# Myeloproliferative neoplasms – from origins to outcomes

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## Abstract

Substantial progress has been made in our understanding of the pathogenetic basis of myeloproliferative neoplasms. The discovery of mutations in *JAK2* over a decade ago heralded a new age for patient care as a consequence of improved diagnosis and the development of therapeutic JAK inhibitors. The more recent identification of mutations in calreticulin brought with it a sense of completeness, with the vast majority of MPN patients now having a biological basis for their excessive myeloproliferation. We are also beginning to understand the processes that lead to acquisition of somatic mutations, and the factors that influence subsequent clonal expansion and emergence of disease. Extended genomic profiling has established a multitude of additional acquired mutations, particularly prevalent in MF where their presence carries prognostic implications. A major goal is to integrate genetic, clinical and laboratory features to identify patients that share disease biology and clinical outcome such that therapies, both existing and novel, can be better targeted.

## Learning objectives

-To understand the central role of aberrant JAK-STAT signaling in driving the myeloproliferative phenotype.

-To appreciate the factors that may influence the earliest stages of MPN emergence.

-To become familiar with the range of additional mutations in MPNs and their role in disease biology.

## Introduction

The myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders characterized, in chronic phase, by an overproduction of differentiated hematopoietic cells. The Philadelphia-negative MPNs include three main diseases: polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (PMF)[1]. The last decade has witnessed dramatic advances in our understanding of the molecular and cellular basis of excessive myeloproliferation, and this has led to the development and therapeutic use of novel targeted treatments, such as JAK inhibitors. More recently, we have begun to accumulate tantalising insights into the origins of these cancers, as well as the factors that drive later disease evolution.

Here, we review our current understanding of the evolution of MPNs, from their origins to the emergence of disease and clinical progression. Our evolutionary narrative begins with a review of the molecular basis of myeloproliferation in an established neoplastic clone. We then address the factors that influence disease phenotypes in chronic phase and the basis for disease progression, following which we discuss what is known about how clonal populations of MPN cells might initially emerge. We conclude by considering the potential implications for clinical practice.

## Phenotypic driver mutations converge on JAK-STAT signalling

The cardinal and mutually exclusive mutations in MPNs occur in *JAK2, CALR* or *MPL*, referred to herein as the 'phenotypic drivers' due to their role in driving the myeloproliferative phenotype. In 2005, a single point mutation resulting in $JAK2^{V617F}$ was identified in the vast majority of patients with PV and half of those with ET or MF[2–5]. JAK2 is intimately associated with the cytoplasmic portions of receptors for key hematopoietic cytokines, such including erythropoietin (EPO), thrombopoietin (TPO) and granulocyte colony-stimulating-factor (G-CSF). Normal JAK2 functions to activate intracellular signalling pathways following ligand binding, however, $JAK2^{V617F}$ is rendered constitutively active. The mutation is understood to result in loss of the normal inhibitory function provided by the pseudokinase (JH2) domain upon the active (JH1) kinase domain - in this model, it is unclear whether the disrupted JH1/JH2 interface occurs within an individual JAK2 molecule, or between JAK2 dimers[6]. The mutation may also result in direct activation of the JH1 domain via an SH2-JH2 linker[7]. Subsequent downstream activation of intracellular signalling occurs via

Signal Transducer and Activator of Transcription (STAT) protein signalling, and to a lesser extent via mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) signalling pathways, which together result in excessive myeloid cell proliferation and differentiation.

Additional genetic aberrations that perturb JAK-STAT signalling are found in *JAK2*-unmutated MPNs. Mutations in the TPO receptor c-MPL at W515, and less commonly at S505, are found in 5-8% of patients with ET and MF[8]. These mutations are understood to result in conformation changes to the receptor that mimic the consequences of TPO binding, such that cytoplasmic JAK2 molecules are brought into close proximity conducive for activation, transphosphorylation and ligand-independent intracellular signalling[9]. As might be expected from the normal function of MPL, these mutations are associated with megakaryocyte proliferation, and disease phenotypes of ET and MF are recapitulated in murine models[8].

In 2013, mutations in calreticulin (CALR) were identified in the majority of *JAK2*- or *MPL*-unmutated patients with ET or MF[10,11]. *CALR* mutations are insertions or deletions affecting the terminal exon of the gene, which all result in a +1 base-pair shift to the amino-acid reading frame of the DNA sequence, such that the mutant protein acquires a novel C-terminus. The identification of such mutations was surprising since CALR was neither a cytokine receptor, nor a known participant in JAK-STAT signalling. Calreticulin is best known for its house-keeping function as a chaperone protein in the endoplasmic reticulum (ER), where it aids the appropriate folding of client protein molecules prior to their trafficking, either to the cell surface or for extracellular secretion[12]. However, knowledge that c-MPL is an ER client protein and that *CALR* mutations were associated exclusively with only ET or MF (hence, resembling *MPL*-mutated MPNs), suggested the possibility that mutant CALR somehow activates MPL. Consistent with this, mutant-CALR can induce an ET/MF phenotype in a variety of mouse models, but only in the presence of MPL[13–17]. The mechanism by which mutant CALR complexes with MPL to result in its constitutive activation remains unclear. The extracellular domain of MPL, along with both the N-terminal and mutant C-terminal of CALR appear necessary for this interaction, which may be facilitated by the newly acquired positive electrostatic charge within the C-terminus[13,15]. It is unclear why mutant CALR complexes preferentially with MPL and whether mutant-CALR mediated MPL activation occurs following transport from the ER to the cell surface, or if aberrant activation occurs intracellularly.

Uncommon variants in phenotypic drivers also lead to constitutive activation of JAK-STAT signalling, such as mutations in exon 12 of *JAK2* and the recently identified non-canonical variant *MPL*$^{S204P}$ [18–20]. Occasionally, other proteins that participate in JAK-STAT signalling (eg., SH2B3 and CBL) are also mutated in MPNs, although they are less frequently found as an isolated somatic mutation in patients[21,22]. Overall, excepting the ~10% of patients with MF or ET that have as yet undiscovered drivers of their disease[10], aberrant activation of intracellular signalling via EPOR and/or MPL remains central to the development of the MPN phenotype, regardless of whether the phenotypic driver mutation is in *JAK2, CALR or MPL* (Figure 1).

## Relationship between phenotypic drivers and clinical presentation

*CALR*-mutated ET patients display a phenotype that is remarkably similar to those with *MPL* mutations. Both disease subgroups are characterised by isolated thrombocytosis and no gender bias at presentation[23,24]. However, *CALR*-mutated patients are significantly younger at presentation than both *MPL*-mutated and *JAK2*-mutated counterparts. This is reminiscent of the disease characteristics of PV patients with *JAK2*$^{V617F}$ and *JAK2*$^{exon12}$ mutations – those with the latter exhibit more marked erythrocytosis and present at a younger age[18], differences which may reflect differential strengths of aberrant signalling via the EPO receptor (EPOR). By analogy, one can speculate that *CALR* mutations may result in higher levels of MPL signalling compared to those induced by heterozygous mutations in *MPL* or *JAK2*. Such a hypothesis accords with the observation that *CALR*-mutated ET patients have an increased risk of myelofibrotic transformations[25], particularly since excessive signalling via the MPL receptor is associated with bone marrow fibrosis in transgenic mice[26] and increased bone marrow reticulin is seen in ET patients with homozygous *MPL* mutations[27]. This would also be consistent with the observation that *CALR* mutations that result in a more extensive mutant C-terminus (Type 1, 52bp-deletion, L367fs*46) are more prevalent in patients with MF[10,11], and result in a more severe phenotype with frequent MF transformation in a retroviral *CALR*-mutant mouse model[14].

