**Pathogenesis of Myeloproliferative Disorders**

Volume 11 of the *Annual Review of Pathology: Mechanisms of Disease*

Jyoti Nangalia\(^1\), Jacob Grinfeld\(^1\), Anthony R Green\(^1\)

\(^1\)Department of Haematology, Cambridge Institute for Medical Research and Wellcome Trust/MRC Stem Cell Institute, University of Cambridge, Cambridge, UK
\(^2\)Department of Haematology, Addenbrooke’s Hospital, Cambridge, UK

Address for correspondence: Professor A. R. Green
Cambridge Institute for Medical Research,
Hills Road, Cambridge, CB2 0XY, UK
E-mail arg1000@cam.ac.uk
Telephone (+44) 1223 336820
Fax (+44) 1223 762670

**Key words (3-5):** Myeloproliferative neoplasms; JAK2; CALR; MPL; epigenetic

**Abstract (150 words)**

Myeloproliferative neoplasms (MPN) are a set of chronic hematopoietic neoplasms with overlapping clinical and molecular features. Recent years have witnessed considerable advances in our understanding of their pathogenetic basis. In addition, due to their protracted clinical course, the evolution to advanced hematological malignancies, and the accessibility of neoplastic tissue, the study of MPNs has provided a window into the earliest stages of tumorigenesis. With the discovery of mutations in *CALR*, the majority of MPN patients now bear an identifiable marker of clonal disease. However, the mechanism by which mutated *CALR* perturbs megakaryopoiesis is currently unresolved. We are beginning to understand better the role of *JAK2*\(^{V617F}\) homozygosity, the function of co-mutations in epigenetic regulators and spliceosome components, and how these mutations cooperate with *JAK2*\(^{V617F}\) to modulate MPN phenotype.
1.1 Introduction

Myeloproliferative neoplasms are chronic hematological disorders with an incidence of 0.5-2 per 100,000 per year, and are characterised by the overproduction of one or more mature myeloid blood cell lineages. Clinical features include splenomegaly, thrombosis or hemorrhage, and around five percent of patients suffer progression to more advanced disease, which can include transformation to acute myeloid leukemia (AML). Descriptions of patients with these disorders have been found dating back to the nineteenth century, and in 1951, William Dameshek coined the term 'myeloproliferative disorders' to group together a number of hematological conditions, including chronic myelogenous leukemia (CML), essential thrombocythemia (ET), polycythemia vera (PV) and myelofibrosis (MF), due to their closely overlapping clinical and laboratory features.

The current World Health Organization (WHO) classification of myeloproliferative neoplasms separates CML, defined by the presence of the Philadelphia (Ph) chromosome (t(9;22)), from the three main Ph-negative myeloproliferative neoplasms (MPN) which are the focus of this chapter – polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF). PV is characterised by erythrocytosis and bone marrow panmyelosis, and is occasionally accompanied by neutrophilia and/or thrombocytosis. Patients with ET display a more isolated thrombocytosis. MF is a more advanced form of MPN associated with bone marrow collagen deposition, often in the context of bone marrow myeloid proliferation, and patients may have peripheral blood cytopenias with leucoerythroblastosis, splenomegaly and constitutional symptoms. In a minority of patients with ET, features of PV can develop over time. In addition, both ET and PV patients can transform to more advanced disease such as MF. AML, a serious complication of these conditions that carries a poor prognosis, occurs rarely and can affect all MPN subtypes (Figure 1).
Figure 1. The Philadelphia-negative myeloproliferative neoplasms (MPN). ET (essential thrombocythemia) and PV (polycythemia vera) are chronic phase MPNs characterised by thrombocytosis and increased erythrocytosis respectively. MF (myelofibrosis) is a more advanced phase of MPNs which can also evolve from ET or PV. MPNs can occasionally transform to AML (acute myeloid leukemia).

In latter parts of the twentieth century, the identification of the Ph chromosome in patients with CML revealed that acquired chromosomal aberrations can underlie cancer \(^4,5\). Subsequent X-chromosome inactivation studies in females with MPNs revealed that these disorders were also clonally derived neoplastic proliferations, but their genetic basis was unclear \(^6\)–\(^10\). It was not until the early twenty-first century, that we began to understand the genetic aberrations in MPNs, and, with the advent of next generation sequencing technologies, MPNs are now one of the best characterised of hematological malignancies. This review discusses the genetic basis of MPNs, our current understanding of the molecular and cellular pathogenic processes involved, and how our knowledge of MPN biology has been harnessed to improve the management of patients.

1.2 Mutations in JAK2 in the majority of MPNs

Dameshak’s recognition of the interrelatedness of the different subtypes of ‘myeloproliferative disorders’ was highly prescient, as in 2005, landmark findings showed that a mutation in the gene \(JAK2\) “janus kinase 2” (chromosome (chr) 9p24) was found in the majority of MPN patients \(^11\)–\(^14\). The somatically acquired point mutation G>T that results in a valine to phenylalanine at the 617 position (V617F) in exon 14 of \(JAK2\) \((JAK2^{V617F})\), together with rare insertions and deletions in exon 12 that were discovered
subsequently\textsuperscript{15}, are found in the vast majority of patients with PV. In addition, JAK2\textsuperscript{V617F} is also found in over half of patients with ET or MF. As a result of this, JAK2 mutation testing has now become firmly embedded as a front line investigation in clinical diagnostic algorithms for patients with a suspected MPN\textsuperscript{3,16,17}. In addition, in a short space of time, the discoveries have led to the development of inhibitors of JAK2. Ruxolitinib, the first food and drug administration (FDA)-approved JAK1/JAK2 inhibitor, has proven to be a valuable addition to the armamentarium of treatments for MF\textsuperscript{18,19}, and additional JAK2 inhibitors are currently undergoing evaluation in clinical trials.

1.2.1 JAK2 in normal hematopoiesis

JAK2 is a member of the Janus Kinase family of cytoplasmic tyrosine kinases (comprising JAK1, JAK2, JAK3 and TYK2), that associate with the intracellular surface of cytokine receptors to mediate downstream signalling in response to ligand binding. JAK2 activation is important for responses to a wide variety of cytokines, such as, erythropoietin (Epo), thrombopoietin (Tpo), granulocyte colony stimulating factor (G-CSF), interleukin-3 and -5 (IL-3 and IL-5), as well as interferons (IFN)\textsuperscript{20}. All of these receptors lack catalytic activity, and therefore, require JAK proteins, with which they interact, to allow signal transduction. Following ligand binding, receptor reconformation or dimerization leads to the trans-phosphorylation of receptor bound cognate JAK2 molecules, which results in JAK2 kinase activation. This rapidly leads to cellular signal transduction through binding, phosphorylation, activation and nuclear translocation of downstream STAT transcription factors, as well as activation of MAP kinase and PI3K/Akt signalling pathways, to result in cell proliferation and differentiation. In myeloid cells, the JAK2-STAT5 signalling pathway is a critical downstream effector of Epo signalling\textsuperscript{21}, and mice deficient in the Epo receptor (EpoR)\textsuperscript{22}, JAK2\textsuperscript{20} or STAT5\textsuperscript{23} die in utero due to ineffective erythropoiesis. Tpo signalling via its receptor MPL also utilises JAK2. However, in addition to STAT5, activation of the MAP kinase pathway and STAT3 have been shown to be important for megakaryocytic differentiation\textsuperscript{24,25}. Granulocytic differentiation, via G-CSF and its receptor G-CSFR, occurs predominantly through JAK1, and to a lesser degree via JAK2\textsuperscript{26}. Thus, in hematopoiesis, JAK2 is a critical mediator for effective erythropoiesis, megakaryopoiesis, and to a lesser extent, granulopoiesis.

