After 10 years of JAK2V617F: Disease biology and current management strategies in polycythaemia vera

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1. Introduction

Polycythaemia vera (PV), one of the Philadelphia-negative myeloproliferative neoplasms (MPNs), is a clonal haematopoietic stem cell disorder characterised by an increased red cell mass, associated with proliferation of erythroid, granulocytic and megakaryocytic elements of the bone marrow [11]. In 2005 the identification of the JAK2V617F mutation elucidated a molecular basis for the disorder in over 95% of patients [2-5], followed two years later by the identification of JAK2 exon 12 mutations in most remaining patients [6,7]. The vast majority of patients with PV therefore carry mutations in a single gene, and a wealth of studies has since illuminated the many molecular and cellular consequences of these mutations.

Whilst JAK2 exon 12 mutations are specific to PV, the JAK2V617F mutation is also found in other myeloid disorders: 50 to 60% of those with essential thrombocythaemia (ET) or primary myelofibrosis (PMF) [2-5], about half of patients with the MDS/MPN entity “refractory anaemia with ring sideroblasts and marked thrombocytosis” (RARS-T) [8], and lower frequencies in AML, other myeloproliferative and myelodysplastic disorders [9,10]. ET and PMF in particular share important clinical features with PV, including the presence of neutrophilia and/or thrombocytosis in some patients, hypercellularity of the bone marrow, frequent splenomegaly, and risks of thrombosis and haemorrhage [1]. In a minority of patients, PV, ET and PMF can all transform into acute myeloid leukaemia (AML) [11-13], those with ET may develop transformation to PV [14], and both ET and PV may transform into secondary myelofibrosis [11,13,14]. The identification of JAK2V617F offered some basis for the clinical similarities between these MPNs, but the molecular mechanisms behind the phenotypic differences between the JAK2V617F-positive disorders has remained the subject of intensive investigation.

The identification of JAK2 mutations in PV led to the introduction of a rapid and non-invasive diagnostic test, with mutation testing now widely available and incorporated into national and international clinical guidelines [1,15-17]. Moreover the development of JAK2 inhibitors has led to a much-needed novel therapeutic avenue for patients with myelofibrosis [18,19], and most recently these drugs have also been shown to benefit certain patients with PV [20]. However, clinical management of PV remains predominantly centred on the reduction of vascular complications and symptoms. In spite of significant advances that have been made in understanding the disease biology of PV, agents that can target the underlying neoplastic clone have remained elusive and the risk of disease transformation, with its almost inevitably poor outcome, remains an important and unmet clinical need.

This review will first examine current understanding of the molecular basis of PV, both in terms of the pathological effects of JAK2 mutations and the factors that may contribute to phenotypic differences between PV and other JAK2-mutated MPNs. Diagnostic criteria and their controversies will be considered. Key prognostic factors in PV will then be examined, followed by a discussion of current management strategies and unresolved clinical challenges.
2. The molecular and cellular effects of JAK2 mutations

JAK2 is one of a family of cytoplasmic tyrosine kinases that mediates signal transduction from cell surface cytokine receptors, including the erythropoietin and thrombopoietin receptors [21]. Binding of ligand to receptor leads to JAK autophosphorylation, receptor transphosphorylation and creation of binding sites for signalling molecules such as STAT proteins. STATs are phosphorylated, translocate to the nucleus and affect target gene transcription. For example, binding of erythropoietin to its receptor (EpoR) causes phosphorylation of JAK2 and STAT5 [22], together with activation of other effectors including MAP kinase and phosphoinositide 3-kinase (PI3K)/Akt pathways [23].

2.1. Biochemical effects of JAK2 mutations

In early studies, the V617F mutation was found to have activating effects on JAK2 signalling activity, cytokine sensitivity and cytokine-dependent survival in cell lines [2–5]. This activation of JAK2 is thought to reflect the location of the mutation within the JH2 “pseudokinase” domain of the protein, which normally has auto-inhibitory effects on the catalytically active JH1 kinase domain [24–27]. Mechanisms may include the interference of the mutation with direct phosphorylation of JH1 by JH2 [28], and cooperation of V617F with other residues in JH2 to enhance JH1 kinase activity [29,30]. Interestingly, recent work on the JAK1 JH2 crystal structure has also suggested that Jak2V617F activation requires a specific conformational change in the SH2-JH2 linker domain [31], which is the area targeted by exon 12 mutations. Exon 12 mutations appear to have similar activating effects to V617F in cell lines, including cytokine independence and constitutive activation of JAK2 and its downstream mediators in the absence of erythropoietin [6].

Several signalling changes appear to mediate the cellular effects of Jak2V617F. Increased phosphorylation of STAT5 has been demonstrated in numerous experimental systems, including cultured erythroid cells and bone marrow trephine biopsies from patients with MPNs, and in Jak2V617F knock-in mouse models [24,32–41]. The particular importance of STAT5 activation in PV is illustrated by the observation that expression of constitutively active STAT5 in erythroid progenitors can drive formation of the same erythropoietin-independent endogenous erythroid colonies that are typical of PV [42]. Moreover it has been shown more recently that Stat5 deletion in either a Jak2V617F knock-in mouse [43] or retroviral bone marrow transplantation model [44] abrogates the marked erythrocytosis that is seen in the presence of Stat5. Other signalling processes that appear to be activated by Jak2V617F include the PI3K/Akt and MAPK/ERK pathways, which have been studied in many experimental systems [25,33,34,36,38,45]. There is evidence that the mutation has direct effects on apoptosis, both through the extrinsic pathway that acts through cell surface death receptors such as Fas [46], and through the intrinsic pathway, with increased levels of the anti-apoptotic protein Bcl-xL and impairment of its normal function in the nucleus and affect target gene transcription. For example, binding of the arginine methytransferase PRMT5 and impairing its histone methylation activity, which may in turn promote growth and erythroid differentiation of haematopoietic cells [59].

There is evidence that Jak2V617F may not only act through intrinsic cellular pathways, but may have important effects through the microenvironment. For example, the mutation has been reported to increase levels of the cytokine TNFα, which is increased in the serum of MPN patients and may paradoxically stimulate growth of JAK2-mutant cells [60]. An important role for the microenvironment was also suggested by two reports that macrophage depletion abrogates erythrocytosis in mouse models of Jak2V617F-driven polycythaemia [61,62].

2.2. Cellular effects of JAK2 mutations

Early studies of the cellular effects of Jak2V617F on haematopoiesis analysed cell populations at different stages of differentiation from primary patient samples, with or without a period of ex vivo culture. These studies were mostly in keeping with an expansion advantage for Jak2V617F-mutant cells at later stages of myeloid differentiation, particularly at low erythropoietin levels and in the presence of homoglycosy for the V617F mutation [63–67]. The effects of JAK2 mutations on haematopoietic cells have been elucidated further using mouse models. Importantly, early work demonstrated that expression of Jak2V617F in retroviral bone marrow transplantation models could cause a myeloproliferative phenotype [2,5], confirming the causal role of the mutation in human MPNs. These models had high, dysregulated levels of Jak2V617F expression, whilst knock-in models allow observation of the phenotypes obtained when the gene is expressed at physiological levels, under the normal regulatory elements of the mouse JAK2 gene. These knock-in models have supported the conclusions from patient studies, showing that Jak2V617F is associated with increased numbers of lineage-restricted progenitors, including those of the megakaryocyte/erythroid and granulocyte/macrophage lineages [38–41,68].

The effects of Jak2V617F on haematopoietic stem cells (HSCs) have been more controversial. Early studies used xenotransplantation experiments, in which CD34+ cells from MPN patients were transplanted into NOD/SCID mice. Whilst these cells could engraft in the mice, the proportion of Jak2V617F-positive cells was frequently reduced in the reconstituted marrow compared to the original cells, arguing against an advantage for Jak2V617F-positive cells over wild-type HSCs [69,70]. In two knock-in models of murine Jak2V617F, an increase in the stem cell-enriched Lin–Sca-1+ Kit+ (LSK) cell compartment was reported [38,68], whilst in another LSK numbers were unchanged [40]. With respect to HSC function, competitive transplantation experiments in one of these models suggested a selective advantage for Jak2V617F-mutant cells in primary transplantation, associated with an increase in HSC proliferation and decrease in apoptosis, but this advantage was not maintained in secondary transplants [68]. Similarly, competitive transplantation experiments in a second model suggested normal early function but a late advantage in primary recipients [40,71], whilst subsequent data have suggested a lack of advantage in secondary recipients [72]. In the one knock-in model with a human Jak2V617F construct there was a quantitative and qualitative HSC defect, with reduced LSK cell numbers and evidence of increased DNA damage, reduced cell cycling, and impaired function in competitive transplantation experiments, which was most marked in cells homozygous for Jak2V617F [39,73]. Further studies of this latter model suggested that the mutation may promote differentiation of HSCs at the expense of self-renewal [74].

