

Pathogenesis of Myeloproliferative Disorders

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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.

Main Text

10,200 words allocation (inc figures/tables each using 300-600 word space)

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 century¹, 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.² 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)³. 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).

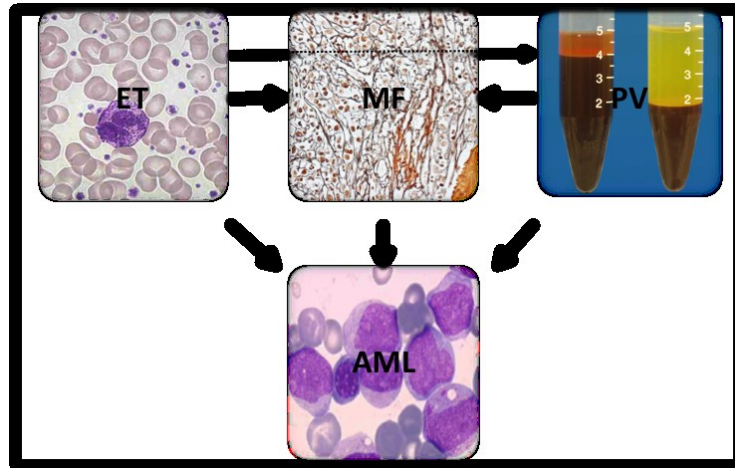


Figure 1. The Philadelphia-negative myeloproliferative neoplasms (MPN). ET (essential thrombocythemia) and PV (polycythemia vera) are chronic phase MPNs characterised by thrombocytosis and increased erythrocytosis respectively. MF (myelofibrosis) is a more advanced phase of MPNs which can also evolve 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 unclear⁶⁻¹⁰. 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 patients¹¹⁻¹⁴. 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

subsequently¹⁵, are found in the vast majority of patients with PV. In addition, *JAK2*^{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^{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^{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)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 re-conformation 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 signalling²¹, and mice deficient in the Epo receptor (EpoR)²², JAK2²⁰ or STAT5²³ 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^{24,25}. Granulocytic differentiation, via G-CSF and its receptor G-CSFR, occurs predominantly through JAK1, and to a lesser degree via JAK2²⁶. 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 response²⁷⁻²⁹. 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* activation³⁰. 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^{31,32}. 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.

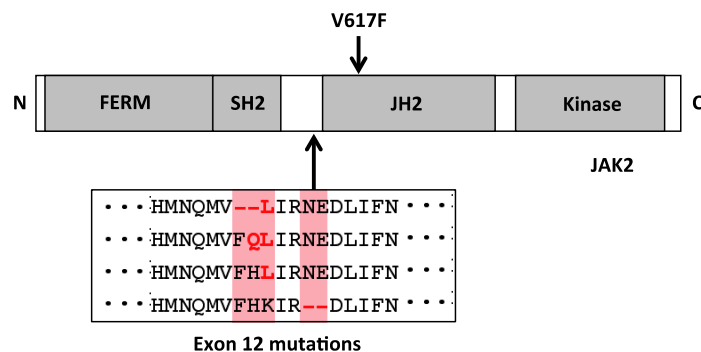


Figure 2. Model depicting the protein domains of *JAK2*. The FERM (4.1 protein, ezrin, radixin, moesin) domain is involved in receptor interaction. The SH2 (Src Homology 2) domain allows interaction with other proteins to aid tyrosine kinase signal transduction. JH2 (JAK homology 2) is the pseudo-kinase domain that negatively regulates the adjacent JH1 kinase domain. V617F is the commonest *JAK2* mutation and locates to the JH2 domain. *JAK2*^{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*^{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*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 linker³². The JH2 domain of JAK2 has also been shown to itself bind ATP and phosphorylate sites that negatively regulate JAK2 kinase activity³³. Disruption of this ATP binding has been shown to abrogate V617F mediated JAK2 kinase activation without affecting wild-type JAK2 function³⁴, 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 JAK2^{V617F}.^{11,12} 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 JAK2^{V617F} expressing Ba/F3 cells (Basel F4 cell line), and erythrocytosis in JAK2^{V617F} expressing transgenic mice^{35,36}. STAT5, in turn, has been reported to be involved in JAK2^{V617F} 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 proliferation³⁷⁻⁴¹.

In addition to canonical signalling via STAT or alternative effector pathways, it is worth noting that JAK2^{V617F} has also been shown to have non-canonical roles in cell function. For example, JAK2^{V617F} 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 proliferation⁴². In addition, JAK2^{V617F} 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 HP1⁴³.

1.2.3 Effect of JAK2^{V617F} on HSCs

The fact that MPNs emerge and are propagated over time requires that JAK2^{V617F} is mutated in a cell that harbours long-term self-renewal capacity. Indeed, JAK2^{V617F} 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 cells⁴⁴⁻⁴⁶. In addition, using conditionally expressing *JAK2*^{V617F} knock-in mouse models, MPN disease propagating cells have been shown to be HSCs with long term self-renewal capacity (LT-HSC)⁴⁷.

However despite these data, there are numerous lines of evidence that suggest that *JAK2*^{V617F} does not impart a strong clonal advantage to HSCs. In patients, *JAK2*^{V617F} allele burdens remain stable over decades, and often at very low levels, rather than gradually increasing^{48,49}. When assessing the CD34+CD38- (containing HSC/progenitor) cell compartment of *JAK2*^{V617F}-mutated MPN patients with PV and ET, significant clonal expansion has not been demonstrated⁵⁰. In addition, CD34+ (containing HSC/progenitor) cells from PV patients harbouring *JAK2*^{V617F} display poor engraftment in NOD/SCID/IL2R-gamma-null mice, compared with wildtype cells^{51,52}. Recently, *JAK2*^{V617F} has also been demonstrated in the blood of normal individuals that lack any overt MPN phenotype⁵³⁻⁵⁵. Furthermore, *JAK2*^{V617F}-mutated LT-HSCs in some conditional *JAK2*^{V617F} knock-in mouse models demonstrate an impairment of self-renewal activity when tested in competitive re-transplantation experiments^{56,57}, and utilizing the same model, *JAK2*^{V617F} has been shown to confer a proliferative advantage to progenitor cells with loss of self-renewal at the level of HSCs⁵⁸.

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 *JAK2*^{V617F}-mutated MPNs. Indeed, *JAK2*^{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^{51,59}. This ability of other mutations to enhance the clonal advantage of *JAK2*^{V617F}- HSCs or progenitors has been demonstrated in studies investigating *TET2*-*JAK2* co-mutations, using either stem/progenitor cells from patients^{60,61}, or in transgenic mouse models^{62,63}.

It is worth noting, however, that in a significant number of *JAK2*^{V617F}-mutated patients, additional somatic mutations have not been demonstrated⁶⁴. This suggests that other factors may modulate stem cell function in the context of *JAK2*^{V617F}, to result in the development of an MPN.

1.2.4 Factors influencing phenotype in *JAK2*^{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 9p24⁶⁶. 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 ET⁶⁷, 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 patients¹⁴. More recently, it has come to light that homozygosity for *JAK2^{V617F}* occurs in both ET and PV patients⁶⁸. 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}* clone⁶⁸.

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 splenomegaly⁶⁹⁻⁷¹. 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 $JAK2^{V617F}$ are supported by transgenic mouse models which have shown that increased expression of $JAK2^{V617F}$ 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 $JAK2^{V617F}$ cells show differing responses to Epo, with only heterozygous $JAK2^{V617F}$ iPS cells capable of producing Tpo independent megakaryocyte colonies⁷³. From murine models, homozygous $JAK2^{V617F}$ 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 $JAK2^{V617F}$ that result in its distinct effects on differentiation remain unknown.