In contrast to *CALR* or *MPL*, mutated *JAK2* is pleiotropic and results in numerous MPN phenotypes also recapitulated in *JAK2*$^{V617F}$ mouse models[28]. A number of factors determine the phenotype that results

following a *JAK2* mutation. First, specific types of *JAK2* mutation, for example, V617I, V617F or mutations in exon 12, are associated with differing degrees of erythrocytosis versus thrombocytosis[18,29]. Recent data demonstrate that this may be due to the differential coupling of certain mutant JAK2 proteins with cytokine receptors[30]. Exon 12 mutation K539I has been shown predominantly to bind EPOR, whereas *JAK2*$^{V617F}$ preferentially complexes with MPL[30]. Secondly, homozygosity for *JAK2*$^{V617F}$ is a key factor in determining the degree of erythrocytosis that subsequently develops, both in patient samples and animal models[31–33]. One can hypothesize that biallelic expression of *JAK2*$^{V617F}$ may be required for the mutant protein to preferentially complex with EPOR, although this is yet to be demonstrated. Whilst clones bearing homozygous *JAK2*$^{V617F}$ can be found as minor subclones in patients with ET, they are clonally dominant in patients with PV[34]. The factors that influence the degree of expansion of homozygous *JAK2*$^{V617F}$ clones are not fully understood, although the order in which *JAK2*$^{V617F}$ is acquired relative to other somatic mutations has been shown to be important[35]. Thirdly, patient specific factors such as iron status, age, gender, coexistent beta-thalassaemia traits, and timing of presentation (relative to the course of the disease in a given patient) may all influence whether a patient harbouring *JAK2*$^{V617F}$ presents with significantly elevated red cell parameters and/or thrombocytosis at diagnosis[36] (Figure 2).

## Genetic interactions influence disease presentation and progression

Several additional genetic interactions shape the molecular, cellular and clinical consequences of phenotypic driver mutations in MPN.

***a. Germline predisposition.*** Constitutional variation that predisposes to MPNs can be classified into two groups: (i) common variants, prevalent in the population, that result in a small predisposition to MPN, and (ii) rare variants, often found in familial MPNs, that have a higher penetrance for disease. Well recognised examples of the former include the *JAK2* 46/1 haplotype, as well as single nucleotide polymorphisms (SNPs) in the gene *TERT,* that confer a ~1.5-3.5 fold increased odds of MPN[37]. Whilst up to 10% of patients are estimated to have additional affected family members, the germline loci responsible for predisposition in familial MPNs have been ascertained in only a handful of pedigrees[38–41]. Two broad mechanisms exist

through which germline variation may impact on MPN incidence. The observation that *JAK2*$^{V617F}$ is often found in *cis* with the 46/1 haplotype in heterozygous individuals, initially raised the possibility that this allele was predisposed to *acquiring JAK2*$^{V617F}$. However, direct evidence to support such DNA hypermutability has, thus far, not been demonstrated. An alternative model contends that certain germline genetic backgrounds are more permissive for an MPN to emerge. Two recent studies provide evidence for the existence of such an interaction. (i) The association between ET and *HBS1L-MYB* SNP rs9376092 is understood to be through reduction in MYB expression, which is a recognised driver of thrombocytosis[42]. (ii) Germline duplication of *ATG2B* and *GSKIP* has been shown to enhance sensitivity of megakaryocyte progenitors to TPO, particularly in the presence of *JAK2*$^{V617F}$, resulting in highly penetrant MPNs within certain families[38]. Given that many additional predisposition loci for MPNs have been recently identified across genes involved in cell senescence (*TERT*), JAK-STAT signalling (*SH2B3*), myeloid differentiation (*GFI1B*), DNA damage and repair (*ATM*, *CHEK2*) and epigenetic regulation (*TET2*), suggests that the germline genetic background of an individual may influence diverse biological cellular functions to accentuate the molecular and cellular consequences of nascent MPN clones following their acquisition of phenotypic driver mutations[43].

***(ii) Additional somatic mutations in MPNs.*** Approximately one third of patients with MPN have additional mutations in known drivers of myeloid malignancies. These mutations alter DNA methylation (*DNMT3A*, *TET2, IDH1/2*), chromatin modifications (*ASXL1*, *EZH2, IDH1/2*), mRNA splicing (*U2AF1*, *SF3B1*, *SRSF2*, *ZRSR2*), and DNA repair (*TP53*)[10]. The presence of certain mutations, such as in *ASXL1*, *SRSF2*, *IDH1*/2 and *EZH2* in patients with MF are associated with an increased risk of leukemic transformation and/or reduced survival[44]. Investigation of the biological effects of these additional mutations reveal common molecular perturbations, as illustrated in the examples below.

Methylation of cytosines at CpG sites is a mechanism through which information establishing stem cell and differentiation states is retained by cells followed cell division. DNMT3A is a *de novo* methyltransferase, and TET2, a member of the TET family of proteins, hydroxymethylates methylcytosines, which may be viewed simplistically as a process of demethylation. Mutations in both genes are prevalent across MPNs, understood to be loss-of-function (or dominant-negative effects for *DNMT3A*$^{R882H}$ [45]), and

result in a hematopoietic stem cell (HSC) advantage in murine models[46–49]. The similarities in loss-of-function consequences despite seemingly opposing functions has recently been reconciled by work demonstrating that DNMT3A cooperates with TET2 to (i) promote the activity of enhancers important in ensuring stem cell fitness through the maintenance of high levels of DNA hydroxymethylation at enhancer centres[50]; and (ii) repress key lineage-specific transcription factors that promote differentiation in hematopoiesis[51]. Mutations in IDH1/2 also affect DNA methylation via the generation of 2-hydroxyglutarate (2HG) that competitively inhibits alpha-KG dependent DNA-hydroxylases, such as TET2 and Jumonji family histone-demethylases[52,53]. Mutant IDH1 has also been linked to increased DNA damage via downregulation of the DNA damage sensor ATM[54]. Knock-in mice for *IDH1*$^{R132H}$ suffer expansion of HSPCs, extramedullary hematopoiesis and anemia[55], in keeping with this mutation being found in advanced phases of MPN.

Perturbation of polycomb repressor complex 2 (PRC2) is another pathogenic mechanism prevalent in MF. EZH2, the enzymatically active subunit of PRC2, compacts chromatin and represses gene transcription via histone H3 (H3K27) trimethylation at lysine 27 (H3K27). In myeloid malignancies, *EZH2* loss-of-function mutations lead to derepression of several target genes, such as the Hox gene family that enhances HSC self-renewal, and Lin28b/ Hmga2 that promote fibrosis and reduce erythropoiesis in a *JAK2*$^{V617F}$ context[56–58]. Loss of EZH2 can also be a consequence of other genetic perturbations, such as loss of heterozygosity at chromosome 7q, as well as mutations in spliceosome components U2AF1 and SRSF2[59,60]. Mutations in ASXL1 are present in up to a quarter of patients with MF, and also leads to reduced PRC2 recruitment, although other additional pathogenic effects have been reported [61–63].

