[24,57] and the *JAK2* V617F allele burden in neutrophils, which are sensitive to activated JAK2, in patients with myeloproliferative neoplasms.[58] Figure S5 in the Supplementary Appendix shows the effect of these variables on the allele burden in hematopoietic stem cells, which are not sensitive to activated JAK2.[54] Clonal dominance occurs when the malignant stem-cell population exceeds the normal one. Clonal dominance drives the disease phenotype, for which the *JAK2* V617F neutrophil allele burden is not a reliable measure,[57] particularly in polycythemia vera, which is characterized by the slow development of clonal dominance. In primary myelofibrosis, clonal dominance is usually present at diagnosis,[57] and in essential thrombocytosis, clonal dominance is rare (Fig. S5 in the Supplementary Appendix). It is clear that host genetic variation,[2] in which sex is an important component,[23] is the major determinant of the myeloproliferative neoplasm phenotype, not specific driver mutations or their allele burdens.

ACUTE LEUKEMIA

Acute myeloid leukemia occurs spontaneously in patients with myeloproliferative neoplasms and has a poor prognosis.[59] Estimates of the incidence of acute myeloid leukemia range from 1.5% in patients with essential thrombocytosis and 7.0% in patients with polycythemia vera[60] to 11% in patients with primary myelofibrosis.[61] However, such estimates are confounded by age-related de novo acute leukemia and chemotherapy; chemotherapy increases the incidence to 20%.[60,62] Acute leukemia in patients with myeloproliferative neoplasms can involve the founding hematopoietic stem-cell clone but more often involves a subclone, as occurs in cases of de novo acute leukemia in patients without such neoplasms.[53]

The cytogenetic landscape of the myeloproliferative neoplasms is relatively limited and does not differ substantially according to the type of neoplasm.[33] Furthermore, driver mutation status is not associated with the time to leukemic transformation or survival after transformation.[32] Disease transformation is associated with older age; acquisition of 9p uniparental disomy; 1q amplification, which involves MDM4, the TP53 inhibitor[63]; and additional cytogenetic abnormalities and mutations.[32,33,64]

Acute leukemia originating in a *JAK2* V617F–negative hematopoietic stem cell is a unique feature of *JAK2* V617F–positive myeloproliferative neoplasms (Fig. S6 in the Supplementary Appendix), occurring in approximately 40% of cases,