B. Differential signalling downstream of $JAK2^{V617F}$

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 $JAK2^{V617F}$ 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 $JAK2^{V617F}$, present across the MPN subtypes. To circumvent some of these confounding issues, a gene expression study comparing wild-type to heterozygous $JAK2^{V617F}$ 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 STAT1⁷⁶. In keeping with this, attenuation of STAT1 activity in a transgenic $JAK2^{V617F}$ mouse model of PV has also demonstrated a skew towards erythropoiesis⁷⁷. Given that some PV patients do not harbour $JAK2^{V617F}$ homozygosity^{48,68}, it is possible that such qualitative differences in heterozygous $JAK2^{V617F}$ 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 *JAK2*^{V617F}-mediated MPN phenotype^{3,78}. Higher mutant allele burdens for *JAK2*^{V617F} have been reported in males compared with females, in keeping with a more prevalent PV phenotype in men⁷⁹. One interpretation of this data is that males may be more likely to develop homozygosity for *JAK2*^{V617F}. However, recent data has shown that homozygosity occurs equally commonly in both genders, and that while homozygosity was associated with males in PV, it was also associated with ET in females⁷¹. This raises the possibility that gender specific factors may differentially affect the degree of expansion of homozygous *JAK2*^{V617F} subclones to influence MPN phenotype. For example, it is possible that following the acquisition of homozygous *JAK2*^{V617F}, 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 levels⁴⁸, 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*⁸⁰, as well as other SNPs such as rs9376092 (*HBS1L/MYB*)⁸¹ 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' haplotype⁸². 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 *JAK2*^{V617F} ('hypermutability hypothesis') or that following acquisition of *JAK2*^{V617F}, 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 diagnosis⁸³. 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 *JAK2*^{V617F} 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 *JAK2*^{V617F}.

D. Role of other mutations

Targeted and whole-exome sequencing have identified additional mutations in some MPN patients and these may modulate *JAK2*^{V617F} 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^{64,84–86}. 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^{85,87}. 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 erythrocytosis⁸⁸.

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).

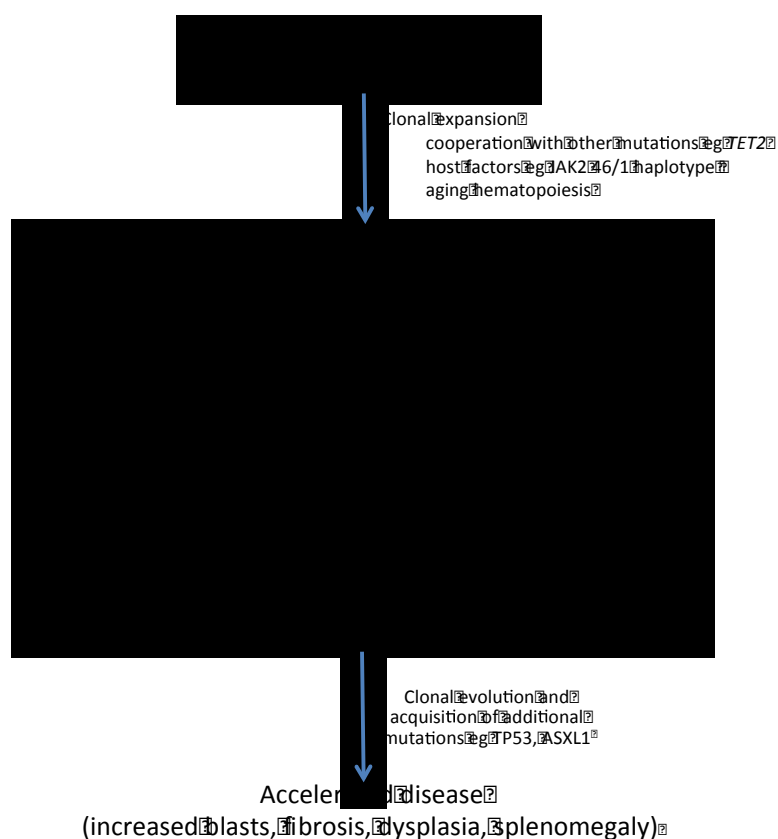


Figure 3. A model depicting the factors that are known to influence the development, and the resulting phenotype, of *JAK2*^{V617F}-mutated MPNs.

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*. Somatic 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^{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 thrombocythemia^{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*^{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 binding⁹³. 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*^{V617F} exhibit disease phenotypes ranging from ET to PV and MF⁴⁷, murine models of *MPL* mutations develop a disease marked by thrombocytosis with features of ET and MF⁹⁴. 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^{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^{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 phenotype⁹⁹.

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 patients¹⁰⁰. In late 2013, two whole-exome sequencing studies of MPN patients, identified recurrent somatic mutations in the gene *CALR*, which encodes the gene calreticulin^{64,101}. These mutations were found in 60-90% of patients with *JAK2* and *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 *JAK2*, *MPL* or *CALR*, most MPN patients had a diagnostic marker for their disease (Figure 4).

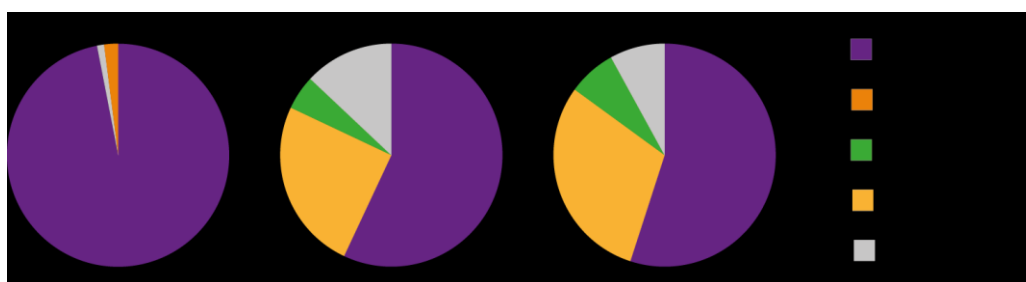


Figure 4. MPN subtypes and the frequency of mutations in *JAK2*, *CALR* or *MPL*.

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, *CALR* mutations have not been demonstrated in lymphoid malignancies, solid tumours or healthy controls^{64,101}. Very rare cases of polycythemia vera have also been found to harbour *CALR* mutations, however, the possibility that these patients harboured an alternative reason for manifesting an erythrocytosis were not excluded¹⁰². Overall, *CALR* mutations demonstrate a striking specificity for *JAK2*-unmutated ET and MF that is highly reminiscent of the disease spectrum associated with mutations in *MPL*.

CALR mutations in MPNs affect the terminal exon of the gene. All mutations are

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 both¹⁰³. 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).