1.2.2 Molecular consequences of JAK2 mutations
The vast majority of JAK2 mutations, and particularly V617F, are located in or around the JH2 domain of the protein, which is also known as the 'pseudokinase' domain because it lacks conserved motifs typically required for kinase activity (Figure 1). The JH2 domain is a critical negative regulator of the upstream JH1 'kinase' domain of the protein. Loss-of-function mutations in, or deletions of, JH2 domains of various JAK proteins (including deletion of JH2 in JAK2) result in increased kinase activity, activation of downstream effectors, and abrogation of a cytokine mediated response. Recently, it has been proposed that JH2 and kinase domains interact intermolecularly to maintain JAK2 in an inactive state. Receptor ligand binding induces separation of this interaction, with subsequent pairing of intermolecular JAK2 kinase domains which results in JAK2 activation. Crystal structure studies of the JH2-JH1 domain of TYK2, as well as molecular modelling studies of JAK2, have also shown that JH2 interacts with JH1 to stabilise it in an inactive state. As a result of these studies, the current model is that the JH2 domain normally functions to inhibit basal JH1 kinase activation in the absence of cytokines, and is required for JAK2 activation in response to cytokines.

Figure 2. Model depicting the protein domains of JAK2. The FERM (4.1 protein, ezrin, radixin, moesin) domain is involved in receptor interaction. The SH2 (Src Homology 2) domain allows interaction with other proteins to aid tyrosine kinase signal transduction. JH2 (JAK homology 2) is the pseudo-kinase domain that negatively regulates the adjacent JH1 kinase domain. V617F is the commonest JAK2 mutation and locates to the JH2 domain. JAK2\textsuperscript{V617F} is found in the vast majority of patients with PV, and over half of patients with ET or MF. Exon 12 mutations are found in many patients with JAK2\textsuperscript{V617F} negative PV but not in patients with ET. The mutations consist of DNA insertions or deletions and affect the SH2-JH2 linker region of the protein. N, N-terminus; C, C-terminus.

Acquisition of V617F results in constitutive tyrosine phosphorylation and activation of JAK2\textsuperscript{11}. At the structural level, V617F, as well as other mutations such as those in Exon
12, are believed to destabilise the JH2-JH1 auto-inhibitory interaction to result in JAK2 hyper-activation, possibly via conformation changes to the SH2-JH2 linker. The JH2 domain of JAK2 has also been shown to itself bind ATP and phosphorylate sites that negatively regulate JAK2 kinase activity. Disruption of this ATP binding has been shown to abrogate V617F mediated JAK2 kinase activation without affecting wild-type JAK2 function, data which may be of future importance for the development of V617F targeted therapies in MPNs. Overall, it is possible that multiple mechanisms contribute to the constitutive activation of JAK2 as a result of V617F.

Overactivation of many participants of JAK-STAT signalling, such as STAT5, STAT3, MAP kinase, ERK1/2 and Akt have been detected in the context of JAK2V617F. Of these, STAT5 has emerged as the critical downstream effector. Inability to activate STAT5 through transcriptional inhibition by shRNA, or by Stat5 deletion, abrogates cytokine independent proliferation of JAK2V617F expressing Ba/F3 cells (Basel F4 cell line), and erythrocytosis in JAK2V617F expressing transgenic mice. STAT5, in turn, has been reported to be involved in JAK2V617F mediated deregulation of transcription of a wide variety of genes which include MYC, PIM, JUNB, ID1, BCL-xL and cell cycle regulators, resulting in increased myeloid lineage differentiation, inhibition of apoptosis, and proliferation.

In addition to canonical signalling via STAT or alternative effector pathways, it is worth noting that JAK2V617F has also been shown to have non-canonical roles in cell function. For example, JAK2V617F has been shown to phosphorylate, and thus reduce the arginine methyltransferase activity of PRMT5 to result in chromatin remodeling, a mechanism which has also been shown to contribute to increased erythroid proliferation. In addition, JAK2V617F has been shown to directly phosphorylate histone H3 at the Y41 position within the nucleus, which results in transcriptional changes via dislocation of the transcription factor HP1.

1.2.3 Effect of JAK2V617F on HSCs

The fact that MPNs emerge and are propagated over time requires that JAK2V617F is mutated in a cell that harbours long-term self-renewal capacity. Indeed, JAK2V617F is detectable in early hematopoietic stem/progenitor cells (HSC) that have been isolated by flow cytometry using cell surface markers characteristic of such populations (CD34+CD38-CD90+Lin-), as well as in multi-lineage myeloid, and in some studies,
lymphoid cells\textsuperscript{14–46}. In addition, using conditionally expressing \textit{JAK2}\textsuperscript{V617F} knock-in mouse models, MPN disease propagating cells have been shown to be HSCs with long term self-renewal capacity (LT-HSC)\textsuperscript{47}.

However despite these data, there are numerous lines of evidence that suggest that \textit{JAK2}\textsuperscript{V617F} does not impart a strong clonal advantage to HSCs. In patients, \textit{JAK2}\textsuperscript{V617F} allele burdens remain stable over decades, and often at very low levels, rather than gradually increasing\textsuperscript{48,49}. When assessing the CD34+CD38- (containing HSC/progenitor) cell compartment of \textit{JAK2}\textsuperscript{V617F}-mutated MPN patients with PV and ET, significant clonal expansion has not been demonstrated\textsuperscript{50}. In addition, CD34+ (containing HSC/progenitor) cells from PV patients harbouring \textit{JAK2}\textsuperscript{V617F} display poor engraftment in NOD/SCID/IL2R-gamma-null mice, compared with wildtype cells\textsuperscript{51,52}. Recently, \textit{JAK2}\textsuperscript{V617F} has also been demonstrated in the blood of normal individuals that lack any overt MPN phenotype\textsuperscript{53–55}. Furthermore, \textit{JAK2}\textsuperscript{V617F}-mutated LT-HSCs in some conditional \textit{JAK2}\textsuperscript{V617F} knock-in mouse models demonstrate an impairment of self-renewal activity when tested in competitive re-transplantation experiments\textsuperscript{56,57}, and utilizing the same model, \textit{JAK2}\textsuperscript{V617F} has been shown to confer a proliferative advantage to progenitor cells with loss of self-renewal at the level of HSCs\textsuperscript{58}.