***(iii) Order of mutation acquisition.*** Akin to other cancers, MPNs are clonally heterogeneous reflecting the ongoing interplay between somatic mutation, cellular adaptation to the changing microenvironment and selection of tumor subclones. The sequence of acquisition of somatic mutations can be inferred from the genotypes of detectable subclones. For instance, if some tumor cells have *JAK2*$^{V617F}$, and others from the same patient bear *JAK2*$^{V617F}$ with an additional somatic mutation, then it indicates that *JAK2*$^{V617F}$ came first. By genotyping individual hematopoietic colonies it has been shown that the order of acquisition of *JAK2*$^{V617F}$, relative to mutations in *TET2* or *DNMT3A,* influences subclonal composition within hematopoietic

stem/progenitor cells (HSPC) and mature cell compartments, disease presentation and clinical outcome[35,64]. In *JAK2*-first patients, the HSPC compartment is dominated by double-mutant cells, and such patients present at a younger age, often with PV[35]. Conversely, in *TET2*-first patients, the HSPC compartment is dominated by single-mutant cells, and such patients present at an older age, usually with ET. Studies of mutation order have also revealed that *DNMT3A* mutations, often thought to be early events, frequently occur after an initial JAK2 mutation. This situation is difficult to detect in the absence of clonal assays since only small numbers of JAK2 single-mutant colonies may be present, probably reflecting their out-competition by double-mutant clones elderly. At least 3 mechanisms, not mutually exclusive, may underlie these observations. (i) The first mutation may alter a cell's response to the second mutation. Thus, the transcriptional response to *TET2* is altered by the presence of a prior $JAK2^{V617F}$ mutation[35]. (ii) The first mutation may alter HSC differentiation and give rise to altered target cell populations in which the second mutation can arise. (iii) The first mutation may alter the number and function of mature progeny and thus affect the bone marrow microenvironment. Further studies are required to address which mechanisms are most important in driving the phenotypic differences in patients with different temporal patterns of mutation acquisition, and whether phenotype in chronic phase, or disease progression, are influenced by the order of acquisition of other pairs of common mutations.

## Disease initiation and clonal outgrowth in MPNs

The conventional view of hematopoiesis envisions a self-renewing pool of long-term repopulating HSCs producing terminally differentiated cells via a series of intermediates. However, recent data from mice suggest that steady state hematopoiesis does not routinely rely on HSCs, but predominantly reflects the successive recruitment of thousands of transiently active progenitors[65,66]. Furthermore, there is now compelling evidence for heterogeneity in the differentiation potential of individual cells within the HSC compartment in mice[67–69].

Direct evidence for a clonal stem-cell origin of MPNs came 40 years ago when Adamson and colleagues demonstrated that females with PV who were heterozygous for two glucose-6-phosphate dehydrogenase alleles expressed only one allele in their blood cells[70]. Both $JAK2^{V617F}$ and *CALR*-mutated cells are readily

detectable in immunophenotypically defined HSPCs and across all myeloid lineages, confirming that these mutations arise in cells close to the apex of the hematopoietic hierarchy[10,71]. It is tempting to speculate that following the acquisition of a driver mutation, platelet-biased HSCs might be particularly prone to giving rise to an MPN, but direct evidence for this is currently lacking. In some studies but not others, *JAK2*$^{V617F}$ has been found in the lymphoid compartments of MPN patients raising the possibility that HSCs with differing degrees of lymphoid potential are targets of somatic mutation in different patients[72–76]. However, alternative explanations include the presence of other somatic mutations, and the long-lived nature of mature lymphocytes which means it may be many years before an HSC mutation becomes detectable in lymphocyte populations. Regardless of the uncertainty over the precise nature of the cell(s)-of-origin, for MPNs to emerge requires that (i) these cells acquire phenotypic driver mutations, and (ii) this results in persistent and significant clonal outgrowth.

*(i) Acquisition of mutations in hematopoiesis.* Human cells accumulate somatic mutations throughout their life time as a result of cell-intrinsic mutational processes and exposure to external mutagens. The accumulated DNA changes in a single cell can be viewed as a 'barcode' that can then be used to estimate tissue-specific mutation rates, trace the cell's developmental origins, and understand the nature of DNA damaging processes. Under normal circumstances, these genetic changes are unique to individual cells, making their study possible only through single-cell interrogation techniques. However, the clonal expansion of tumors renders these aberrations detectable in bulk tissue. Study of these mutation spectra in cancer reveal many mutational patterns, so called 'mutation signatures', that in turn inform us of the biological processes that drive mutation acquisition in normal tissues as well as in cancer[77,78].

Compared to solid tumors, hematopoietic malignancies are relatively mutation sparse. A key study by Ley and colleagues demonstrated that the mutation rate in acute myeloid leukemia (AML) was not elevated relative to age-matched hematopoietic progenitors from healthy individuals, suggesting that most mutations present in AML reflect those acquired prior to oncogenic transformation[79]. Two mutational patterns have been recognised to contribute to background mutation acquisition in myeloid cells. The first ('signature 1') is understood to be the consequence of spontaneous deamination of methylcytosines, which

results in an age-associated genome-wide accumulation of C>T transition mutations at CpG dinucleotides[77,79]. The second pattern of mutations (signature 5) exhibits various nucleotide changes at low frequency, but with a small bias towards T>C mutations occurring on the transcribed DNA strand[77]. The aetiology of this mutation signature is not currently known. Both mutational signatures are observed ubiquitously across somatic tissues as well as in the germline[80].

Whether these background mutational processes are sufficient to explain the acquisition of specific oncogenic mutations remains the subject of ongoing research. Two observations suggest that the frequency of acquisition of oncogenic drivers may occur at higher rates than conventionally thought. First, some patients with MPN have been shown to harbour multiple oncogenic driver mutations in ancestrally unrelated clones, suggesting that these mutations can arise multiple independent times in the same individual[81,82]. Second, in a recent study of familial MPN, two-thirds of carriers of a germline duplication (involving *ATG2B* and *GSKIP)* developed an MPN harbouring a somatic mutation in *JAK2*, *CALR* or *MPL*. The germline duplication enhanced the outgrowth of mutant cells, and there was no without co-existent evidence of hypermutability that might otherwise have increased mutation acquisition in these patients[38]. Together these data suggest that *acquisition* of phenotypic driver mutations in cells capable of initiating MPNs might be relatively frequent, but that clones either remain small (and thus undetected in analyses of cell populations), or do not normally survive, unless additional conditions facilitate their clonal expansion.