Interestingly the concept of a Jak2V617F-induced HSC defect is consistent with other evidence from the human MPNs. For example, allelic burdens for Jak2V617F are low in many patients [64] and may remain stable over years [75], and it was reported that Jak2V617F-positive cells did not expand in the recipient of an allogeneic stem cell transplant from a Jak2V617F-positive donor [76]. Conversely it was reported that a
**JAK2V617F** mutation, found in a family with hereditary thrombocytosis in the absence of additional MPN-associated molecular abnormalities, was associated with increased numbers of phenotypic HSCs and an advantage in xenotransplantation assays [77]. However this mutation also showed qualitative signalling differences to V617F and may be associated with a different HSC phenotype, and moreover the constitutional nature of the V617I variant would obviate the requirement for a competitive advantage in human disease. The concept that oncogenes may not impart a stem cell advantage is also consistent with data from other malignancies. For example, the BCR-ABL fusion gene has been associated with reduced HSC self-renewal in vitro [78], in NOD/SCID transplantation experiments [79] and in secondary transplants from a transgenic mouse model [80], and oncogene-induced senescence is a recognised barrier to malignant progression in certain haematological and non-haematological neoplasms [81,82].

In summary, numerous studies have contributed to a body of knowledge regarding the biochemical and cellular consequences of JAK2 mutations. However, it has remained less clear how the various signalling perturbations contribute to the specific clinical features of MPNs, and which are the other factors that distinguish between the different JAK2-mutant MPN phenotypes. For example, STAT5 activation appears to play an important role in erythropoiesis, the defining feature of PV, since knockdown of Stat5 in JAK2V617F-driven models of erythropoiesis abrogates this aspect of the phenotype [43,44]. By contrast Stat5 deletion did not prevent development of myelofibrosis in a Jak2V617F retroviral bone marrow transplantation model [44]. Moreover studies of haematopoietic cells from MPN patients found that patterns of pSTAT3, pSTAT5, pERK1/2 and pAkt correlated better with MPN subtype rather than with the presence or burden of the JAK2V617F mutation [83], highlighting a potential role for additional molecular mechanisms in these signalling changes.

### 3. Factors determining phenotype in PV vs other JAK2-mutated MPNs

In 2005, analysis of ET patients in the PT1 trial demonstrated that in comparison to JAK2V617F-negative patients, JAK2V617F-positive patients showed higher haemoglobin levels, neutrophil counts, more bone marrow erythropoiesis and granulopoiesis, lower platelet counts, mean corpuscular volume (MCV), serum erythropoietin and ferritin, and more frequent PV transformation [84]. Subsequent studies confirmed these associations [14,85–88], suggesting that JAK2V617F-positive ET has some characteristics of a mild form of PV, as well as a similar incidence of thrombosis [89]. These clinical similarities between PV and JAK2V617F-positive ET are mirrored by certain biological similarities (e.g. a low frequency of driver mutations additional to JAK2V617F [90]) and are in keeping with a model in which the two disorders form a phenotypic continuum [84]. However there are also important differences between PV and JAK2V617F-positive ET, which have prompted speculation and investigation of the factors that push an individual's phenotype towards one or other disorder. These factors include the presence and size of clones homozgyous for the JAK2V617F mutation; the presence of additional mutations and their subclonal hierarchy in relation to JAK2V617F; germline genetic factors; differences in gene expression; and other constitutional factors including age and gender. We discuss these factors in more detail below.

#### 3.1. The role of JAK2V617F homozygosity in PV phenotype

A “homozygous” JAK2V617F sequence (>50% mutant) in granulocyte DNA was originally identified in 25–30% of those with PV, 9–20% with PMF, and 0–3% with ET [2–5]. Clinical studies next investigated how JAK2V617F allele burden, a measure of “gene dosage” for the mutation that is most often measured in granulocyte DNA, correlated with certain clinical features. Initial studies divided PV patients into “homozygous” (<50% mutant allele) and “homozygous” (>50% mutant allele) groups, whilst subsequent studies analysed mutant allele burden as a continuous variable. Together these studies indicated that within PV, higher JAK2V617F allele burdens were associated with higher haemoglobin levels, higher white cell counts and lower platelet counts, together with other features suggesting a “more extreme” PV phenotype (lower MCV, lower serum ferritin and erythropoietin, more splenomegaly, more pruritus and more need for cytoreductive therapy) [13,63,64,91–95]. These data suggested that a higher JAK2V617F allele burden might promote a more “PV-like”, rather than “ET-like”, phenotype. Within ET, higher JAK2V617F allele burdens were associated with some of the same features as in PV (higher white cell counts and more splenomegaly), but in contrast to PV there was an association with higher platelet counts [63,64,88]. This may reflect a limitation of measuring allele burden in bulk granulocyte DNA: the assays did not demonstrate the contribution of JAK2V617F-heterozygous and homozygous cells to the overall mutant allele burden.

Whilst these early patient studies showed a correlation between JAK2V617F allele burden and PV phenotype, mouse models have since been used to establish the causal nature of this relationship. One transgenic mouse model was consistent with the concept that a phenotype of ET or PV could result from lower and higher levels of JAK2V617F expression levels, respectively, suggesting a role for gene dosage in determining MPN phenotype [96]. However the same switch from ET to PV was not seen with increasing gene dosage in other transgenic models [97,98], and all transgenic models are complicated by variable transgene activation between cells.

More recently the development of knock-in mouse models has allowed study of JAK2V617F when expressed at physiological levels under its normal regulatory elements [38–41,68]. Four knock-in models with a heterozygous murine mutant JAK2 allele developed a phenotype consistent with PV with erythropoietin, leucocytosis, splenomegaly, variable thrombocytosis and increased megakaryocyte-erythroid progenitors [38,40,41,68]. One of these studies reported generation of a homozygous-mutant mouse, which showed more pronounced leucocytosis, thrombocytosis and splenomegaly but reduced or unchanged haemoglobin levels compared to heterozygous mice [38]; similar phenotypes were observed in a hemizygous mouse with deletion of the wild-type JAK2 allele [99]. By contrast in a fifth model with a human JAK2V617F allele, mice heterozygous for the mutation showed a moderate thrombocytosis with minimal erythropoiesis, normal spleen size and plasma erythropoietin levels, whilst homozygosity was associated with marked erythropoiesis and reduced platelet counts, significant splenomegaly and suppressed plasma erythropoietin levels. These features closely parallel those of human ET and PV, respectively, and provided direct genetic evidence that homozygosity for human JAK2V617F can be associated with a switch from ET-like disease to a PV-like phenotype [73]. The reasons behind the phenotypic variation between the different knock-in models remain unclear however, and may reflect differences in technical aspects of the targeting strategies or inherent differences in the mutant human and mouse proteins.

Further analysis of the precise role of JAK2V617F homozygosity in human PV has utilised genotyping of individual erythroid colonies grown from MPN patient samples. This approach circumvents the limitations of allele burden studies in bulk granulocyte DNA, by investigating the presence and frequency of homozygosity at the level of single precursors. An early study of BFU-E colonies grown in high erythropoietin conditions found that while virtually all PV and ET patients produced both wild-type and mutant colonies, JAK2V617F-homozygous colonies were detectable in almost all of the PV patients, but in none of the cohort with ET [100]. Subsequent studies largely supported these observations [63,64], but raised the possibility that JAK2V617F homozygous erythroid colonies could be found in a small number of patients with ET [63,64,101], whilst PV patients lacking JAK2V617F homozygous colonies were also described [63,64]. A large study of PV and ET patients then analysed the genotypes of erythroid colonies grown in low erythropoietin conditions, aiming to select for even the
smallest homozygous-mutant clones. This study confirmed that JAK2V617F-homozygous precursors could be identified from approximately 80% of PV patients [102]. A higher proportion of JAK2V617F-homozygous erythroid precursors, relative to heterozygous precursors, was associated with more pronounced PV-like clinical features, again supporting a causal role for homozygosity in this phenotype [103]. However small homozygous-mutant clones could also be identified reproducibly in approximately half of ET patients [102]. Moreover many patients with PV and ET were shown to have acquired JAK2V617F homozygosity on multiple occasions in independent subclones, but PV was distinguished by the expansion of one dominant homozygous-mutant subclone.

Together these data have demonstrated that in the 80% of PV patients with significant JAK2V617F-homozygous clones, these may have a causal role in the PV phenotype. However mitotic recombination occurs at the JAK2 locus remarkably frequently; this observation remains unexplained, although JAK2V617F itself has also been associated with increased homologous recombination and DNA replication stress [54, 56]. Moreover acquisition of homozygosity is not sufficient to drive a phenotype of PV, but the important step appears to be whether a homozygous-mutant clone expands sufficiently to impact on the disease phenotype. Further work has aimed to investigate what factors may drive the expansion of individual subclones, together with the additional factors that may influence phenotype in the presence of a particular balance of JAK2V617F-homozygous and -heterozygous subclones.

3.2. Other mutations and PV phenotype

Since the identification of JAK2V617F, a range of other mutations have been identified in MPNs. Mutations in CALR, MPL and LNK are found predominantly in JAK2-unmutated MPNs and seem likely to have parallel effects on cellular signalling that drive the specific MPN phenotype. By contrast many of the other mutations (Table 1) are found in other myeloid malignancies as well as MPNs and seem likely to contribute to disease through less specific effects on HSC survival or self-renewal [104]. Amongst the MPNs these mutations tend to be commonest in myelofibrosis and/or blast-phase disease with low frequencies in PV, but nonetheless they are candidates for driving expansion of JAK2-mutant subclones in PV and ET. For example, TET2 and DNMT3A mutations have been identified patients with PV who lack JAK2V617F-homozygous precursors [105,106]. These patients tend to have particularly large homozygous-mutant subclones, perhaps accounting for the development of sufficient erythrocytosis to meet a diagnosis of PV rather than ET [63,102]. It is likely that these additional mutations may contribute to the phenomenon of “clonal dominance”, a description used for patients with a similar JAK2V617F allele burden in CD34+ progenitor cells compared to neutrophils [64]. This feature is particularly seen in myelofibrosis but is also commoner in patients with PV compared to those with ET, the latter tending to show lower JAK2V617F allele burdens in CD34+ progenitor cells compared to more mature granulocytes [107].