As a results of these data, it has been proposed that additional somatic mutations, acquired either at the level of HSCs or progenitors, confer the self-renewal advantage required to maintain \textit{JAK2}\textsuperscript{V617F}-mutated MPNs. Indeed, \textit{JAK2}\textsuperscript{V617F}-mutated HSC/progenitor cells from in patients with MF, who are known to harbour greater numbers of concomitant somatic mutations, do show clonal expansion as well as displaying better engraftment in NOD/SCID/IL2R-gamma-null mice\textsuperscript{51,59}. This ability of other mutations to enhance the clonal advantage of \textit{JAK2}\textsuperscript{V617F}-- HSCs or progenitors has been demonstrated in studies investigating \textit{TET2-JAK2} co-mutations, using either stem/progenitor cells from patients\textsuperscript{60,61}, or in transgenic mouse models\textsuperscript{62,63}.

It is worth noting, however, that in a significant number of \textit{JAK2}\textsuperscript{V617F}-mutated patients, additional somatic mutations have not been demonstrated\textsuperscript{64}. This suggests that other factors may modulate stem cell function in the context of \textit{JAK2}\textsuperscript{V617F}, to result in the development of an MPN.

\textbf{1.2.4 Factors influencing phenotype in \textit{JAK2}\textsuperscript{V617F}-mutated MPNs}
\( JAK2^{V617F} \) is associated with a spectrum of hematological phenotypes, ranging from an infrequent finding in normal individuals with no blood count abnormalities, to any one of ET, PV, or MF. It is also rarely seen in patients with chronic myelomonocytic leukemia (CMML) and refractory anemia with ringed sideroblasts and thrombocytosis (RARS-T)\(^65\). A number of genetic and physiological factors have been shown to modulate the clinical phenotype mediated by \( JAK2^{V617F} \).

### A. Influence of homozygosity for \( JAK2^{V617F} \)

\( JAK2^{V617F} \) is commonly found as a homozygous mutation in MPNs, and occurs following mitotic recombination and uniparental disomy at the \( JAK2 \) locus on chromosome 9p24\(^66\). Previously, clonal analysis of hematopoietic colonies from MPN patients had established that homozygosity for \( JAK2^{V617F} \) was found in PV patients but not those with ET\(^67\), and this data was in keeping with the observation that PV patients harboured a higher mutant allele fraction of \( JAK2^{V617F} \) in peripheral blood, compared with ET patients\(^14\). More recently, it has come to light that homozygosity for \( JAK2^{V617F} \) occurs in both ET and PV patients\(^68\). In fact, patients have been found to each harbour multiple acquisitions of homozygosity, with each homozygous \( JAK2^{V617F} \) subclone distinguishable, using microsatellite screening, by its distinct chromosomal breakpoint and length of 9p24 loss-of-heterozygosity (LOH)\(^68\). Interestingly, in PV patients, dominance of one of these homozygous clones is seen, whereas in ET patients, these homozygous subclones remain minor in comparison to the dominance of the heterozygous \( JAK2^{V617F} \) clone\(^68\).

The proportion of homozygosity for \( JAK2^{V617F} \) also correlates with more extreme features of PV, such as a higher hemoglobin and white count, and more marked splenomegaly\(^69\)–\(^71\). These observations hold true both in studies where levels of homozygous \( JAK2^{V617F} \) versus heterozygous \( JAK2^{V617F} \) are determined by analysis of individual hematopoietic colonies, as well as in studies that have measured the overall mutant allele fraction of \( JAK2^{V617F} \) by quantitative PCR, to infer the presence and proportion of homozygosity. Using the latter methodology, it is worth noting that whilst greater than 50% mutant allele fractions imply the presence of cells harbouring homozygous \( JAK2^{V617F} \), allele burdens of less than 50% allele burdens may be representative of heterozygous cells, homozygous cells or a combination of both. Regardless of this ambiguity, these studies have established the notion that homozygosity for \( JAK2^{V617F} \) drives erythropoiesis, whereas heterozygosity for \( JAK2^{V617F} \) drives thrombopoiesis.
The distinct effects of differential ‘doses’ of \( \text{JAK2}^{V617F} \) are supported by transgenic mouse models which have shown that increased expression of \( \text{JAK2}^{V617F} \) results in a phenotype shift from ET to PV\(^{56,72}\). Furthermore, data from induced pluripotent stem (iPS) cells developed from MPN patients harbouring either heterozygous or homozygous \( \text{JAK2}^{V617F} \) cells show differing responses to Epo, with only heterozygous \( \text{JAK2}^{V617F} \) iPS cells capable of producing Tpo independent megakaryocyte colonies\(^{73}\). From murine models, homozygous \( \text{JAK2}^{V617F} \) has been shown to result in stronger activation of downstream STATs, ErK1/2 and Akt\(^{56,74}\). However, the exact downstream consequences of heterozygous versus homozygous \( \text{JAK2}^{V617F} \) that result in its distinct effects on differentiation remain unknown.

**B. Differential signalling downstream of \( \text{JAK2}^{V617F} \)**

Studies of patient bone marrow trephines and CD34+ cells have reported differential activation of JAK-STAT signaling components that correlate with MPN subtype. Results of these studies are variable but overall show increased STAT5 and STAT3 activation in PV and MF, compared with ET\(^{50,75}\). This suggests that there may be qualitative differences in \( \text{JAK2}^{V617F} \) mediated signaling across disease phenotypes. However, due to the bulk analysis of samples, that comprise both tumor and normal tissue, one cannot exclude the possibility that these differences may, at least in part, reflect the differential consequences of the varying proportions of tumor burden, or the different genotypes for \( \text{JAK2}^{V617F} \), present across the MPN subtypes. To circumvent some of these confounding issues, a gene expression study comparing wild-type to heterozygous \( \text{JAK2}^{V617F} \) hematopoietic colonies from PV and ET patients (thereby controlling for interpatient differences) has shown that whilst STAT5 activation is common to both patient groups, ET patients also demonstrate activation of STAT1\(^{76}\). In keeping with this, attenuation of STAT1 activity in a transgenic \( \text{JAK2}^{V617F} \) mouse model of PV has also demonstrated a skew towards erythropoiesis\(^{77}\). Given that some PV patients do not harbour \( \text{JAK2}^{V617F} \) homozygosity\(^{48,68}\), it is possible that such qualitative differences in heterozygous \( \text{JAK2}^{V617F} \) mediated signalling may also play a part in determining MPN phenotype.

