EXPERT REVIEWS

Anagrelide: 20 years later

Expert Rev. Anticancer Ther. 9(1), 37–50 (2009)

Ashkan Emadi and Jerry L Spivak†

†Author for correspondence
Johns Hopkins University School of Medicine, Traylor 924, 720 Rutland Avenue, Baltimore, MD 21205, USA
Tel.: +1 410 955 5454
Fax: +1 410 614 0854
jlspivak@jhmi.edu

Thrombocytosis is a common feature of chronic myeloproliferative disorders (MPD) and may be asymptomatic or associated with transient microvascular vaso-occlusive symptoms or large vessel arterial or venous thrombosis. Failure of either the hematocrit or the platelet count to correlate with thrombotic events is a peculiar conundrum of the MPDs. Asymptomatic thrombocytosis in young MPD patients with no cardiovascular risk factors does not require treatment. It is also undisputed that lowering the platelet count reduces the incidence of microvascular events in MPD patients. At the same time, no study to date has demonstrated that platelet count reduction prolongs survival in MPD patients. Agents such as hydroxyurea, busulfan, IFN-α and anagrelide, have been used to reduce an elevated platelet count and decrease thrombohemorrhagic events in at-risk patients with thrombocytosis associated with an MPD. When treatment is required, it makes sense to use drugs that are not myelotoxic or mutagenic. Based on the Primary Thrombocythaemia 1 study, hydroxyurea is the treatment of choice for thrombocytosis-associated transient ischemic attacks. However, hydroxyurea does not prevent venous thrombosis, is not more effective in preventing arterial thrombosis than anagrelide and its long-term safety is not established. Therefore, unless curative therapy is planned, one should use the least myelotoxic agent when platelet count reduction is required. In this regard, anagrelide can be considered a first-line drug. With regard to long-term safety of anagrelide, the EMEA has required close monitoring of the safety points identified in future Periodic Safety Update Reports and in a Post Authorisation Safety Study in the EU, which will focus especially on cardiovascular events and acute leukemia. In this article, we review anagrelide pharmacology, the physiology of thrombopoiesis, the differential diagnosis of thrombocytosis and the management of patients with an elevated platelet count.

Keywords: anagrelide • essential thrombocytosis • myeloproliferative disorder

Platelets constitute the first line of defense against hemorrhage when the integrity of the vessel wall has been compromised. As a corollary, a decrease in platelet number or impairment of platelet function, if severe, creates a hemorrhagic diathesis. Paradoxically, a marked increase in platelet number in a myeloproliferative disorder (MPD), such as polycythemia vera (PV), primary myelofibrosis (PMF) or essential thrombocytosis (ET), can also create a hemorrhage diathesis by consumption of circulating high-molecular-weight von Willebrand multimers [1], while paradoxically also causing microvascular spasm or transient obstruction [2], leading to syndromes such as erythromelalgia [3], ocular migraine [4,5] or transient ischemic attacks [6]. However, to date, no study has shown a definite correlation between the height of the platelet count and major vessel thrombosis, although this association is frequently imputed clinically. Thrombocytosis is far less common than thrombocytopenia, and the means for establishing its etiology, as well as the mechanisms for its vascular effects and their management, are still matters of debate. Until two decades ago, when the imidazoquinazoline, anagrelide, was introduced [7], chemotherapy or plateletpheresis were the only means for reducing platelet number. However, despite two decades of scrutiny, there is still no consensus regarding the role of anagrelide in the management of thrombocytosis. In this article, we discuss anagrelide pharmacology, the physiology of thrombopoiesis, the differential diagnosis of thrombocytosis and the therapeutic use of anagrelide based on published experience with this drug over the past 20 years.

Chemistry

Anagrelide (6,7-dichloro-1,5-dihydroimidazo [2,1-β] quinazolin-2(1H)-one; molecular weight: 256) is a hydrochloric imidazoquinazoline