A recent finding is that it is not only the combination of mutations that is important in determining subclonal balance and phenotype, but also the order of their acquisition. In a series of patients carrying both JAK2 and TET2 mutations, acquisition of mutant JAK2 prior to TET2 was more likely to be associated with a phenotype of PV than ET, and as expected this correlated with a higher proportion of JAK2V617F-homozygous colonies [108]. The double-mutant clone was larger in these patients at the level of haematopoietic stem and progenitor cells (HSPCs), in contrast to the “TET2-first” group in which TET2-single-mutant HSPCs predominated, and “JAK2-first” patients also had a higher risk of thrombosis. Certain other mutations may not only promote the expansion of JAK2-mutant clones but may also contribute directly to PV phenotype. An example is that of truncating NF-E2 mutations that were identified in a small number of patients with JAK2V617F-positive PV, as well as some with myelofibrosis [109]. These mutations are associated with a proliferative advantage in haematopoietic colony assays and cell lines. Moreover a murine bone marrow transplantation model showed erythrocytosis, thrombocytosis and neutrophilia in the presence of an NF-E2 mutation, but the combination with JAK2V617F led to an even more pronounced erythrocytosis than JAK2V617F alone, suggesting a cooperative effect on phenotype.

The factors contributing to clonal balance are frequently unclear however. There is evidence to suggest that in some patients, additional mutations may not account for differential subclonal expansion. In two PV patients with multiple JAK2V617F-homozygous subclones, an additional “driver” mutation was identified by exome sequencing in the dominant homozygous-mutant subclone [110]. However these mutations were not specific to the dominant subclone and were each present in at least one minor homozygous-mutant subclone. These findings raise the possibility that in some patients, stochastic mechanisms may account for the relative sizes of JAK2-mutant clones and therefore for differences in phenotype.

3.3. Germline factors in the phenotype of JAK2-mutated MPNs

Germline JAK2 mutations affecting V617 (V617F) [77] and other residues outside the pseudokinase domain [111,112] have recently been reported to cause hereditary thrombocytosis, and all have been suggested to have weaker consequences for JAK2 function than V617F [77,111,112]. Equivalent mutations have not been identified in patients with acquired ET or PV, however.

Germline factors are important in genetic predisposition to acquired MPNs: a specific constitutional JAK2 haplotype, designated 46/1, is strongly associated with the development of JAK2V617F-positive MPNs [113,114], as well as with JAK2 exon 12-mutated and MPL-mutated disease [115–117]. These findings suggested that either the specific haplotype is associated with an increased risk of developing a pathogenic JAK2 mutation, or that the acquisition of a mutation is more likely to lead to clinical disease in the presence of the 46/1 haplotype [118]. A small study of PV patients suggested that homozygosity for the 46/1 haplotype was associated with a greater likelihood of increasing allele burden over time [119], raising the possibility that this genotype may promote the development or expansion of JAK2V617F-homozygous clones. A study in the Chinese population also suggested that the 46/1 haplotype is associated with a higher haemoglobin and white cell count at diagnosis in ET and PMF, and with higher platelet counts in PMF [120], although other studies have not found associations with clinical features [118].

More recently, extensive genome-wide association studies have identified a number of other novel single-nucleotide polymorphisms (SNPs) associated with MPNs. A germline variant in the TERT gene was associated with MPNs but not with disease phenotype or with the presence of the JAK2V617F mutation [121,122], and recently additional variants related to the TET2 and SH2B3 gene loci have been described...
A particularly interesting observation was that a SNP located between the HBS1L and MYB genes showed an association with CALR- and MPL-mutant MPNs, but within JAK2-mutated patients showed a significantly different allele frequency between ET and PV [122]. The risk allele was found to specifically increase the risk of ET but not PV and was associated with significantly lower MYB expression in haematopoietic colonies; moreover MYB-knockdown mice show a transplantable ET-like disease [124]. These findings, together with other studies [125–127], suggest a potentially important role of germline factors in determining disease phenotype within JAK2-mutant MPNs.

3.4. Differential gene expression in JAK2-mutant PV and ET

A number of studies have investigated differences in gene expression between the different MPNs. One novel approach was to use clonally-derived erythroid cells from patients with PV and ET, in each case comparing JAK2V617F-heterozygous mutant cells to a wild-type sample from the same patient and thus controlling for inter-individual variation in gene expression [128]. In ET but not in PV, JAK2-mutant cells showed upregulation of interferon target genes compared to wild-type cells, associated with increased activation of pSTAT1, which was absent in PV patients. Functional experiments suggested that differential activation of STAT1 could contribute to altered megakaryocytic and erythroid differentiation. These findings are also consistent with data from a JAK2V617F transgenic mouse model, in which loss of Stat1 resulted in more marked erythrocytosis and reduction of thrombocytopoiesis compared to normal Stat1 levels [129]. Interestingly this study found that serum interferon-gamma levels were increased in the mice with thrombocytopoiesis and also in patients with ET, with lower levels in those with PV. These findings raise the possibility that varying levels of interferon-gamma might account for differences in signalling seen between PV and ET patients. However, the molecular explanation for these data in patients remains elusive and it is unclear if this phenomenon may relate to acquired or constitutional differences in interferon biology.

Within PV, a microarray-based study of CD34+ peripheral blood cells suggested that gene expression could be used to differentiate between two groups of PV patients who also showed differences in disease duration, haemoglobin level, thromboembolic events, splenomegaly, exposure to chemotherapy, leukemic transformation and survival [130]. These findings were independent of JAK2V617F allele burden and raise the possibility that differences in gene expression between patients with PV may contribute to phenotypic heterogeneity, although the genetic or epigenetic mechanisms are uncertain. Dereguulation of microRNA expression has also been implicated in the biology of PV [131] and a small study suggested that perturbations in microRNA expression may also differ between PV and ET patients [132].

In summary, a number of factors are likely to play a role in determining a precise phenotype PV or ET in the presence of a JAK2 mutation. There are likely to be many other germline genetic influences. For example, we have studied two patients with beta-thalassaemia trait in whom colony assays have revealed large JAK2V617F-homozgyous clones, typical of PV (unpublished data). However in both cases a phenotype of ET is evident clinically, a phenomenon that seems likely to reflect a constraining effect of the beta-globin abnormalities on the development of erythrocytosis. Age may influence the phenotype obtained and gender may be particularly important, possibly through modulating the phenotypic effects of large JAK2V617F-homozgyous clones [103,133]. It has also been reported that gender may be associated with additional phenotypic features in PV including age of presentation, presence of splenomegaly and thrombotic events [133,134]. Genome-wide association studies in normal individuals have identified a number of genetic loci associated with full blood count parameters [135–138], confirming for example that variants in genes related to iron metabolism may influence haemoglobin levels [139,140]. Given that haemoglobin and platelet levels are polygenic traits in the normal population, they are likely to be similarly influenced by genetic determinants in patients with MPNs. The timing of presentation to medical care and institution of therapy may also be important, since many PV patients show a thrombocytopoiesis during the evolution of their disease and could gain a diagnostic label of ET if they receive cytoreduction before the full PV phenotype is allowed to develop [141]. Together all of these data support a model in which JAK2-mutant PV and ET form a biological continuum, with the precise phenotype determined by a range of discrete and continuous variables (Fig. 1).

3.5. JAK2 exon 12 mutations in PV

Over 20 different mutations in exon 12 of JAK2 have been reported in PV but have not been described in ET. Exon 12 mutations tend to be associated with a particular clinical phenotype: compared to PV patients with JAK2V617F, patients are younger, with higher haemoglobin concentrations, lower white cell and platelet counts, and isolated bone marrow erythroid hyperplasia without granulocytic or megakaryocytic morphological abnormalities [6,142]. In a series of 106 patients with exon 12 mutations, 64% presented with isolated erythrocytosis, with 15% having additional leucocytosis, 12% additional thrombocytopoiesis and 9% both, with a similar incidence of thrombosis, myelofibrosis, acute leukaemia and death to those with JAK2V617F [142]. By contrast to JAK2V617F-positive PV, patients tend to carry predominantly heterozygous-mutant clones, with small homozygous-mutant clones detectable in 40–50% of patients (similar to ET) [102].

The mechanism for the marked, and relatively isolated, erythrocytosis seen in patients with JAK2 exon 12 mutations is not entirely clear. Some in vitro studies [6], but not others [143], have suggested that these mutations might be associated with more marked activation of JAK2 than V617F. Expression profiling has demonstrated that STAT1 activation is preserved in the presence of exon 12 mutations and that attenuated STAT1 signalling is therefore not the explanation for erythrocytosis in these patients [144].