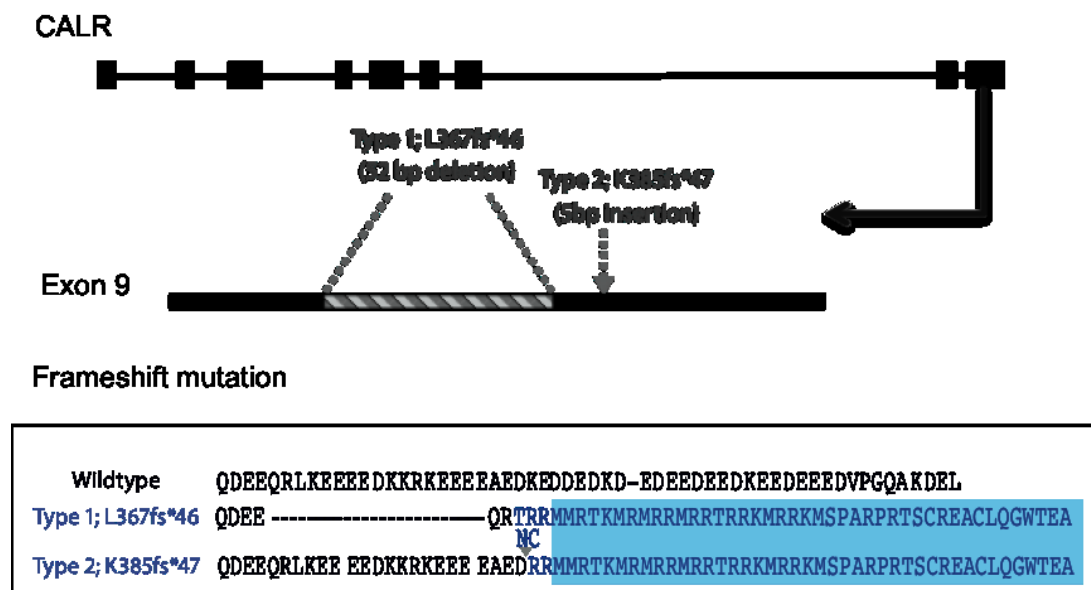


Figure 5. *CALR* mutations. The intron-exon structure is shown along the top of the picture. The terminal exon 9 is affected by two common mutations: a 52 base pair deletion (Type 1 or L367fs*46) or a 5 base pair insertion (Type 2 or K387fs*47). Both mutations result in a shift in the reading frame of 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^{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^{64,101}. In addition, mutant calreticulin no longer harbours the low-affinity high-capacity calcium binding potential of the wildtype function¹⁰⁶, and it is possible that this alteration disrupts ER function in a way that perturbs megakaryopoiesis (Figure 6).

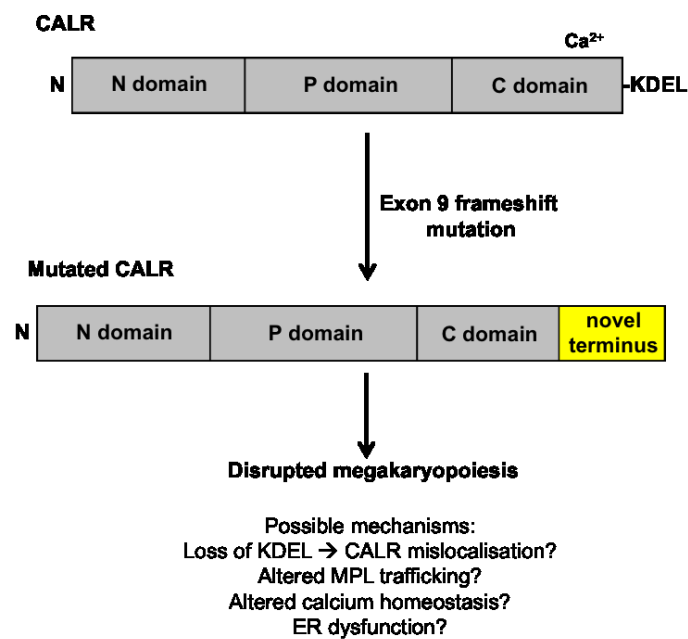


Figure 6. Model depicting the domains of CALR and the predicted effect of CALR mutations. Mutant CALR results in the generation of a novel C-terminus that lacks KDEL as well as calcium binding sites. N, N-terminus; N domain, Lectin binding domain; P domain, proline rich domain; C domain, calcium binding 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 arrays¹⁰⁷ and transfected cell lines¹⁰¹

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 *JAK2*^{V617F}. The presence of multiple subclones was also supported by the finding that the percentage of granulocytes carrying *JAK2*^{V617F} 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*, *IDH1/2*^{60,113-115}, chromatin remodeling, such as *EZH2*, *ASXL1*^{116,117}, 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

Gene	PV (%)	ET (%)	MF (%)
JAK2 (V617F)	95-97	50-60	50-60
JAK2 (Exon 12)	1-2	0	0
CALR	0	25	30
MPL	0	3-5	5-10
CBL	-	0-2	5-10
SH2B3	2	2-6	3-6
ASXL1	2	5-10	10-35
EZH2	1-2	1-2	7-10
IDH1/IDH2	2	1	5
DNMT3a	5-10	2-5	8-12
TET2	10-20	4-5	10-20
SF3B1	2	2	4
SRSF2	-	-	4-17
U2AF1	<1	<1	1-8
ZRSR2	<1	<1	<1
TP53	1-2	1-2	1-2

1.5.1 Mutations in *DNMT3A* and *TET2*

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

Mutations in *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 *DNMT3A*, R882H has recently been demonstrated to confer a dominant negative effect on wildtype DNMT3A. Serial transplantation studies using transgenic mouse models with knock-out of *DNMT3A* has demonstrated that loss of DNMT3A results in hematopoietic stem cell compartment expansion¹¹⁹. In keeping with these data, a study of human AML has shown that *DNMT3A* mutations are acquired early in tumourigenesis, and the pre-leukemic HSCs harbouring mutated *DNMT3A* display a self-renewal advantage over wildtype HSCs¹²⁰, confirming murine data that acquisition of mutations in *DNMT3A* result in a stem cell advantage. In MPNs, *DNMT3A* mutations can occur prior to, or following, the acquisition of mutations in *JAK2*¹²¹ but clinically, studies

to date have not demonstrated any associations between *DNMT3A* mutations and alterations to MPN phenotype or outcome, and analysis of larger patient cohorts is required. It is likely that *DNMT3A* mutations function to confer a clonal advantage to MPN mutant clones, particularly, given data suggesting that *JAK2^{V617F}* results in a self-renewal impairment at the level of HSCs. However, mutations in *DNMT3A* may also confer a myeloproliferative phenotype, as suggested by murine models of R882H that display myeloid proliferation with thrombocytosis^{122,123}.

Loss of function *TET2* mutations are found in 5-17% of MPN patients^{60,124}. In terms of function, murine models with attenuated *TET2* display HSC compartment expansion in a manner similar to that seen with mutations in *DNMT3A*. However, loss of *TET2* results in obvious myelo-monocytic proliferation and phenotypic features resembling chronic myelomonocytic leukemia¹²⁵. In fact, patients with myelodysplastic/myeloproliferative (MDS/MPN) disorders often harbour biallelic loss of *TET2* either through acquisition of compound *TET2* mutations, or loss of heterozygosity at chr4q24^{60,126}. Patients with mutated *TET2* have been shown to display reduced levels of 5-hmc^{84,127}. Clinically, one study has shown *TET2* mutations to be associated with an increased risk of leukemic transformation and shorter survival. However, other studies have not found such clinical associations^{121,128}. Interestingly, both *TET2* and *DNMT3A* mutations have been found in normal elderly females who display clonal hematopoiesis in the absence of any blood count parameter abnormalities¹²⁹. This finding, in conjunction with data from murine models, suggests that *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 *JAK2^{V617F}* as well as loss of *TET2* shows that *TET2* may well function to confer a stem cell clonal advantage to *JAK2*-mutated cells and help propagate and accelerate disease phenotype^{62,130}.