**C. Additional patient factors**

The observation that PV is more common in males whereas ET is more frequent in females suggests that gender specific factors, such as androgen and estrogen levels,
and/or iron status in premenopausal women, may also influence JAK2V617F-mediated MPN phenotype. Higher mutant allele burdens for JAK2V617F have been reported in males compared with females, in keeping with a more prevalent PV phenotype in men. One interpretation of this data is that males may be more likely to develop homozygosity for JAK2V617F. However, recent data has shown that homozygosity occurs equally common in both genders, and that while homozygosity was associated with males in PV, it was also associated with ET in females. This raises the possibility that gender specific factors may differentially affect the degree of expansion of homozygous JAK2V617F subclones to influence MPN phenotype. For example, it is possible that following the acquisition of homozygous JAK2V617F, subclonal expansion could be promoted by male androgens to result in PV in males, whereas constrained by premenopausal iron deficiency in females to result in ET. Similarly, other constraints on erythropoiesis, for example, germline thalassemia traits, chronic renal insufficiency, or low endogenous Epo levels, may also contribute to the development of an ET phenotype, rather than PV. Of interest, studies of germline polymorphisms in ET and PV patients have also identified potential sites within JAK2 and EpoR, as well as other SNPs such as rs9376092 (HBS1L/MYB) that are associated with specific MPN subtypes.

About 50% of the population attributable risk of developing an MPN can be accounted for by the presence of a JAK2 constitutional 46/1 or ‘GGCC’ haplotype. Carriers of this haplotype, either in a heterozygous or homozygous state, show an increased incidence of MPNs. However, it is unclear, whether the risk allele leads to a more frequent acquisition of JAK2V617F (‘hypermutability hypothesis’) or that following acquisition of JAK2V617F, patients with the allele are more likely to develop an MPN (‘fertile ground hypothesis’).

In addition to the JAK2 46/1 haplotype, another germline sequence variant (rs2736100_C) in the gene TERT has also been reported in the Icelandic population to be associated with an increased risk of MPN, as well as higher blood count indices in the absence of an overt MPN diagnosis. Neither of these germline changes have been shown to be associated with a specific MPN subtype. However, recent studies that have demonstrated the presence of JAK2V617F within normal individuals (haplotype status unknown) who lack any clinical features of MPN, raise the possibility that the presence or absence of such germline factors may influence whether any downstream pathological consequences follow acquisition of JAK2V617F.

D. Role of other mutations
Targeted and whole-exome sequencing have identified additional mutations in some MPN patients and these may modulate JAK2V617F phenotype, as will be discussed later in this review. For example, mutations in ASXL1 are prevalent in MF, and TP53 loss as well as IDH1/2 mutations are common at the time of leukemic transformation. Mutations in SRSF2, IDH1/2, EZH2 and U2AF1 have all been shown to be more prevalent in MF patients, in whom their presence carries a poor prognosis in terms of overall survival and leukemic progression. There is also evidence that coexisting mutations may modulate MPN phenotype during chronic phase disease. For example, in patients with PV, heterozygous truncating mutations in NF-E2 have been shown to enhance wildtype NF-E2 function and promote erythrocytosis.

In summary, a number of factors have been identified to be important in determining the phenotype, if any, that results following acquisition of a JAK2 mutation (Figure 3).

Figure 3. A model depicting the factors that are known to influence the development, and the resulting phenotype, of JAK2V617F-mutated MPNs.
1.3 Other mutations affecting JAK-STAT signalling in a minority of MPNs

Up to 50-60% of ET and MF patients do not harbour mutations in JAK2. Somatically acquired mutations in the gene MPL (Myeloproliferative leukemia virus oncogene; 1p34), have been shown to be present in roughly four percent of ET patients and up to ten percent of patients with MF\textsuperscript{89,90}. MPL encodes for the receptor of Tpo, the main megakaryopoiesis stimulating cytokine, thus explaining the lack of these mutations in PV. MPL mutations commonly affect the W515 position in exon 10, but other mutations are also rarely seen, such as S505N, which is also found as a germline change in some patients with familial thrombocytemia\textsuperscript{91,92}. In the vast majority of patients, JAK2 and MPL mutations are found in a mutually exclusive manner, which suggests that MPL mutations coopt similar molecular processes to those resulting from JAK2\textsuperscript{V617F} in causing an MPN. The juxtamembrane tryptophan residue W515 in MPL is required to maintain an inactive receptor state in the absence of Tpo binding\textsuperscript{93}. W515 mutations result in loss of this function with resultant constitutive activation of downstream signaling pathways, in a manner reminiscent of the downstream consequences of V617F in JAK2. But whilst different murine models of JAK2\textsuperscript{V617F} exhibit disease phenotypes ranging from ET to PV and MF\textsuperscript{47}, murine models of MPL mutations develop a disease marked by thrombocytosis with features of ET and MF\textsuperscript{94}. These differences in disease phenotype in animal models mimic the clinical spectrum of MPNs with which these different mutations are associated, and are probably reflective of the differing expression patterns, and cytokine selectivity of JAK2 and MPL in hematopoietic progenitors.

There are additional targets within the JAK-STAT pathway that can also be infrequently affected by somatic mutations in MPNs. SH2B3 (LNK), an inhibitor of EPO and TPO signalling, harbours loss of function mutations in 2-6% of all MPN subtypes and is associated with increased JAK-STAT signalling\textsuperscript{95,96}. In addition, CBL mutations, which are found in 5-10% of MF patients, as well as secondary AML or other myeloid malignancies, abrogate the ubiquitin ligase activity of wildtype CBL, resulting in reduced degradation of tyrosine kinases and prolonged activation of intracellular signalling\textsuperscript{84,97,98}. These mutations are not specific to JAK2 or MPL-unmutated MPN patients, and there is data to support that their presence alters clinical phenotype\textsuperscript{99}.

