CLINICAL INVESTIGATION

Essential thrombocythemia: new advances in an old disease

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Essential thrombocythemia (ET), a Philadelphia-negative myeloproliferative neoplasm, is characterized by thrombocytosis, megakaryocytic hyperplasia and vascular complications. It has been recognized as a distinct clinical entity since the 1930s but our understanding of its pathogenesis and available therapies remained static for many years. The last decade has witnessed a proliferation of research interest and clinical trials both for ET and related diseases. Much of this work stemmed from the seminal discovery of the JAK2V617F mutation in 2005, which has germinated a far greater understanding of the genetic and epigenetic complexity of ET. Targeted therapies are already in clinical use and newer agents are in clinical trials. This review focuses on the recent advances in therapy as well as diagnosis, pathogenesis and risk stratification. However, there are still unanswered questions about this disease, including the management of low- and intermediate-risk patients as well as the factors that predict disease progression.

Keywords: essential thrombocythemia • JAK • novel therapies

Essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF) are three distinct clinical entities, classified together as myeloproliferative neoplasms (MPNs). The JAK2V617F mutation is highly prevalent across the MPNs and its discovery in 2005 has created considerable interest in research into the biology and pathogenesis of MPNs. There has been a significant shift in the paradigm of diagnosis and treatment of these conditions. For example, the umbrella term is no longer myeloproliferative disorders, as the name of the disease class has been changed to MPNs in order to reflect their clonal nature [1]. Interestingly, there has also been a resurgence of interest in old drugs, predominantly interferon, as well as the development of novel targeted treatments, including JAK and histone deacetylase (HDAC) inhibitors. Ruxolitinib (INCB18424 or JAKAVI) was the first JAK inhibitor to be tested in clinical trials and has recently received approval by the US FDA for the treatment of myelofibrosis (MF) in the USA.

Pathogenesis

Karyotype analysis is not usually performed in suspected cases of ET, but literature review suggests abnormalities in 10% of cases. This figure is likely to be an over representation in a group of highly selected patients. Karyotypic abnormalities are more commonly seen in MF and carry prognostic significance. The most important advance in the understanding of the pathogenesis of ET has undoubtedly been, as we have already alluded, the seminal discovery of the JAK2V617F mutation. This discovery, and the plethora of work that stemmed from it, has given us a much better insight into the molecular pathogenesis of

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MPNs. The JAK2V617F mutation is present in 97% of patients with PV and 50–60% of patients with ET and PMF [2–5]. A further 3–5% of patients with ET have one of a number of mutations in *MPL*, the gene encoding the thrombopoietin receptor, resulting in its autonomous activation and, as a direct consequence, constitutively activating JAK2.

The JAK2V617F mutation is an acquired mutation involving a change from G to T in exon 14 of JAK2 that results in the substitution of the normal valine residue at position 617 by the more bulky phenylalanine. JAK2 is a nonreceptor tyrosine kinase (has the ability to transfer a phosphate group from ATP to a tyrosine residue) that plays an essential role in signal transduction from several cytokine receptors that are essential for normal myelopoiesis, including EpoR, the thrombopoietin receptor *MPL* and G-CSFR. Wild-type JAK2 assumes an inactive conformation until binding of specific ligands to their receptors; for example, Epo to EpoR, which then results in a conformational change in JAK2. Activated JAK2 phosphorylates specific tyrosine kinase residues, which lead to downstream signaling cascades involving STAT, MAPK and PI3K proteins. The JAK2V617F mutation results in the constitutive activation of JAK2 in the absence of cytokine receptor stimulation (i.e., in the absence of binding to cogent ligand) and uncontrolled downstream signaling. This is sufficient to produce a MPN phenotype *in vivo* when JAK2V617F is expressed in murine bone marrow cells [6]. Two further important gains of function have been attributed to JAK2V617F. The first relates to SOCS3, a potent negative regulator of EPO signaling. SOCS3 is thought to target JAK2 for degradation by binding to its catalytic loop. JAK2V617F escapes this degradation and is actually enhanced by SOCS3, probably through its ability to hyperphosphorylate and stabilize SOCS3 [7]. The second gain of function relates to the phosphorylation and consequential functional impairment of PRMT5 by JAK2V617F, which binds more strongly than wild-type JAK2. PRMT5 is an arginine methyltransferase, important in the negative control of hematopoietic colony formation and erythroid differentiation. JAK2V617F abrogates this function and thereby potentiates myeloproliferation [8].

MPL mutations, as already discussed, also cause indirect dysregulation of JAK2 signaling. They most commonly result in an amino acid substitution at tryptophan 515 of the thrombopoietin receptor, resulting in W515L, W515K or W515A [9]. The normal function of this amino acid is to prevent autonomous activation of the receptor, but this function is abrogated by the mutation, which results in constitutive activation of JAK2 [10]. Mutations in S505N are seen infrequently and also result in *MPL* activation.