 10.1586/14737140.9.1.37 ISSN 1473-7140

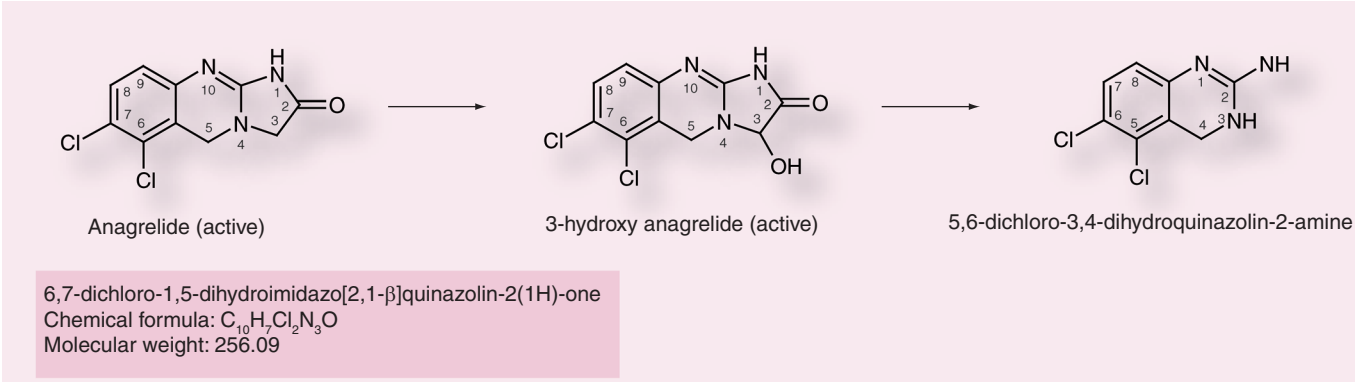

Figure 1. Anagrelide and its metabolic pathway in healthy volunteers.

(Figure 1), which was initially developed in 1979 [7]. It is non-hygroscopic, water insoluble and poorly soluble in organic solvents (except dimethylsulfoxide and dimethyl fumarate).

Compounds containing an imidazoquinazolin nucleus not only exhibit platelet aggregation inhibitory activity [201] but also positive inotropic [8,202,203] and bronchodilatory effects [204].

Anagrelide is primarily metabolized in the liver and excreted mainly through the kidneys. Its two major metabolites are 3-hydroxy anagrelide (6,7-dichloro-3-hydroxy-1,5-dihydro-imidazo[2,1-β]quinazolin-2-one) and 2-amino-5,6-dichloro-3,4-dihydroquinazoline (Figure 1), which is made after dissociation of the two carbonyl groups (C2 and C3) [9,301]. 3-hydroxy anagrelide is equipotent with the parent drug in its *in vitro* effects on megakaryocytopoiesis and platelet count reduction but is 40-times more potent as a phosphodiesterase (PDE) III inhibitor and, therefore, as an inotrope, chronotrope and vasodilator [9].

Pharmacology

Pharmacokinetics

An intravenous formulation of anagrelide was never developed, presumably because of the chronic nature of essential thrombocytosis. This has precluded direct measurement of its absolute oral bioavailability. However, there are studies that have provided valuable insights into anagrelide metabolism. Oral administration of a single dose of ^{14}C-anagrelide, equivalent to 1 mg of free base, in five healthy, fasting men, resulted in approximately 70% absorption from the GI tract and recovery in the urine, mainly as metabolites [10].

Based on limited data, between doses of 0.5 and 2 mg, anagrelide kinetics are linear [302]. In fasting healthy subjects, 1 mg of anagrelide was rapidly absorbed with a C_{max} of 5 ng/ml at approximately 1 h (t_{max}), which decreased rapidly during the first 6–8 h,and then declined more slowly, with an estimated terminal elimination half-life of approximately 3 days [10]. The plasma half-life was short (1.3–1.5 h). Anagrelide does not accumulate in plasma after repeated administration [303].

When the drug was taken with food, bioavailability (based on the AUC from time zero to infinity [AUC_∞]) was reduced by 14% on average, C_{max} decreased by 45%, t_{max} was delayed to 2 h and half-life increased to 1.8 h [11].

Anagrelide's pharmacokinetics were different between healthy volunteers and ET patients. Mean AUC_∞ was 11 ng.h/ml for healthy volunteers as opposed to 19.5 ng.h/ml for ET patients. Both C_{max} and t_{max} were higher in ET patients compared with healthy volunteers (C_{max}: 6.2 ng/ml; t_{max}: 2 h vs C_{max}: 5 ng/ml; t_{max}: 1.3 h) [12]; however, the reasons for this are unknown.

Metabolism

Less than 1% of anagrelide is recovered in the urine, and severe renal impairment (creatinine clearance <30 ml/min) had no significant effects on anagrelide pharmacokinetics [303]. The mean recovery of the 2-amino-5,6-dichloro-3,4-dihydroquinazoline metabolite (Figure 1) in urine was approximately 18–35% of the administered dose [302].