***(ii) Factors influencing clonal outgrowth of cells with phenotypic driver mutations.*** Approximately half of patients with a *JAK2*$^{V617F}$-mutated MPN in chronic phase lack additional somatic mutations in oncogenic drivers. However, studies in mice suggest that whilst *JAK2*$^{V617F}$ is capable of driving an MPN phenotype, it may be less able to initiate clonal expansion in an individual HSC. Three different knock-in mouse models have shown that *JAK2*$^{V617F}$-HSCs are either no better, or they are less adept than wildtype HSCs in initiating clonal expansion, as assayed by competitive repopulation transplantation assays[28]. Furthermore, transplantation of single *JAK2*$^{V617F}$-HSCs into recipient mice only rarely results in an MPN phenotype[83]. Therefore, additional factors may be required for an initiating *JAK2* mutation to result in an expanded mutant clone. These may include interaction with the constitutional genetic background of a patient, or a

physiological stochastic expansion of individual HSCs that is independent of the driver mutation. Moreover, there is increasing evidence that ageing and the bone marrow microenvironment may play important roles.

Ageing is a strong risk factor for MPN. Recent studies demonstrate that with increasing age clonal hematopoietic expansions occur in the absence of overt haematological disease[84–87]. Mutations leading to clonal hematopoiesis (CH) commonly involve *DNMT3A*, *TET2*, *ASXL1, PPM1D,* but also *JAK2*$^{V617F}$. Interesting, mutations in *CALR* have not yet been reported in CH. This may be a reflection of the inherent challenges in detecting such mutations from standard exome sequencing due to poor sequencing coverage and alignment efficiency over the mutated region. However, it also raises the possibility that *CALR* mutations have a higher penetrance for the development of overt disease. Several observations suggest that CH is not simply a reflection of the increased risk of mutation acquisition with age, but that *ageing* itself favors clonal expansion. First, CH is rare under the age of 40, but increases exponentially in the elderly; a pattern inconsistent with a linear rate of somatic mutation accumulation over time[79]. Secondly, some mutations (eg *SF3B1*) are only associated with CH more advanced decades of life[87]; it would be unlikely that specific mutations are acquired at different rates in different age groups. Thirdly, no known oncogenic drivers can be identified from analysis of whole-exome or whole-genome sequencing in up to half of individuals with CH, suggesting that oncogene-independent mechanisms such as clonal drift or reduced bone marrow genetic diversity, can lead to clonal expansion[86,88]. It is recognised that HSCs undergo a wide range of age-related biochemical and functional changes and it seems plausible that some of these may favor the outgrowth of cells that acquire a driver mutation[89]. Moreover, both cell-intrinsic and environmental influences are likely to play a role.

Several lines of evidence point to the importance of environmental effects. Medical interventions, immune dysregulation and inflammation have all been shown to alter selective pressures for mutant clones within the bone marrow. In *TP53*-mutated therapy-related MDS, *TP53*-mutated cells have been demonstrated to pre-exist at low levels prior to any exposure to therapy, but only outgrow in the context of post-chemotherapy bone marrow[90]. Furthermore, CH is prevalent in the bone marrow of patients with aplastic anemia, where one can envisage that immune-mediated destruction of normal bone marrow cells favors selection for clones more adept at evading cell death, such as those bearing *PIG-A* mutations, deletion of the major histocompatibility locus on chr 6p or mutations in TP53-inhibitor *PPM1D*[91]. There is also

increasing evidence that inflammation has a key role in promoting MPN initiation and influencing disease evolution. The secretion of pro-inflammatory cytokines by bone marrow stromal cells, such as interleukin(IL)-6, IL-33, fibroblast growth factor (FGF), C-X-C motif ligand 10 (CXCL10), IL-33 and tumor necrosis factor alpha (TNFα), have been shown to preferentially promote the expansion of *JAK2*$^{V617F}$-mutated clones[92–94]. Furthermore, elevated levels of NFE-2 (itself induced by inflammatory cytokine IL-1beta), has been shown to drive MPN proliferation and progression through modulation of inflammatory cascades including elevated IL-8 expression[95,96]. Overall, it remains unclear exactly how a single phenotypic driver mutation initiates a clonal expansion that will evolve into an overt MPN, but current evidence suggests that the constitutional genetic background, ageing process, and tumor microenvironment are key factors, with each exerting cell-intrinsic and environmental effects (Figure 3).

## Clinical implications and future directions

The JAK inhibitor Ruxolitinib has been a valuable addition to the therapeutic armamentarium for MPNs, in particular for patients with MF suffering from splenomegaly and/or disease related symptoms[97,98]. However, allogeneic stem cell transplantation (ASCT) still remains the only potentially curative treatment for MPNs, an approach limited by age-related comorbidities and high treatment related mortality. Demonstrable disease modifying activity for conventional therapeutic agents is lacking. Ruxolitinib and Interferon alpha have been associated with reductions in allele-burdens of phenotypic drivers mutations in some cases, but molecular response is variable and unpredictable. Amongst the multitude of novel agents tested in clinical trials, many have shown clinical responses but only in a minority of patients, or there are dose-limiting toxicities[99]. Patient heterogeneity may be a key factor contributing to the lack of demonstrable clinical efficacy for many agents. Therefore, a major challenge is how we use our emerging understanding of the pathogenetic basis of MPNs to identify groups of patients with shared disease biology and clinical outcome, such that both existing therapies, as well as novel agents, can be better targeted to specific patient groups. Novel paradigms for both disease classification and predictions of clinical outcome may be needed to meet this goal.

The 2016 revision to the World Health Organization classification of MPNs retains the traditional distinction between PV, ET and MF, and distinguishes between pre-fibrotic and fibrotic MF[1]. However, such a conceptual schema has significant weaknesses. First, there is a fundamental challenge associated with defining diseases as discrete entities on the basis of continuous variables, such as hemoglobin level or amount of bone marrow fibrosis[100]. Secondly, there is emphasis on the identification of cytological nuances (especially megakaryocyte morphology) that are subjective in their interpretation and which have been shown to have poor inter-observer consensus[101–103]. Thirdly, despite the importance of somatic mutations, there remains substantial molecular overlap between, and heterogeneity within, MPN categories[10]. Furthermore, there is significant overlap between the spectrum of mutations found in MPN and myelodysplasia (MDS), and it is unclear whether patients with MF who lack mutations in *JAK2, CALR* or *MPL*, have disease biology more akin to MDS than MPN. Accurate classification is critical as it predetermines the therapeutic strategy employed by clinicians. Therefore, there is a rationale for large-scale sequencing studies that incorporate patients with MPN, MDS and related myeloid conditions, combined with clinical and laboratory parameters, in order to provide a more biological basis for the way we classify these disorders in the future.

Another major goal for the future is to identify, at an early stage, those patients at risk of a poor outcome. The International Prognostic Scoring System (IPSS) [104] has been valuable when considering the timing of ASCT in patients with MF. However, there are several ongoing challenges. First, current prognostic scoring systems apply to patients with MF, and there are no predictive models for use in patient with chronic phase MPN. Secondly, multiple scoring systems are required for patients with MF, depending on whether risk stratification is performed at diagnosis or later[105], whether MF is primary or post-ET/post-PV[106–108], and the age of the patient[105]. Thirdly, both the IPSS and Dynamic IPSS (DIPSS) rely on potentially subjective scores for the presence or absence of constitutional symptoms. Recently, patient age together with *JAK2*, *CALR* and *MPL* status, have been used to build a simple yet objective predictive model for patients with MF[109]. However, such a model does not take into account the presence of additional somatic mutations that strongly associate with outcome[110]. Comprehensive sequencing of patients, combined with clinical parameters and longitudinal follow up, are now required in order to elucidate how the various genetic and

non-genetic factors contribute to the different possible outcomes for MPN patients. Such a 'knowledge bank' approach has been developed in AML[111], and has the potential to deliver a universal and clinically relevant model for predicting outcome for all patients with MPN.