Mutations in epigenetic regulators have become quite frequently described across the MPN subgroups but their functional consequences are poorly understood and they are also found in other myeloid conditions such as myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). There is an increasing number of these, as discussed below. Such epigenetic mutations are most frequently seen in the gene *TET2*, which is postulated to have a role in the regulation of transcription and lineage commitment [11,12]. Mutations in *ASXL1* occur in chronic MPNs as well as blastic phase disease. Its role is unclear but is thought to relate to the control of chromatin structure [13]. Mutations in *IDH1* and *IDH2* are seen occasionally in chronic MPNs, but again are more frequent at leukemic transformation and may involve the same pathway as *TET2*. Mouse cells expressing mutant *IDH1/2* demonstrate impaired hemopoietic differentiation and greater expression of early progenitor markers [14]. *EZH2* is a well-characterized gene that is involved in stem cell renewal by epigenetic repression of genes involved in cell-fate decisions. *EZH2* mutations although rare in ET, are well described in MF and are inactivating. *DNMT3A* mutations have also been described but are rare occurrences [15]. In terms of likely pathogenic roles, JAK2 and *MPL* mutations appear to exert a phenotype-modifying effect and are distinctly associated with the MPNs, while *TET2*, *IDH1/2* and *ASXL1* do not (Table 1).

Genotype–phenotype correlations

JAK2V617F allele burden is emerging as an important contributor to MPN clinical phenotype with higher burdens seen in the entities PV and MF, where homozygosity for the mutation is common; and lower allele levels seen in ET, where homozygosity rarely occurs [16,17]. Homozygosity for JAK2V617F results from mitotic recombination with the breakpoints spread along chromosome 9p. In PV, the prevalence of homozygosity for JAK2 increases with time, which suggests that stem cells bearing the malignant clone have a survival advantage. Hematocrit, leucocyte count and lactate dehydrogenase levels have been found to directly correlate with the volume of JAK2V617F RNA present and those with the highest levels were more likely to have splenomegaly, pruritus and a greater risk of transformation [18]. This evidence supports the concept that higher allelic burden correlates with a more symptomatically advanced or accelerated disease across the MPN disease spectrum, as well as a more 'PV-like' phenotype.

A fundamental question that the field has been focusing upon is how one mutation can cause such

different clinical phenotypes. It has been postulated that increased signaling through JAK2V617F may be responsible for the PV phenotype, however, attempts to identify different signaling consequences in PV and ET have not yielded consistent results [19]. Interestingly, recent data have demonstrated a significant difference in STAT1 signaling between the diseases. In ET, JAK2V617F induces simultaneous activation of both STAT5 and STAT1 signaling pathways. Activation of phosphorylated STAT1 constrains erythroid and promotes megakaryocytic differentiation. In PV, reduced pSTAT1 response to JAK2V617F removes the 'brake' on erythropoiesis, thus allowing the development of an overt erythrocytosis and also reduces megakaryopoiesis [20]. This indicates that the clinical consequences of the JAK2V617F mutation in terms of hemopoiesis may reflect a balance between STAT5 and STAT1 activation.

However, this is not the end of the story, as the genetics of MPNs have proven to be unexpectedly complex. Recent evidence suggests that JAK2 is not the initiating event in proliferation, despite the fact that JAK2V617F can induce an MPN-like disease in mouse models and that there is in fact a pre-JAK2V617F phase. Support for this theory comes from several lines, including JAK2-mutant MPN cases that transform to a JAK2 wild-type AML, suggesting that the mutation alone is insufficient to cause a clinical phenotype [18]. In addition, in familial cases of MPNs, the JAK2 mutation is an acquired event, thus suggesting that the inherited predisposition is unrelated to the JAK2 mutation [21]. Mutations in the *THPO* gene and *MPL* gene can cause hereditary thrombocytosis. The presence of these germline mutations and the clinical phenotype suggests that these mutations are the drivers of the disease, but in nonhereditary ET other factors are undoubtedly at play. Follow up of these patients is advised as progression to MF and AML has been reported [22]. Recently a germline JAK2V617I mutation has been described in a kindred relationship with hereditary thrombocytosis [23]. The JAK2 locus through the 46/1 haplotype increases the risk of MPN by an as yet unknown mechanism. More direct evidence comes from single cell-derived clones that exhibit multiple mutations, in particular *TET2* mutations and del(20q), which have been shown to precede JAK2V617F [24,25]. This suggests that a primary transformation event occurs in a stem cell, which had not yet acquired the JAK2 mutation. It is likely that factors other than JAK2 status contribute to the final MPN phenotype. Potential candidate factors include gender, genetic instability, host polymorphisms, epigenetic factors and additional, as yet unidentified, somatic mutations (Figures 1 & 2).

Table 1. Approximate frequencies of acquired mutations in myeloproliferative neoplasms.

Gene	Mutation	Protein	Incidence (%)			
			PV	ET	MF	BP
JAK2	Exon 14, V617F	JAK2	95	60	60	50
	Exon 12		1–2	–	–	?
MPL	Exon 10, W515K/l/A, S505N	TpoR	–	3–5	5–10	?
TET2	Inactivating	TET2	10–20	5	10–20	20
IDH1/2	IDH1-R132 IDH2-R140	IDH1/2	Rare	Rare	5	20–35
ASXL1	Inactivating	ASXL1	2–5	2–5	13–20	20
EZH2	Inactivating	EZH2	1–3	1	5–10	20

BP: Blast phase; ET: Essential thrombocythemia; MF: Myelofibrosis; PV: Polycythemia vera.
Adapted from [12].

