Myelofibrosis 2012: it's complicated

Harper G. Hubbeling, Dale M. Frank and Elizabeth O. Hexner

Abstract: Major advances in myeloproliferative neoplasms in the last decade have cast light on their complexity. The identification of $JAK2^{V617F}$ briefly promised a unifying mechanism of pathogenesis with a single pathway that could be efficiently targeted. Instead, there have been major advances in understanding acquired and background genetic and epigenetic contributors to this group of disorders, with refined risk prediction models and experimental therapeutics that have provided a more nuanced model of disease. In aggregate these observations likely explain the heterogeneity of these disorders and their generally unpredictable response to therapy. Molecular studies, beginning with the identification of $JAK2^{V617F}$, have led to a concept of MPN subtypes existing on a continuum, and additional discoveries such as *TET2* and *EZH2* mutations have provided the molecular underpinnings to begin to explain overlapping phenotypes in myeloid malignancies more generally. In many ways the pace of molecular discovery is outstripping our ability to integrate these observations into clinical care, both in terms of molecular diagnostics and medical decision making. This review will attempt to summarize, within a clinical context, our evolving understanding of myeloproliferative neoplasms. It focuses on biology, histopathology, prognostic scoring systems, stem cell transplantation as well as selected clinical/preclinical therapeutic observations.

Keywords: essential thrombocytosis, hematopoietic stem-cell transplantation, interferon, JAK2 V617F, myelofibrosis, myeloproliferative neoplasms, polycythemia vera, TET2

'I think the next century will be the century of complexity.' (Stephen Hawking, January 2000)

Introduction

In 1951, when William Dameshek proposed that chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocytosis (ET) and myelofibrosis (MF) were related disorders, 'variable manifestations of proliferative activity of bone marrow cells perhaps due to a hitherto undiscovered stimulus', the concept was, for some, considered radical. That MF patients receiving blood transfusions to correct cytopenias might be suffering from the same underlying aberration as PV patients dependent on blood letting? That myeloproliferative and myelodepletive afflictions were merely variations on a common theme? This was understandably a fanciful proposition [Dameshek, 1951].

A decade later, Dr Nowell and Dr Hungerford identified a one-to-one relationship between the Philadelphia chromosome and CML, and the subsequent characterization of the BCR-ABL fusion protein supported the concept that dysregulated tyrosine kinase activity might be pathogenic, that Dr Dameshek's, 'hitherto undiscovered stimulus', may have finally been discovered, at least for CML [Nowell and Hungerford, 1960]. Indeed, tyrosine kinase dysregulation is today accepted as the single unifying feature of myeloproliferative neoplasms (MPN), a family of biologically and clinically related disorders now expanded to encompass diverse and relatively rare disorders including systemic mast cell disorders and chronic eosinophilic leukemia, both of which have well-characterized tyrosine kinase activation pathways [Swerdlow *et al.* 2008].

The specifics of the tyrosine kinase activation pathways in MF, PV and ET (the Philadelphia chromosome negative or 'classical' MPN), however, remained mysterious for decades. Then, in 2005, four groups identified a somatic activating point mutation in *JAK2* in the majority of

Correspondence to:
Elizabeth O. Hexner, MD
Department of Medicine and Abramson Cancer Center, University of Pennsylvania, 3400 Civic Center Boulevard Philadelphia, PA, 19104 USA.
elizabeth.hexner@uphs.upenn.edu

Harper G. Hubbeling, BS
Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Dale M. Frank, MD
Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA
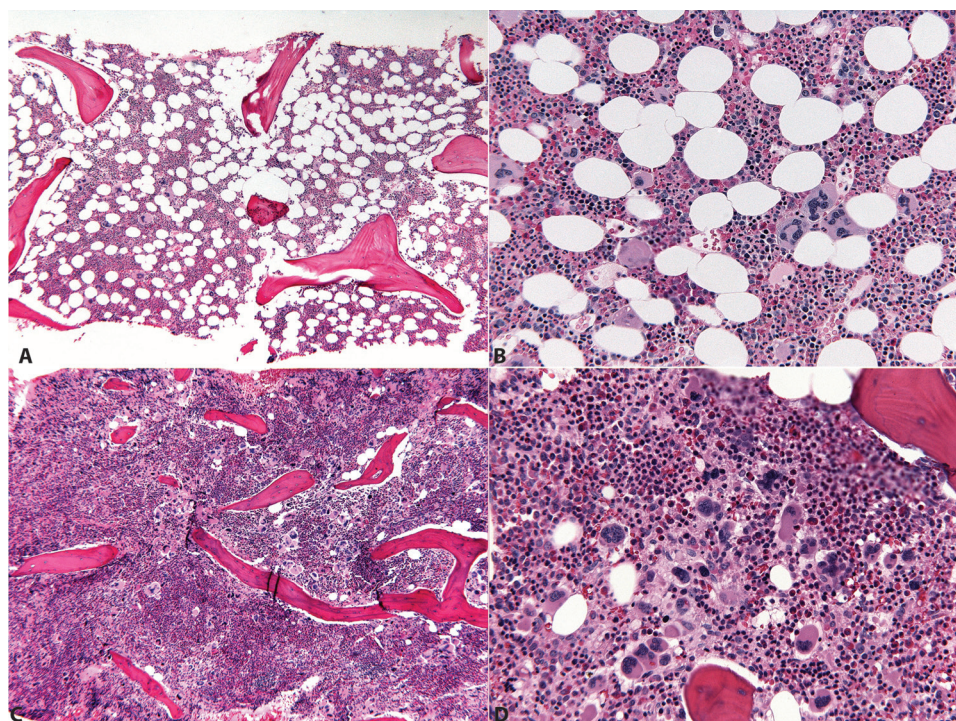
Figure 1. Morphologic findings in essential thrombocytosis (ET) and myelofibrosis (MF).
(A), (B) A 39-year-old woman with *JAK2*[V617F+] ET. Mildly hypocellular (50%) marrow (5× H&E) with balanced trilineage hematopoiesis featuring singly scattered and focally clustered normal sized and large megakaryocytes with widely spaced ('staghorn') nuclei (20× H&E). (C), (D) A 55-year-old woman with *JAK2*[V617F+] MF. Markedly hypercellular (95% cellularity) marrow (5× H&E) with granulocytic hyperplasia and prominent aggregates of smaller cytologically atypical megakaryocytes with hypolobated 'cloudlike' nuclei (20× H&E).