1.4 Mutations in CALR in MPNs

Following the discoveries of mutations in JAK2 and MPL, around forty percent of patients
with ET and MF still lacked a clonal marker. Diagnosing these patients in the clinical setting would invariably require bone marrow biopsies and multiple investigations. Researchers hypothesized that other candidates within the JAK-STAT signaling pathway may be involved but no such abnormalities were identified. With the advent of next generation sequencing technologies, it soon became possible to perform whole exome or whole genome sequencing of patient samples, as well as of single tumour cells. Single cell whole exome sequencing of a JAK2-unmutated MPN revealed that these patients did harbour a clonal proliferation as somatic mutations were identified, however, the mutations were not found to be recurrent amongst other patients\textsuperscript{100}. In late 2013, two whole-exome sequencing studies of MPN patients, identified recurrent somatic mutations in the gene \textit{CALR}, which encodes the gene calreticulin\textsuperscript{64,101}. These mutations were found in 60-90\% of patients with \textit{JAK2} and \textit{MPL}-unmutated ET or MF, and not in patients with PV. This discovery now meant that, with the exception of 10-15\% of ET or MF patients negative for mutations in \textit{JAK2}, \textit{MPL} or \textit{CALR}, most MPN patients had a diagnostic marker for their disease (Figure 4).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{mpn_subtypes.png}
\caption{MPN subtypes and the frequency of mutations in \textit{JAK2}, \textit{CALR} or \textit{MPL}.}
\end{figure}

\textit{CALR} mutations have also been infrequently found in myelodysplasia (MDS), refractory anemia with ringed sideroblasts and thrombocytosis (RARS-T) and, very rarely, in cases of atypical chronic myeloid leukemia or chronic myelomonocytic leukemia (CMML). Thus far, \textit{CALR} mutations have not been demonstrated in lymphoid malignancies, solid tumours or healthy controls\textsuperscript{64,101}. Very rare cases of polycythemia vera have also been found to harbour \textit{CALR} mutations, however, the possibility that these patients harboured an alternative reason for manifesting an erythrocytosis were not excluded\textsuperscript{102}. Overall, \textit{CALR} mutations demonstrate a striking specificity for \textit{JAK2}-unmutated ET and MF that is highly reminiscent of the disease spectrum associated with mutations in \textit{MPL}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{calr_mutation.png}
\caption{CALR mutations in MPNs affect the terminal exon of the gene. All mutations are}
\end{figure}
insertions or deletions, and 85% of patients harbour one of two common mutations: a 52-bp deletion known as Type 1 (c.1092_1143del; L367fs*46; 44-53% of cases) or a 5-bp insertion known as Type 2 (c.1154_1155insTTGTC; K385fs*47; 32-42% of cases) (Figure 4). The remaining 15% of patients harbour alternative insertions, deletions or a combination of both\textsuperscript{103}. Intriguingly, regardless of the exact mutation in CALR, the effect of the mutation is to shift the reading frame of the mRNA by 1 base pair, which leads to the coding of a novel amino-acid peptide sequence distal to the site of the mutation, and the generation of a mutant CALR protein with a novel C-terminus. This is in contrast to other genes affected by insertions or deletion mutations where there is often premature truncation of the protein sequence, with resultant loss-of-function. The fact that all CALR mutations lead to the generation of one particular terminal amino-acid sequence strongly suggests that these mutations are gain-of-function, much like mutations in JAK2 or MPL (Figure 5).

Figure 5. CALR mutations. The intron-exon structure is shown along the top of the picture. The terminal exon 9 is affected by two common mutations: a 52 base pair deletion (Type 1 or L367fs*46) or a 5 base pair insertion (Type 2 or K387fs*47). Both mutations result in a shift in the reading frame of the mRNA to result in a new amino acid sequence (blue amino acids) and a common novel peptide sequence (blue shading in bottom panel).

Unlike JAK2 or MPL that are proteins involved in cytokine receptor signaling in hematopoiesis, calreticulin is an endoplasmic reticulum (ER) chaperone protein. CALR ensures the proper folding of newly synthesized glycoproteins within the ER and has a C-
terminal –KDEL amino acid motif, that ensures its retention within the ER. However, CALR has also been implicated in a number of other roles both within and outside of the ER, including calcium homeostasis, immunogenic cell death, proliferation and apoptosis\textsuperscript{104,105}. How these functions of CALR may relate to the pro-megakaryocytic proliferation seen in the context of the mutated protein is unknown. Mutant calreticulin no longer has a –KDEL motif, but studies that have looked at any potential mislocalization of the mutant protein have not yielded consistent results\textsuperscript{64,101}. In addition, mutant calreticulin no longer harbours the low-affinity high-capacity calcium binding potential of the wildtype function\textsuperscript{106}, and it is possible that this alteration disrupts ER function in a way that perturbs megakaryopoiesis (Figure 6).

Figure 6. Model depicting the domains of CALR and the predicted effect of CALR mutations. Mutant CALR results in the generation of a novel C-terminus that lacks KDEL as well as calcium binding sites. N, N-terminus; N domain, Lectin binding domain; P domain, proline rich domain; C domain, calcium binding C-terminal domain; KDEL, endoplasmic reticulum retention signal. Possible mechanisms by which the generation of mutated CALR may disrupt megakaryopoiesis are listed below the protein.

Preliminary evidence using gene-expression arrays\textsuperscript{107} and transfected cell lines\textsuperscript{101}
suggested that mutant calreticulin may also lead to overactivation of JAK2-STAT5, however, a study of a CALR-mutated cell line has found no activation of this pathway, or sensitivity to JAK-inhibitor treatment108. The downstream consequences of mutant CALR are currently under investigation and some possible mechanisms include the alteration of the structure, trafficking or activation status of the TPO receptor MPL, or alternatively, activation of megakaryopoiesis through altered calcium signalling. Interestingly, mutant CALR expression has been shown to be restricted to megakaryocytes in patient bone marrow trephines109, data which helps explain why these mutations are predominantly associated with MPN subtypes such as ET or MF that are typified by abnormal megakaryopoiesis109.

1.5 Clonal complexity

MPNs had always been viewed as neoplasms with a relatively simple genomic landscape. This was in part because classical cytogenetic analyses had always shown that chromosomal aberrations were infrequent in MPNs compared with other hematological malignancies, with only occasional patients harbouring regions of chromosomal deletions, such as, del20q, del13q, trisomy 8 and del9p110. The first suggestion of clonal complexity in MPNs came with findings that JAK2-mutated MPNs often transformed to JAK2-unmutated AML111. This supported the existence of other mutant subclones in MPN, that were either independent of JAK2 mutations or had occurred prior to acquisition of JAK2V617F. The presence of multiple subclones was also supported by the finding that the percentage of granulocytes carrying JAK2V617F in some patients with ET and PV was smaller than the percentage of clonal granulocytes as determined by X-chromosome inactivation patterns112. Exome sequencing studies have since shown that MPN patients actually harbour multiple somatically acquired mutations: PV and ET patients harbour on average 6-7 mutations per patient, and MF patients have a greater number of mutations in keeping with it being a more advanced phase of disease64. In addition to mutations in JAK2, MPL and CALR, that are highly specific for MPNs, a number of genes, often mutated in myeloid malignancies in general, are also mutated in MPNs. These other mutations affect genes involved in DNA methylation, such as TET2, DNMT3A, IDH1/260,113–115, chromatin remodeling such as EZH2, ASXL1116,117, or mRNA splicing, such as, SF3B1, U2AF1, SRSF2. Table 1 lists the frequencies of such mutations across the different MPN subtypes, alongside mutations in the genes previously discussed.