Clinical presentation

The incidence of ET has been estimated as 2.53 cases per 100,000 population making it the most common of the MPNs [26]. This figure is likely to have increased in recent years, given the widespread use of routine laboratory testing and the reduction in the WHO diagnostic platelet-count threshold for investigating to exclude for ET, from 600 to 450 × 10^9/l. Most patients present asymptomatically and often remain so for many years. The disease can manifest at all ages with a median presentation at 60 years and, interestingly, there is a slight female preponderance. Vascular events are the most common clinical complication, including thrombosis (arterial more commonly than venous) and microvascular occlusion [27]. Neurological symptoms are a common finding in ET, including cephalalgia, visual disturbance and dizziness [28]. These symptoms may improve with antiplatelet therapy and for this reason microvascular occlusion secondary to platelet aggregation is felt to be causative. Aquagenic pruritus is an uncommon symptom but can be very disabling. It is more prevalent in JAK2V617F-positive patients and raises the possibility of PV as a diagnosis.

Diagnosis

The diagnostic pathway for ET has changed significantly in recent years and now reflects the molecular developments in this disease. We have clearly defined, easily accessible markers for clonality and upfront testing for JAK2V617F is now standard practice. This has undoubtedly facilitated the timely achievement of a robust diagnosis in those 50% of ET patients who harbor mutations. However, for those who do not, this remains a diagnosis of exclusion. The WHO diagnostic criteria were updated in 2007 and adopted a lower platelet threshold of 450 × 10^9/l but place significant

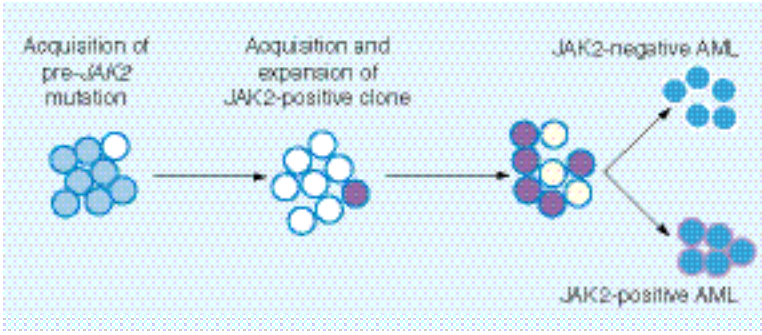

Figure 1. Is acquisition of JAK2 a secondary event in acute myeloid leukemia? JAK2 does not appear to be the disease-initiating event, as evidenced by the evolution to JAK2-negative AML.
AML: Acute myeloid leukemia.

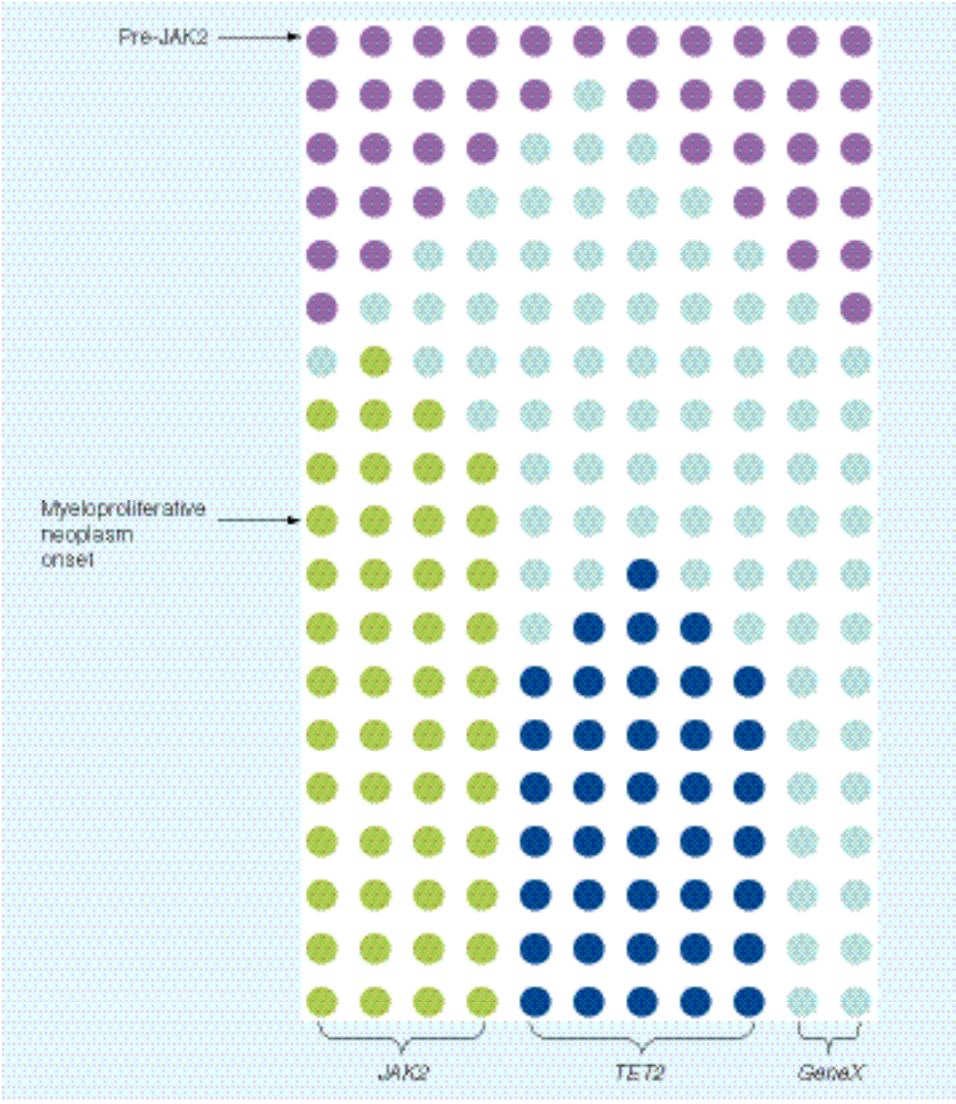

Figure 2. Biclonality of myeloproliferative neoplasms. An as yet unknown gene (*GeneX*) may predate clinically detectable disease and presence of *JAK2/TET2* mutations.

emphasis on specific histological features [29]. In 2010, the British Committee for Standards in Hematology proposed a modification of these diagnostic criteria, as shown in Box 1, acknowledging difficulties in the recognition of specific histological features associated with ET and early PMF [30].