patients with these disorders [Baxter *et al.* 2005; James *et al.* 2005; Kralovics *et al.* 2005; Levine *et al.* 2005]. JAK2 is a nonreceptor tyrosine kinase that associates with cytokine receptors and signals canonically through STAT3 and STAT5, cytoplasmic effector proteins that can dimerize and translocate to the nucleus. More than 95% of patients with PV and approximately 60% of patients with ET and MF were found to have a G to T substitution in exon 14 of *JAK2*, leading to a valine to a phenylalanine change at position 617 (*JAK2*[V617F]). The mutation is in the pseudokinase domain of JAK2, which is thought to deregulate its autoinhibitory activity, although the exact mechanism by which it leads to constitutive activation remains incompletely understood. However, while targeting BCR-ABL has radically changed the natural history and treatment paradigms for CML, targeted therapies based on the discovery of *JAK2*[V617F] have had less impressive results. In addition to the pharmacology and target itself, this is most likely a reflection of the clinical heterogeneity and the biologic complexity of MPN associated with *JAK2*[V617F].

This review is an attempt to tackle some of those complexities and their clinical (particularly their therapeutic) implications, focusing in large part on the entity of MF.

Diagnostic and histopathologic considerations

The *sine qua non* of MF, whether evolved from PV/ET or primary myelofibrosis (PMF), is fibrous disruption of the marrow space, typically identified by a reticulin stain, and in more advanced states of collagen fibrosis, a trichrome stain. Cytokines elaborated by the malignant clone lead to reactive stromal hyperplasia, and can also cause severe constitutional symptoms in afflicted patients. Less commonly, an early manifestation of PMF, termed prefibrotic MF, lacks marked fibrosis. Prefibrotic MF often presents with an isolated thrombocytosis, and therefore can be difficult to distinguish clinically and histologically from ET (Figure 1). In fact, making this distinction often lacks immediate clinical consequences but does have prognostic significance. True ET has a low likelihood of progression to post-ET

MF, while prefibrotic MF is considered the MF prodrome. Prefibrotic MF is distinguished from ET by a constellation of bone marrow morphologic features: an ET bone marrow should be normocellular or only slightly hypercellular for age, while prefibrotic MF is typically hypercellular with expanded left-shifted granulopoiesis and decreased erythropoiesis. Perhaps the most important, and controversial, distinguishing features between these two entities are the morphology and geographic distribution of the aberrant cells held responsible for these disorders: the megakaryocytes. The megakaryocytes of ET are frequently large or giant with hyperlobated 'staghorn' nuclei, while those of prefibrotic MF are more variably sized (often small) and cytologically bizarre, with maturation defects and hypolobated 'cloudlike' nuclei. In ET, megakaryocytes are scattered singly and in small clusters throughout the marrow, while in prefibrotic MF megakaryocytes are packed densely into large aggregates and found in close proximity to the endosteum and vascular sinuses. Whether these morphologic distinctions, incorporated into the most recent (2008) World Health Organization diagnostic criteria, are truly reproducible and prognostically significant has been a matter of some debate [Spivak and Silver, 2008]. However a recent systematic re-evaluation suggests reasonable concordance and true prognostic utility for the new criteria [Thiele *et al.* 2011].

Clinically, MF can be associated with either increased or decreased white blood cell and platelet counts, but is typically associated with anemia and splenomegaly in symptomatic patients. It is also important to note that, like PV and ET, these patients have a propensity towards clotting [Barbui *et al.* 2010]. While this is historically well recognized in PV and ET, it was only recently established for MF, with age (over 60) and possibly $JAK2^{V617F}$ and/or elevated white blood cell count contributing to thrombotic risk. Unlike for PV and ET, primary and secondary prevention strategies/recommendations are not well established for MF [Barbui *et al.* 2011]. Vexing issues of portal hypertension, cytopenias and bleeding frequently complicate anticoagulation, cytoreductive and antiplatelet therapy in MF patients.

Prognostic scoring systems in MF: how precise can we be?

While the prognosis can be poor in MF, it is variable, ranging from a median survival of 1 to more than 15 years, depending on a variety of factors. Thus a more precise prognostic system is critical for medical decision making, particularly when aggressive treatments such as stem-cell transplantation are being considered. A short review of the evolution of prognostic scoring systems for MF illustrates the evolution of our understanding of prognostic factors. The first widely used systematic stratification of risk groups among MF patients, the Lille Score, was put forth in 1996 [Dupriez *et al.* 1996]. This initial system considered two factors, hemoglobin level and leukocyte count, touting a 'simple prognostic model for survival'. Its simplicity was attractive, and indeed anemia remains one of the most predictive factors determining survival. The Lille classification system grouped patients into three risk brackets, low, intermediate and high, observing associated median survivals of 93, 26 and 13 months, respectively. But the power of the Lille system to accurately discriminate between those patients with 'intermediate' and 'high' risk levels was quickly called into question. In 1997, Cervantes and colleagues demonstrated that when applied to a new patient group the Lille scoring system could not in fact distinguish between intermediate- and high-risk patients, and proposed their own new system, adding one new prognostic element, constitutional symptoms, and replacing white blood cell count with peripheral blood blast count [Cervantes *et al.* 1997]. The original Cervantes system classified patients simply into low- and high-risk brackets, and while it held up over time, its weakness turned out to be not in its capacity to accurately fulfill its prognostic aims, but in distinguishing between shades of risk. Defining and refining intermediate- and high-risk groups is exceptionally useful for tailoring treatment options.