Interestingly, the effects of *TET2* and *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 *TET2* and *JAK2*-mutated MPN patients, the order in which these mutations were acquired was found to be associated with patient age at presentation, presence of *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 *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 *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 (H3K27me_{2/3}) 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 transformation¹³¹. Deletion of the *EZH2* homologue in murine models results in an MDS/MPN phenotype which is exacerbated by concurrent deletion of *TET2*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^{84,133}.

ASXL1 (Addition of Sex Combs Like 1) is a mediator of PRC2 function and also interacts with the nuclear deubiquitinating enzyme BAP1¹³⁴. 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^{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 splenomegaly¹³⁶. This model also displayed a block to erythroid differentiation and HSC expansion, features that are reminiscent of many patients with MF¹³⁶.

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 decisions¹³⁷.

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-hydroxyglutarate¹³⁸, a protein that has been shown to inhibit histone

demethylation and attenuate hematopoietic differentiation¹³⁹. In addition, alpha-ketoglutarate has also been shown to inhibit normal TET2 function¹⁴⁰. 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^{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^{142,143}. Recently, it has been confirmed that these mutations result in altered mRNA splicing^{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-T¹⁰¹. 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 MDS⁸⁷.

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 time¹²¹. In addition, mutations in epigenetic regulators have been shown to occur both early and late in tumorigenesis¹²¹. While we are beginning to understand the implications of mutation order for *TET2* and *JAK2* mutations in MPNs⁶¹, 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 pedigree¹⁴⁷. Interestingly, affected family members also display the same spectrum of somatically acquired mutations in *JAK2*, *MPL* or *CALR*^{148,149}. Why these family members have an increased risk of developing these neoplasms is unclear. In one such pedigree, mutations in *RBBP6* were found and appeared to account for the germline predisposition to acquiring

MPNs¹⁵⁰. 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^{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*^{V617F}. From these studies, it became clear that *JAK2*^{V617F}-mutated ET patients displayed many of the hallmarks of PV: patients had higher hemoglobins and white counts and reduced platelets compared with *JAK2*^{V617F}-unmutated ET patients, as well as panmyelosis on bone marrow examination^{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*^{V617F}¹⁵². Therefore, the current model is that *JAK2*-mutated PV or ET may be better viewed as one disease continuum¹⁵⁴. 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*^{V617F} PV and ET patients are very similar¹⁵⁵, 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*^{V617F}-mutated counterparts^{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 ET⁹¹. However, no differences in clinical outcome have, thus far, been demonstrated for this small subgroup of MPN patients in either ET or MF^{156,158}. Interestingly, homozygosity for *MPL* mutations is also observed, although, at a far lower frequency than that observed for *JAK2*^{V617F}, and such patients have been shown to display increased marrow fibrosis with a higher rate of transformation to MF¹⁵⁹.

Clinically, *CALR*-mutated ET is associated with a higher platelet count, lower hemoglobin and lower leucocyte count at presentation, compared with *JAK2*-mutated counterparts^{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*^{V617F} patients with ET show a female predominance, *CALR*-mutated ET affects males and females equally^{154,160}. Interestingly, some studies have shown that *CALR*-mutated ET have a reduced incidence of thrombosis compared with *JAK2*^{V617F}-mutated patients, which remains significant after taking into account their younger age¹⁶¹, however, another study has shown no such favorable impact¹⁶². It is likely that what is observed reflects the known increased risk of thrombosis imparted by *JAK2*^{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 ET¹⁵⁵.

In MF, compared with *JAK2*^{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^{163,164}. These patients also have a better prognosis in terms of overall survival and leukemia free survival compared with *JAK2*^{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^{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 MPNs¹⁶⁵. 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.

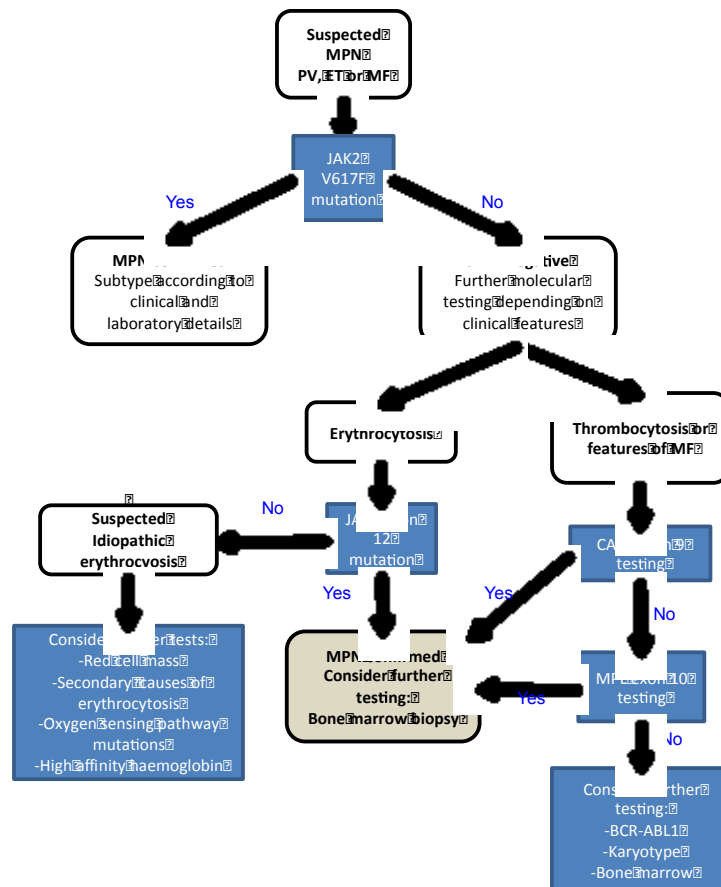


Figure 7. Diagnostic clinical algorithm including molecular testing for MPNs

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*^{154,160,163}, 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 transformation⁸⁷. 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 patients¹⁶⁷.

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 phenotype¹⁶⁸. 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 inhibitor⁸⁶.

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 *JAK2*, *MPL* or *CALR*, but patients also often harbour other somatically acquired mutations as well homozygosity for *JAK2^{V617F}*. We are beginning to understand how additional mutations, particularly those affecting *TET2*, interact with *JAK2^{V617F}* to influence MPN phenotype, and work investigating the role of many other mutations in MPNs is ongoing. The identification of mutations in *CALR* have filled a large gap in our understanding of the genetic basis of *JAK2^{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. *JAK2*, *MPL* and *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.

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 *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 *JAK2^{V617F}* may allow for it to be specifically targeted in the future.