Thus, normally ET should be considered when there is an unexplained, persistent thrombocytosis of >450 × 10^9/l. Iron studies are mandatory in any suspected ET case, to distinguish a reactive thrombocytosis due to iron deficiency and in the JAK2V617F patients, to exclude PV that has been masked by iron deficiency. JAK2V617F-positive ET patients, as a cohort, have higher hemoglobin and neutrophil counts, lower platelet counts and EPO levels and display a phenotype more akin to PV. They also have a higher incidence of venous thrombosis and transformation to PV [31].

Those patients with a thrombocytosis who are *JAK2* wild-type should be tested for a mutation in *MPL*. *JAK2*/*MPL*-negative ET remains a diagnosis of exclusion and a search for causes of a reactive thrombocytosis, as well as other clonal causes including MDS is needed to secure a diagnosis. In retrospective studies of unselected patients with thrombocytosis, less than 20% of patients were found to have a clonal hematological cause [32,33]. The most common causes for a reactive thrombocytosis include infection, malignancy and chronic inflammation. Careful clinical examination, testing for CRP and bone marrow histology are recommended in these cases. Chronic myeloid leukemia can present infrequently with an isolated thrombocytosis and for this reason, patients who lack *JAK* or *MPL* mutations, or who have an abnormal myelogram, should be considered for *BCR–ABL1* testing. Careful examination of the blood film is required to exclude the classical features of MF, and indeed MDS, which are leucoerythroblastosis and splenomegaly, since some subtypes of MDS may present with a thrombocytosis (MDS with isolated 5q minus) and also harbor a JAK2V617F mutation; for

Box 1. Diagnostic criteria for essential thrombocythemia.

WHO diagnostic criteria for essential thrombocythemia, as proposed in 2007 [29]

Diagnosis requires meeting all four criteria:

- Sustained platelet count ≥450 × 10^9/l during the work-up period
- Bone marrow biopsy specimen showing proliferation, mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes; no significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis
- Not meeting WHO criteria for PV, PMF, CML, MDS, or other myeloid neoplasm
- Presence of an acquired mutation or clonal marker or no reactive cause for thrombocytosis

British Committee for Standards in Haematology diagnostic criteria, as proposed in 2010 [30]

Diagnosis requires A1–A3 or A1 + A3–A5:

- A1: Sustained platelet count ≥450 × 10^9/l
- A2: Presence of an acquired pathogenic mutation (e.g., in the *JAK2* or *MPL* genes)
- A3: No other myeloid malignancy, especially PV†, PMF‡, CML§ or MDS¶
- A4: No reactive cause for thrombocytosis and normal iron stores
- A5: Bone marrow aspirate and trephine biopsy showing increased megakaryocyte numbers displaying a spectrum of morphology with predominant large megakaryocytes with hyperlobulated nuclei and abundant cytoplasm. Reticulin is generally not increased (grades 0–2/4 or grade 0/3)

†Excluded by a normal hematocrit in an iron-replete patient.

‡Indicated by presence of significant bone marrow fibrosis, grades ≥2/3 or 3/4 reticulin and palpable splenomegaly, blood film abnormalities (circulating progenitors and tear drop cells) or unexplained anemia.

§Excluded by absence of BCR–ABL1 fusion from bone marrow or peripheral blood.

¶Excluded by absence of dysplasia on blood film and bone marrow.

CML: Chronic myelogenous leukemia; MDS: Myelodysplasia; PMF: Primary myelofibrosis; PV: Polycythemia vera.

example, patients with refractory anemia and ring sideroblasts.

The concept of early-stage or prefibrotic MF is a contentious issue and this entity is said to be frequently and readily misdiagnosed as ET [34–36]. Prefibrotic MF was initially said to present with thrombocytosis in the absence of anemia, splenomegaly, leucoerythrocytosis or bone marrow fibrosis but has dysplastic megakaryocytes, which are a typical feature of overt MF. The recognition of such an entity has been criticized for being subjective, lacking reproducibility and long-term outcome data. For example, in a study involving three expert hematopathologists and 370 ET bone marrow biopsy specimens, no differences could be discerned between patients labeled as having 'prefibrotic MF' or 'true ET' in clinical and laboratory features at presentation, transformation to overt MF or survival [37]. However, the most recent WHO criteria also incorporate a requirement for clinical features compatible with PMF to be present before making this diagnosis and, in a recent paper with central review by a single pathologist, this has shown some clinical correlations [38]. However, other authors and analyses have described the same difficulties in robust application and lack of clinical consequence. Histological features, such as reticulin, appear to be reproducible but the finer features, such as megakaryocytic nuclear abnormalities, are subjective and here is where the difficulty lies. In a multicenter study, six pathologists from five countries reviewed 102 marrow trephines from patients with a clonal thrombocytosis. The percentage of unclassifiable MPNs rose from 2 to 28%. This was mostly attributable to histological MF cases that did not fill the complete diagnostic criteria. Considerable problems arise if our diagnostic criteria are too stringent and fail to classify patients appropriately and instead confine them to the miscellaneous 'MPN unclassifiable' subgroup [39].