Table 1. Frequency of somatic mutations in MPNs
1.5.1 Mutations in $\text{DNMT3A}$ and $\text{TET2}$

Methylation of cytosines in CpG dinucleotides, is an important epigenetic regulatory mechanism controlling the expression of genes. $\text{DNMT3A}$ (DNA methyltransferase 3A) carries out de novo methylation, and $\text{TET2}$ (Ten-eleven translocation methylcytosine dioxygenase 2) demethylates DNA by converting 5-methylcytosine to 5-hydroxymethylcytosine (5-hmc).

Mutations in $\text{DNMT3A}$ were first discovered in AML, and are also known to be mutated in around ten percent in MPNs$^{84,118}$. The commonest mutation occurs at position R882, although loss of function mutations (for example, frameshift truncation insertions or deletions, or nonsense mutations) also occur. In keeping with the loss of function spectrum of mutations in $\text{DNMT3A}$, R882H has recently been demonstrated to confer a dominant negative effect on wildtype $\text{DNMT3A}$. Serial transplantation studies using transgenic mouse models with knock-out of $\text{DNMT3A}$ has demonstrated that loss of $\text{DNMT3A}$ results in hematopoietic stem cell compartment expansion$^{119}$. In keeping with these data, a study of human AML has shown that $\text{DNMT3A}$ mutations are acquired early in tumourigenesis, and the pre-leukemic HSCs harbouring mutated $\text{DNMT3A}$ display a self-renewal advantage over wildtype HSCs$^{120}$, confirming murine data that acquisition of mutations in $\text{DNMT3A}$ result in a stem cell advantage. In MPNs, $\text{DNMT3A}$ mutations can occur prior to, or following, the acquisition of mutations in $\text{JAK2}$$^{121}$ but clinically, studies
to date have not demonstrated any associations between \textit{DNMT3A} mutations and alterations to MPN phenotype or outcome, and analysis of larger patient cohorts is required. It is likely that \textit{DNMT3A} mutations function to confer a clonal advantage to MPN mutant clones, particularly, given data suggesting that \textit{JAK2}^{V617F} results in a self-renewal impairment at the level of HSCs. However, mutations in \textit{DNMT3A} may also confer a myeloproliferative phenotype, as suggested by murine models of R882H that display myeloid proliferation with thrombocytosis\textsuperscript{122,123}.

Loss of function \textit{TET2} mutations are found in 5-17\% of MPN patients\textsuperscript{60,124}. In terms of function, murine models with attenuated \textit{TET2} display HSC compartment expansion in a manner similar to that seen with mutations in \textit{DNMT3A}. However, loss of \textit{TET2} results in obvious myelo-monocytic proliferation and phenotypic features resembling chronic myelomonocytic leukemia\textsuperscript{125}. In fact, patients with myelodysplastic/myeloproliferative (MDS/MPN) disorders often harbour biallelic loss of \textit{TET2} either through acquisition of compound \textit{TET2} mutations, or loss of heterozygosity at chr4q24\textsuperscript{60,126}. Patients with mutated \textit{TET2} have been shown to display reduced levels of 5-hmc\textsuperscript{84,127}. Clinically, one study has shown \textit{TET2} mutations to be associated with an increased risk of leukemic transformation and shorter survival. However, other studies have not found such clinical associations\textsuperscript{121,128}. Interestingly, both \textit{TET2} and \textit{DNMT3A} mutations have been found in normal elderly females who display clonal hematopoesis in the absence of any blood count parameter abnormalities\textsuperscript{129}. This finding, in conjunction with data from murine models, suggests that \textit{TET2} mutations may confer a clonal advantage at the level of the HSC rather than driving the overproduction of erythroid and/or megakaryocyte cells that is characteristic of MPNs. Data from murine mouse models with \textit{JAK2}^{V617F} as well as loss of \textit{TET2} shows that TET2 may well function to confer a stem cell clonal advantage to \textit{JAK2}-mutated cells and help propagate and accelerate disease phenotype\textsuperscript{62,130}.

Interestingly, the effects of \textit{TET2} and \textit{DNMT3A} mutations on HSC self renewal may function to alter MPN phenotype over and above that of simply providing a stem cell advantage to MPN clones. In a study of \textit{TET2} and \textit{JAK2}-mutated MPN patients, the order in which these mutations were acquired was found to be associated with patient age at presentation, presence of \textit{JAK2}^{V617F} homozygosity, incidence of thrombotic complications, as well as disease phenotype of ET versus PV at presentation. Therefore, it appears that the ability of a \textit{JAK2}^{V617F} homozygous subclone to expand, may also be related to the nature of competing clones within the bone marrow, as well as the other mutations already present within the cell at the time when \textit{JAK2}^{V617F} homozygosity is acquired.
1.5.2 Mutations affecting histone methylation

In addition to DNA methylation, gene expression is also regulated by histone modifications. The polycomb repressive complex 2 (PRC2) is a transcriptional repressor and acts through the methylation of lysine 27 at histone H3 (H3K27me2/3) which in turn leads to gene silencing and compaction of chromatin. The H3K27 methyltransferase EZH2, which is a member of the enzymatic component of the PRC2 complex, is also mutated at a frequency of 3-13% in MPNs. These mutations are found most frequently in MF, where they represent a poor prognostic marker associated with lower overall survival and increased risk of leukemic transformation\textsuperscript{131}. Deletion of the EZH2 homologue in murine models results in an MDS/MPN phenotype which is exacerbated by concurrent deletion of TET2\textsuperscript{132}. Other PRC2 components are SUZ12, EED and JARID2, but these genes are only rarely found to be mutated in patients with MPNs, and their clinical and pathological significance is currently unknown\textsuperscript{84,133}.

ASXL1 (Addition of Sex Combs Like 1) is a mediator of PRC2 function and also interacts with the nuclear deubiquitinating enzyme BAP1\textsuperscript{134}. Loss of function mutations in ASXL1 are prevalent in MDS, CMML and MF. In MF, ASXL1 mutations are found in up to a quarter of patients and they are associated with a more severe anemia and an inferior survival\textsuperscript{64,84,87,135}. Disruption of the gene in murine models results in a phenotype with features of both MDS and MF: anemia, leucopenia, morphological dysplasia, extramedullary hemopoiesis and splenomegaly\textsuperscript{136}. This model also displayed a block to erythroid differentiation and HSC expansion, features that are reminiscent of many patients with MF\textsuperscript{136}.

Overall, impairment of PRC2 function appears to be a very important pathogenic mechanism in malignant myelopoiesis. Interestingly, whilst the PRC2 complex is often affected by loss of function in myeloid malignancies, the spectrum of mutations affecting this complex is very different to that found in lymphoid malignancies. In the latter, gain of function mutations (eg Y641 of EZH2) have been reported, suggesting that differential PRC2 activity is important in driving myeloid-lymphoid fate decisions\textsuperscript{137}.