1. Bennett J. Case of hypertrophy of the spleen and liver in which death took place from suppuration of the blood. *Edinb Med Surg J.* 1845;64:413–423.
2. Dameshek W. Some speculations on the myeloproliferative syndromes. *Blood.* 1951;6(4):372–5.
3. Swerdlow SH, Campo E, Harris NL, et al. WHO classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2008.
4. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature.* 1973;243(5405):290–293.
5. Nowell PC, Hungerford DA. Chromosome studies in human leukemia. II. Chronic granulocytic leukemia. *J. Natl. Cancer Inst.* 1961;27:1013–1035.
6. Adamson JW, Fialkow PJ, Murphy S, Prchal JF, Steinmann L. Polycythemia vera: stem-cell and probable clonal origin of the disease. *N. Engl. J. Med.* 1976;295(17):913–916.
7. Jacobson RJ, Salo A, Fialkow PJ. Agnogenic myeloid metaplasia: a clonal proliferation of hematopoietic stem cells with secondary myelofibrosis. *Blood.* 1978;51(2):189–94.
8. Fialkow PJ, Faguet GB, Jacobson RJ, Vaidya K, Murphy S. Evidence that essential thrombocythemia is a clonal disorder with origin in a multipotent stem cell. *Blood.* 1981;58(5):916–9.
9. Tsukamoto N, Morita K, Maehara T, et al. Clonality in chronic myeloproliferative disorders defined by X-chromosome linked probes: demonstration of heterogeneity in lineage involvement. *Br. J. Haematol.* 1994;86(2):253–258.
10. El Kassar N, Hetet G, Li Y, Brière J, Grandchamp B. Clonal analysis of haemopoietic cells in essential thrombocythaemia. *Br. J. Haematol.* 1995;90(1):131–137.
11. James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature.* 2005;434(7037):1144–8.
12. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell.* 2005;7(4):387–97.
13. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet.* 2005;365(9464):1054–61.
14. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med.* 2005;352(17):1779–90.
15. Scott LM, Tong W, Levine RL, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med.* 2007;356(5):459–68.
16. Harrison CN, Butt N, Campbell P, et al. Modification of British Committee for Standards in Haematology diagnostic criteria for essential thrombocythaemia. *Br. J. Haematol.* 2014;167(3):421–423.
17. McMullin MF, Reilly JT, Campbell P, et al. Amendment to the guideline for diagnosis and investigation of polycythaemia/erythrocytosis. *Br. J. Haematol.* 2007;138(6):821–822.
18. Harrison C, Kiladjian J-J, Al-Ali HK, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *N. Engl. J. Med.* 2012;366(9):787–798.
19. Verstovsek S, Mesa RA, Gotlib J, et al. A Double-Blind, Placebo-Controlled Trial of Ruxolitinib for Myelofibrosis. *N. Engl. J. Med.* 2012;366(9):799–807.
20. Parganas E, Wang D, Stravopodis D, et al. Jak2 Is Essential for Signaling through a Variety of Cytokine Receptors. *Cell.* 1998;93(3):385–395.
21. Grebien F, Kerenyi MA, Kovacic B, et al. Stat5 activation enables erythropoiesis in the absence of EpoR and Jak2. *Blood.* 2008;111(9):4511–4522.
22. Wu H, Liu X, Jaenisch R, Lodish HF. Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor. *Cell.* 1995;83(1):59–67.

23. Kerenyi MA, Grebien F, Gehart H, et al. Stat5 regulates cellular iron uptake of erythroid cells via IRP-2 and TfR-1. *Blood*. 2008;112(9):3878–3888.
24. Rouyez MC, Boucheron C, Gisselbrecht S, Dusanter-Fourt I, Porteu F. Control of thrombopoietin-induced megakaryocytic differentiation by the mitogen-activated protein kinase pathway. *Mol. Cell. Biol.* 1997;17(9):4991–5000.
25. Miyakawa Y, Oda A, Druker BJ, et al. Thrombopoietin induces tyrosine phosphorylation of Stat3 and Stat5 in human blood platelets. *Blood*. 1996;87(2):439–446.
26. Shimoda K, Feng J, Murakami H, et al. Jak1 plays an essential role for receptor phosphorylation and Stat activation in response to granulocyte colony-stimulating factor. *Blood*. 1997;90(2):597–604.
27. Chen M, Cheng A, Candotti F, et al. Complex effects of naturally occurring mutations in the JAK3 pseudokinase domain: evidence for interactions between the kinase and pseudokinase domains. *Mol. Cell. Biol.* 2000;20(3):947–956.
28. Yeh TC, Dondi E, Uze G, Pellegrini S. A dual role for the kinase-like domain of the tyrosine kinase Tyk2 in interferon-alpha signaling. *Proc. Natl. Acad. Sci. U. S. A.* 2000;97(16):8991–8996.
29. Saharinen P, Silvennoinen O. The pseudokinase domain is required for suppression of basal activity of Jak2 and Jak3 tyrosine kinases and for cytokine-inducible activation of signal transduction. *J Biol Chem.* 2002;277(49):47954–63.
30. Brooks AJ, Dai W, O'Mara ML, et al. Mechanism of activation of protein kinase JAK2 by the growth hormone receptor. *Science*. 2014;344(6185):1249783.
31. Lupardus PJ, Ultsch M, Wallweber H, et al. Structure of the pseudokinase-kinase domains from protein kinase TYK2 reveals a mechanism for Janus kinase (JAK) autoinhibition. *Proc. Natl. Acad. Sci. U. S. A.* 2014;111(22):8025–8030.
32. Shan Y, Gnanasambandan K, Ungureanu D, et al. Molecular basis for pseudokinase-dependent autoinhibition of JAK2 tyrosine kinase. *Nat. Struct. Mol. Biol.* 2014;21(7):579–584.
33. Ungureanu D, Wu J, Pekkala T, et al. The pseudokinase domain of JAK2 is a dual-specificity protein kinase that negatively regulates cytokine signaling. *Nat. Struct. Mol. Biol.* 2011;18(9):971–976.
34. Hammarén HM, Ungureanu D, Grisouard J, et al. ATP binding to the pseudokinase domain of JAK2 is critical for pathogenic activation. *Proc. Natl. Acad. Sci. U. S. A.* 2015;112(15):4642–4647.
35. Funakoshi-Tago M, Tago K, Abe M, Sonoda Y, Kasahara T. STAT5 activation is critical for the transformation mediated by myeloproliferative disorder-associated JAK2 V617F mutant. *J. Biol. Chem.* 2010;285(8):5296–5307.
36. Yan D, Hutchison RE, Mohi G. Critical requirement for Stat5 in a mouse model of polycythemia vera. *Blood*. 2012;119(15):3539–3549.
37. Wernig G, Gonneville JR, Crowley BJ, et al. The Jak2V617F oncogene associated with myeloproliferative diseases requires a functional FERM domain for transformation and for expression of the Myc and Pim proto-oncogenes. *Blood*. 2008;111(7):3751–3759.
38. Da Costa Reis Monte-Mór B, Plo I, da Cunha AF, et al. Constitutive JunB expression, associated with the JAK2 V617F mutation, stimulates proliferation of the erythroid lineage. *Leukemia*. 2009;23(1):144–152.
39. Garçon L, Rivat C, James C, et al. Constitutive activation of STAT5 and Bcl-xL overexpression can induce endogenous erythroid colony formation in human primary cells. *Blood*. 2006;108(5):1551–4.
40. Wood AD, Chen E, Donaldson IJ, et al. ID1 promotes expansion and survival of primary erythroid cells and is a target of JAK2V617F-STAT5 signaling. *Blood*. 2009;114(9):1820–1830.
41. Gautier E-F, Picard M, Laurent C, et al. The cell cycle regulator CDC25A is a target for JAK2V617F oncogene. *Blood*. 2012;119(5):1190–1199.