As consistent evidence is lacking to suggest that these patients with a putative diagnosis of prefibrotic MF have a more accelerated disease pathway, they should perhaps be treated as ET in the absence of overt MF. A second difficulty arises from the not insubstantial volume of ET patients who have increased reticulin in their marrow at diagnosis, but lack any other features of MF. The PT-1 trial has shown that these patients, who account for 15–20% of the cohort, are at an increased risk of thrombosis and disease progression but interestingly, have no change in overall survival. These patients should be treated for standard ET, but common sense would suggest that they and those with putative prefibrotic MF might benefit from closer monitoring for progression [40].

Prognosis & risk stratification

With regards to survival, the life-expectancy of patients with ET was found to be no different from that of the general population [41]. Evolution to MF affects a proportion of ET patients but the prevalence

Box 2. Criteria for the diagnosis of post-essential thrombocythemia myelofibrosis.

Diagnosis requires A1 and A2 plus any two B criteria:

- A1: Bone marrow fibrosis ≥3 (on 0–4 scale)
- A2: Previous diagnosis of essential thrombocythemia
- B1: New palpable splenomegaly or ≥5 cm increase in spleen size
- B2: Unexplained anemia with a ≥2 g/dl decrease from baseline
- B3: Leucoerythroblastic blood film
- B4: Tear-drop poikilocytes
- B5: Constitutional symptoms†
- B6: Histological evidence of extramedullary hematopoiesis

†Drenching night sweats, weight loss of >10% over 6 months, unexplained fever >37.5°C or diffuse bone pain.

Adapted from [19].

varies widely, reflecting differences in definition and study design. Retrospective studies suggest prevalence rates of 6–15% at 15 years [42]. Transformation to post-ET MF is not just defined by accumulation of reticulin fibrosis but must also be evidenced by accompanying clinical and laboratory features (Box 2).

Morbidity in ET is predominantly related to thrombosis and to a lesser extent, hemorrhage. Age and previous thrombosis are the most predictive factors for a thrombotic event and JAK2V617F-positive ET patients have a higher incidence of venous and overall thrombosis [43]. Therapeutic decisions are most often based on risk assessment and absolute platelet numbers; however, the relationship between platelet count and thrombotic complications is not well established. The most widely accepted definition of high-risk ET is a patient who fits any of the following criteria: over 60 years of age; prior thrombosis; hemorrhage related to disease; or platelet count of >1500 × 10^9/l [44].

Recent evidence suggests a correlation between leucocyte count and thrombotic risk through monocyte activation and expression of tissue factor and P-selectin [45]. Myelosuppressive agents impacting the granulocyte lineage, as well as platelet production, are more effective in reducing thrombosis in ET than drugs selectively inhibiting the megakaryocyte maturation, which supports this theory [46]. There is a clearer association with hemorrhage and platelet counts in excess of 1500 × 10^9/l. There is some evidence to suggest that this is due to an acquired von Willebrands disease as a consequence of platelet-mediated increased proteolysis of the large vWf multimers [47]. However, bleeding occurs in the absence of disrupted vWf profile and routine testing of vWf is not recommended as it does not predict hemorrhage.

Modification of cardiovascular risk is important as risk factors for atherosclerosis such as diabetes, hypertension and hypercholesterolemia may also predict for thrombosis. Mutational status and fibrosis will require further study before being incorporated into a risk stratification model, but enough evidence now