Thus, at the request of the International Working Group for MPN Research and Treatment (IWG-MRT), Cervantes and colleagues developed an entirely new prognostic system (International Prognostic Scoring System [IPSS]) using five prognostic factors: hemoglobin (<100 g/l), leukocyte count (>25 × 10³/l), constitutional symptoms, circulating blasts (>1%) and, additionally, age (>65 years). This hybrid of Lille and the previous Cervantes system, with the addition of age, proved capable of accurately establishing median survival for four clearly distinct risk groups among MF patients: low (135 months), intermediate-1 (95 months), intermediate-2 (48 months) and high (27 months) [Cervantes *et al.* 2009]. In 2010, the IPSS system evolved further with

the development of dynamic IPSS (DIPSS), which considers the same five factors as time-dependent covariates, and allows assessment of MF patients at any time during their clinical course, not merely at initial diagnosis. The DIPSS also established that risk of leukemic transformation increases with increasing risk categories [Passamonti *et al.* 2010]. Clinically, establishing validity over time, either for reassuring patients who are stable or for changing treatment if the prognostic category changes, can be a useful tool.

Prognostic implications for genetic events in myelofibrosis

Cytogenetics
The studies that produced the IPSS suggested that cytogenetic abnormalities contributed independently to prognosis for patients in intermediate-risk groups, although they did not formally incorporate cytogenetic criteria into the IPSS. More recently, detailed mapping of commonly affected genomic regions (on chromosomes 3, 7 and others) has identified single target genes and correlated lesions with disease progression [Klampfl *et al.* 2011]. Likewise, a tumor suppressor gene (L3MBTL1) found on chromosome 20, one of the most commonly deleted regions in PV specifically and MPN generally, was recently shown to be important in erythroid fate decisions, which suggested that haploinsufficiency could promote erythroid differentiation [Perna *et al.* 2010]. Clinically, the most recent prognostic refinement, DIPSS Plus, incorporates cytogenetic abnormalities, thrombocytopenia and transfusion-dependent anemia as additional risk factors for shortened survival. Thus, at the expense of simplicity, we are becoming better able to define risk categories in MF [Gangat *et al.* 2011]. Interestingly, the criteria coming into use in MF scoring systems increasingly resemble those used in myelodysplastic syndromes (MDS); these similarities likely reflect the often overlapping molecular underpinnings of these two heterogeneous disorders.

JAK2^{V617F}: dose matters
JAK2^{V617F} gene 'dose' has emerged as an important patterning event for the MPN phenotype, particularly with respect to PV and ET (see below). Dose also likely contributes to prognosis, both with respect to thrombotic risk (PV and ET) and to survival. This topic has been recently extensively reviewed in this journal [Vannucchi *et al.* 2011]. Notably low allele burden in PMF was observed to be associated with a 'myelodepletive' phenotype, i.e. bone marrow failure, associated low blood counts and increased incidence of infection with poor overall survival, when compared with those patients with PMF and high allele burdens, who tend to have a more truly myeloproliferative phenotype (constitutional symptoms, splenomegaly) [Guglielmelli *et al.* 2009; Tefferi *et al.* 2008]. That myelodepletive PMF may on some level represent a distinct biologic group is a fascinating observation, but it is also an important consideration in the interpretation of clinical trials: myelodepletive patients are more likely to be excluded from clinical trials because of cytopenias, so generalizing outcome data to this population is problematic [Verstovsek *et al.* 2010]. What does this suggest about biology? Does the utility of low mutant allele burden as a surrogate for worse outcome suggest that *JAK2*^{V617F} is a passenger mutation, or a 'late hit' in a disease driven by other factors? Or is this mutation an early event which, in the proper context, dysregulates growth and/or predisposes to genomic instability? Additional studies should help to clarify the underlying biology, and ideally would include detailed analyses of patients over time. Thus far, modern prognostic systems do not take into account the presence, absence, or overall burden of the *JAK2*^{V617F} mutation. How these (and other predisposing genetic factors) relate to pathogenesis, clinical presentation, and prognosis is an active area of investigation [Bejar *et al.* 2011; Guglielmelli *et al.* 2011b].

The JAK2^{V617F} mutation: of mice and MPN
Multiple mouse models have been used to study the role of *JAK2*^{V617F} in MPN, and have demonstrated the phenotypic variation associated with gene dosage. Initial studies relied on overexpression models, using retrovirally transduced bone marrow transplantation. These initial models made the fundamental observation that the *JAK2*^{V617F} mutation alone was sufficient to recapitulate many of the clinicopathologic features of human PV progressing to MF. Mice transplanted with *JAK2*^{V617F} transduced bone marrow display elevated hemoglobin/hematocrit, leukocytosis, and megakaryocyte hyperplasia followed by extramedullary hematopoiesis, splenomegaly and reticulin fibrosis in the bone marrow. Other

somewhat more nuanced observations arose from these initial models as well. Wernig and colleagues noted that the effects of $JAK2^{V617F}$ bone marrow transplants were markedly different between mouse strains (Balb/c mice showing much more elevated leukocyte counts, more splenomegaly and more reticulin fibrosis than C57B1/6 mice), hinting that strain-specific modifiers might explain the phenotypic pleiotropism of MF in humans [Wernig *et al.* 2006].

The next wave of mouse models went beyond proving the basic sufficiency of the $JAK2^{V617F}$ mutation in recapitulating PV/MF and examined the dose-dependent nature of its effect. Using assorted transgenic expression systems to achieve various levels of constitutive $JAK2^{V617F}$ expression, these models revealed that while a high level of $JAK2^{V617F}$ expression generated PV-MF-like symptoms as observed previously, a low level of $JAK2^{V617F}$ expression phenocopied essential thrombocytosis. This new understanding of $JAK2^{V617F}$ as a dose-dependent contributor to myeloproliferative disorders was perhaps the first credible, biologic explanation of how diseases with such variable, even sometimes opposite, pathologies could be due to the same genetic aberration. Meanwhile a parallel story was emerging in humans: simple but elegant genotyping of colony assays from cells of patients with PV and ET showed that ET patients lack progenitors homozygous for $JAK2^{V617F}$, while at least some homozygous clones are typically found in individuals with PV [Scott *et al.* 2006]. A conditional transgenic mouse with a human version of the $JAK2^{V617F}$ gene under the control of the mouse *Jak2* promoter develops mild elevations in hemoglobin and platelet counts [Li *et al.* 2010]. Interestingly, in contrast to other transgenic models, these mice demonstrate a decrease in both the size and function of the stem/progenitor cell compartment, a deficiency that does not manifest immediately, but requires prolonged exposure (26 weeks) to mutant $JAK2$. Stem cells display increased DNA damage, decreased cell cycling and impaired apoptotic responses. Taken together, these findings might account for the functional competitive disadvantage observed for these stem cells compared with their wild-type counterparts in primary and secondary transplantation experiments. One wonders whether the same mechanisms might account for the bone marrow failure observed in advanced 'myelodepletive' myelofibrotic disorders.