3.6. Factors contributing to disease transformation from PV: myelofibrosis and acute myeloid leukaemia

Whilst JAK2V617F is also found in 50 to 60% of those with PMF, it appears likely that additional factors contribute to this more complex, heterogeneous phenotype. Transformations of ET and PV to secondary myelofibrosis are well recognised and patients diagnosed with “primary” myelofibrosis may show evidence of a prior undiagnosed chronic-phase MPN [145], supporting the concept of myelofibrosis as an accelerated phase of disease. It is clear that additional genetic lesions are important. Exome sequencing studies have demonstrated a higher average number of somatic mutations in patients with myelofibrosis compared to those with PV or ET [90], and consistent with this, most of the other recurrent mutations identified in chronic-phase MPNs are also commonest in PMF (Table 1). Moreover the spectrum of genetic lesions in PMF shows increasing overlap both with secondary AML and with poor prognostic subgroups of other myeloid malignancies. Many of the genes affected are predicted to alter DNA and/or histone modifications through a variety of mechanisms, or to affect the RNA splicing machinery.

The role of JAK2V617F in progression of chronic-phase PV to AML is particularly complex. In individuals in whom JAK2V617F is detectable in the leukaemic blasts, the mutation may have contributed to disease evolution by causing increased DNA damage [54] and an impaired apoptotic response to DNA damage [49]. Other patients with JAK2V617F-positive chronic MPNs may however develop JAK2V617F-negative AML [146,147]. A variety of molecular lesions have been implicated to have a specific role in leukaemic transformation, including mutations in TP53, RUNX1, c-CBL, IKZF1, NRAS and KRAS [104,147,148].
4. Diagnostic criteria for PV

Clinical assessment of a patient with suspected PV should aim to identify possible alternative causes of erythrocytosis. Examples include secondary erythrocytosis associated with respiratory disease, renal disease or obstructive sleep apnoea, relative erythrocytosis that reflects a contracted plasma volume, or congenital causes of erythrocytosis. Investigations are described in guidelines such as those published by the British Committee for Standards in Haematology (BCSH) [15,149], but an essential laboratory investigation is screening for JAK2V617F (and, if necessary, exon 12 mutations), for which guidelines are also available [150].

Where an MPN is suspected, criteria have been published by the World Health Organisation (WHO) or BCSH to define those patients who should be diagnosed with PV (Table 2) [1,15,151]. An essential component in both cases is the presence of an erythrocytosis, but the parameters and thresholds used to define this key criterion remain controversial [152]. The WHO criteria have been recently updated and now suggest the use of absolute haemoglobin level, haematocrit, or red cell mass [151], whilst the BCSH criteria depend on either haematocrit or red cell mass [15]. Although scintigraphic measurement of red cell mass would be helpful [158], it is not universally available and is not absolutely required in either set of criteria. However, haemoglobin or haematocrit levels do not provide a perfect surrogate for red cell mass and their use in diagnostic thresholds for PV have been questioned [153,154]. This issue relates to unpredictable changes in plasma volume in PV, especially in the presence of splenomegaly [155–157], and it has been suggested that more systematic measurement of red cell mass would be helpful [158]. Iron deficiency may further complicate the clinical presentation. The term “masked PV” has been used for those patients whose bone marrow morphology is in keeping with a diagnosis of PV but who do not meet haemoglobin- or haematocrit-based thresholds for erythrocytosis [159]. These patients may represent an important group since they were reported to have higher rates of myelofibrotic transformation, acute leukaemia and thrombosis with inferior survival, compared to those with overt PV [159–161], although this was not confirmed in all studies [162]. Interestingly it was found that the use of BCSH criteria resulted in a smaller proportion of patients falling into this category compared to the 2008 WHO criteria [163], which is reflected in the 2016 WHO criteria (Table 2) [151]. However these criteria have not been validated prospectively [164].

In clinical practice, the issue of defining erythrocytosis is most relevant in distinguishing PV from ET in the presence of a JAK2 mutation. Both WHO and BCSH criteria for ET require the exclusion of PV and therefore, to at least some extent, depend on the same thresholds used to define erythrocytosis. A study of patients with PV (overt or masked) and ET suggested that the most optimal cut-offs to distinguish between JAK2-mutated ET and PV are a haemoglobin level of 165 g/l in males and 160 g/l in females, or haematocrit of 45% in males and 48% in females [163], which is reflected in the 2016 WHO criteria [151]. However these criteria have not been validated prospectively [164].

The BCSH and WHO criteria also differ in the importance given to bone marrow morphology. Although the classical histological features of PV are identifiable in most patients (Fig. 2), the use of histology to determine diagnosis within the MPNs, as recommended by the WHO, remains controversial. Indeed BCSH criteria allow a diagnosis of PV to be made in the presence of erythrocytosis and a JAK2 mutation without a bone marrow biopsy. In one study of 272 bone marrow biopsies, blinded histological evaluation by seven haematopathologists resulted in a diagnostic consensus in only 53% of patients, and even where a histological...
consensus was obtained, the concordance rate with the clinician’s diagnosis was only 71% [165]. There are therefore ongoing uncertainties about the reliability of histology in discriminating between PV and JAK2-mutated ET in routine clinical practice. WHO criteria also retain serum erythropoietin level as a minor criterion for the diagnosis of PV, although this has been shown to add little to the accuracy of PV diagnosis in the era of JAK2 mutation testing [166].

Although PV and JAK2-mutated ET are discriminated predominantly on the basis of pragmatic thresholds for red cell mass, haemoglobin or haematocrit levels, it is clear that these thresholds are somewhat arbitrary and some patients with JAK2V617F-positive ET will be more “similar” to PV than others. Moreover this dichotomous classification does not fully capture the phenotypic heterogeneity within and overlap between the two groups of patients. As discussed above, JAK2V617F-positive PV and ET can be considered as two ends of a biological continuum [84] in which specific phenotypic features (blood count parameters, and perhaps others such as spleen size and thrombotic risk) may be influenced by the balance between clones of different JAK2 genotype together with genetic and environmental modifiers (Fig. 1). This concept of a disease spectrum rather than a dichotomy raises questions as to whether it is appropriate to divide treatment studies and guidelines according to current diagnostic criteria, in which JAK2V617F-positive and -negative ET are considered together, and quite separate to PV [16, 149]. Interestingly one report suggested that higher rates of thrombosis in masked PV might reflect a lower intensity of treatment as compared to overt PV [161]. This raises the possibility that a more unified diagnostic (and management) approach to those with JAK2-mutated MPNs (PV or ET) might improve outcomes, but this has not yet been tested in clinical trials.

5. Prognosis in PV

5.1. Factors predictive of overall survival and vascular events

Patients with PV have a reduced life expectancy in comparison to an age-matched healthy population and compared to patients with ET. A study of 396 consecutive PV patients demonstrated a 15-year survival of 65%, compared to 73% in ET [167], while a multi-centre study of 1545 PV patients showed a median survival of 14.1–18.9 years [168]. Age and thrombotic history have been identified as independent poor prognostic factors for overall survival in several studies [168,169] and leucocytosis, abnormal karyotype and a leucoerythroblastic blood smear have also been shown to confer an adverse prognosis [168].

Venous and arterial thromboses are the leading causes of mortality in patients with PV and are the presenting feature in 20% [170]. After diagnosis, the rate of thrombotic events has been estimated as 3.4–5.5 per 100 patients per year, with age at diagnosis and history of prior thrombotic episodes being most strongly predictive of further events [169, 170]. The European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) study also found that a smoking history carried a small but significant risk for thrombosis (hazard ratio 1.9), but there is little convincing evidence that other factors traditionally associated with cardiovascular risk, such as hypertension, hyperlipidaemia or diabetes mellitus, play a significant role in the context of PV [171].

A correlation between haematocrit and risk of thrombosis was first described in a retrospective report in 1978 [172], leading to the recommendation that the haematocrit should be maintained below 0.45. As well as being associated with increased blood viscosity, a raised haematocrit may also contribute to increased platelet activation due to displacement towards the vessel wall, and may be associated with red cell aggregates [173]. Polycythemia Study Group (PVSG)-01 and 05, a large randomised controlled study initiated in 1967, allowed further indirect assessment of the impact of haematocrit on thrombotic risk, since a fall in thrombotic events in the venesection-only arm was observed once the target haematocrit was reduced from 0.52 to 0.45 [174,175]. However the use of the haematocrit as a therapeutic target was only validated formally in the recent CytoPV study [176]. This prospective study randomised 365 patients with PV to intensive (target haematocrit <0.45) or less intensive (target haematocrit 0.45–0.50) treatment, with the choice of therapy (venesection, cytoreduction or both) at the investigator’s discretion. With a median follow-up of 31 months, 9.8% of patients in the low-intensity arm met the primary endpoint of death from cardiovascular or major thrombotic events compared to 2.7% in the intensive treatment arm. Although this lends more definitive support to a haematocrit target of <0.45, it remains unclear whether an even lower threshold might offer a further reduction in thrombotic events. A number of centres advocate different targets for males and females – i.e. <0.45 and <0.42, respectively - based on differences in steady-state physiological levels [155,177]. This is reasonable but currently there is no prospective or retrospective evidence to support the use of different targets.