Anagrelide undergoes significant first-pass hepatic metabolism to form its active metabolite, 3-hydroxy anagrelide (Figure 1) [301]. A single 1-mg oral dose of anagrelide in ten subjects with moderate hepatic impairment revealed a 2.2-fold increase in half-life (half-life of 3.3 vs 1.5 h) and an eightfold increase in AUC_∞ (83.8 vs 10.8 ng/h/ml) [301]. Since anagrelide is primarily metabolized by cytochrome P450 (CYP)1A2, its elimination can be slowed by CYP1A2 inhibitors, such as grapefruit juice.

Pharmacodynamics

In vitro studies

Essential thrombosis is associated with an approximately three- to fourfold increase in the megakaryocytic mass as a result of the increase in both megakaryocyte (MK) volume and number. MK diameter, cell ploidy and mean platelet turnover rate were also increased [13]. Anagrelide reversibly disrupts MK maturation in a dose-dependent fashion by influencing the post-mitotic phases of MK development. Inhibitory effects *in vitro* were observed on MK ploidy, size and cytoplasmic maturation [14,15]. *In vitro*, anagrelide and its active metabolite 3-hydroxy anagrelide (Figure 1), independent of PDE III inhibition, specifically target the MK differentiation program [9]. MK number may also be decreased by anagrelide [13] but not to the degree, for example, caused by hydroxyurea *in vitro* [16].

Studies of the effects of anagrelide metabolites on MK behavior have been inconclusive. A water-soluble anagrelide metabolite, 2-amino-5,6-dichloro-3,4-dihydroquinazoline (Figure 1), which

lacks an imidazo side-group, at concentrations ranging from 10 to 500 ng/ml, blocked *in vitro* MK migration by 20–40%. It also selectively inhibited *in vitro* MK maturation, resulting in a 50% decrease in the total number of $CD41^+$ MK [17] and compared with anagrelide, the metabolite was 50-times more potent. However, in another side-by-side comparison of the effects of anagrelide and its 2-amino-5,6-dichloro-3,4-dihydroquinazoline metabolite (Figure 1), on human cord blood-derived $CD34^+$ cells stimulated with thrombopoietin (TPO), the metabolite at concentrations up to 430 ng/ml showed no evidence of activity on the expression of the MK differentiation marker CD61, MK size or polyploidization. In sharp contrast, anagrelide was active at doses as low as 2.5 ng/ml, with half maximal inhibition (ID_{50}) observed at 25 ng/ml [18].

It has been suggested that anagrelide, in a dose-dependent manner, reduced MK proliferation by specific inhibition of thrombopoietin-mediated intracellular signaling since it failed to inhibit IL-3 stimulated MK proliferation in the same cells [19]. Anagrelide's effects also appear to be species-specific because the drug failed to inhibit murine thrombopoiesis [19].

At *in vitro* doses of 1 µM, much higher than those required for platelet count reduction (10 nM), anagrelide blocked platelet aggregation by inhibition of platelet cAMP-PDE III [9].

In vivo *studies*

The lack of animal models for the thrombocytopenic effect of anagrelide has limited *in vivo* pharmacodynamic studies [20]. In ET patients, anagrelide reduced the platelet turnover rate but did not affect platelet survival [13,21]. Proliferation of megakaryocytic-committed progenitor cells (CFU-M) *in vivo* was not affected by anagrelide, although high concentrations of anagrelide inhibited CFU-M *in vitro* [21]. In a study of the effect of anagrelide on *in vivo* MK proliferation and maturation in ET, it was found that anagrelide exerted its selective thrombocytopenic effect by decreasing both MK proliferation and size [13]. In another study of *in vivo* megakaryocytopoiesis, anagrelide caused a reduction in MK number and volume with modal ploidy of 16N, resulting in a 50% reduction in MK mass [22].

A comparative immunohistochemical and morphometric study of MK in 20 early-stage primary myelofibrosis patients with thrombocytosis, taking either hydroxyurea or anagrelide, demonstrated the frequent occurrence of promegakaryoblasts and micromegakaryocytes in both treatment groups. These were identified by CD61 expression in pre- and post-treatment bone marrow biopsies. However, hydroxyurea, but not anagrelide, was associated with significant dysplastic changes [23].

In a prospective study, 17 PMF patients received anagrelide for a median of 2 years with bone marrow examinations at baseline and after 6 and 12 months of treatment. Regardless of the platelet count reduction by anagrelide, bone marrow MK number increased in 12 patients who had bone marrow cellularity adequate enough to estimate MK number after 6 months of therapy. MK expression of TGF-β and PDGF, as assessed by histochemistry, was not consistently affected. No patient had a grade change of two or more in either bone marrow fibrosis or osteosclerosis. The authors concluded that anagrelide interfered with MK maturation rather than proliferation. No patient had a clinically appreciable benefit on the basis of treatment with respect to anemia, transfusion requirements or organomegaly, and the lack of a clinical therapeutic effect was presumed to be due to anagrelide's inability to alter MK expression of TGF-β or PDGF [24].