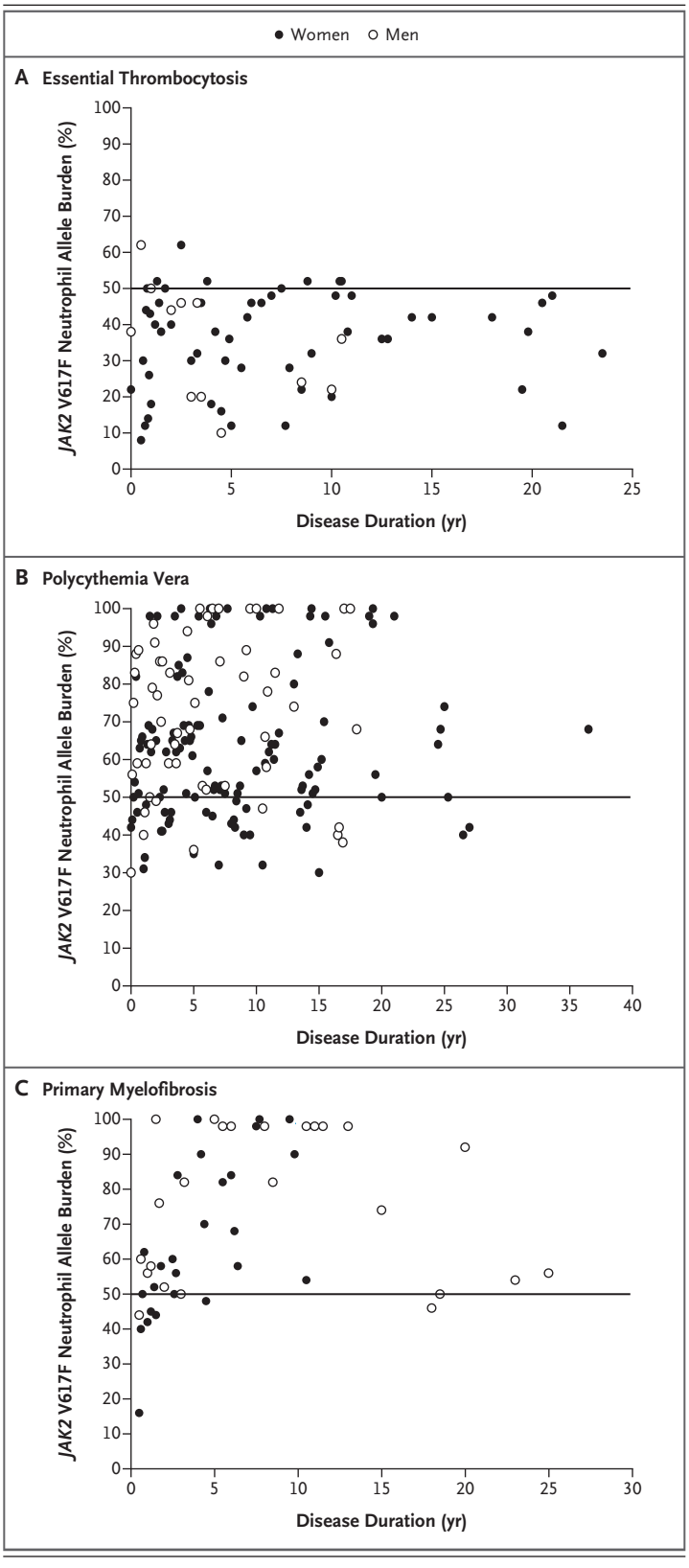


Figure 2. Sex, Disease Duration, and the *JAK2* V617F Neutrophil Allele Burden in Essential Thrombocytosis, Polycythemia Vera, and Primary Myelofibrosis.

The relationship among sex, disease duration, and the neutrophil allele burden is complex in patients with *JAK2* V617F–positive essential thrombocytosis, polycythemia vera, or primary myelofibrosis. Essential thrombocytosis is characterized by a neutrophil allele burden of less than 50%, which is constant during the course of the disease, with no difference in allele burden between men and women, even though the disease is more common in women. In polycythemia vera, the neutrophil allele burden is often greater than 50% at diagnosis or subsequently increases over time to more than 50% because of uniparental disomy, but not in all patients, and the burden is usually greater in men than in women. In primary myelofibrosis, the neutrophil allele burden is usually greater than 50% in most patients at diagnosis and is higher in women than in men, even though primary myelofibrosis is more common in men.

most often in chronic-phase polycythemia vera and essential thrombocytosis.[65]

Like acute leukemia in patients with myeloid neoplasms,[66] acute leukemia in those with myeloproliferative neoplasms can be classified by its mutations as de novo (*DNMT3A*, *NPM1*, and *RUNX1*), secondary to the myeloproliferative neoplasm (*SRSF2*, *EZH2*, and *ASXL1*), or treatment-related (*TP53*, del5q, del7/7q, and del17p), regardless of disease phase or driver mutation. Most worrisome is treatment-related acute leukemia, since it is preventable. Chemotherapy neither averts disease transformation and thrombosis nor prolongs survival, as compared with supportive care.[60,62,67] Rather, chemotherapy facilitates the selection of drug-resistant stem-cell subclones.[68]

DIAGNOSIS

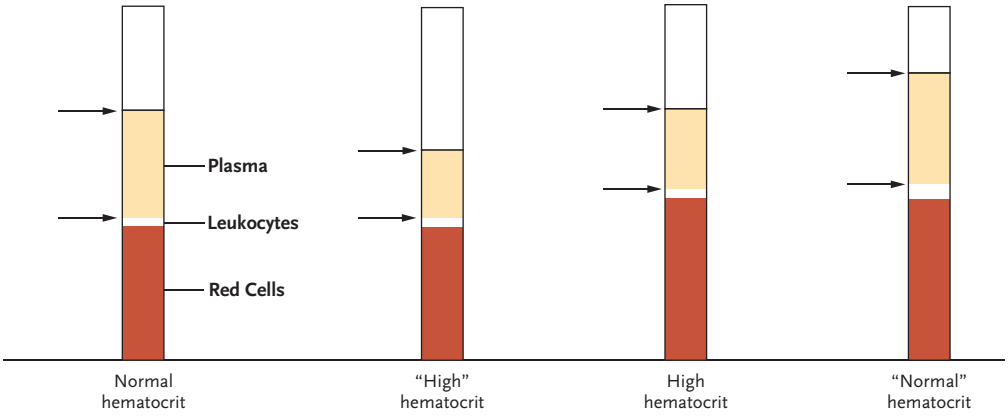
JAK2, *CALR*, and *MPL* mutations are not mutually exclusive, they are not exclusive to a particular myeloproliferative neoplasm, and their absence does not preclude any of these neoplasms. A positive mutation assay establishes the presence of a hematopoietic stem-cell disorder, not its identity, and surrogate markers such as the serum erythropoietin level[69] or bone marrow histologic features cannot provide specificity, except that myelodysplasia can be ruled out on the basis of histologic features.[70,71] All three myeloproliferative neoplasms may be manifested as isolated thrombocytosis, whereas polycythemia vera, the ulti-