Interpretation of the CytoPV study is confounded by the fact that patients in the lower-intensity arm tended to have higher white cell counts than those in the intensive arm, most likely reflecting differences in the use of cytoreductive drugs. This is important because leucocytosis may itself be a risk factor for thrombosis. In ET, a leucocyte count >15 x 10^9/L was identified as an independent predictor both of overall survival and thrombosis in a study of 322 patients [178], and the prospective PT1 study also found a linear relationship between leucocyte count and the estimated hazard ratio for thrombosis [179]. Proposed mechanisms for this association include the activation of platelets by granule-derived proteases, and direct interactions via CD11b - CD42b or -CD63b [173,180]. Data are more limited in the context of PV however. Analysis of the prospective ECLAP cohort did demonstrate an increased thrombotic risk in patients with a leucocytosis (HR 1.71), mainly reflecting an increased risk of myocardial infarction [171], although no association was seen in a retrospective analysis that included 153 PV patients [181].
There have been mixed reports regarding the significance of *JAK2V617F* homozygosity in determining thrombotic risk both in PV and ET. Tefferi et al. [95] compared rates of thrombosis between 13 *JAK2V617F*-“homozygous” and 45 *JAK2V617F*—“heterozygous” patients and found no difference, with a later larger study reaching a similar conclusion [13]. However, these were both retrospective studies that distinguished between homozygous and heterozygous patients using semi-quantitative techniques. Subsequently *JAK2V617F* allele burden was assessed using quantitative real-time PCR in diagnostic samples from a cohort of 173 PV patients, with a median follow-up of 24 months [91]. The 18% of patients with mutant allele burdens of >75% had greater rates of cardiovascular events at the time of, and following, diagnosis, as well as greater numbers of thrombotic events post-diagnosis. Allele burden remained the only independent variable predictive of cardiovascular events in a multivariate analysis that included age, prior thrombosis and leucocytosis.

In summary, age and thrombotic history remain established risk factors for thrombosis in PV, whilst leucocytosis and *JAK2V617F* allele burden may have significance and warrant further investigation in prospective studies. Interestingly there has been no evidence, either in ET or PV, that the degree of thrombocytosis correlates with thrombotic risk. In fact, in ET a U-shaped relationship has been demonstrated between platelet count and the risk of haemorrhage [179], which is to be explained by the development of an acquired von Willebrand syndrome at high platelet counts (generally >1000 × 10⁹/l) [182]. In PV, analysis of the ECLAP cohort demonstrated a prior bleeding history as the only independent predictor for haemorrhage [169], while data from the German Study Alliance Leukaemia MPN registry, which includes 142 patients with PV, identified prior thrombosis, splenomegaly and heparin use as risk factors for bleeding in univariate analyses [183].

5.2. Factors predictive of transformation to myelofibrosis or acute leukaemia

Factors that have been associated with increased risk of myelofibrotic transformation in PV include the presence of increased bone marrow fibrosis at diagnosis [184] and a diagnostic white cell count >15 × 10⁹/l [185]. *JAK2V617F* allele burden was associated with risk of disease transformation in a prospective study of 338 patients with PV [93]; 2% of patients transformed to secondary myelofibrosis, all of whom had allele burdens >50%, and this association remained significant on multivariate analysis. Although the latter study did not identify an association between allele burden and progression to AML, the frequency of this endpoint was low (3%). The incidence of leukaemic transformation varies widely between studies, and has been reported as high as up to >30% in trials with follow-up of >20 years [186]. Studies with higher rates of transformation to AML have implicated leucocytosis, age and abnormal karyotype as additional risk factors [168,187]. There is also a strong association with the use of certain cytoreductive agents (see Section 6.4).

Although a number of somatic mutations have been associated with progression to myelofibrosis or acute leukaemia (see Section 3.6), it is less clear whether the mutational profile at diagnosis can predict transformation events or other outcome measures. A number of studies have addressed this question in primary myelofibrosis, but only one study to date has comprehensively investigated other MPN subtypes. Lundberg et al. [188] screened for mutations in 104 genes in a 197 patient cohort that included 94 patients with PV. Of these, only mutations of *JPH3* and *TET2* were associated with increased rates of leukaemic transformation.

6. Management of PV

The aims of treatment in PV are to reduce disease-related symptoms and the risk of venous and arterial thrombosis, while minimising treatment-related toxicity, haemorrhagic risk and, in particular, the risk of progression to post-PV myelofibrosis and acute leukaemia. As such management largely adopts a risk-adapted approach, targeting cytoreductive treatment towards those at higher thrombotic or haemorrhagic risk. Although, with the exception of smoking, there is no association between established cardiovascular risk factors and thrombotic risk in the context of PV, it is generally accepted that these should be targeted regardless of other specific management steps.

6.1. Use of anti-platelet agents

Since platelet activation seems to be a central mechanism in MPN-associated thrombosis [173,189] and there is evidence of increased platelet thromboxane biosynthesis in PV [190], there has been interest in the use of anti-platelet agents, in particular aspirin, for thromboprophylaxis in MPNs. High-dose aspirin (900 mg) or dipyridamole (225 mg) was used in the venesection arm of the PVSG-05 trial (which was a comparator for pipobroman), but this was discontinued early due to excessive gastrointestinal bleeds and deaths from haemorrhage [175]. This initially led to the avoidance of aspirin in patients with MPNs, but subsequently the use of low-dose aspirin was assessed in the ECLAP trial [191]. This multicentre, double-blind trial randomised 518 high- or low-risk PV patients without a specific indication for aspirin to receive daily aspirin 100 mg or placebo, with a mean follow-up period of three years. The composite primary endpoint of non-fatal myocardial infarction, non-fatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes was met in 7.9% of the placebo-treated group and 3.2% of the aspirin treated group (p = 0.03), with overall incidences of thrombotic events of 15.5% and 6.7% respectively (p = 0.003). No significant differences were seen in the incidence of major, or minor bleeding. It is worth noting that this study was terminated early due to poor recruitment and only one third of the target number of patients were actually recruited; as such it may have been under-powered to detect significant differences in bleeding complications [192]. Nonetheless this study established a role for low-dose aspirin in primary prevention of thrombotic events in PV and did not demonstrate a significant increase in haemorrhagic events, leading to the widespread use of aspirin across all risk groups in PV, as well as in ET [16]. This approach is further supported by a recent retrospective study in low-risk ET that showed a reduction in venous events in *JAK2*-mutated patients who received antiplatelets, compared to observation alone (although an increase in bleeding, without a reduction in thrombosis, was seen in *CALR*-mutated ET patients and it is now unclear that universal aspirin use remains appropriate in this latter group) [193]. A Cochrane meta-analysis [194] included both the ECLAP study and an earlier study by Gruppo Italiano Studio Policitemia that assessed the safety of low-dose aspirin (40 mg), and included thrombotic/embolic events as secondary endpoints [195]. The reduction in thrombosis-related mortality did not reach significance in this analysis, but there was no significant increase in haemorrhagic complications. This review also concluded that aspirin is safe to use in PV. However, these and other authors have highlighted the need for further research into which subgroups might benefit most or might be at greater risk of bleeding complications, as well as into alternatives, for example thienopyridine anti-platelet agents such as clopidogrel, which might be used in patients intolerant of or resistant to aspirin [192].

6.2. Venesection

As discussed above, a number of studies have indicated that thrombotic risk is significantly reduced when the haematocrit is maintained below 0.45 and that venesection of 250–500 ml per session can be sufficient to achieve this target. This is achieved both by immediate reduction in haematocrit by the removal of red cells with compensatory expansion of the plasma volume, but also by the longer term induction of an iron-deficient state that constrains erythropoiesis. Furthermore, venesection alone compared favourably compared to radiophosphorus
(\(^{32}\)P) and chlorambucil in the PVSG trials, which randomised 431 patients to these three treatments with median survivals of 13.9, 11.8 and 8.9 years respectively. However, higher rates of early thrombotic events were observed in the venesection-only arm, particularly in patients over 70 [196]. This has led to the recommendation that venesection (rather than cytoreduction) may be adequate to control haematocrit in low-risk patients but raised the question of whether cytoreductive agents have a particularly important role in the high-risk group. Conversely the fact that a haematocrit target of 0.52 was initially used in this trial, and subsequently reduced to 0.45, does makes its interpretation problematic. Venesection is generally well tolerated and avoids potential toxicities associated with cytoreductive drugs but necessitates regular outpatient visits (in some cases it may need to be performed daily until the haematocrit is range). Other potential problems include difficult venous access, anxiety regarding the use of needles, and symptoms resulting from iron deficiency (such as fatigue, cognitive impairment or restless legs) or cardiovascular instability/vasovagal events (where isovalaemic venesection may be more appropriate).

6.3. Historical perspective: cytoreduction using radiophosphorus or alkylating agents

\(^{32}\)P has been used for cytoreduction since the 1940s and has the advantage that only intermittent treatments are required, but has long been associated with increased risk of leukaemic transformation. A number of early trials therefore addressed whether other myelosuppressive, predominantly alkylating, agents could provide an alternative. However, even greater rates of leukaemic transformation were seen with the use of chlorambucil in the PVSG trials (6% with \(^{32}\)P and 11% with chlorambucil, compared to 1% in the venesection arm) [186]. The European Organisation for Research on Treatment of Cancer (EORTC) group randomised 293 patients to \(^{32}\)P or busulfan [197]. No differences were seen in rates of leukemic transformation (1–2% with median follow-up of 8 years), but the busulfan-treated group had longer durations of remission and lower rates of thrombosis and non-haematological malignancies.