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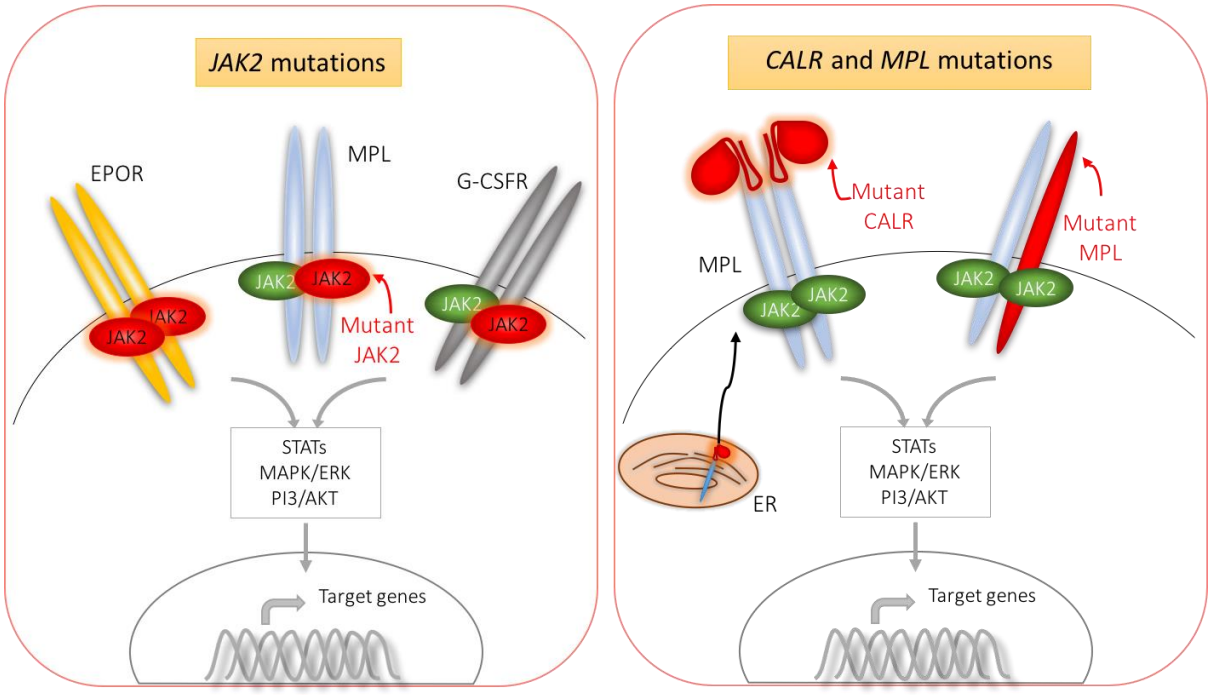


**Figure 1. Mutations in *JAK2*, *CALR* and *MPL* drive excessive myeloproliferation via constitutively active signalling downstream of JAK2.** JAK2 associates with the cytoplasmic portion of a variety of receptors, such as those for erythropoietin (EPOR), thrombopoietin (MPL) and granulocyte/macrophage colony-stimulating factor (G-CSFR). JAK2 is also activated in response to additional cytokines (eg., growth hormone and interleukin-5) (not shown). **Left panel**. Mutant JAK2, shown in red, is constitutively active and leads to variable levels of erythroid, megakaryocytic and to a lesser degree granulocytic proliferation and differentiation. It is unclear whether mutant JAK2 dimerises with mutant or wildtype JAK2 with respect to the individual receptors. **Right panel.** Mutations in *CALR* and *MPL* result in aberrant activation of signalling downstream of the MPL receptor. Mutant CALR complexes with MPL in the endoplasmic reticulum (ER). Both mutations in CALR and MPL result in receptor dimerization and activation of JAK2. STATs, Signal Transducer and Activator of Transcription; MAPK/ERK, mitogen-activated protein kinases/extracellular signal-regulated kinases; PI3/AKT, phosphoinositide 3-kinase/serine/threonine kinase Akt.

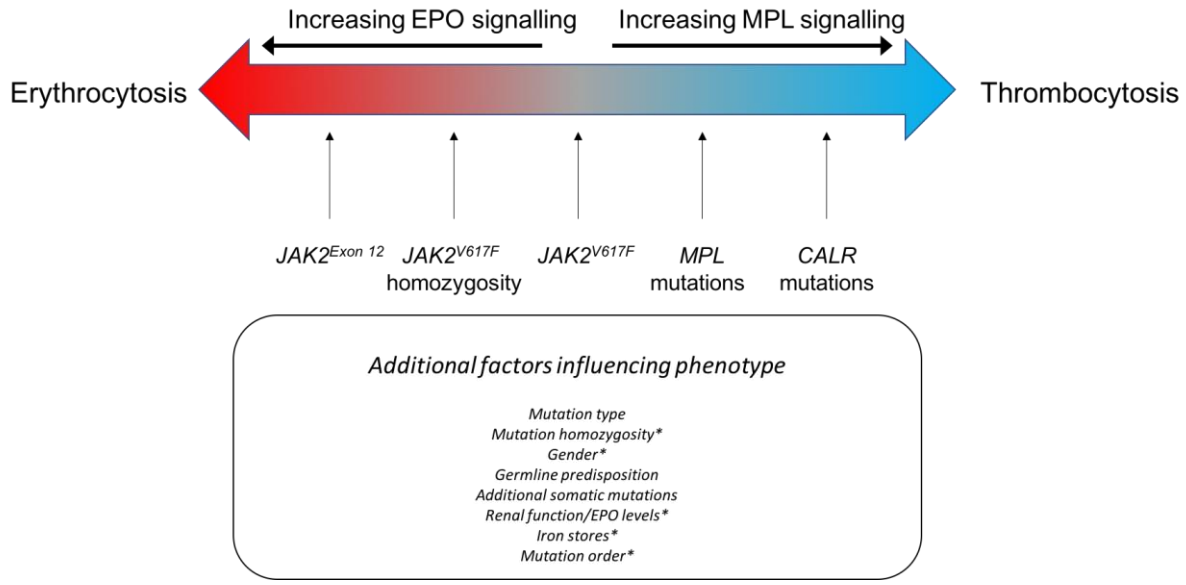


**Figure 2. Clinical presentation in chronic phase and relationship to phenotypic driver mutation.** PV and ET are modelled as a disease spectrum along a biological continuum where different genetic lesions skew the clinical phenotype from that of thrombocytosis to that of additional erythrocytosis (+/- leucocytosis). *CALR* mutations result in excessive MPL signalling, in a manner similar to that resulting from *MPL* mutations. *JAK2* mutations signal downstream of multiple cell surface receptors including MPL, and are thus associated with thrombocytosis but also erythrocytosis and leucocytosis. The exact nature of the phenotypic driver mutation, germline genetic background and additional somatic mutations influence disease phenotype. *In the context of $JAK2^{V617F}$, specific factors modulate the balance between erythrocytosis and thrombocytosis, such as gender, mutation homozygosity, and patient specific factors such as erythropoietin (EPO) levels, renal function and iron status.