Mutations in the genes IDH1 and IDH2 have also been shown to deregulate histone methylation. These proteins are enzymes that catalyze the conversion of isocitrate to alpha-ketoglutarate. However, in the presence of mutations, the enzymes catalyze conversion to 2-hydroxyglutarate\textsuperscript{138}, a protein that has been shown to inhibit histone
demethylation and attenuate hematopoietic differentiation\textsuperscript{139}. In addition, alpha-ketoglutarate has also been shown to inhibit normal TET2 function\textsuperscript{140}. IDH1/2 mutations are present found predominantly in MF, where they confer a poorer prognosis, as well as in 20% of blast phase MPN patients\textsuperscript{131,132}.

1.5.3 Mutations affecting mRNA splicing

In MDS, mutations in genes that deregulate mRNA splicing, for example, SF3B1, SRSF2, U2AF1 and ZRSR2 are common. SF3B1 mutations are frequent in RARS while SRSF2 mutations are most highly recurrent in CMML\textsuperscript{142,143}. Recently, it has been confirmed that these mutations result in altered mRNA splicing\textsuperscript{144–146}, although, generation of murine models for these mutations have thus far proven unsuccessful. In MPNs, mutations in this group of genes affect about 5% of patients, with SF3B1 mutations most frequent in the MDS/MPN overlap syndrome RARS-T\textsuperscript{101}. Some of these mutations carry a poor prognosis for patients with MF. Interestingly, these mutations are highly prevalent in triple-negative MF patients, raising the possibility that this subgroup of patients may actually represent patients with MDS\textsuperscript{87}.

All the mutations discussed thus far are not necessarily present in the same tumor subclone. Analyses of hematopoietic colonies, that can be grown from the peripheral blood of MPN patients, to effectively allow interrogation of these tumours at a single-cell level and reconstruction of tumour ‘histories’, has revealed that patients often harbour multiple tumour subclones that coexist and remain stable over long periods of time\textsuperscript{121}. In addition, mutations in epigenetic regulators have been shown to occur both early and late in tumorigenesis\textsuperscript{121}. While we are beginning to understand the implications of mutation order for \textit{TET2} and \textit{JAK2} mutations in MPNs\textsuperscript{61}, the significance of mutation order for other genes is still unclear.

1.6 Familial MPNs

It has been estimated, that up to 5% of MPN patients have a family history of MPNs, as defined by the presence of at least two cases within a family pedigree\textsuperscript{147}. Interestingly, affected family members also display the same spectrum of somatically acquired mutations in \textit{JAK2}, \textit{MPL} or \textit{CALR}\textsuperscript{148,149}. Why these family members have an increased risk of developing these neoplasms is unclear. In one such pedigree, mutations in \textit{RBBP6} were found and appeared to account for the germline predisposition to acquiring
MPNs\textsuperscript{150}. It is worth noting that these families are not to be confused with those affected by hereditary erythrocytosis or thrombocytosis. In the latter cases, myeloproliferation is non-clonal or non-neoplastic, and is caused by a spectrum of inherited germline mutations in genes known to affect erythropoietin or thrombopoietin signalling\textsuperscript{82,151}.

### 1.7 Clinical impact of our current understanding of the pathogenesis of MPNs

Considerable advances have been made in understanding the molecular and cellular consequences of the various genetic lesions that are found in MPNs. We are beginning to understand how multiple genetic abnormalities may interact. In addition to understanding the phenotypic consequences of genomic abnormalities in MPNs, our knowledge of the molecular aberrations in MPNs has witnessed clinical utility in three areas: diagnosis, prognosis and targeted therapy.

1.7.1 Genotype-phenotype correlations for mutations in JAK2, MPL and CALR

Prior to the discoveries of mutations in MPL or CALR, numerous studies assessed the clinical phenotypes of MPN patients to assess for any disease related features of JAK2\textsuperscript{V617F}. From these studies, it became clear that JAK2\textsuperscript{V617F}-mutated ET patients displayed many of the hallmarks of PV: patients had higher hemoglobins and white counts and reduced platelets compared with JAK2\textsuperscript{V617F}-unmutated ET patients, as well as panmyelosis on bone marrow examination\textsuperscript{152,153}. In addition, they displayed a propensity to transform to PV, particularly in females. This latter finding may, in part, be attributed to reduced iron deficiency, and subsequent reduced constraints on erythropoiesis, in post menopausal females. Prospective analysis of a large ET cohort, taking into account factors known to affect thrombotic risk, such as gender, age, treatment and previous thrombosis, has shown an increased risk of venous thrombosis attributable to JAK2\textsuperscript{V617F} \textsuperscript{152}. Therefore, the current model is that JAK2-mutated PV or ET may be better viewed as one disease continuum\textsuperscript{154}. Factors discussed previously, such as levels of JAK2 homozygosity or allele burden, differential signalling, other mutations as well as host factors (e.g. levels of iron stores, erythropoietin, gender, genetic modifiers) may modulate the MPN phenotype that results. Indeed, long term survival and rates of thrombosis in JAK2\textsuperscript{V617F} PV and ET patients are very similar\textsuperscript{155}, in keeping with the closely related biological features of these clinical entities.
In terms of the clinical features of MPL-mutated MPNs, studies have shown that these patients are, on average, older, have higher platelet counts and lower hemoglobin levels at diagnosis, than JAK2\textsuperscript{V617F}-mutated counterparts\textsuperscript{91,156,157}. In ET, MPL mutations are also associated with higher platelet counts and reduced bone marrow cellularity with reduced erythropoiesis, when compared to JAK2-mutated ET\textsuperscript{91}. However, no differences in clinical outcome have, thus far, been demonstrated for this small subgroup of MPN patients in either ET or MF\textsuperscript{156,158}. Interestingly, homozygosity for MPL mutations is also observed, although, at a far lower frequency than that observed for JAK2\textsuperscript{V617F}, and such patients have been shown to display increased marrow fibrosis with a higher rate of transformation to MF\textsuperscript{159}.

Clinically, CALR-mutated ET is associated with a higher platelet count, lower hemoglobin and lower leucocyte count at presentation, compared with JAK2-mutated counterparts\textsuperscript{154,160}. Patients present at a younger age, which may be due to the earlier investigation prompted by a more severe thrombocytosis on blood count testing. Whilst JAK2\textsuperscript{V617F} patients with ET show a female predominance, CALR-mutated ET affects males and females equally\textsuperscript{154,160}. Interestingly, some studies have shown that CALR-mutated ET have a reduced incidence of thrombosis compared with JAK2V617F-mutated patients, which remains significant after taking into account their younger age\textsuperscript{161}, however, another study has shown no such favorable impact\textsuperscript{162}. It is likely that what is observed reflects the known increased risk of thrombosis imparted by JAK2\textsuperscript{V617F} which comprises the majority of CALR-unmutated patients. Importantly, no differences in survival have thus far been demonstrated for the different mutation subgroups in ET\textsuperscript{155}.