Pipobroman, a piperazine derivative structurally similar to many alkylating agents, has also been assessed as a first line agent in a number of trials and demonstrated rates of haematological remission of >92%, but rates of leukemic transformation by 10 years of 5–14% [187,198,199]. In the French Polycythemia Study Group (FPSG) study 292 patients aged under 65 years were randomised to hydroxychemamide or pipobroman [186,200]. Although there was no observed difference in thrombotic or haemorrhagic events, rates of transformation to AML or myelodysplasia were significantly higher in patients treated with pipobroman (52% compared to 24% by 20 years). This was associated with a median overall survival of 15.4 years in the pipobroman arm compared to 20.3 years in the hydroxychemamide arm [186].

The rates of leukemic transformation in alkylator- or \(^{32}\)P-treated or untreated patients are inconsistent between trials, which may reflect differences in patient selection or treatment intensity. These associations were assessed more systematically in the larger ECLAP trial, where the leukaemia transformation rate by 1.8 per 100 patients per year in alkylator-treated patients compared to 0.29 per 100 patients per year in patients treated with venesection alone, or interferon-alpha [201]. Similarly Teferi et al. reported an overall incidence of AML of 2.3% by 10 years, with significantly higher rates seen in patients treated with single agent \(^{32}\)P, pipobroman, chlorambucil or combined pipobroman and busulfan therapy [168].

6.4. Hydroxychemamide use in PV and leukaemogenic potential

Given the associations between leukaemia transformation and alkylating agents, the use of hydroxychemamide, a ribonucleoside reductase inhibitor that interferes with DNA replication and may also act as a nitric oxide donor, has been explored as an alternative. An early phase 2 study demonstrated its tolerability and efficacy in a cohort of 118 PV patients [202]. There has been a relative paucity of phase 3 data examining the use of hydroxychemamide in PV, although the FPSG trial described above showed comparable efficacy in preventing vascular events as compared to pipobroman [200]. However subsequent data from randomised trials in ET have been informative in establishing hydroxychemamide as a safe and effective first-line agent for the prevention of vascular events in high-risk MPNs. Cortelazzo et al. randomised 114 high-risk ET patients to hydroxychemamide or no myelosuppressive treatment with a median follow-up of 27 months, and reported vascular events in 24% of the untreated arm but only 3.6% of the hydroxychemamide-treated arm [203]. In the high risk arm of the PTI trial, 806 ET patients were randomised to aspirin plus anagrelide, an inhibitor of cyclic AMP phosphodiesterase that blocks megakaryocytic differentiation and proliferation, or hydroxychemamide plus aspirin [111]. Patients in the anagrelide arm were more likely to reach the composite primary end-point of arterial or venous thrombosis, serious haemorrhage or death from thrombotic or haemorrhagic causes, and showed higher rates of myelofibrotic transformation, arterial thrombosis and serious haemorrhage.

The risk of leukaemia associated with hydroxychemamide therapy has been more controversial. From trials such as the FPSG – 65 study it is clear that the risk of AML is lower with hydroxychemamide than with alkylating agents, but even in this study a surprisingly high AML/ MDS transformation rate of 24.2% was seen in the hydroxychemamide arm at 20 years (16.6% in patients who had only received hydroxychemamide and no other therapy) [186]. Nonetheless AML is part of the natural history of PV and a major issue has been the lack of trials in PV comparing hydroxychemamide to venesection alone, or to other “non-leukaemogenic agents”. Analysis of the ECLAP cohort showed no difference in the rate of transformation in venesection/interferon-treated patients compared to those treated with hydroxychemamide [201]. This was also the case in a Swedish case-control study that included 162 patients with transformation [204] and another cohort of 1545 PV patients [168]. Further evidence against a significant leukaemogenic effect of hydroxychemamide comes from an analysis of hprt locus mutations and “illegitimate” VDJ recombination events in untreated patients, hydroxychemamide-treated MPN patients and hydroxychemamide-treated patients with sickle cell anaemia [205]. No difference was seen in mutation rates between MPN patients treated with prolonged hydroxychemamide exposure compared to those with short exposure or untreated controls. However, it is worth noting that assessment of VDJ recombination is only of relevance in T cells, and whether the conclusions of this analysis can be extended to myeloid or haematopoietic stem/progenitor cells is unclear.

Although the available data do not conclusively support a direct leukaemogenic effect of hydroxychemamide monotherapy, combination therapy with other agents may increase the risk of AML. In the FPSG trial of patients older than 65 years, patients were randomised to \(^{32}\)P or hydroxychemamide maintenance [206]. A higher actuarial risk of leukaemic transformation was observed in the latter arm, despite the potential to reduce the radiation dose with hydroxychemamide maintenance. In the long-term follow-up of the Italian ET study described above, higher rates of leukaemic transformation and of other secondary malignancies were also seen when hydroxychemamide was used sequentially following busulfan, but were much rarer in those treated with hydroxychemamide alone [207].

In summary, hydroxychemamide is an effective treatment to achieve haematological responses and reduce vascular events in MPNs. There is evidence that the leukaemic risk associated with hydroxychemamide is much lower than that seen with many other myelosuppressive agents, specifically alkylating agents and \(^{32}\)P, but this risk may be increased if hydroxychemamide is used in combination or sequentially with these agents. The European Leukaemia Network (ELN) have generated response criteria for the treatment of PV [208] (Table 3), which depend largely on features of PV biology rather than the development of

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et al. assessed 76 patients with PV, 28 of early myelo other effective non-leukaemogenic agents.haps surprisingly, control of haematocrit did not correlate with throm-
ination of myelo and restoration of polyclonal haematopoiesis[211,212]. Furthermore, resistance to hydroxycarbamide, as de
ulceration, skin cancers, pneumonitis or gastrointestinal side effects
complications. Approximately 11% of patients fail to achieve an ade-
quate response with hydroxycarbamide, and a further 14% will discon-
tinue due to intolerance, such as development of mucocutaneous ulceration, skin cancers, pneumonitis or gastrointestinal side effects [209]. Resistance to hydroxycarbamide, as defined according to ELN criteria [210] (Table 4), has been shown to identify a subset of patients with increased risk of death and transformation to acute leukaemia or myelofibrosis in one retrospective study [209]. In particular, the lack of resolution in leucocytosis was associated with worse overall survival, and lack of platelet control with risk of thrombosis and bleeding. Perhaps surprisingly, control of haematocrit did not correlate with thrombotic risk. Hydroxycarbamide-resistant patients therefore represent a difficult group of patients to manage and this has driven the search for other effective non-leukaemogenic agents.

6.5. Interferon-alpha and anagrelide in PV

Recombinant interferon alpha is an attractive agent for first-line treatment in PV. It has no known leukaemogenic potential, can achieve good haematocrit and platelet count control, and has been shown to selectivity inhibit the growth of the malignant clone leading to a reduc-
tion of JAK2V617F burden or reversion of cytogenetic abnormalities, and restoration of polyclonal haematopoiesis [211,212]. Furthermore, it can antagonise platelet-derived growth factor and impair fibroblast differentiation, providing possible theoretical mechanisms for an inhibi-
tion of myelofibrotic transformation, supported by some evidence in early myelofibrosis [213]. To date however, trials evaluating interferon have predominantly been small, single-arm studies and there have been no randomised controlled trials allowing comparison with other agents such as hydroxycarbamide. A literature review of trials performed in PV has provided evidence for the efficacy and tolerability of interferon for a total of 349 patients across 16 studies [214], although a formal meta-analysis was not possible due to differences in the drug (interferon alpha 2a/2b, pegylated/unpegylated) and response criteria used. This analysis demonstrated that on average 60% of patients were able to discontinue venesection, although this was as high as 97% in some studies, and patients generally suffered low rates of thrombosis. Treatment was limited by discontinuation rates of 30–40%, with the main side effects including fatigue, headache, pyrexia, myalgia and neuropsychiatric symptoms. This tolerability profile, together with the need for subcutaneous administration, are the main factors limiting use of interferon in practice.

Pegylated interferons are those that are bound by urethane or amide bonds to a polyethylene glycol molecule. This property prolongs their half-lives so that weekly rather than 24–48 hourly dosing is possible, and the reduced peak dose may potentially reduce toxicity. Three studies have assessed response rates and tolerability using pegylated interferons in PV. Kuriakose et al. assessed 76 patients with PV, 28 of whom were treated with recombinant interferon-alpha-2b, 18 with pegylated interferon-alpha-2a (Pegasys), 8 with hydroxycarbamide and 19 with other agents (imatinib, dasatinib, 32P and busulfan) [215]. Haematological responses were seen in 89.3%, 88.9% and 59.3% in the interferon-alpha-2b, pegylated interferon and non-interferon groups respectively. Discontinuation rates were 16.1% in the non-pegylated interferon arm, compared to 5.6% with pegylated treatment. Low rates of molecular response were observed across all arms (15.2% of interferon-treated patients having a partial molecular response) and were independent of haematological response. By contrast, Pegasys treatment in two other studies of 40 and 37 PV patients was associated with molecular responses in 54% and 90% of patients respectively and complete molecular responses (i.e. undetectable JAK2V617F) in 14% and 24%, with haematological responses in 80% and 100% [212,216]. Five patients in the former study had a sustained complete molecular and haematological response after discontinuing Pegasys, and no thrombotic events were observed in the study. Discontinuation rates were 10% and 24.3% respectively, suggesting pegylated interferons may be a viable alternative to other interferon preparations or to hydroxycarbamide as a first-line treatment.