mate phenotype of *JAK2* mutations and, in rare cases, *CALR* mutations,[72] may be characterized by isolated erythrocytosis, leukocytosis, splenomegaly, and even myelofibrosis.[69]

Since each myeloproliferative neoplasm can evolve into the others, diagnosis is a moving target. For example, *JAK2* V617F–positive essential thrombocytosis evolves into polycythemia vera in women more often than in men,[24] whereas *JAK2* V617F–positive[24] or *CALR* type 1–positive[73] essential thrombocytosis in men is more likely to evolve into secondary myelofibrosis; polycythemia vera evolves into myelofibrosis, and primary myelofibrosis evolves into polycythemia vera. Quantification of the driver-mutation allele burden at diagnosis provides a baseline for assessment of clonal evolution (Fig. 2, and Fig. S5 in the Supplementary Appendix).

Erythrocytosis in polycythemia vera, unlike hypoxic erythrocytosis, usually induces plasma-volume expansion,[69] masking the true hematocrit (Fig. 3). In many patients, especially women,[74,75] the hematocrit appears to be normal. Because polycythemia vera is the most common myeloproliferative neoplasm, has the most protean manifestations, and is associated with the highest risk of thrombosis, its identification is paramount when a myeloproliferative neoplasm is a diagnostic consideration. Moreover, because of phenotypic mimicry, polycythemia vera must be ruled out to establish the diagnosis of essential thrombocytosis or primary myelofibrosis, unless an *MPL* mutation is involved.[74-76]

A hematocrit or erythrocyte count above the normal range for sex, in conjunction with a *JAK2* or *CALR* mutation, establishes the diagnosis of neoplastic erythrocytosis, even in the absence of leukocytosis, thrombocytosis, and splenomegaly;



	Plasma-Volume Contraction			Secondary Erythrocytosis			Polycythemia Vera		
	52-Yr-old man taking replacement androgens			50-Yr-old man with a pulmonary arterial venous fistula			18-Yr-old man with hepatic-vein thrombosis		
	Expected	Observed	Deficit or excess	Expected	Observed	Deficit or excess	Expected	Observed	Deficit or excess
Red-Cell Mass (ml)	2370	2661	+291	1924	3982	+2058	2058	3210	+1152
Plasma Volume (ml)	3393	1824	–1569	2919	2489	–430	3061	5674	+2613
Total Blood Volume (ml)	5763	4455		4843	6481		5119	8884	
Hematocrit (%)	41.1	59.3		39.7	61.4		40.0	36.1	

Figure 3. Effect of Changes in Plasma Volume and Red-Cell Mass on the Venous Hematocrit.

Plasma-volume contraction alone causes an apparent increase in the hematocrit, even though the red-cell mass is unchanged and is indistinguishable on the basis of the hematocrit from an increase in red-cell mass due to hypoxia, since with hypoxia, an increase in erythropoiesis is associated with plasma-volume contraction because maintaining a constant blood volume is the body's default program. In polycythemia vera, however, as the red-cell mass increases, the plasma volume usually increases, masking the erythrocytosis or its extent, a situation further confounded by splenomegaly or pregnancy. Thus, as illustrated in the examples below the graph, without an independent determination of both the red-cell mass and plasma volume, it is not possible, on the basis of the hematocrit, to distinguish plasma-volume contraction from absolute erythrocytosis or even the presence of erythrocytosis, assuming normocytic red cells, if polycythemia vera is a diagnostic consideration. The arrows indicate the plasma volume.