How does *JAK2^V617F* associated MPN arise, who is at risk, and are there known environmental contributors?

While mouse models clearly show that $JAK2^{V617F}$ is sufficient for the development of an MPN phenotype, many lines of evidence suggest that this mutation might be neither the sole nor initiating event in MPN pathogenesis. The existence of rare families predisposed to developing MPN point to a heritable factor; it is notable that $JAK2^{V617F}$ is often present in affected family members, but always as an acquired mutation, and that both $JAK2^{V617F}$ and $JAK2$ wild-type MPN can exist within a single kindred. By contrast, elegant cytogenetic and clonal hierarchy studies within a single patient with acquired disease have confirmed multiple separate acquisitions of $JAK2^{V617F}$ in different clones [Olcaydu *et al.* 2009a]. In 2009, three groups identified a germline haplotype that increased the risk of acquisition of $JAK2^{V617F}$ MPN approximately four-fold. Unexpectedly, a single nucleotide polymorphism (SNP) mapped to the 3' portion of the $JAK2$ gene itself on chromosome 9 and typically occurred *in cis* with the acquired $JAK2^{V617F}$ mutation [Jones *et al.* 2009; Kilpivaara *et al.* 2009; Olcaydu *et al.* 2009a]. Interestingly, subsequent studies confirmed not only the observed association between this SNP, commonly referred to as the 46/1 $JAK2$ haplotype, with $JAK2^{V617F}$, but also noted an association between 46/1 and MPN-associated exon 12 mutations in $JAK2$ and, more surprisingly, MPN-associated mutations in Mpl, the thrombopoietin-receptor gene located on chromosome 1 [Jones *et al.* 2010; Olcaydu *et al.* 2009b]. Overall, $JAK2$ 46/1 is estimated to contribute approximately half of the heritable risk of MPN [Campbell, 2009]. Other more circumstantial observations suggest that environmental factors may also influence the acquisition of $JAK2^{V617F}$: a possible cluster of cases of PV in Southeastern Pennsylvania in a pattern that overlaps both with the distribution of waste/coal power plants and a major superfund site provides indirect evidence for an association with toxic exposure [Seaman *et al.* 2009]. $JAK2^{V617F}$ has also been shown to be present in a disproportionately high number of cases of therapy-related leukemia [Schnittger *et al.* 2007]. A mechanistic understanding of how these genetic and environmental factors lead to the acquisition of this mutation might one day provide insights into the prevention of the diseases associated with it.

What are the other important molecular events in MPN?

Within the JAK/STAT pathway

Recurrent activating mutations in Mpl, the thrombopoietin receptor, have been identified in a subset of patients with MF and ET. Mpl mutations rarely occur together with $JAK2^{V617F}$ [Pikman *et al.* 2006]. Mouse models of Mpl^{515} mutations demonstrate elevated platelet counts and develop MF with a relatively short latency [Koppikar *et al.* 2010]. The identification of Mpl mutations is consistent with the general model that MPN are diseases characterized by dysregulation of the cytokine receptor/JAK/STAT axis and explains, to some extent, why MF or ET without $JAK2^{V617F}$ is often phenotypically indistinct from $JAK2^{V617F}$ positive disease. By contrast, while the overwhelming majority (>95%) of patients with PV have $JAK2^{V617F}$, many (if not all) of those without the mutation have mutations elsewhere in the *JAK2* gene, typically exon 12, and exon 12-mutated PV is phenotypically distinct, usually presenting with an isolated erythrocytosis. How the different amino acid substitutions in JAK2 lead to an altered clinical presentation is not well established [Scott *et al.* 2007].

Mutations in the adaptor protein, LNK, are one example of JAK2/STAT pathway dysregulation stemming from the loss of function of a negative regulator of signaling. LNK structure/function relationships have been recently comprehensively reviewed in this journal [Oh, 2011]. LNK is an adapter protein that associates with both Mpl and JAK2 in its active form, and attenuates STAT signaling. The absence of LNK results in myeloproliferation in mouse models [Tong *et al.* 2005; Velazquez *et al.* 2002]. Missense mutations in LNK have recently been identified and characterized in two patients with MPN, one with MF and one with ET, and the mutations lead to either complete or partial loss of inhibitory activity, respectively. Interestingly STAT phosphorylation appeared to be increased in stem/progenitor cells from these patients [Oh *et al.* 2010].

Going nuclear

$JAK2^{V617F}$ may also have noncanonical oncogenic functions. Mutant *JAK2* can enter the nucleus, where it has recently been shown to directly phosphorylate histones and alter the expression of the leukemic oncogene lmo2 [Dawson *et al.* 2009]. $JAK2^{V617F}$ has also been implicated in other potentially oncogenic epigenetic modifications. PRMT5, a methyltransferase, more effectively binds mutant *JAK2* than wild type, both in the cytoplasm and nucleus. This interaction decreases PRMT5 activity, leading to myeloproliferation [Liu *et al.* 2011]. Taken together, it appears that $JAK2^{V617F}$ may create genomic instability and/or epigenetic alterations relevant to the pathogenesis of MPN.

TET2

One clue to other adaptive mutations, also with epigenetic relevance, emerged with the identification of *TET2* mutations in MPN. TET2 is an enzyme that modifies DNA, one of three known proteins that hydroxylates 5-methylcytosine in genomic DNA. The activity of TET2 appears to be sensitive to metabolic perturbations and important for growth regulation. *TET2* mutations are present in approximately 8% of patients with MPN (with or without $JAK2^{V617F}$), 20% of patients with MDS, 12% of patients with acute myelogenous leukemia (AML), and a rather striking 42% of patients with chronic myelomonocytic leukemia (CMML) [Abdel-Wahab *et al.* 2009]. With this mutation cropping up in both proliferative and depletive myeloid disorders, *TET2* presents itself as a potentially 'unifying' genetic aberration in myeloid malignancies. As we unravel the causes and effects of *TET2* dysfunction in these disorders, we may begin to understand how these diseases can be situated at opposing ends of the same spectrum. Mutations in *TET2* appear to have adverse prognostic significance in AML, but are not clearly established as correlating with risk in MPN at this point.