Phase 3 clinical trials comparing interferons with hydroxycarbamide are ongoing and may further inform the choice of first-line agents in future. These include the PROUD-PV study (NCT01949805), in which patients were randomised to either hydroxycarbamide or pegylated interferon alpha-2b (PEG-intron), as well as DALIAH (NCT01387763) and NCT01259856, which compare hydroxycarbamide to Pegasys and/or PEG-intron. Further molecular characterisation may also help target the use of interferons: there is evidence that sensitivity to interferon may correlate with the presence of JAK2V617F-homozygous clones [217] and, conversely, that clones carrying non-JAK2 mutations (e.g. TET2) may be more resistant to interferon [218,219].

Anagrelide selectively targets platelet production and therefore its role in PV is limited. However, it has demonstrated responses when used in combination with hydroxycarbamide in a cohort of hydroxycarbamide-resistant patients, suggesting combination therapy may be a possibility as a second-line option [220]. Anagrelide has the advantage of being non-leukaemogenic but its use is further limited by the increased potential for myelofibrotic transformation [11] and its side effect profile which includes tachycardias, fluid retention and headaches.

6.6. JAK2 inhibitors in PV

Ruxolitinib is a JAK1/JAK2 inhibitor currently approved for clinical use in myelofibrosis and in patients with PV resistant or intolerant of hydroxycarbamide. A Phase 2 trial first assessed its use in a cohort of 34 PV patients who were resistant or intolerant to hydroxycarbamide.
with a median follow-up of 152 weeks [221]. Haematological response (by ELN criteria) was achieved in 97% of patients with 61% achieving a durable response (no requirement for venesection) for 144 weeks. 70% of patients with splenomegaly showed responses in spleen size within 24 weeks.

This was followed by the Phase 3 RESPONSE trial comparing ruxolitinib to best available therapy in 222 PV patients with both hydroxyurea/carbamide resistance/intolerance and splenomegaly [20]. The primary composite end-point of haematocrit control and reduction in spleen size of at least 35% was met in 20.9% of the ruxolitinib arm and 9.9% of the standard therapy arm, with 23.6% and 8.9% of patients, respectively, achieving a complete response by ELN criteria. Ruxolitinib was particularly effective in improving constitutional symptoms, as was seen in previous trials in myelofibrosis [18,19]. Although the trial was not sufficiently powered to detect a difference, there was a low rate of thrombosis in the ruxolitinib arm (1 patient, compared to 6 in the standard therapy arm). Overall ruxolitinib had a good safety profile: anaemia was seen in 43.6% of patients and thrombocytopenia in 24.5% but grade III/IV toxicity was only seen in 1.8% and 5.4% respectively, compared to 0% and 3.6% in the standard treatment arm. The most commonly reported side effects were headache, fatigue, diarrhoea, muscle spasms and dizziness and the rate of discontinuation due to toxicity was low (3.6%). These studies therefore indicate a role for ruxolitinib in achieving haematological control in hydroxyurea/carbamide-resistant/intolerant patients with PV and also suggest that ruxolitinib may offer additional advantages in terms of reduction of splenomegaly and symptom control. However, the generalisability of the results is limited by the highly selected patient group; in particular the presence of significant splenomegaly. The RESPONSE-2 and MAJIC studies are comparing ruxolitinib to best available therapy in hydroxyurea/carbamide-resistant or -intolerant patients without a requirement for splenomegaly and their results should therefore be more generalisable to routine practice.

6.7. Histone deacetylase inhibitors and other agents in PV

Histone deacetylase (HDAC) inhibitors have been shown to have anti-tumour activity in AML and multiple myeloma, and also have anti-angiogenic properties. A phase 2 study assessed the use of vorinostat in a 63 patient cohort that included 44 patients with PV. Although patients achieved complete resolution of pruritus (present initially in 19% of patients), improvement in splenomegaly with resolution in 23% of patients and a decrease in JAK2V617F burden in 65% of patients, 44% discontinued treatment during the 24-week trial period due to adverse events that included severe fatigue, diarrhoea, vomiting and weight loss [222]. Givinostat, which has been shown in vitro to selectively inhibit JAK-STAT signalling and proliferation of JAK2-mutant primary cells and cell lines [223], was tolerated better in a pilot study of 12 PV, 1 ET and 16 myelofibrosis patients [224]. Although patients also experienced diarrhoea (62%), nausea (10%), fatigue (7%) and abdominal pain (17%), these were Grade I-II in almost all cases and only one patient discontinued treatment because of drug-related adverse events. Seven of the thirteen PV or ET patients showed a clinical response by ELN criteria. This study was followed by a trial of 50 or 100 mg givinostat in combination with hydroxyurea/carbamide in a cohort of 44 patients who were non-responsive to maximal doses of hydroxyurea/carbamide [225]. The combination was generally well tolerated, with only 18% discontinuing treatment within 24 weeks. Responses were seen in 55% of patients on 50 mg and 50% on 100 mg givinostat, with resolution of pruritus in 64% and 67%, respectively. These studies suggest a potential role for HDAC inhibitors in disease control and symptomatic management of hydroxyurea/carbamide-resistant patients.

There are a number of other agents currently under investigation for hydroxyurea/carbamide-resistant patients. These include inhibitors of signalling downstream of JAK2 (such as TGR-1202, a PI3K-delta inhibitor), CEP-701 (lestaurtinib, a FLT3 inhibitor), RG7388 (a selective inhibitor of p53-MDM2 binding), AUY922 (an inhibitor of heat-shock protein 90) and Imelrestat (a telomerase inhibitor that has shown some efficacy in ET and myelofibrosis [226,227]).

6.8. Symptom burden and control in PV

Beyond the morbidity and mortality related to PV-related complications such as thromboses, haemorrhage and disease transformation, PV itself carries a significant symptomatic burden. This can be formally quantified using the MPN symptom assessment form (MPN-SAF), where severity of a number of symptoms are scored from 1 to 10, and from which a total symptom score (TSS, out of 100) can be calculated as a global measure of morbidity [228]. This score was found to correlate well with other measures of quality of life, with PV patients in a large international study having a mean TSS of 21.3, compared to 18.7 for those with ET and 25.7 for those with myelofibrosis [229]. Fatigue showed the greatest severity in patients with PV (mean 4.4,10, present in 88%), followed by itch (62%), concentration problems (65%), early satiety (64%) and inactivity (61%), and the incidence and severity of all of these symptoms was higher for patients with PV than those with ET.

The RESPONSE trial reported significant improvements in constitutional symptoms with ruxolitinib compared to standard therapy, particularly those related to cytokines such as pruritus, sweats, fatigue and muscle aches [20]. The effects on symptoms and quality of life were described further in a post-hoc analysis that used a number of tools including the MPN-SAF and EORTC QLQ-C30 quality of life questionnaire [230]. Ruxolitinib treatment was associated with a fall in TSS and with improvements in other global measures of quality of life, social, physical and emotional functioning, while patients in the standard treatment arm showed a deterioration on average in all these measures. More specifically the EORTC QLQ-C30 demonstrated improvements in fatigue, pain, anorexia, dyspnoea, diarrhoea and constipation in the ruxolitinib arm but not the standard treatment arm, while insomnia improved in both treatment arms. The symptomatic benefit of ruxolitinib was further assessed in the RELIEF study, a double blind, double-dummy switch study which randomised symptomatic patients who were otherwise well controlled on hydroxyurea/carbamide, with no venesection requirement or splenomegaly, to either ruxolitinib and hydroxyurea/carbamide-placebo, or hydroxyurea/carbamide at the same dose/schedule and ruxolitinib-placebo [231]. Both ruxolitinib- and hydroxyurea/carbamide-treated patients showed mean improvements in cytokine-related total symptom scores, although a significant difference between these arms was not observed.

Pruritus is particularly an issue in PV and is seen in 62% of patients compared to 46% of those with ET and 50% with MF [229]. It can be associated with discomfort, problems sleeping, social embarrassment, mood changes, intolerance of bathing, depression and even suicidal ideation [232]. Antihistamines are widely prescribed but the literature is inconsistent on whether they are beneficial. Phototherapy has been used in small numbers of patients to good effect, and there is also some evidence for the use of selective serotonin reuptake inhibitor anti-depressant agents (reviewed in Saini et al. [232]). A review in 2000 of 11 reports of use of interferon alpha found that 81% of the 80 PV patients evaluated showed improvement in pruritus on interferon treatment, as well as improvements in splenomegaly in 77% [233]. Most recently there is not only evidence that ruxolitinib can significantly improve the pruritus associated with PV [20,230], but also that HDAC inhibitors may prove to be useful in this setting [225].