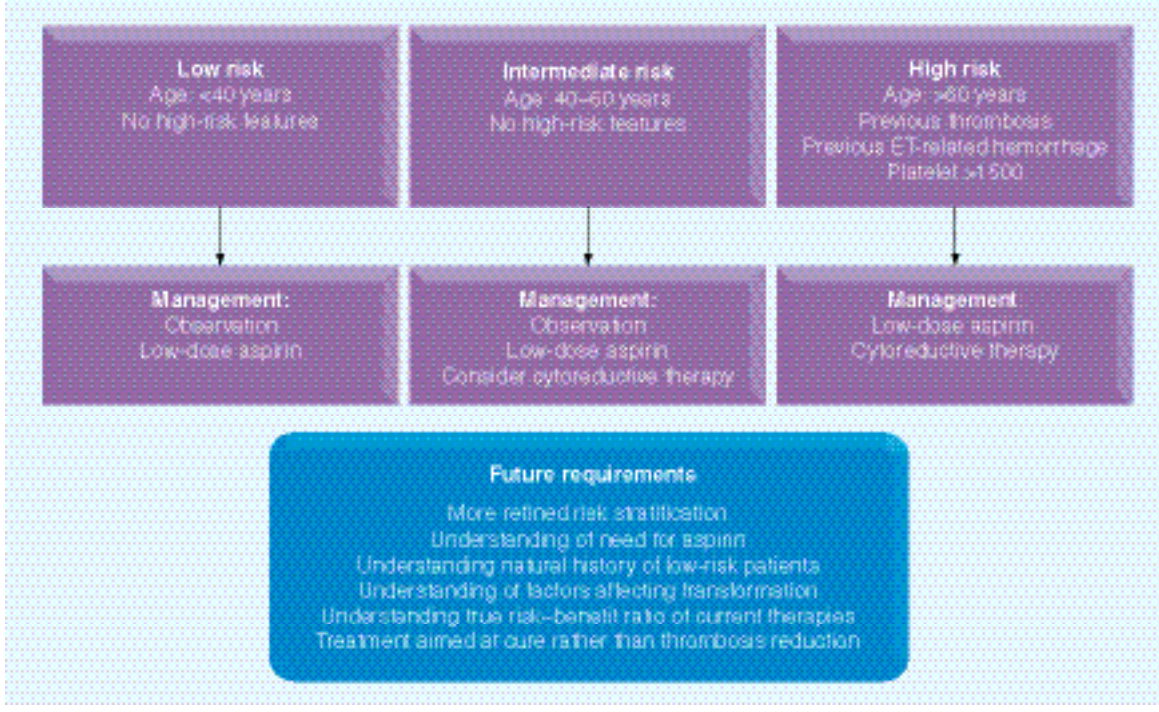

Figure 3. Therapeutic algorithm for essential thrombocythemia. A risk-adaptive approach to management of essential thrombocythemia.
ET: Essential thrombocythemia.

exists to regard leucocytosis as a risk factor and to consider it when making therapeutic decisions.

Treatment strategies

As thrombosis and hemorrhage are the main causes of morbidity in ET, treatment is conventionally aimed at reducing the risk of these complications, ideally without increasing the risk of transformation. Figure 3 shows a treatment algorithm for ET. All patients should have their cardiovascular risk assessed and treatment instigated for these factors where appropriate. In particular, smoking cessation should be advised and blood pressure control optimized. Recently there have been some interesting data regarding the use of statins. Statins have been shown to possess anti-inflammatory, immunomodulatory, antioxidant and growth inhibitory properties. Preclinical data suggest that statins inhibit proliferation and induce apoptosis of malignant cells and also inhibit angiogenesis and inflammation [48,49]. Their use should be considered in ET patients with dyslipidemia and probably merits investigation in ET patients as a separate cohort.

For low-risk disease, consensus opinion is that all patients should receive low-dose aspirin unless this is contraindicated. The evidence is extrapolated from the ECLAP study in PV, which showed a reduced thrombotic risk in high-risk patients [50]. However, the effectiveness of antiplatelet therapy as primary prophylaxis in low-risk ET is not proven. In a study of 300 low-risk ET patients, 198 were treated with an antiplatelet as monotherapy and 102 with observation alone. Thrombotic rates were only different in those groups who were JAK2 positive or those with cardiovascular risk factors, suggesting observation alone may be an adequate option in low-risk patients [51]. For those patients who are intolerant of aspirin or who develop gastrointestinal bleeding, clopidogrel – a thienopyridine-class antiplatelet agent – is a reasonable alternative. In patients with a platelet count >1500 × 10^9/l, antiplatelet therapy is relatively contraindicated due to the increased risk of hemorrhage.

The evidence is scanty for a group of so-called 'intermediate-risk ET'. In the UK these are defined as those patients who are aged 40–60 years with no high-risk features, whereas elsewhere this is defined as those patients <60 years with cardiovascular risk factors [52]. Other experts might define intermediate-risk ET by, for example, the presence of vascular risk factors or a strong family history, so this group lacks a clear definition of clinical features as well as a clear evidence base for its treatment. The ongoing intermediate risk arm of the PT-1 trial compares hydroxycarbamide and aspirin with aspirin alone in this patient population (defined as per UK guidance) and should elucidate the best therapeutic option for these patients, as well as more accurately determining risks associated with hydroxycarbamide therapy [101].

For high-risk patients, cytoreductive therapy is clearly indicated and hydroxycarbamide remains the first-line agent in conjunction with low-dose aspirin. Hydroxycarbamide was compared with no cytoreduction in 114 high-risk ET patients and was found to reduce the thrombotic rate from 24 to 3.6% [53]. Hydroxycarbamide has been shown to be superior to anagrelide in terms of prevention of vascular events and transformation to MF in high-risk ET patients [46]. Intolerance can develop and is manifested by cytopenias, oral or leg ulceration and gastrointestinal upset (Box 3). There is still some concern regarding the leukemogenicity of hydroxycarbamide and it should therefore be avoided in the younger population, where interferon is the preferred treatment modality. For patients who are intolerant or resistant to hydroxycarbamide as defined by the European LeukaemiaNet response criteria (Box 2), anagrelide is a reasonable second-line agent [54]. Importantly, the use of a second agent known to be associated with leukemia after the use of hydroxycarbamide – for example busulfan – is associated with appreciable risk of leukemia.

Anagrelide acts by selectively inhibiting megakaryocyte differentiation, thereby reducing the platelet count. The starting dose is 500 µg two- to three-times a day. Side effects include palpitations, headache, arrhythmias, fluid retention, gastrointestinal upset and pulmonary fibrosis. There is an increased incidence of marrow fibrosis in patients receiving anagrelide treatment [45]. It is reversible in a minority of cases and for this reason surveillance marrows are recommended every 2–3 years [27].