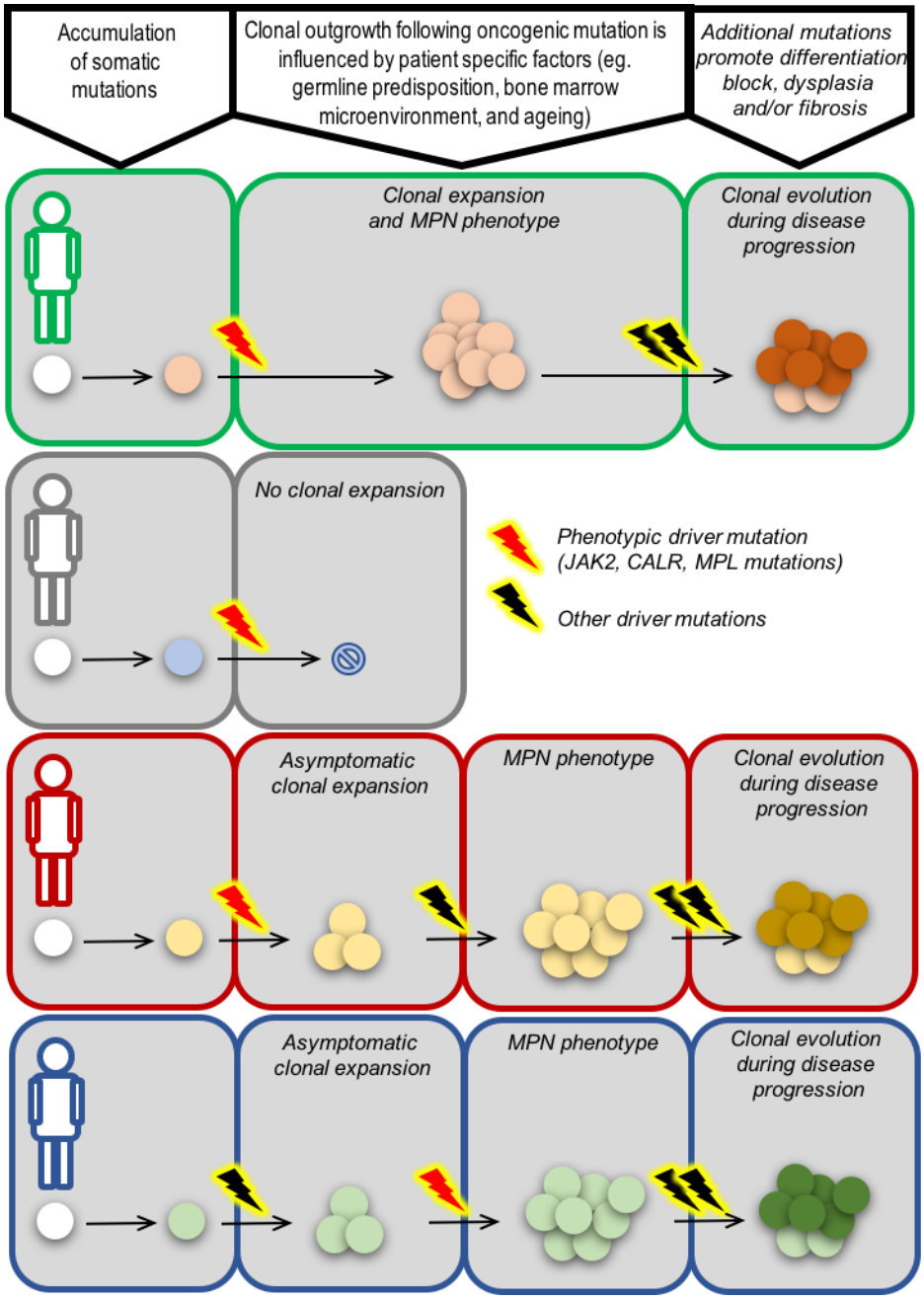


**Figure 3. From origins to outcomes.** Different evolutionary paths to MPN and disease progression in four patients, each with their unique genetic background (green, grey, red, blue). In the first patient (green), a phenotypic driver mutation is acquired in a hematopoietic stem cell (HSC) results in clonal expansion and the emergence of an MPN phenotype as a consequence of favourable cell-intrinsic and/or environmental factors. The MPN in this context has no additional oncogenic driver mutations, as is common for patients in chronic phase. Additional driver mutations, such as those that perturb polycomb repressor 2 function (*EZH2*, *ASXL1* mutations), spliceosome components (*SRSF2*, *SF3B1*, *U2AF1*), or DNA damage repair (*TP53*), can lead to cells gaining a further clonal advantage and disease progression. In the second patient (grey), the cell-

intrinsic and/or environmental context is not favourable, and a cell acquiring a phenotypic driver mutation does not have a clonal advantage relative to competing normal cells. In some circumstances, a phenotypic driver mutation may be insufficient to result in abnormal blood counts and an overt MPN but can instead result in a clonal expansion. Additional mutations or cell-extrinsic changes may be required to result in emergence of disease (patient in red). Finally, in some patients, phenotypic driver mutations may not be the first event. Clonal hematopoiesis as a result of mutations in, for example, *TET2*, *DNMT3A*, *ASXL1* may be the required backdrop for a phenotypic driver mutation to result in an overt MPN (patient in blue).

References


1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127(20):2391–405.

2. 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.

3. 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.

4. 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.

5. 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.

6. Silvennoinen O, Hubbard SR. Molecular insights into regulation of JAK2 in myeloproliferative neoplasms. Blood 2015;125(22):3388–92.

7. Leroy E, Dusa A, Colau D, et al. Uncoupling JAK2 V617F activation from cytokine-induced signalling by modulation of JH2 αC helix. Biochem J 2016;473(11):1579–91.

8. 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.

9. Varghese LN, Defour J-P, Pecquet C, Constantinescu SN. The Thrombopoietin Receptor: Structural Basis of Traffic and Activation by Ligand, Mutations, Agonists, and Mutated Calreticulin. Front Endocrinol 2017;8:59.

10. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med 2013;369(25):2391–405.

11. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med 2013;369(25):2379–90.

12. 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):651–66.

13. Chachoua I, Pecquet C, El-Khoury M, et al. Thrombopoietin receptor activation by myeloproliferative neoplasm associated calreticulin mutants. Blood 2016;127(10):1325–35.

14. Marty C, Pecquet C, Nivarthi H, et al. Calreticulin mutants in mice induce an MPL-dependent thrombocytosis with frequent progression to myelofibrosis. Blood 2016;127(10):1317–24.