42. Liu F, Zhao X, Perna F, et al. JAK2V617F-mediated phosphorylation of PRMT5 downregulates its methyltransferase activity and promotes myeloproliferation. *Cancer Cell*. 2011;19(2):283–294.
43. Dawson MA, Bannister AJ, Göttgens B, et al. JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. *Nature*. 2009;461(7265):819–822.
44. Jamieson CHM, Gotlib J, Durocher JA, et al. The JAK2 V617F mutation occurs in hematopoietic stem cells in polycythemia vera and predisposes toward erythroid differentiation. *Proc. Natl. Acad. Sci*. 2006;103(16):6224–6229.
45. Delhommeau F, Dupont S, Tonetti C, et al. Evidence that the JAK2 G1849T (V617F) mutation occurs in a lymphomyeloid progenitor in polycythemia vera and idiopathic myelofibrosis. *Blood*. 2007;109(1):71–7.
46. Ishii T, Bruno E, Hoffman R, Xu M. Involvement of various hematopoietic-cell lineages by the JAK2V617F mutation in polycythemia vera. *Blood*. 2006;108(9):3128–34.
47. Li J, Kent DG, Chen E, Green AR. Mouse models of myeloproliferative neoplasms: JAK of all grades. *Dis. Model. Mech*. 2011;4(3):311–317.
48. Dupont S, Masse A, James C, et al. The JAK2 V617F mutation triggers erythropoietin hypersensitivity and terminal erythroid amplification in primary cells from patients with polycythemia vera. *Blood*. 2007;110(3):1013–21.
49. Gale RE, Allen AJ, Nash MJ, Linch DC. Long-term serial analysis of X-chromosome inactivation patterns and JAK2 V617F mutant levels in patients with essential thrombocythemia show that minor mutant-positive clones can remain stable for many years. *Blood*. 2007;109(3):1241–3.
50. Anand S, Stedham F, Beer P, et al. Effects of the JAK2 mutation on the hematopoietic stem and progenitor compartment in human myeloproliferative neoplasms. *Blood*. 2011;118(1):177–81.
51. James C, Mazurier F, Dupont S, et al. The hematopoietic stem cell compartment of JAK2V617F-positive myeloproliferative disorders is a reflection of disease heterogeneity. *Blood*. 2008;112(6):2429–38.
52. Ishii T, Zhao Y, Sozer S, et al. Behavior of CD34+ cells isolated from patients with polycythemia vera in NOD/SCID mice. *Exp Hematol*. 2007;35(11):1633–40.
53. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N. Engl. J. Med*. 2014;371(26):2477–2487.
54. Jaiswal S, Fontanillas P, Flannick J, et al. Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes. *N. Engl. J. Med*. 2014;
55. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat. Med*. 2014;20(12):1472–1478.
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;
57. Li J, Spensberger D, Ahn JS, et al. JAK2 V617F impairs hematopoietic stem cell function in a conditional knock-in mouse model of JAK2 V617F-positive essential thrombocythemia. *Blood*. 2010;116(9):1528–1538.
58. Kent DG, Li J, Tanna H, et al. Self-renewal of single mouse hematopoietic stem cells is reduced by JAK2V617F without compromising progenitor cell expansion. *PLoS Biol*. 2013;11(6):e1001576.
59. Wang X, Prakash S, Lu M, et al. Spleens of myelofibrosis patients contain malignant hematopoietic stem cells. *J. Clin. Invest*. 2012;122(11):3888–3899.
60. Delhommeau F, Dupont S, Della Valle V, et al. Mutation in TET2 in myeloid cancers. *N Engl J Med*. 2009;360(22):2289–301.
61. Ortmann CA, Kent DG, Nangalia J, et al. Effect of mutation order on myeloproliferative neoplasms. *N. Engl. J. Med*. 2015;372(7):601–612.

62. Chen E, Schneider RK, Breyfogle LJ, et al. Distinct effects of concomitant Jak2V617F expression and Tet2 loss in mice promote disease progression in myeloproliferative neoplasms. *Blood*. 2015;125(2):327–335.
63. Kameda T, Shide K, Yamaji T, et al. Loss of TET2 has dual roles in murine myeloproliferative neoplasms: disease sustainer and disease accelerator. *Blood*. 2015;125(2):304–315.
64. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N. Engl. J. Med.* 2013;369(25):2391–2405.
65. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013;
66. Kralovics R, Guan Y, Prchal JT. Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. *Exp Hematol*. 2002;30(3):229–36.
67. Scott LM, Scott MA, Campbell PJ, Green AR. Progenitors homozygous for the V617F mutation occur in most patients with polycythemia vera, but not essential thrombocythemia. *Blood*. 2006;108(7):2435–7.
68. Godfrey AL, Chen E, Pagano F, et al. JAK2V617F homozygosity arises commonly and recurrently in PV and ET, but PV is characterized by expansion of a dominant homozygous subclone. *Blood*. 2012;120(13):2704–2707.
69. Tefferi A, Lasho TL, Schwager SM, et al. The clinical phenotype of wild-type, heterozygous, and homozygous JAK2V617F in polycythemia vera. *Cancer*. 2006;106(3):631–5.
70. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Clinical profile of homozygous JAK2 617V>F mutation in patients with polycythemia vera or essential thrombocythemia. *Blood*. 2007;110(3):840–846.
71. Godfrey AL, Chen E, Pagano F, et al. Clonal analyses reveal associations of JAK2V617F homozygosity with hematologic features, age and gender in polycythemia vera and essential thrombocythemia. *Haematologica*. 2013;98(5):718–721.
72. Tiedt R, Hao-Shen H, Sobas MA, et al. Ratio of mutant JAK2-V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. *Blood*. 2008;111(8):3931–40.
73. Saliba J, Hamidi S, Lenglet G, et al. Heterozygous and homozygous JAK2(V617F) states modeled by induced pluripotent stem cells from myeloproliferative neoplasm patients. *PloS One*. 2013;8(9):e74257.
74. Akada H, Akada S, Hutchison RE, Mohi G. Loss of wild-type Jak2 allele enhances myeloid cell expansion and accelerates myelofibrosis in Jak2V617F knock-in mice. *Leukemia*. 2014;28(8):1627–1635.
75. Teofili L, Martini M, Cenci T, et al. Different STAT-3 and STAT-5 phosphorylation discriminates among Ph-negative chronic myeloproliferative diseases and is independent of the V617F JAK-2 mutation. *Blood*. 2007;110(1):354–9.
76. Chen E, Beer PA, Godfrey AL, et al. Distinct clinical phenotypes associated with JAK2V617F reflect differential STAT1 signaling. *Cancer Cell*. 2010;18(5):524–35.
77. Duek A, Lundberg P, Shimizu T, et al. Loss of Stat1 decreases megakaryopoiesis and favors erythropoiesis in a JAK2-V617F-driven mouse model of MPNs. *Blood*. 2014;123(25):3943–3950.
78. Jensen MK, de Nully Brown P, Nielsen OJ, Hasselbalch HC. Incidence, clinical features and outcome of essential thrombocythaemia in a well defined geographical area. *Eur J Haematol*. 2000;65(2):132–9.
79. Stein BL, Williams DM, Wang NY, et al. Sex differences in the JAK2 V617F allele burden in chronic myeloproliferative disorders. *Haematologica*. 2010;95(7):1090–7.
80. Pardananani A, Fridley BL, Lasho TL, Gilliland DG, Tefferi A. Host genetic variation contributes to phenotypic diversity in myeloproliferative disorders. *Blood*. 2008;111(5):2785–9.