microcytic erythrocytosis, if present, provides a useful clue.[77] With iron deficiency, the hemoglobin level cannot be substituted for the hematocrit diagnostically and should not be used for therapeutic guidance,[78] since erythrocytosis, not the hemoglobin level, determines blood viscosity[79] (Fig. S7 in the Supplementary Appendix). When the hematocrit is apparently normal, especially in patients with splenomegaly, only red-cell mass and plasma-volume measurements can distinguish polycythemia vera from its companion myeloproliferative neoplasms[69,74]; marrow histologic features have no role in this situation.[70] Molecular diagnostic assays for these disorders are currently lacking, though data are available for the development of such assays.[23,37-39]

Finally, a serious consequence of using predetermined hematocrit or hemoglobin levels diagnostically is conflation of polycythemia vera with *JAK2* V617F–associated essential thrombocytosis.[75,78] This inflates the thrombosis rate in *JAK2* V617F–positive essential thrombocytosis relative to the rate in essential thrombocytosis associated with a *CALR* mutation, because *CALR* mutations rarely cause erythrocytosis.[72]

THERAPY

Cure is the ultimate objective of cancer therapy, but the hallmark of myeloproliferative neoplasms is their chronicity. On the basis of retrospective data unadjusted for sex, age, driver mutation, or therapy, life expectancy for patients with essential thrombocytosis is normal, whereas the median survival for patients with polycythemia vera and patients with primary myelofibrosis is 27 years and 14 years, respectively.[1] Thus, strategies for accurate risk assessment are essential to maximize therapeutic benefits and avoid unnecessary toxic effects. The therapeutic goals for patients with myeloproliferative neoplasms are symptom alleviation and prevention of thrombosis and transformation to myelofibrosis or acute leukemia.

POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTOSIS

For polycythemia vera and essential thrombocytosis, current therapeutic guidelines stipulate that an age of 65 years or more and a history of thrombosis put patients at high risk for complications, apart from the role of sex, driver or other mutations, allele burdens, and the fact that thrombosis in polycythemia vera is provoked and related only to the hematocrit.[80,81] Furthermore, except for hepatic-vein thrombosis in young women,[82] complications in patients with polycythemia vera do not differ according to age,[83] but longevity data notwithstanding, chemotherapy is recommended in both diseases.[84] Yet prospective, controlled clinical trials[60,62] and a large retrospective study[67] have shown that neither chemotherapy nor phosphorus-32 for the treatment of polycythemia vera prevents thrombosis or prolongs survival, and both treatments are associated with an increased risk of leukemic transformation. In patients with essential thrombocytosis, hydroxyurea alleviates transient ischemic attacks but does not prevent either arterial or venous thrombosis and is not otherwise more effective than anagrelide or aspirin,[85,86] even though it normalizes both the platelet and leukocyte counts. Moreover, attempts to achieve hematologic remission with hydroxyurea have failed to prolong survival.[87]

Dameshek's advice nearly 50 years ago is still apt: "There is a tendency in medical practice — by no means limited to hematologists — to treat almost any condition as vigorously as possible. In hematology, this consists in attempting to change an abnormal *number* — whether this number is the hematocrit, white cell count or platelet count — to get normal values, whether the patient needs it or not!"[88]

Treatment of polycythemia vera relies on phlebotomy. The target hematocrit is below 45% in men and below 42% in women.[80] The iron deficiency due to phlebotomy can aid in the control of erythrocyte production and rarely needs to be treated unless symptoms in other systems interfere with the quality of life.[89]

Asymptomatic patients with essential thrombocytosis require no therapy[90]; platelet counts exceeding 1 million per cubic millimeter can cause mild acquired von Willebrand's disease as a result of platelet proteolysis of high-molecular-weight von Willebrand multimers, but unprovoked hemorrhage is uncommon.[91] If reduction of the platelet count is necessary, pegylated interferon is preferable to hydroxyurea in patients younger than 65 years of age.[84] In both polycythemia vera and essential thrombocytosis, aspirin is usually effective for microvascular episodes such as ocular migraine, transient ischemic attacks,[92] and erythromelalgia due to hyperactive platelets.[93] Aspirin has no antithrombotic

benefit in the absence of cardiovascular risk factors.[94]

MYELOFIBROSIS

In patients with secondary myelofibrosis due to either essential thrombocytosis or polycythemia vera, as well as in patients with primary myelofibrosis, longevity is compromised by extramedullary hematopoiesis, marrow failure, and leukemic transformation, regardless of the driver mutation. In patients with primary myelofibrosis, *CALR* type 1 mutations may offer a survival advantage but not freedom from leukemic transformation.[95] Current prognostic scoring systems for myelofibrosis,[96,97] which predict median survival and need for therapeutic intervention, are based on the primary myelofibrosis phenotype. These systems are inexact for secondary myelofibrosis[98] and do not account for the influence of driver or other mutations on survival or leukemic transformation.