15. Elf S, Abdelfattah NS, Chen E, et al. Mutant Calreticulin Requires Both Its Mutant C-terminus and the Thrombopoietin Receptor for Oncogenic Transformation. Cancer Discov 2016;6(4):368–81.

16. Shide K, Kameda T, Yamaji T, et al. Calreticulin mutant mice develop essential thrombocythemia that is ameliorated by the JAK inhibitor ruxolitinib. Leukemia 2017;31(5):1136–44.

17. Balligand T, Achouri Y, Pecquet C, et al. Pathologic activation of thrombopoietin receptor and JAK2-STAT5 pathway by frameshift mutants of mouse calreticulin. Leukemia 2016;30(8):1775–8.

18. 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.

19. Milosevic Feenstra JD, Nivarthi H, Gisslinger H, et al. Whole-exome sequencing identifies novel MPL and JAK2 mutations in triple-negative myeloproliferative neoplasms. Blood 2016;127(3):325–32.

20. Cabagnols X, Favale F, Pasquier F, et al. Presence of atypical thrombopoietin receptor (MPL) mutations in triple-negative essential thrombocythemia patients. Blood 2016;127(3):333–42.

21. 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):904–8.

22. Lasho TL, Pardanani A, Tefferi A. LNK mutations in JAK2 mutation-negative erythrocytosis. N Engl J Med 2010;363(12):1189–90.

23. 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 2014;123(10):1544–51.

24. 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):1552–5.

25. Al Assaf C, Van Obbergh F, Billiet J, et al. Analysis of Phenotype and Outcome in Essential Thrombocythemia with CALR or JAK2 mutations. Haematologica 2015;

26. Villeval JL, Cohen-Solal K, Tulliez M, et al. High thrombopoietin production by hematopoietic cells induces a fatal myeloproliferative syndrome in mice. Blood 1997;90(11):4369–83.

27. 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):4388–95.

28. Li J, Kent DG, Chen E, Green AR. Mouse models of myeloproliferative neoplasms: JAK of all grades. Dis Model Mech 2011;4(3):311–7.

29. Mead AJ, Chowdhury O, Pecquet C, et al. Impact of isolated germline JAK2V617I mutation on human hematopoiesis. Blood 2013;121(20):4156–65.

30. Yao H, Ma Y, Hong Z, et al. Activating JAK2 mutants reveal cytokine receptor coupling differences that impact outcomes in myeloproliferative neoplasm. Leukemia 2017;

31. 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.

32. Li J, Kent DG, Godfrey AL, et al. JAK2V617F homozygosity drives a phenotypic switch in myeloproliferative neoplasms, but is insufficient to sustain disease. Blood 2014;123(20):3139–51.

33. 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):1627–35.

34. 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):2704–7.

35. Ortmann CA, Kent DG, Nangalia J, et al. Effect of mutation order on myeloproliferative neoplasms. N Engl J Med 2015;372(7):601–12.

36. Grinfeld J, Godfrey AL. After 10 years of JAK2V617F: Disease biology and current management strategies in polycythaemia vera. Blood Rev 2017;31(3):101–18.

37. Rumi E, Cazzola M. Advances in understanding the pathogenesis of familial myeloproliferative neoplasms. Br J Haematol 2017;

38. Saliba J, Saint-Martin C, Di Stefano A, et al. Germline duplication of ATG2B and GSKIP predisposes to familial myeloid malignancies. Nat Genet 2015;47(10):1131–40.

39. Harutyunyan AS, Giambruno R, Krendl C, et al. Germline RBBP6 Mutations In Myeloproliferative Neoplasms. Blood 2013;122(21):267–267.

40. Loscocco GG, Mannarelli C, Pacilli A, et al. Germline transmission of LNKE208Q variant in a family with myeloproliferative neoplasms. Am J Hematol 2016;91(9):E356–E356.

41. Rumi E, Harutyunyan AS, Pietra D, et al. *LNK* mutations in familial myeloproliferative neoplasms. Blood 2016;128(1):144–5.

42. 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.

43. Hinds DA, Barnholt KE, Mesa RA, et al. Germ line variants predispose to both JAK2 V617F clonal hematopoiesis and myeloproliferative neoplasms. Blood 2016;128(8):1121–8.

44. Vannucchi AM, Lasho TL, Guglielmelli P, et al. Mutations and prognosis in primary myelofibrosis. Leukemia 2013;27(9):1861–9.

45. 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):442–54.

46. Challen GA, Sun D, Jeong M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet 2012;44(1):23–31.

47. Guryanova OA, Lieu YK, Garrett-Bakelman FE, et al. Dnmt3a regulates myeloproliferation and liver-specific expansion of hematopoietic stem and progenitor cells. Leukemia 2016;30(5):1133–42.

48. 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):2620–5.

49. 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):304–15.

50. Rinaldi L, Datta D, Serrat J, et al. Dnmt3a and Dnmt3b Associate with Enhancers to Regulate Human Epidermal Stem Cell Homeostasis. Cell Stem Cell 2016;19(4):491–501.

51. Zhang X, Su J, Jeong M, et al. DNMT3A and TET2 compete and cooperate to repress lineage-specific transcription factors in hematopoietic stem cells. Nat Genet 2016;48(9):1014–23.

52. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 2009;462(7274):739–44.

53. 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):474–8.

54. Inoue S, Li WY, Tseng A, et al. Mutant IDH1 Downregulates ATM and Alters DNA Repair and Sensitivity to DNA Damage Independent of TET2. Cancer Cell 2016;30(2):337–48.

55. Sasaki M, Knobbe CB, Munger JC, et al. IDH1(R132H) mutation increases murine haematopoietic progenitors and alters epigenetics. Nature 2012;488(7413):656–9.

56. Sashida G, Wang C, Tomioka T, et al. The loss of Ezh2 drives the pathogenesis of myelofibrosis and sensitizes tumor-initiating cells to bromodomain inhibition. J Exp Med 2016;213(8):1459–77.

57. Yang Y, Akada H, Nath D, Hutchison RE, Mohi G. Loss of Ezh2 cooperates with Jak2V617F in the development of myelofibrosis in a mouse model of myeloproliferative neoplasm. Blood 2016;127(26):3410–23.

58. Shimizu T, Kubovcakova L, Nienhold R, et al. Loss of Ezh2 synergizes with JAK2-V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis. J Exp Med 2016;213(8):1479–96.

59. Khan SN, Jankowska AM, Mahfouz R, et al. Multiple mechanisms deregulate EZH2 and histone H3 lysine 27 epigenetic changes in myeloid malignancies. Leukemia 2013;27(6):1301–9.

60. Kim E, Ilagan JO, Liang Y, et al. SRSF2 Mutations Contribute to Myelodysplasia by Mutant-Specific Effects on Exon Recognition. Cancer Cell 2015;27(5):617–30.

61. 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):2641–59.

62. Li Z, Zhang P, Yan A, et al. ASXL1 interacts with the cohesin complex to maintain chromatid separation and gene expression for normal hematopoiesis. Sci Adv 2017;3(1):e1601602.

63. Dey A, Seshasayee D, Noubade R, et al. Loss of the tumor suppressor BAP1 causes myeloid transformation. Science 2012;337(6101):1541–6.