For the older patient with ET, pulsed busulfan can provide excellent control of counts and is very well tolerated. The doses can often be administered

Box 3. European LeukemiaNet criteria for resistance or intolerance to hydroxycarbamide in essential thrombocythemia.

Resistance

- Platelet count >600 × 10^9/l after 3 months of at least 2 g/day of hydroxycarbamide (2.5 g/day in patients with a bodyweight >80 kg)
- Platelet count >400 × 10^9/l and white blood cell count <2.5 × 10^9/l at any dose of hydroxycarbamide
- Platelet count >400 × 10^9/l and hemoglobin <10 g/dl at any dose of hydroxycarbamide

Intolerance

- Presence of leg ulcers or other unacceptable mucocutaneous manifestations at any dose of hydroxycarbamide or hydroxycarbamide-related fever

Adapted from [58].

intermittently and at long intervals, which is convenient for the elderly patient who finds clinic visits difficult. There is a well-established increased risk of leukemic transformation and therefore should be reserved only for this patient cohort [55].

Interferon: old drug, new data

Interferon should be considered in the young patient or female patients planning a family. It can be difficult to tolerate because of its side-effect profile, which includes fatigue, flu-like symptoms and mood disturbance but, these concerns withstanding, it can provide excellent control of proliferative symptoms. It has no leukemogenic potential and is not teratogenic. There has recently been interesting data regarding reduction in JAK2V617F allelic burden with specific types of interferon and some patients have achieved complete molecular responses [56]. In a study of pretreated ET patients, 39 received pegylated IFN-α2A (PEGASYS) and of these, 92% had a hematological response and 86% had a complete response. Major molecular responses were seen in 13% and complete molecular responses in a further 13%. Treatment discontinuation was low at 10%, suggesting that this agent is better tolerated than previously reported [48]. Interferon appears to act selectively on the JAK2V617F malignant clone in both ET and PV, possibly acting through a STAT1-independent pathway [17]. A future development may be the use of JAK2V617F allelic burden as a marker of minimal residual disease. However, a recent update from a French group demonstrated that, in selected PV patients, while the JAK2V617F clone was reduced by PEGASYS, the *TET-2* mutant clones were apparently not affected [57]. Current trials underway include a Phase III study of PEGASYS versus hydroxycarbamide in newly diagnosed high-risk ET patients and PEGASYS in patients who are intolerant or resistant to hydroxycarbamide [102,103].

Investigational agents

Although our current therapeutic strategy is effective for most patients, there is a substantial cohort who become refractory or intolerant of therapy, have been shown to have a poor prognosis and pose a therapeutic challenge [58]. There is clearly a role for experimental agents for these patients. Two such agents are JAK2 inhibitors and HDAC inhibitors.

JAK2 inhibitors

JAK2 inhibitors are beginning to be tested in high-risk ET patients who have been intolerant or resistant to standard treatments. They have been shown to control proliferation but no data are available in terms of their ability to control the vascular complications that are the hallmark of the disease or the long-term effects of continuous JAK inhibition [59]. The bulk of evidence for these agents relates to MF where they have been shown to be highly effective at reducing spleen size and controlling the troublesome symptoms of sweating, pruritis and weight loss. Although follow up is short, there is evidence that ruxolitinib, a JAK1 and JAK2 inhibitor, is having an impact on the natural history of the disease and early data does indicate a survival advantage [60]. Verstovsek *et al.* have demonstrated hematological responses in hydroxycarbamide-intolerant or -resistant ET patients with ruxolitinib [59]. A total of 49% of enrolled subjects' normalized platelet counts to the upper limit of normal after a median of 0.5 months and the drug was well tolerated. CEP701, another JAK inhibitor, has been trialled in PV and ET patients with good control of proliferative symptoms but considerable gastrointestinal toxicity [61].

HDAC inhibitors

HDAC inhibitors have also been investigated in MPNs. Most data relate to the agents vorinostat, givinostat and panobinostat. HDACs are enzymes involved in the remodeling of chromatin and have a key role in the epigenetic regulation of gene expression. HDACs also promote the acetylation of other nonhistone proteins, including HSP90, a chaperone for various proteins conferring protection from ubiquitin-directed proteasomal degradation. Altered expression of HDACs is present across the MPN spectrum, making these enzymes interesting therapeutic targets [62]. *In vitro* studies investigating the use of ITF2357 (givinostat), a HDAC inhibitor, in JAK2V617F-mutant cell lines from ET and PV patients, showed preferential reduction in the mutant clone with preservation of the wild-type cells [63]. Givinostat was evaluated in the treatment of JAK2 V617F-positive PV, ET and PMF. It was given orally for 24 weeks at a starting dose of 50 mg twice daily with a median treatment duration of 20 weeks [64]. Among 13 PV/ET patients, one complete, six partial and four no responses were documented at the study end, while two patients went off-study prematurely. Concomitant hydroxycarbamide was required in four patients to achieve full control. Strikingly, pruritus disappeared in most patients and reduction of splenomegaly was observed in 75% of PV/ET patients. Results to date show that these drugs are not as well tolerated as standard therapy but they do appear to have a profound effect on symptoms, as well as reducing spleen size, and a modest reduction in JAK2 allelic burden by reverse transcriptase PCR. Their role in the future is likely to be in combination

with JAK inhibitors and Phase I clinical trials are currently underway in MF patients with a combination of panobinostast and ruxolitinib [104]. There appears to be a synergistic cytotoxic effect against human myeloproliferative neoplastic cells.