Missense mutations in *TET2* occur at different sites, but collectively lead to partial or complete loss of function through inhibition of catalytic activity. Mutations in *TET2* can precede the acquisition of other mutations, including $JAK2^{V617F}$ [Abdel-Wahab *et al.* 2009; Delhommeau *et al.* 2009]. Much of the initial study of *TET2* in MPN was dedicated to understanding a potential relationship between mutations in this gene and those in *JAK2*. In a small study, Delhommeau and colleagues found that the $JAK2^{V617F}$ mutation was preceded by mutations in *TET2*, although acquisition of $JAK2^{V617F}$ prior to *TET2* may also be possible. These observations lead to an emerging paradigm which suggests that it is not only the specific combination of molecular events but also the

sequence of their acquisition that contributes to phenotype, progression and risk in these disorders.

In this vein, Delhommeau and colleagues looked at xenografts of primary CD34+ cells from MPN patients positive for *JAK2*^{V617F} with or without *TET2* mutations. They found a preferential expansion of human *JAK2*^{V617F} cells with a *TET2* mutation (with myeloid skewing) over time, and a concomitant loss of *JAK2*^{V617F} cells lacking a *TET2* mutation. These findings suggested the possibility that the *TET2* mutation in these patients occurred in a functional stem-cell compartment, and that it conferred competitive self-renewal properties upon these cells and their offspring.

Not surprisingly, genotypic heterogeneity appears to be associated with varying sensitivity of different clones to clinical interventions. A subsequent case report detailed a response to treatment with interferon and noted a differential effect on different subclones, with the disappearance of combined *JAK2*^{V617F}/*TET2*-mutated clones and the persistence of their *JAK2* wild-type/*TET2*-mutant counterparts [Kiladjian *et al.* 2010].

More recently, mouse models have been engineered with loss of function mutations in *TET2*, and have clarified its transforming role in hematopoietic malignancies [Moran-Crusio *et al.* 2011; Quivoron *et al.* 2011]. *TET2*-mutant homozygotes demonstrate increases in the stem/progenitor cell compartment in bone marrow and spleen, and develop a myeloproliferative/myelodysplastic syndrome reminiscent of CMML. Qualitatively, stem/progenitor cells possess increased self-renewal capacity both *ex vivo* and in competitive serial transplantation experiments. These differences were attenuated but maintained in *TET2* haploinsufficient mice, which may be more clinically relevant, given that patients overwhelmingly maintain one wild-type allele.

Therapeutic considerations
JAK2^{V617F} alone in mouse models can recapitulate the MPN phenotype, but inhibiting *JAK2* alone does not reverse the disease.

Tyrosine kinase inhibitors clearly have activity in patients with MF, although parallels with early clinical studies of imatinib in CML do not appear to hold. JAK2 inhibitors have been tested most extensively in advanced MF [Dolgin, 2011; Verstovsek *et al.* 2010], and the early normalization of blood counts that is almost invariably the outcome with initial treatment of CML in chronic phase is rarely seen [Druker *et al.* 2006]. Likewise, the degree of fibrosis appears to be unchanged with treatment, with only modest, if any, reductions in mutant clonal burden. In fact, the primary activity of JAK2 inhibitors in treated patients appears to be an early and sometimes dramatic reduction in spleen size, and (perhaps more impressively) improvements in quality of life that track with changes in cytokine and chemokine profiles. Our mechanistic understanding of the response (or lack thereof) to these agents is incomplete. JAK2, unlike ABL kinase, is indispensable for normal hematopoiesis [Ghaffari *et al.* 2001] and may therefore be a target with a finite and limited therapeutic index. The dysregulated, overactive JAK/STAT pathway in MPN cells (or their more generalized proliferative disarray) must account for selective sensitivity to inhibition, and may explain why the growth of cells from patients with and without the mutant allele is inhibited in preclinical studies [Hexner *et al.* 2008; Weinstein *et al.* 1997]. There are no published peer-reviewed data for small molecule inhibitors specific for the JAK2^{V617F} mutant kinase, although in principle they exist in the preclinical pipeline [Dolgin, 2011]. Moreover, the published clinical trials themselves analyze biomarkers such as cytokine profiles and changes in allele burden [Pardanani *et al.* 2011; Verstovsek *et al.* 2010], but to date do not provide pharmacodynamic evidence for inhibition of the JAK2 kinase (or its downstream effectors) in treated patients. Thus, it remains formally possible that the failure to completely reverse the disease process is simply due to a failure of the drug to hit its intended target. Other schools of thought challenge the paradigm that increasing the specificity of the kinase inhibitor will improve efficacy, and instead support the concept that targeting multiple pathways within a malignant clone (or targeting nonclonal bystander cells) will enhance activity. For example, the clinical activity that has been observed with respect to changes in cytokine profiles and splenomegaly in MF could in part be attributable to JAK1 inhibition in lymphocytes or other cells within the spleen. This 'clean' *versus* 'dirty' drug hypothesis becomes more complicated to test or apply when one takes into account the general dearth of information available for most proprietary compounds. It is the opinion of these authors that

rationally designed small molecule inhibitors should offer the greatest degree of specificity and that when possible clinical studies should include pharmacodynamic studies to measure target inhibition. This strategy would offer the most potent therapeutic intervention while minimizing off-target effects; multitarget inhibition could be achieved through combinations of small molecule inhibitors or with biologics such as interferon.

Interferon-α: how does it work?