With respect to risk stratification, it appears that mutations in *ASXL1*, *EZH2*, *SRSF2*, or *IDH1*/*2*, with or without a driver mutation, in patients with primary myelofibrosis are independent risk factors for shortened survival[20]; in patients with secondary myelofibrosis, only *SRSF2* is associated with shortened survival.[99] Patients without myelofibrosis may also be at risk of leukemic transformation if they acquire a *TP53* mutation, even at a subclonal level.[7,63]

TARGETED THERAPIES

Chemotherapy has traditionally been used to control intractable pruritus and ocular migraine, as well as extramedullary hematopoiesis associated with myelofibrosis, but it does not avert the need for splenectomy or splenic irradiation. Now, however, there are two effective, nongenotoxic therapies to address these problems: ruxolitinib and interferon.

Ruxolitinib, an inhibitor of JAK1 and JAK2, durably alleviates symptoms, reduces splenomegaly, corrects blood counts,[100] and is effective in patients with hydroxyurea-refractory polycythemia vera.[101] Suppression of inflammatory cytokine production and hematopoietic progenitor-cell proliferation appear to be the major effects of ruxolitinib. Hematopoietic stem cells are not appreciably affected, and neither is leukemic transformation. Whether the presence of additional mutations impairs the effectiveness of ruxolitinib is disputed.[102,103]

Interferon is currently the only agent that specifically targets hematopoietic stem cells in patients with myeloproliferative neoplasms[104]; its pegylated derivative alleviates symptoms, reduces splenomegaly, and induces hematologic remission. Durable complete molecular remission has been achieved in 18% of patients with polycythemia vera or essential thrombocytosis,[105,106] and marrow fibrosis has been ameliorated in some patients with primary myelofibrosis.[107] The influence of nondriver mutations on the effectiveness of interferon is unclear.[106,108] Neither interferon nor its pegylated derivative is uniformly effective in all patients, and clinically significant side effects, such as immunosuppression, myelotoxicity, and neurotoxicity,[109] limit the use of these drugs in some patients.

BONE MARROW TRANSPLANTATION

Bone marrow transplantation is the only curative therapy for the myeloproliferative neoplasms,[110] but several questions remain unanswered. It is unclear whether full allogeneic or haploidentical transplantation should be performed, and there is uncertainty about the conditioning regimen. The most important question, given transplantation-related mortality and the chronicity of myeloproliferative neoplasms, is when to intervene in patients other than those with high-risk myelofibrosis.

FUTURE CONSIDERATIONS

There are two challenges in future therapy for myeloproliferative neoplasms: accurate genetic, as opposed to phenotypic, identification of patients at risk for disease transformation, and eradication of neoplastic hematopoietic stem cells to prevent leukemic transformation. With regard to both challenges, since few oncogenes are recurrently mutated in these disorders and other mechanisms, including cytogenetic and epigenetic abnormalities, are involved in transformation, gene-expression profiling is likely to be the most informative approach for defining risk and identifying molecular pathways for targeted therapy.[23]

While new therapies targeting hematopoietic stem cells in myeloproliferative neoplasms are being developed, efforts should be focused on when and how to use the three treatments currently documented as effective — ruxolitinib, pegylated interferon, and bone marrow transplantation — alone or in combination and possibly with epigenetic-modifying drugs to eradicate neoplastic hematopoietic stem cells.

We do not understand why only a minority of patients have a molecular remission with pegylated interferon or what the biologic basis for ruxolitinib failures is. Answers to these questions will come only from prospective, randomized clinical trials combined with molecular analysis to define genomic abnormalities in patients. With respect to new treatment directions, hematopoietic stem cells are most vulnerable in their bone marrow niches (Fig. S4 in the Supplementary Appendix). Consequently, targeting MPL (the thrombopoietin receptor) or its ligand (thrombopoietin) could lead to fruitful, nongenotoxic therapeutic strategies.[111,112]

Dr. Spivak reports receiving consulting fees from Incyte and holding a patent on a genetic assay to determine prognosis in patients with polycythemia vera (PCT/US2013/069192). No other potential conflict of interest relevant to this article was reported.

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