In MF, compared with JAK2\textsuperscript{V617F}-mutated patients, CALR-mutated patients again present at a younger age, with higher platelet counts and lower leucocyte counts, but with reduced anemia and transfusion dependency\textsuperscript{163,164}. These patients also have a better prognosis in terms of overall survival and leukemia free survival compared with JAK2\textsuperscript{V617F}-mutated patients. Furthermore, triple-negative patients (those patients lacking mutations in JAK2, MPL or CALR) have the worst prognosis and a more severe anemia. These findings are likely to be of significant importance in the future risk stratification of patients with MF\textsuperscript{155,154,160}.

1.7.2 Improvements in patient diagnosis

Following the discovery of mutations in JAK2 and MPL, the WHO diagnostic criteria were
revised to incorporate molecular testing as a first line tool of investigation for patients with suspected MPNs. The new diagnostic criteria for PV, for example, has been hugely simplified as a consequence of these revisions. The new WHO revision will also incorporate CALR testing for patients with suspected ET and MF. JAK2, MPL and CALR mutation screening are already firmly embedded in clinical diagnostic algorithms and a typical workflow is depicted in Figure 7.

![Figure 7. Diagnostic clinical algorithm including molecular testing for MPNs](image)

**1.7.3 Prognostic impact of mutational status**

For patients with ET or PV, studies looking comprehensively at molecular determinants of prognosis are still lacking, and risk stratification systems in these MPN subtypes are currently still based on clinical criteria. However, CALR mutations are known to be predictive of reduced thrombosis in ET, compared to those with mutated JAK2, and this may be relevant for future risk stratification, or antithrombotic prophylaxis, of patients.

Within MF, mutations in a number of genes, such as ASXL1, SRSF2, IDH1/2 and EZH2
have also been shown to carry prognostic value in MF, where they are associated with poorer survival and increased leukemic transformation\(^{87}\). The number of such ‘high risk’ mutations has also been shown to inversely correlate with median survival (ranging from a 12 year median survival for MF patients with no such mutations, and 2 year median survival for those with 2 or more mutations)\(^{166}\). MF patients with ‘high risk’ mutations are now being assessed as part of clinical studies to establish whether such patients may benefit from early intervention with alternative therapies. Early data indicates that treatment with JAK2 inhibitors such as Ruxolitinib may alter the natural history of such high risk patients\(^{167}\).

During AML transformation of a preceding MPN, cytogenetic abnormalities are often complex. In addition, biallelic loss of TP53 is a common feature, which occurs either through acquisition of independent mutations on both TP53 alleles, or via monoallelic mutation followed by 17p LOH\(^{86,121}\). Indeed, expression of JAK2\(^{V617F}\) combined with Tp53 loss in a murine model results in a fully penetrant AML phenotype\(^{168}\). Interestingly, in patients with AML transformation that lack mutations in TP53, alternative routes that effectively lead to inhibition of p53 activity have been demonstrated to be coopted by tumour cells, such as amplification of chromosome 1q which contains MDM4, a potent p53 inhibitor\(^{86}\).

Large scale molecular characterisation of prospective MPN cohorts is required to establish these clinical associations further, and the hope is that such information can then be used to guide treatment and inform patients in the future.

1.7.4 Targeted therapy

Based on our knowledge of the genetic and molecular aberrations in MPNs, a number of therapeutic targets have emerged and are being tested in clinical trials. By far the most successful of all these agents have been JAK inhibitors, that emerged rapidly following the discovery of JAK2\(^{V617F}\) mutations in MPNs. Ruxolitinib, the first FDA approved JAK inhibitor is proving to be a very useful adjunct to the treatment armamentarium for patients with MF, and is particularly useful at alleviating MF-related symptoms and splenomegaly. Interestingly, JAK2\(^{V617F}\) status does not predict response to JAK inhibitors, as JAK2-unmutated patients with MF can be successfully treated with such agents. This suggests that JAK inhibitors do not specifically target mutant tumor subclones. In keeping with this notion, reductions in mutant allele fractions of JAK2\(^{V617F}\) following JAK
inhibitor have not been convincingly demonstrated.

1.8 Conclusions and future directions

Despite MPNs representing early pre-leukemic neoplasms, evidence of intra-tumor clonal heterogeneity and complexity are emerging. The vast majority of patients with MPNs have a mutation in \textit{JAK2, MPL} or \textit{CALR}, but patients also often harbour other somatically acquired mutations as well homozygosity for \textit{JAK2}\textsuperscript{V617F}. We are beginning to understand how additional mutations, particularly those affecting \textit{TET2}, interact with \textit{JAK2}\textsuperscript{V617F} to influence MPN phenotype, and work investigating the role of many other mutations in MPNs is ongoing. The identification of mutations in \textit{CALR} have filled a large gap in our understanding of the genetic basis of \textit{JAK2}\textsuperscript{V617F}-unmutated MPNs, but has raised the very important question of how a mutant endoplasmic reticulum chaperone protein can lead to dysregulated cell signalling and excessive megakaryopoiesis.

The management of patients has evolved concurrently with advances in our understanding of pathogenesis. \textit{JAK2, MPL} and \textit{CALR} testing are being utilized effectively for clinical diagnosis. However, 10-15% of patients with ET or MF do not harbour such mutations, and our understanding of pathogenic basis of these subgroups is far from complete. Clonality studies, whole genome sequencing and functional analysis of hematopoietic cells have the potential to lend further insights in these patients. Given that mutations commonly seen in myelodysplasia, such as mutations in epigenetic regulators and spliceosome genes, have been documented in triple-negative MF patients, and that these patients have been shown to carry a poor clinical prognosis, careful consideration is required as to what myeloid classification and therapy is most appropriate for such patients. We now have many molecular markers of prognosis for MF patients, and future work may also involve using this information to test alternative therapeutic strategies in these patients.

\textit{JAK} inhibitors are proving to be a very useful treatment option for MF-related symptoms, however, dose-limiting anemia, thrombocytopenia and an inability to delay disease progression are potential areas of improvement for newer agents. Mutant \textit{CALR} holds future promise as a tumor specific therapeutic target given its neomorphic C-terminus and selective expression in megakaryocytes. In addition, it is hoped that continued advances in our understanding of the structural consequences of \textit{JAK2}\textsuperscript{V617F} may allow for it to be specifically targeted in the future.


56. Li J, Kent DG, Godfrey AL, et al. JAK2V617F-homozygosity drives a phenotypic switch between myeloproliferative neoplasms in a murine model, but is insufficient to sustain clonal expansion. Blood. 2014;