Thus far, interferon appears to be a unique agent that can, over time, significantly decrease JAK^{V617F} clonal burden and induce complete molecular responses with prolonged drug exposure [Kiladjian *et al.* 2008]. A great deal is known about the pleiotropic actions of interferons generally, but their effects on the particular pathway(s) of interest in MPN are not well understood [Kiladjian *et al.* 2011]. Interferons act directly on hematopoietic stem and progenitor cells, and are well known clinically to induce cytopenias. Interferon-α has recently been shown to induce murine stem cells to enter into the cell cycle, and IFN receptors may mediate self-renewal and stem-cell exhaustion/senescence [Essers *et al.* 2009; Sato *et al.* 2009]. Interferon also modulates the humoral immune response and acts upon T cells, NK cells, macrophages and dendritic cells. These bystander/immunomodulatory effects may be a key effector mechanism in MPN responses. The tumor neoantigen MPD6, for example, was found to be upregulated in cells from patients with PV in response to interferon treatment [Xiong *et al.* 2007]. Interferons may also modify the stem-cell niche, and are known to be anti-angiogenic. When these diverse effects are considered *in toto*, interferon may well be the prototypic 'dirty drug', arguably a more appropriate role for a biologic agent than for small molecule inhibitors.

The most promising clinical data for the use of interferon in MPN are in patients with early PV and ET; historically, trials in patients with MF have been more problematic, largely due to tolerability and, more specifically, lowering of blood counts and worsening of constitutional symptoms. That said, the pegylated formulation of IFN-2α (Pegasys™) appears to be a promising improvement both in potency and tolerability, even potentially in the MF population [Ianotto *et al.* 2009]: an important milestone given that interferon may also be the unique conventional therapeutic agent that can reverse and/or retard progression of bone marrow fibrosis [Silver *et al.* 2011]. Nonetheless, overcoming the tolerability hurdles in more advanced forms of MF is ambitious, and probably unrealistic. In PV, a major international randomized study currently underway comparing pegylated interferon with hydroxyurea has the potential to change standard practice; the study might also provide informative data with respect to progression to MF.

Hypomethylating agents and histone deacetylase (HDAC) inhibitors

5-azacitadine, a DNA methyltransferase inhibitor, has demonstrated clinical activity in patients with dysplasia or leukemia evolved from MPN [Thepot *et al.* 2010]. This provides further indirect clinical evidence that the epigenetic disarray proposed to play a major role in these diseases, and conferred in part upon malignant clones by mutations in *TET2*, *EZH2*, *ASLX1*, *PRMT5*, or *JAK2* itself, can be modified for clinical benefit. Likewise histone deacetylase (HDAC) inhibitors have been explored in MPN, with apparent clinical activity. The single largest study ($n = 29$) tested Givinostat, an oral HDAC inhibitor, in an open-label pilot study in Italy [Rambaldi *et al.* 2010]. ET, PV and MF patients were included in the study, designed with a relatively short (24-week) exposure to drug. Freedom from phlebotomy and reduction in both spleen size and pruritis was seen in a subset of patients, with a possible modest reduction in JAK^{V617F} for PV and ET but not MF patients. Approximately half of patients enrolled had MF, but only a minority completed the study, apparently a function of the level of illness of this patient population rather than any specific toxicity of the agent. Taken together, epigenetic modulation appears to be a promising therapeutic avenue; several investigators have proposed combining HDAC inhibitors or DNA methyltransferase inhibitors with JAK2 inhibitors although this has not been extensively modeled preclinically.

HSP90 inhibitors

HSP90, a chaperone protein known to directly bind to and stabilize proteins, including tyrosine kinases generally and JAK2 specifically, has been elegantly studied as an alternative drug target [Marubayashi *et al.* 2010]. PU-H71 is particularly promising, with favorable pharmacokinetic and pharmacodynamic properties. Marubayashi

and colleagues showed that PU-H71 degrades JAK2, interrupting downstream pathways with specificity for *JAK2* mutant cells, without disturbing JAK2 in normal tissues. This mutant specificity has been attributed to PU-H71's prolonged and selective retention in mutant tissues. In mice PU-H71 treatment reduced white blood cell count and hematocrit levels, lowered cellularity in the bone marrow, improved extramedullary hematopoiesis and reduced clonal burden of disease.

Stem-cell transplantation in MPN: when, how, and what are the obstacles?

The principle

Allogeneic hematopoietic stem-cell transplantation (HSCT) remains the only known curative intervention for MPN. In principle, it replaces the diseased hematopoietic stem-cell compartment with a healthy 'organ' and provides the recipient with a new immune system, which has the potential to eradicate otherwise chemoresistant disease, and can protect its new host over the long run against emerging disease via immune surveillance.

Who needs it?

HSCT is also arguably one of the riskiest interventions in modern medicine, so careful patient selection is of paramount importance. Transplantation for PV and ET, overall associated with a normal or near-normal life expectancy, is not indicated. On the other hand, stem-cell transplantation should be an initial consideration for all patients with MF when first evaluated, and is the treatment of choice for high-risk symptomatic younger patients. However, with MF more typically presenting in the sixth or seventh decades, decisions with respect to stem-cell transplantation are rarely straightforward. The scarcity of truly effective conventional therapeutic options introduces even more complexity into clinical decision making in this setting. In addition, initial and unusually high rates of transplant-related mortality in studies using conventional myeloablative approaches in MF patients [Guardiola *et al.* 1999] have led to their exclusion in most prospective studies of alternative or novel conditioning/immunosuppressive regimens or donor sources. It is difficult, therefore, to know whether incremental advances in HSCT apply to patients with MF. And yet, in the right patient, HSCT is

clearly feasible and often therapeutic. What is fascinating and tantalizing in such cases is the capacity for this approach to restore normal trilineage hematopoiesis in a grossly perturbed marrow microenvironment, with rapid and striking reversal of the fibrosis that is the hallmark of this neoplasm (Figure 2).

Table 1 summarizes a series of retrospective studies that have been published within the last 5 years. Several notable findings with respect to outcomes include confirmation that performance status <90% and the presence of circulating blasts adversely influence survival, and that Lille score is not clearly predictive of transplant outcome [Ballen *et al.* 2010]. A small case series from a single center observed excellent outcomes and low morbidity with sirolimus, an mTOR inhibitor, and tacrolimus as graft-*versus*-host disease (GVHD) prophylaxis [Snyder *et al.* 2010]. It bears mentioning that mTOR inhibitors are one of many classes of investigational therapies shown to have activity in MPN [Guglielmelli *et al.* 2011a]. A multicenter study found no significant differences with respect to resolution of fibrosis in reduced intensity compared with myeloablative stem cell transplantation [Gupta *et al.* 2009], a remarkable testimony to the principle of transplantation as immunotherapy.