81. Tapper W, Jones AV, Kralovics R, et al. Genetic variation at MECOM, TERT, JAK2 and HBS1L-MYB predisposes to myeloproliferative neoplasms. *Nat. Commun.* 2015;6:6691.
82. Jones AV, Cross NCP. Inherited predisposition to myeloproliferative neoplasms. *Ther. Adv. Hematol.* 2013;4(4):237–253.
83. Oddsson A, Kristinsson SY, Helgason H, et al. The germline sequence variant rs2736100_C in TERT associates with myeloproliferative neoplasms. *Leukemia.* 2014;
84. Brecqueville M, Rey J, Bertucci F, et al. Mutation analysis of ASXL1, CBL, DNMT3A, IDH1, IDH2, JAK2, MPL, NF1, SF3B1, SUZ12, and TET2 in myeloproliferative neoplasms. *Genes. Chromosomes Cancer.* .
85. Green A, Beer P. Somatic mutations of IDH1 and IDH2 in the leukemic transformation of myeloproliferative neoplasms. *N Engl J Med.* 362(4):369–70.
86. Harutyunyan A, Klampfl T, Cazzola M, Kralovics R. p53 Lesions in Leukemic Transformation. *N. Engl. J. Med.* 2011;364(5):488–490.
87. Vannucchi AM, Lasho TL, Guglielmelli P, et al. Mutations and prognosis in primary myelofibrosis. *Leukemia.* 2013;27(9):1861–1869.
88. Jutzi JS, Bogeska R, Nikoloski G, et al. MPN patients harbor recurrent truncating mutations in transcription factor NF-E2. *J. Exp. Med.* 2013;210(5):1003–1019.
89. Pikman Y, Lee BH, Mercher T, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med.* 2006;3(7):e270.
90. Pardanani AD, Levine RL, Lasho T, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood.* 2006;108(10):3472–6.
91. Beer PA, Campbell PJ, Scott LM, et al. MPL Mutations in Myeloproliferative Disorders: Analysis of the PT-1 Cohort. *Blood.* 2008;112(1):141–149.
92. Chaligné R, Tonetti C, Besancenot R, et al. New mutations of MPL in primitive myelofibrosis: only the MPL W515 mutations promote a G1/S-phase transition. *Leukemia.* 2008;22(8):1557–1566.
93. Staerk J, Lacout C, Sato T, et al. An amphipathic motif at the transmembrane-cytoplasmic junction prevents autonomous activation of the thrombopoietin receptor. *Blood.* 2006;107(5):1864–71.
94. Pecquet C, Staerk J, Chaligné R, et al. Induction of myeloproliferative disorder and myelofibrosis by thrombopoietin receptor W515 mutants is mediated by cytosolic tyrosine 112 of the receptor. *Blood.* 2010;115(5):1037–1048.
95. Oh ST, Simonds EF, Jones C, et al. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. *Blood.* 2010;116(6):988–992.
96. Lasho TL, Pardanani A, Tefferi A. LNK mutations in JAK2 mutation-negative erythrocytosis. *N Engl J Med.* 363(12):1189–90.
97. Sanada M, Suzuki T, Shih L-Y, et al. Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. *Nature.* 2009;460(7257):904–908.
98. Grand FH, Hidalgo-Curtis CE, Ernst T, et al. Frequent CBL mutations associated with 11q acquired uniparental disomy in myeloproliferative neoplasms. *Blood.* 2009;113(24):6182–6192.
99. Schwaab J, Ernst T, Erben P, et al. Activating CBL mutations are associated with a distinct MDS/MPN phenotype. *Ann. Hematol.* 2012;91(11):1713–1720.
100. Hou Y, Song L, Zhu P, et al. Single-cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm. *Cell.* 2012;148(5):873–885.
101. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N. Engl. J. Med.* 2013;369(25):2379–2390.
102. Broséus J, Park J-H, Carillo S, Hermouet S, Girodon F. Presence of calreticulin mutations in JAK2-negative polycythemia vera. *Blood.* 2014;blood–2014–06–583161.

103. Guglielmelli P, Nangalia J, Green AR, Vannucchi AM. CALR mutations in myeloproliferative neoplasms: Hidden behind the reticulum. *Am. J. Hematol.* 2014;89(5):453–456.
104. Michalak M, Groenendyk J, Szabo E, Gold LI, Opas M. Calreticulin, a multi-process calcium-buffering chaperone of the endoplasmic reticulum. *Biochem. J.* 2009;417(3):651–666.
105. Chao MP, Majeti R, Weissman IL. Programmed cell removal: a new obstacle in the road to developing cancer. *Nat. Rev. Cancer.* 2012;12(1):58–67.
106. Shivarov V, Ivanova M, Tiu RV. Mutated calreticulin retains structurally disordered C terminus that cannot bind Ca(2+): some mechanistic and therapeutic implications. *Blood Cancer J.* 2014;4:e185.
107. Rampal R, Al-Shahrour F, Abdel-Wahab O, et al. Integrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasm pathogenesis. *Blood.* 2014;123(22):e123–133.
108. Kollmann K, Nangalia J, Warsch W, et al. MARIMO cells harbor a CALR mutation but are not dependent on JAK2/STAT5 signaling. *Leukemia.* 2014;
109. Vannucchi AM, Rotunno G, Bartalucci N, et al. Calreticulin mutation-specific immunostaining in myeloproliferative neoplasms: pathogenetic insight and diagnostic value. *Leukemia.* 2014;
110. Milosevic JD, Kralovics R. Genetic and epigenetic alterations of myeloproliferative disorders. *Int. J. Hematol.* 2013;97(2):183–197.
111. Campbell PJ, Baxter EJ, Beer PA, et al. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. *Blood.* 2006;108(10):3548–3555.
112. Kralovics R, Teo SS, Li S, et al. Acquisition of the V617F mutation of JAK2 is a late genetic event in a subset of patients with myeloproliferative disorders. *Blood.* 2006;108(4):1377–80.
113. Pardanani A, Lasho TL, Finke CM, et al. IDH1 and IDH2 mutation analysis in chronic- and blast-phase myeloproliferative neoplasms. *Leukemia.* 2010;24(6):1146–1151.
114. Abdel-Wahab O, Pardanani A, Rampal R, et al. DNMT3A mutational analysis in primary myelofibrosis, chronic myelomonocytic leukemia and advanced phases of myeloproliferative neoplasms. *Leukemia.* 2011;25(7):1219–1220.
115. Stegelmann F, Bullinger L, Schlenk RF, et al. DNMT3A mutations in myeloproliferative neoplasms. *Leuk. Off. J. Leuk. Soc. Am. Leuk. Res. Fund UK.* 2011;25(7):1217–1219.
116. Ernst T, Chase AJ, Score J, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat. Genet.* 2010;42(8):722–726.
117. Carbuccia N, Murati A, Trouplin V, et al. Mutations of ASXL1 gene in myeloproliferative neoplasms. *Leukemia.* 2009;23(11):2183–2186.
118. Ley TJ, Ding L, Walter MJ, et al. DNMT3A Mutations in Acute Myeloid Leukemia. *N. Engl. J. Med.* 2010;363(25):2424–2433.
119. Challen GA, Sun D, Jeong M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat. Genet.* 2012;44(1):23–31.
120. Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature.* 2014;506(7488):328–333.
121. Lundberg P, Karow A, Nienhold R, et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. *Blood.* 2014;123(14):2220–2228.
122. Xu J, Wang Y-Y, Dai Y-J, et al. DNMT3A Arg882 mutation drives chronic myelomonocytic leukemia through disturbing gene expression/DNA methylation in hematopoietic cells. *Proc. Natl. Acad. Sci. U. S. A.* 2014;111(7):2620–2625.