64. Nangalia J, Nice FL, Wedge DC, et al. DNMT3A mutations occur early or late in patients with myeloproliferative neoplasms and mutation order influences phenotype. Haematologica 2015;100(11):e438-442.

65. Sun J, Ramos A, Chapman B, et al. Clonal dynamics of native haematopoiesis. Nature 2014;514(7522):322–7.

66. Busch K, Klapproth K, Barile M, et al. Fundamental properties of unperturbed haematopoiesis from stem cells in vivo. Nature 2015;518(7540):542–6.

67. Benz C, Copley MR, Kent DG, et al. Hematopoietic stem cell subtypes expand differentially during development and display distinct lymphopoietic programs. Cell Stem Cell 2012;10(3):273–83.

68. Sanjuan-Pla A, Macaulay IC, Jensen CT, et al. Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy. Nature 2013;502(7470):232–6.

69. Yamamoto R, Morita Y, Ooehara J, et al. Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. Cell 2013;154(5):1112–26.

70. 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):913–6.

71. 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):6224–9.

72. 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.

73. Pardanani A, Lasho TL, Finke C, et al. Extending Jak2V617F and MplW515 mutation analysis to single hematopoietic colonies and B and T lymphocytes. Stem Cells 2007;25(9):2358–62.

74. 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.

75. Butcher CM, Hutton JF, Hahn U, et al. Cellular origin and lineage specificity of the JAK2V617F allele in polycythemia vera. Blood 2007;109(1):386–7.

76. Larsen TS, Christensen JH, Hasselbalch HC, Pallisgaard N. The JAK2 V617F mutation involves B- and T-lymphocyte lineages in a subgroup of patients with Philadelphia-chromosome negative chronic myeloproliferative disorders. Br J Haematol 2007;136(5):745–51.

77. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature 2013;500(7463):415–21.

78. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013;499(7457):214–8.

79. Welch JS, Ley TJ, Link DC, et al. The origin and evolution of mutations in acute myeloid leukemia. Cell 2012;150(2):264–78.

80. Rahbari R, Wuster A, Lindsay SJ, et al. Timing, rates and spectra of human germline mutation. Nat Genet 2016;48(2):126–33.

81. Beer PA, Jones AV, Bench AJ, et al. Clonal diversity in the myeloproliferative neoplasms: independent origins of genetically distinct clones. Br J Haematol 2009;144(6):904–8.

82. Nangalia J, Nice FL, Wedge DC, et al. DNMT3A mutations occur early or late in patients with myeloproliferative neoplasms and mutation order influences phenotype. Haematologica 2015;haematol.2015.129510.

83. Lundberg P, Takizawa H, Kubovcakova L, et al. Myeloproliferative neoplasms can be initiated from a single hematopoietic stem cell expressing JAK2-V617F. J Exp Med 2014;211(11):2213–30.

84. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014;371(26):2488–98.

85. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med 2014;20(12):1472–8.

86. 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):2477–87.

87. McKerrell T, Park N, Moreno T, et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. Cell Rep 2015;10(8):1239–45.

88. Zink F, Stacey SN, Norddahl GL, et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. Blood 2017;

89. Pang WW, Schrier SL, Weissman IL. Age-associated changes in human hematopoietic stem cells. Semin Hematol 2017;54(1):39–42.

90. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature 2015;518(7540):552–5.

91. Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic Mutations and Clonal Hematopoiesis in Aplastic Anemia. N Engl J Med 2015;373(1):35–47.

92. Fleischman AG, Aichberger KJ, Luty SB, et al. TNFα facilitates clonal expansion of $JAK2^{V617F}$ positive cells in myeloproliferative neoplasms. Blood 2011;118(24):6392–8.

93. Mager LF, Riether C, Schürch CM, et al. IL-33 signaling contributes to the pathogenesis of myeloproliferative neoplasms. J Clin Invest 2015;125(7):2579–91.

94. Manshouri T, Estrov Z, Quintás-Cardama A, et al. Bone marrow stroma-secreted cytokines protect JAK2(V617F)-mutated cells from the effects of a JAK2 inhibitor. Cancer Res 2011;71(11):3831–40.

95. Kaufmann KB, Gründer A, Hadlich T, et al. A novel murine model of myeloproliferative disorders generated by overexpression of the transcription factor NF-E2. J Exp Med 2012;209(1):35–50.

96. Wehrle J, Seeger TS, Schwemmers S, Pfeifer D, Bulashevska A, Pahl HL. Transcription factor nuclear factor erythroid-2 mediates expression of the cytokine interleukin 8, a known predictor of inferior outcome in patients with myeloproliferative neoplasms. Haematologica 2013;98(7):1073–80.

97. 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):787–98.

98. 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.

99. Vannucchi AM, Harrison CN. Emerging treatments for classical myeloproliferative neoplasms. Blood 2017;129(6):693–703.

100. Beer PA, Erber WN, Campbell PJ, Green AR. How I treat essential thrombocythemia. Blood 2011;117(5):1472–82.

101. Buhr T, Hebeda K, Kaloutsi V, Porwit A, Van der Walt J, Kreipe H. European Bone Marrow Working Group trial on reproducibility of World Health Organization criteria to discriminate essential thrombocythemia from prefibrotic primary myelofibrosis. Haematologica 2012;97(3):360–5.

102. Wilkins BS, Erber WN, Bareford D, et al. Bone marrow pathology in essential thrombocythemia: interobserver reliability and utility for identifying disease subtypes. Blood 2008;111(1):60–70.

103. Brousseau M, Parot-Schinkel E, Moles M-P, Boyer F, Hunault M, Rousselet M-C. Practical application and clinical impact of the WHO histopathological criteria on bone marrow biopsy for the diagnosis of essential thrombocythemia versus prefibrotic primary myelofibrosis. Histopathology 2010;56(6):758–67.

104. Cervantes F, Dupriez B, Pereira A, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. Blood 2009;113(13):2895–901.

105. Passamonti F, Cervantes F, Vannucchi AM, et al. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). Blood 2010;115(9):1703–8.

106. Hernández-Boluda J-C, Pereira A, Gómez M, et al. The International Prognostic Scoring System does not accurately discriminate different risk categories in patients with post-essential thrombocythemia and post-polycythemia vera myelofibrosis. Haematologica 2014;99(4):e55–7.

107. Gowin K, Coakley M, Kosiorek H, Mesa R. Discrepancies of applying primary myelofibrosis prognostic scores for patients with post polycythemia vera/essential thrombocytosis myelofibrosis. Haematologica 2016;101(10):e405–6.

108. Passamonti F, Giorgino T, Mora B, et al. A clinical-molecular prognostic model to predict survival in patients with post polycythemia vera and post essential thrombocythemia myelofibrosis. Leukemia 2017;

109. Rozovski U, Verstovsek S, Manshouri T, et al. An accurate, simple prognostic model consisting of age, JAK2, CALR, and MPL mutation status for patients with primary myelofibrosis. Haematologica 2017;102(1):79–84.

110. 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;28(7):1494–500.

111. Gerstung M, Papaemmanuil E, Martincorena I, et al. Precision oncology for acute myeloid leukemia using a knowledge bank approach. Nat Genet 2017;49(3):332–40.