The largest prospective multicenter study to evaluate transplantation for MF was conducted through the European Group for Blood and Marrow Transplantation (EBMT) using a reduced intensity strategy [Kroger *et al.* 2009b]. Using the combination of fludarabine, busulfan (10 mg/kg) and antithymocyte globulin with a standard prophylactic immunosuppressive regimen, 98% of patients engrafted, with a nonrelapse mortality of 16% at 1 year. In addition, the estimated 5-year overall survival was 67%. Older age (over 55) and a mismatched donor adversely influenced survival. Subsequent *post hoc* analyses showed that *JAK2*[V617F] negative disease also carried adverse prognostic significance [Alchalby *et al.* 2010]. These generally favorable results were mirrored histologically in those patients who had serial bone marrow biopsies following transplantation. These studies showed near or complete resolution of fibrosis in 69% and 93% of patients by day 100 and day 365, respectively (see Figure 2 for a representative example of bone marrow morphology before and following reduced intensity transplantation). Not surprisingly, high-risk disease was more likely to relapse
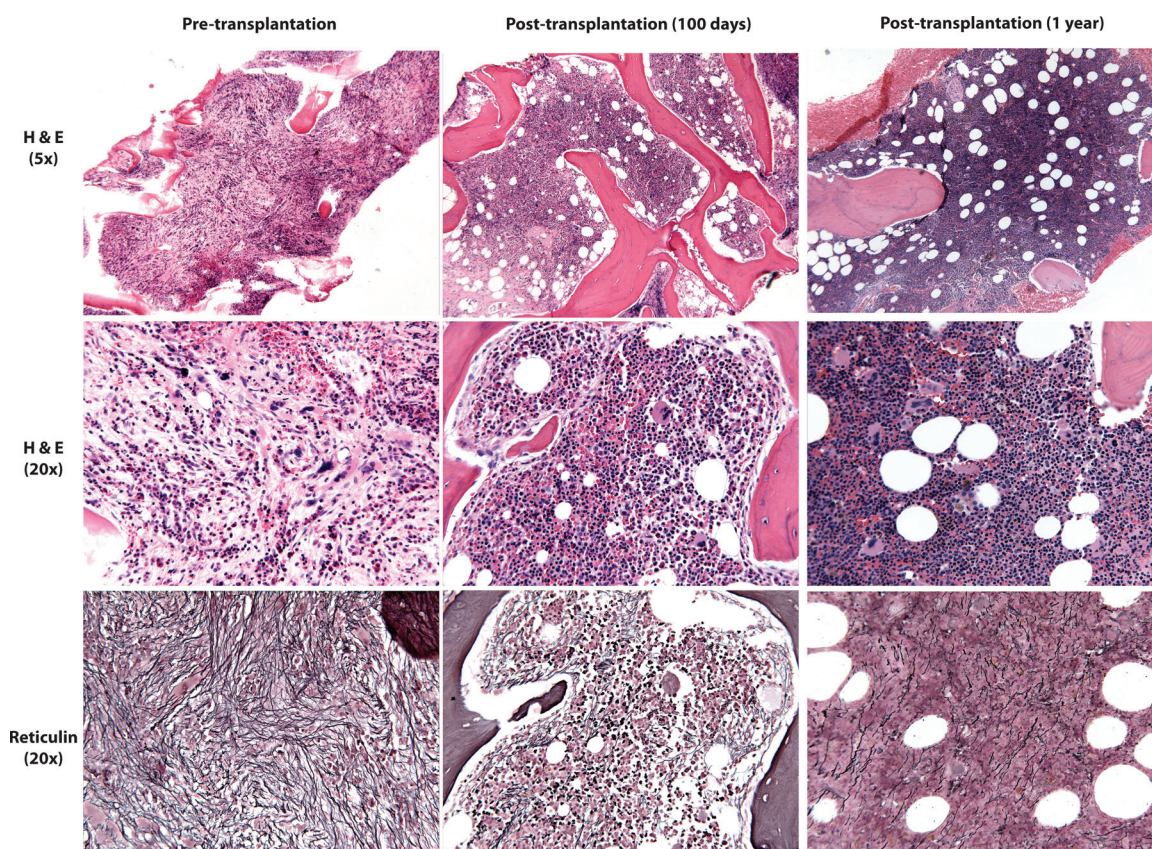
Figure 2. Reversal of fibrosis following reduced intensity hematopoietic stem-cell transplantation (HSCT). A 63-year-old woman with primary myelofibrosis (PMF). *Pre-transplant:* H&E sections (5×) show markedly fibrotic marrow. While myelopoiesis and especially erythropoiesis are decreased, megakaryopoiesis is relatively increased and cytologically atypical (20×), with numerous clustered forms that range from small and hypolobated to large and bizarre. A reticulin stain (20×) highlights extensive diffuse reticulin fibrosis. *Day 100 post-transplant:* H&E sections (5×) show a substantial decrease in fibrosis, with marrow that is hypercellular for age (80%) and demonstrates normal trilineage hematopoiesis. Megakaryocytes are decreased in number and cytologically unremarkable (20×). A reticulin stain shows only mild and variable fibrosis (20×). *One year post-transplant:* H&E sections (5×) show marrow that is hypercellular for age (80%), with normal trilineage hematopoiesis (20×). A reticulin stain shows only mild and variable fibrosis (20×).

post-transplantation. An additional notable finding from the multivariable analysis was that a history of splenectomy was associated with a higher risk of relapse. While a history of splenectomy may simply be a surrogate for higher risk disease, this finding has important practical as well as hypothesis-generating implications. In practice, it puts to rest the concept that massive splenomegaly precludes engraftment, and implies that splenectomy need not be performed prior to transplantation. However, in the speculative realm, as with the results of JAK2 inhibitor studies, this observation raises questions: what, functionally, does the spleen support or provide in this disorder? And what is its function in response to treatment?