123. Russler-Germain DA, Spencer DH, Young MA, et al. The R882H DNMT3A Mutation Associated with AML Dominantly Inhibits Wild-Type DNMT3A by Blocking Its Ability to Form Active Tetramers. *Cancer Cell*. 2014;25(4):442–454.
124. Langemeijer SMC, Kuiper RP, Berends M, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. *Nat. Genet*. 2009;41(7):838–842.
125. Abdel-Wahab O, Tefferi A, Levine RL. Role of TET2 and ASXL1 mutations in the pathogenesis of myeloproliferative neoplasms. *Hematol. Oncol. Clin. North Am*. 2012;26(5):1053–1064.
126. Jankowska AM, Szpurka H, Tiu RV, et al. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. *Blood*. 2009;113(25):6403–6410.
127. Ko M, Huang Y, Jankowska AM, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature*. 2010;468(7325):839–843.
128. Tefferi A, Pardanani A, Lim K-H, et al. TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. *Leukemia*. 2009;23(5):905–911.
129. Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat. Genet*. 2012;44(11):1179–1181.
130. Kameda T, Shide K, Yamaji T, et al. Loss of TET2 has dual roles in murine myeloproliferative neoplasms: disease sustainer and disease accelerator. *Blood*. 2015;125(2):304–315.
131. Guglielmelli P, Biamonte F, Score J, et al. EZH2 mutational status predicts poor survival in myelofibrosis. *Blood*. 2011;118(19):5227–5234.
132. Muto T, Sashida G, Oshima M, et al. Concurrent loss of Ezh2 and Tet2 cooperates in the pathogenesis of myelodysplastic disorders. *J. Exp. Med*. 2013;210(12):2627–2639.
133. Score J, Hidalgo-Curtis C, Jones AV, et al. Inactivation of polycomb repressive complex 2 components in myeloproliferative and myelodysplastic/myeloproliferative neoplasms. *Blood*. 2012;119(5):1208–1213.
134. Dey A, Seshasayee D, Noubade R, et al. Loss of the tumor suppressor BAP1 causes myeloid transformation. *Science*. 2012;337(6101):1541–1546.
135. Tefferi A, Guglielmelli P, Lasho TL, et al. CALR and ASXL1 mutations-based molecular prognostication in primary myelofibrosis: an international study of 570 patients. *Leukemia*. 2014;
136. Abdel-Wahab O, Gao J, Adli M, et al. Deletion of Asxl1 results in myelodysplasia and severe developmental defects in vivo. *J. Exp. Med*. 2013;210(12):2641–2659.
137. Yap DB, Chu J, Berg T, et al. Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. *Blood*. 2011;117(8):2451–2459.
138. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2009;462(7274):739–44.
139. Lu C, Ward PS, Kapoor GS, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature*. 2012;483(7390):474–478.
140. Figueroa ME, Abdel-Wahab O, Lu C, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell*. 2010;18(6):553–567.
141. Tefferi A, Lasho TL, Abdel-Wahab O, et al. IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis. *Leukemia*. 2010;24(7):1302–1309.
142. Papaemmanuil E, Cazzola M, Boultonwood J, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N. Engl. J. Med*. 2011;365(15):1384–1395.
143. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature*. 2011;478(7367):64–69.

144. Madan V, Kanojia D, Li J, et al. Aberrant splicing of U12-type introns is the hallmark of ZRSR2 mutant myelodysplastic syndrome. *Nat. Commun.* 2015;6:6042.
145. Shirai CL, Ley JN, White BS, et al. Mutant U2AF1 Expression Alters Hematopoiesis and Pre-mRNA Splicing In Vivo. *Cancer Cell.* 2015;27(5):631–643.
146. Visconte V, Avishai N, Mahfouz R, et al. Distinct iron architecture in SF3B1-mutant myelodysplastic syndrome patients is linked to an SLC25A37 splice variant with a retained intron. *Leukemia.* 2015;29(1):188–195.
147. Rumi E, Passamonti F, Della Porta MG, et al. Familial chronic myeloproliferative disorders: clinical phenotype and evidence of disease anticipation. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* 2007;25(35):5630–5635.
148. Lundberg P, Nienhold R, Ambrosetti A, et al. Somatic mutations in calreticulin can be found in pedigrees with familial predisposition to myeloproliferative neoplasms. *Blood.* 2014;123(17):2744–2745.
149. Rumi E, Harutyunyan AS, Pietra D, et al. CALR exon 9 mutations are somatically acquired events in familial cases of essential thrombocythemia or primary myelofibrosis. *Blood.* 2014;123(15):2416–2419.
150. Harutyunyan AS, Giambruno R, Krendl C, et al. Germline RBBP6 Mutations In Myeloproliferative Neoplasms. *Blood.* 2013;122(21):267–267.
151. Pianta A, Liu K, Lundberg P, et al. Hereditary Thrombocytosis Caused By a Novel Germ-Line Mutation In The Gelsolin Gene. *Blood.* 2013;122(21):265–265.
152. Campbell PJ, Scott LM, Buck G, et al. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. *Lancet.* 2005;366(9501):1945–53.
153. Antonioli E, Guglielmelli P, Pancrazzi A, et al. Clinical implications of the JAK2 V617F mutation in essential thrombocythemia. *Leuk. Off. J. Leuk. Soc. Am. Leuk. Res. Fund U K.* 2005;19(10):1847–9.
154. Rumi E, Pietra D, Ferretti V, et al. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. *Blood.* 2013;
155. Tefferi A, Guglielmelli P, Larson DR, et al. Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. *Blood.* 2014;124(16):2507–2513.
156. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Characteristics and clinical correlates of MPL 515W>L/K mutation in essential thrombocythemia. *Blood.* 2008;112(3):844–7.
157. Guglielmelli P, Pancrazzi A, Bergamaschi G, et al. Anaemia characterises patients with myelofibrosis harbouring Mpl mutation. *Br J Haematol.* 2007;137(3):244–7.
158. Pardananani A, Guglielmelli P, Lasho TL, et al. Primary myelofibrosis with or without mutant MPL: comparison of survival and clinical features involving 603 patients. *Leukemia.* 2011;25(12):1834–1839.
159. Rumi E, Pietra D, Guglielmelli P, et al. Acquired copy-neutral loss of heterozygosity of chromosome 1p as a molecular event associated with marrow fibrosis in MPL-mutated myeloproliferative neoplasms. *Blood.* 2013;121(21):4388–4395.
160. Rotunno G, Mannarelli C, Guglielmelli P, et al. Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocythemia. *Blood.* 2014;123(10):1552–1555.
161. Trifa AP, Popp RA, Cucuianu A, et al. CALR versus JAK2 mutated essential thrombocythaemia – a report on 141 patients. *Br. J. Haematol.* 2014;n/a–n/a.
162. Gangat N, Wassie E, Lasho T, et al. Mutations and thrombosis in essential thrombocythemia: prognostic interaction with age and thrombosis history. *Eur. J. Haematol.* 2014;
163. Tefferi A, Lasho TL, Finke CM, et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. *Leukemia.* 2014;

164. Rumi E, Pietra D, Pascutto C, et al. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. *Blood*. 2014;124(7):1062–1069.
165. Spivak JL, Silver RT. The revised World Health Organization diagnostic criteria for polycythemia vera, essential thrombocytosis, and primary myelofibrosis: an alternative proposal. *Blood*. 2008;112(2):231–9.
166. Guglielmelli P, Lasho TL, Rotunno G, et al. The number of prognostically detrimental mutations and prognosis in primary myelofibrosis: an international study of 797 patients. *Leukemia*. 2014;
167. Guglielmelli P, Biamonte F, Rotunno G, et al. Impact of mutational status on outcomes in myelofibrosis patients treated with ruxolitinib in the COMFORT-II study. *Blood*. 2014;123(14):2157–2160.
168. Rampal R, Ahn J, Abdel-Wahab O, et al. Genomic and functional analysis of leukemic transformation of myeloproliferative neoplasms. *Proc. Natl. Acad. Sci. U. S. A.* 2014;111(50):E5401–5410.