6.9. Management of special situations in PV: pregnancy and splanchic thrombosis

Recommendations on the management of PV in pregnancy are limited by its low incidence (median age at presentation is 60 and there is a male predominance) and the fact that most reports include only small numbers of patients and are retrospective. The experience in ET shows that myeloproliferative conditions carry a significant risk to the mother
and fetus. A literature review of 291 pregnancies in 190 women with ET revealed a first trimester miscarriage rate of 39% and a live birth rate of only 61% [234], while the rates of maternal thrombosis and haemorrhage were 3% and 2%, respectively. In PV a review of 36 pregnancies in 18 patients reported a neonatal survival rate of only 50% [235]. Three mothers suffered thrombotic complications, one a large post-partum haemorrhage, four developed pre-eclampsia and there was one maternal death.

On the basis of this limited experience, the following recommendations have been suggested [234,235]. Patients should continue on aspirin 50–100 mg and thromboembolic deterrent (TED) stockings should be worn throughout pregnancy and for six weeks post-partum. A weekly full blood counts should be performed until 24 weeks gestation and 2 weekly thereafter to ensure adequate haematocrit control, and iron supplementation should be avoided. Patients over 35 years old, with previous pregnancy complications or microcirculatory events, additional thrombophilic factors (such as a positive lupus anticoagulant) or platelet count over $1000 \times 10^9/l$ should be considered high risk and therefore should be considered for additional low molecular weight heparin (LMWH) or cytoreduction. Cytoreduction, in particular, would be indicated if there is inadequate control of the haematocrit or a rising platelet count, while the use of LMWH is supported by experience of patients with multiple other thrombotic risk factors in pregnancy, outside of the context of PV [236]. Those with a previous thromboembolic or haemorrhagic event are considered at highest risk, in which case both low molecular weight heparin and cytoreduction would be indicated. No studies have addressed the optimal haematocrit in pregnancy. Given the expansion in plasma volume in this state, resulting in a significantly lower haematocrit for a given red cell mass, a target of <0.36 has been recommended by some [155].

Where indicated, prophylactic doses of subcutaneous LMWH are used (for example, 5000 units dalteparin or 40 mg enoxaparin daily) and monitoring of anti-Xa levels are recommended every 2–3 months. Ultrasound scanning is recommended at weeks 12, 20, 26, 30 and 36 and atelectomy is Doppler at 20 and 24 weeks, and evidence of placental insufficiency would be an indication to escalate LMWH treatment or to consider cytoreduction. There is evidence, predominantly from animal studies, that splanchnic vein thromboses may be associated with expansion of plasma volume [238].

6.10. Summary of recommendations for the management of PV

Together the available evidence indicates that the use of low-dose aspirin and achievement of a haematocrit target of <0.45 (and potentially even lower in some contexts) are effective strategies for reducing thrombotic risk in patients with PV. These measures are therefore recommended for all patients, regardless of the presence of additional risk factors. Venection is a safe and generally well-tolerated therapy for haematocrit control.

Cytoreduction is indicated for high-risk patients – those over the age of 60 and/or those with a history of previous thrombosis – or for patients intolerant of or inadequately controlled on venesection, those with intractable symptoms such as pruritus, evidence of progressive myeloproliferation such as worsening splenomegaly or leucocytosis (itself a risk factor for thrombosis) or with progressive thrombocytosis (and therefore at increased risk of haemorrhage) [239]. Patients with SVT require cytoreduction as well as anticoagulation and it is uncertain whether more stringent treatment targets would result in better outcomes in this group. There is insufficient evidence to determine whether cytoreductive agents are beneficial in patients at intermediate risk – i.e. those under 60 with additional risk factors such as diabetes mellitus – but the management of these patients should include modification of risk factors and a lower threshold for considering cytoreductive agents.

First-line cytoreduction should be either with recombinant interferon alpha or hydroxycarbamide. Interferon alpha has the advantages that it is not associated with leukaeamictic transformation, has been shown to reduce the JAK2 mutant allele burden and potentially reduce disease progression in myelofibrosis, and can be effective in reducing pruritus. However its significant side effects limit its use in older patients and it is contraindicated in those with a history of autoimmune or psychiatric conditions. Pegylated interferons may be better tolerated and require less frequent dosing. Hydroxycarbamide does not share these advantages and there still is a lack of certainty about potential leukaeamogenicity, but it is generally better tolerated. Therefore it is generally the drug of choice in older patients (over 50 years) and should be used with caution in patients with previous exposure to leukaeamogenic agents. Patients failing either first-line agent can be changed to the remaining agent if tolerated. Further options at this point would include JAK inhibitor therapy if available, busulfan or pipobroman (which carry an increased risk of disease transformation and should be reserved for patients over 75 years), anagrelide (for thrombocytosis) or a trial of other experimental agents.

7. Conclusions

Since the identification of the JAK2V617F mutation in the majority of patients with PV, significant progress has been made in elucidating the mechanisms by which this genetic abnormality causes disease. The identification of canonical and non-canonical downstream pathways has demonstrated the diverse and cellular effects of this single mutation on haematopoietic precursors and how they contribute to MPN phenotype, particularly that of erythrocytosis. It remains less clear however how JAK2V617F affects haematopoietic stem cell function and whether other advantageous genetic lesions are generally involved in the persistence of chronic-phase disease.

The question of how the single JAK2V617F mutation can be associated with multiple different clinical phenotypes has also been widely investigated. Myelofibrosis is characterised by a wider spectrum and heavier burden of other mutations, and appears to represent a more accelerated phase of disease. By contrast JAK2V617F-positive ET and PV have characteristics of a biological continuum and there is evidence that a number of continuous and discrete variables may determine the exact clinical phenotype and diagnosis. This clinical spectrum of
JAK2V617F-positive ET/PV and its differences from JAK2-negative ET are not yet fully reflected in international diagnostic criteria, management guidelines or clinical trials.

Together the biological data indicate that disease phenotype and persistence in JAK2-mutated PV reflect a broad diversity of molecular perturbations. Moreover the mechanisms that determine the most clinically damaging events – those of disease transformation – are even more complex and are likely to depend on additional, heterogeneous genetic lesions. This complexity accounts for the relative lack of progress in development of therapies that affect the underlying disease biology and in particular the size of the JAK2-mutant clone and risk of disease transformation. It is clear that inhibition of JAK2 in PV and related disorders does not result in the same clonal response that is seen with inhibitors of BCR-ABL in chronic myeloid leukaemia. Although there are promising suggestions that newer agents such as pegylated interferons may specifically target those of disease transformation, the challenge is now to elucidate which characteristics of a patient and their disease account for and predict response to these treatments. Moreover no agent has yet been shown to prevent or delay disease progression. In the meantime, management of PV remains centred on prevention of cardiovascular complications and studies continue to clarify the roles of specific cytoreductive agents and their respective treatment targets. Nonetheless important areas such as the use of additional thrombotic risk markers, the impact of novel agents (e.g. JAK2 inhibitors) on thrombosis, and management of JAK2V617F-associated splanchic vein thrombosis, require further study in prospective trials.

7.1. Practice points
• Optimisation of cardiovascular risk factors and aspirin 75–100 mg daily (unless contraindicated) should be employed for all patients.
• A target haematocrit of <0.45 should be used for all risk groups. Some centres advocate lower targets in females and in certain high-risk situations. Venesection alone may be sufficient to achieve this and is preferable in low-risk patients. Given the evidence for a biological continuum between JAK2-mutated ET and PV and the possibility of masked PV, there is an argument for maintaining haematocrit <0.45 in all JAK2-mutated patients.
• Consider interferon-alpha for low-risk patients if intolerant of venesection or symptomatic, particularly with pruritus.
• Cytoreduction with interferon-alpha or hydroxycarbamide is indicated in high-risk patients (age > 60 and/or previous thrombosis) and should be considered in cases with progressive myeloproliferation (rising white cell count, splenomegaly), uncontrolled thrombocytosis or contraindications to venesection.
• In patients with an inadequate response, alternative first-line agents can be attempted and patients should be considered for clinical trials. In older patients (e.g. >75 years) busulphan or pipobroman may be considered.
• Individualised molecular characterisation (such as JAK2V617F allele burden and presence of additional somatic mutations e.g. TE12 and TP53) may in future offer improved risk stratification and inform the choice of therapeutic agent.

7.2. Research agenda
• Effects of JAK2V617F on haematopoietic stem cells and mechanisms by which disease clones persist in chronic-phase PV
• Prospective evaluation of leucocytosis and JAK2V617F allele burden as possible additional markers of thrombotic risk in PV
• Role of additional genetic lesions in disease transformation and in identifying patients as candidates for novel therapies
• Effects of interferon-alpha, JAK2 inhibitors and other novel therapies on thrombotic risk, JAK2 mutant burden and risk of disease transformation
• Relationship between PV and JAK2V617F-positive ET, as compared to JAK2-negative ET, in clinical practice, especially the role of haematocrit in thrombotic risk in JAK2V617F-positive ET
• Treatment targets in very high risk patients, especially those with splanchic vein thrombosis and for other subgroups, e.g. males vs. females, pregnancy.
• The use of novel agents or first-line JAK inhibitors in high-risk patients.

Conflict of interest statement
JG has no conflicts of interest to declare. ALG has received speaker honoraria from Novartis and Shire.

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16

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