How can we use minimal residual disease monitoring?

Subsequent analyses of the EBMT cohort demonstrated that quantitative measurement of JAK2^{V617F} predicted survival, with an undetectable level at 6 months representing an important milestone [Alchalby *et al.* 2010]. Furthermore, minimal residual disease-triggered pre-emptive donor leukocyte infusions can re-induce molecular remissions following HSCT [Kroger *et al.* 2009a]. Taken together, the observed immunotherapeutic effect of HSCT in MF is particularly striking when one considers the accepted dogma, that successful reduced intensity transplantation relies heavily on a minimal disease state prior to transplantation, and the grim reality, that because

Table 1. Retrospective analyses of hematopoietic stem-cell transplantation, 2007 to present.

Study	MA (n)	RIC (n)	OS (%)	NRM (%)	PFS (%)	Engraftment CI/median day	Comments
[Lissandre, 2011]	15	24	60	54	30	15 days (PMN) 19 days (Plt)	Multicenter (France), 1994–2008, inc 2ary AML, 1/29 1st graft failure.
[Robin, 2011]	46	101	39	32	39	90%/18 days	Registry, France, (SFGM-TC) 1997–2008, inc 2nd AML
[Samuelson, 2011]	5	25	45 (22 months)	30 (3 years)	40 (3 years)	90%	30 patients aged 60–78 (median 65) years, 1999–2007
[Ciurea, 2010]	3	9	49 (31 months)	29 (2 years)	ND	100%, 13 days (PMN) 21.5 days (Plts)	Single center (MD Anderson), *all with leukemic transformation;* 1994–2008
[Ballen *et al.* 2010]	229	60	37 (sib, 5 years) 30 (URD, 5 years)	35 (sib, 5 years) 50 (URD, 5 years)	33 (sib, 5 years) 27 (URD, 5 years)	95%, 18 days (sib) 83% (URD)	Registry, 1989–2002 (CIBMTR); performance status <90% and presence of PB blasts predicted worse DFS; Lille score not predictive.
[Snyder *et al.* 2010]	0	23	55.6 (CsA/MMF, 2 years); 92.9 (Tac/Rap, 2 years)	33.3 (CsA/MMF, 100 days) 0 (Tac/Rap, 100 days)	ND	16.5 days (PMN) 18 days (plt)	Single center (City of Hope); 2000–2008;cohort 1: CsA/MMF; cohort 2: Tac/Rap
[Gupta *et al.* 2009]	23	23	48 (MA, 3 years) 68 (RIC, 3 years)	48 (MA, 3 years) 27 (RIC, 3 years)	43 (MA, 3 years) 58 (RIC, 3 years)	18/21 days (PMN) 17–23 days (plt)	Multicenter, 3 Canadian, 4 European centers 1998–2005; compares MA with RIC; no significant differences in time to resolution of fibrosis RIC versus MA
[Patriarca, 2008]	48	52	42	35 (1 years) 43 (3 years)	35	87% CI at 90 days	26 Italian centers (GITMO) between 1986 and 2006
[Kerbauy, 2007]	95	9	61 (5 years)	34 (5 years)	ND	19 days	Single center (Seattle); Patients conditioned with targeted BU/CY improved survival (68%) than other regimens

MA, myeloablative conditioning; RIC, reduced intensity conditioning; OS, overall survival; NRM, nonrelapse mortality; PFS, progression-free survival; CI, cumulative incidence; CsA, cyclosporine A; Tac, tacrolimus; Rap, sirolimus; sib, sibling; URD, unrelated donor; ND, not described.

of a dearth of truly effective therapies, dense disease at time of transplantation is the rule for patients with MF [Sorror *et al.* 2005].

So, who to transplant?

Refinements in prognostic systems can identify those patients at highest risk for dying of disease, and those for whom the risks of transplantation are justified. Age alone is probably a crude indicator of risk, and additional tools, such as the HSCT comorbidity index can help stratify the risk of transplant-related mortality based upon other factors [Sorror *et al.* 2005]. Individually, patients need to be well informed about the process and willing to accept the risks of HSCT. Globally, transplantation centers and cooperative groups could become more actively engaged in facilitating the participation of patients with MF in general transplantation protocols.

Conclusions

As clinicians who follow and treat patients with this group of disorders, we are often bewildered either by their stability or by their frequently unpredictable evolution. We can start to construct stories as we imagine the sequential accumulation of molecular events. Consider the patient with stable PV, whose spleen enlarges, white blood cell count increases and phlebotomy requirements cease, who loses weight and over months to years develops anemia. Does this evolution represent the sequential acquisition of an *Asxl1* mutation [Stein *et al.* 2011] in an epigenetically disrupted stem-cell compartment? Consider the *de novo* PMF patient, who presents seemingly overnight with frank and severe bone marrow failure. Is this the low $JAK2^{V617F}$ allele burden patient with numerous other chromosomal or molecular genetic abnormalities? Consider the patient who appears to evolve overnight to leukemia from PV without an interceding fibrotic stage, and bypassing all of our prognostic scoring systems. Does this represent an outgrowth of a pre-$JAK2^{V617F}$ primitive clone with a new mutation in p53, or dysregulated $JAK2^{V617F}$ itself wreaking nuclear havoc [Beer *et al.* 2010; Dawson *et al.* 2009]? Only carefully constructed translational studies can verify or dispel these fictionalized accounts.

The discovery of $JAK2^{V617F}$ unified and deepened our understanding of classical MPN, and began a cascade of subsequent molecular breakthroughs. Some initially suggested a reclassification of disease on molecular terms [Campbell and Green, 2006]. Ultimately this may be inevitable (and appropriate), but at this point in time the accumulating molecular data can seem to obscure rather than clarify our understanding of clinical phenotypes and prognosis. We increasingly recognize clinical heterogeneity within subgroups, molecularly defined or otherwise. In short, we are gaining a granular understanding of these disorders; the greatest challenge (and opportunity) may be in developing proper tools for integrating these findings in a responsible and 'user friendly' way into clinical investigation and practice.

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The authors declare no conflicts of interest in preparing this article.

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