Special patient cohorts

Pregnancy

There is an increased incidence of miscarriage and pregnancy complications such as pre-eclampsia, intrauterine growth retardation and intrauterine death in patients with ET [65]. These patients are also at an increased risk of thrombosis during their pregnancy and postpartum. For these reasons, aspirin is recommended throughout pregnancy and low-molecular weight heparin for 6 weeks postpartum. Women who have had a previous thrombosis or pregnancy complication are considered higher risk and should be managed in a high-risk maternity clinic with access to uterine Doppler artery scanning. These women should receive aspirin and low-molecular-weight heparin throughout the pregnancy and should be considered for cytoreduction. The agent of choice is interferon but plateletpheresis can also be considered. Hydroxycarbamide is teratogenic in non-human mammals and should therefore be avoided. It is also associated with reduced spermatogenesis in animal models. It is therefore our practice to stop hydroxycarbamide in male patients planning to conceive.

Perioperative management

Surgery poses additional risks to ET patients and their platelet counts should be optimized prior to all elective major surgery. Aspirin should be stopped 7–10 days pre-operatively and thromboprophylaxis should be administered according to local policy. For ET patients not receiving cytoreductive therapy,

Executive summary

Pathogenesis
- The genetics of myeloproliferative neoplasms (MPNs) has proven to be unexpectedly complex.
- In terms of likely pathogenic roles, *JAK2* and *MPL* mutations appear to exert a phenotype-modifying effect and are distinctly associated with MPNs while *TET2*, *IDH1/2* and *ASXL1* do not.

Genotype–phenotype correlation
- JAK2V617F allele burden is emerging as an important contributor to the MPN clinical phenotype.
- Higher allelic burden appears to correlate with a more symptomatically advanced or accelerated disease.
- The clinical consequences of the JAK2V617F mutation in terms of hemopoiesis may reflect a balance between STAT5 and STAT1 activation.
- Recent evidence suggests that JAK2 is not the initiating event in proliferation.

Clinical presentation
- Essential thrombocythemia (ET) is the most common MPN with an estimated incidence of 2.53 cases per 100,000 population and patients usually present asymptomatically.

Diagnosis
- The diagnostic pathway for ET has changed significantly in recent years and now reflects the molecular developments in this disease.
- Controversy remains with regard to the diagnosis of prefibrotic myelofibrosis and its differentiation from true ET.

Prognosis & risk stratification
- The life expectancy of patients with ET was found to be no different from that of the general population.
- Recent evidence suggests a correlation between leucocyte count and thrombotic risk.

Treatment strategies
- As thrombosis and hemorrhage are the main causes of morbidity in ET, conventional treatment is aimed at reducing the risk of these complications, ideally without increasing the risk of transformation.
- The evidence is scanty for intermediate-risk ET and results from the PT-1 intermediate arm should help guide management.
- Interferon can cause a reduction in JAK2V617F allelic burden and some patients have achieved complete molecular responses.
- A substantial cohort of patients become refractory or intolerant of therapy and there is clearly a role for experimental agents for these patients.
- JAK inhibitors have been shown to control proliferation but no data are available in terms of their ability to control the vascular complications.

Special patient cohorts
- There is an increased incidence of miscarriage and pregnancy complications in women with ET and the treatment of choice for high-risk patients is interferon.
- Platelet counts should be optimized prior to all elective major surgery.

consideration should be given to a temporary course of treatment preoperatively and should be decided on a case-by-case basis.

■ Budd-Chiari

Thrombotic hepatic venous outflow obstruction causing the clinical syndrome known as Budd-Chiari is most commonly seen in PV but can also occur in ET patients. Nearly 60% of cases of Budd-Chiari have JAK2V617F detected [66]. These patients pose particular difficulty because of their increased hemorrhagic risk due to the development of esophageal varices and their thrombotic risk conferred by the MPN. Cautious anticoagulation is advised in this cohort with regular endoscopy and aggressive treatment of new varices. These patients should be followed up in a unit with an interest in liver disease.

■ Thrombosis with normal platelet count

A small cohort of MPN patients will present with a thrombosis and a normal blood count. This has become a more common presentation as a result of JAK2V617F testing in patients exhibiting unprovoked thromboses or thromboses at unusual sites. These patients are particularly challenging. They cannot be classified by standard criteria. By definition they are high-risk, having had a thrombosis, and warrant cytoreduction but there is no target to cytoreduce nor an internationally agreed threshold to achieve with cytoreductive therapy. Anticoagulation is the mainstay of treatment and close follow up for proliferation is indicated.

Future perspective

Despite the wealth of new knowledge and expansion of our drug armamentarium, the overall management of ET is unlikely to change substantially over the coming years and it remains to be seen where the new targeted therapies fit into the treatment algorithm. Long-term follow up is required for investigational agents to show superiority to our standard therapy, both in terms of thrombotic risk and proliferation. Pegylated interferon may well challenge hydroxycarbamide as the first-line agent if it proves to be as effective at prevention of thrombotic complications. Patients who are intolerant or resistant to standard therapy or those with progressive disease will certainly benefit from the newer agents. We look forward to clearer guidance on how best to manage our intermediate-risk ET patients. Laboratory-based research will hopefully clarify how one mutation can lead to three distinct diseases and make it easier to distinguish them in ambiguous patients. There is no doubt that this is an exciting time in the world of MPNs.

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