On the other hand, in a study in ET patients, anagrelide normalized VEGF and platelet factor 4 [25], as well as markers of platelet-related endothelial activation, prothrombin fragment 1 plus 2, plasmin/α2-antiplasmin complex, plasminogen activator inhibitor-1 and tissue factor pathway inhibitor [26].

In another study, decreased platelet PDGF-A and PDGF-B, increased TGF-β1, and normal basic FGF (bFGF) mRNA levels were reported before treatment. During treatment, mRNA levels remained decreased for PDGF-A, were increased for PDGF-B and normal for TGF-β1. In untreated patients, protein expression of PDGF paralleled its mRNA levels, while different patterns of RNA and protein were found for TGF-β1 and bFGF [27].

Owing to PDE III-inhibitory effect, anagrelide causes positive inotropic effects and vasodilation by decreasing peripheral vascular resistance [15] and increasing microvascular area [28]. This undoubtedly explains some of anagrelide's side effects, such as headache, fluid retention, dizziness, orthostatic hypotension, tachycardia, arrhythmias and, occasionally, congestive heart failure.

Clinical efficacy

Platelet-lowering efficacy

Anagrelide was initially developed as a platelet-specific anti-aggregating agent. However, its ability to reduce the platelet count at doses lower than its anti-aggregating effect and its safety profile led to US FDA approval in 1988 as an orphan drug for the treatment of MPD patients with thrombocytosis.

Several small-to-moderate-size, nonrandomized, retrospective and prospective observational studies [20,29–43] have documented the platelet-reduction effect of anagrelide in ET, PV, PMF and chronic myelogenous leukemia (CML). In most studies, one-to two-thirds of the patients had received prior therapy with hydroxyurea, busulfan, chlorambucil, IFN-α, pipobroman or radiophosphorus (^{32}P). Although some patients were symptomatic, a number of trials also included a large number of asymptomatic patients. Anagrelide was generally started at 1–2 mg/day at divided doses and maintenance doses were adjusted to achieve the target platelet count. In general, response was defined as a reduction in platelet count to less than 500–600 × 10^9/l or a greater than 50% decrease in platelet count. The platelet-lowering efficacy was 60–90% in ET, and the response was rapid with most patients reaching the treatment goal within a few weeks.

In a large prospective, multi-institutional Phase II study (Anagrelide Study Group), 577 MPD patients with thrombocytosis (335 with ET, 68 with PV, 114 with CML and 60 with undifferentiated MPD) were enrolled, and 424 were treated with anagrelide (0.5–1.0 mg four-times a day) for more than 1 month [44]. In total, 88 of the 424 patients received hydroxyurea and anagrelide concomitantly. Response rates (a reduction of the platelet count from pretreatment levels by 50% or to below 600 × 10^9/l) were reported to be 94 and 85% in evaluable patients

with ET and PV, respectively. However, out of 335 ET patients, only 262 (78%) were actually evaluable and, of these, 247 (73%) responded to treatment. In the PV group, out of 68 patients, 47 (69%) were evaluable and 40 (59%) responded to treatment. The median time to response after starting anagrelide was 11 days in ET patients and 15 days in PV patients.

The Anagrelide Study Group continued accrual and subsequently reported on 942 MPD patients (546 with ET and 113 with PV) who had been treated with anagrelide for a minimum of 4 years [45]. Table 1 shows their response to anagrelide. A better platelet reduction response to anagrelide in ET than PV was a common finding in this and many other trials of this drug [20,37,40,41].

A retrospective analysis of an open-label, multicenter, international trial of 3660 anagrelide-treated patients was performed to assess efficacy and long-term safety and specifically the leukemogenic potential [46]. Of the patients enrolled, 81% had previously received other myelosuppressive agents. With maximum follow-up over 7 years, anagrelide achieved platelet control in over 75% of patients and did not increase the conversion to acute leukemia during the treatment interval.

Essential thrombocytosis is very rare in the pediatric age range but in a small study of three children, anagrelide achieved adequate control of thrombocytosis with acceptable toxicity during long-term use [47].

Reduction of symptoms

The most common symptoms in ET are of microvascular origin and include headache, visual disturbances, light-headedness, acral dysesthesia and erythromelalgia. There are a few nonrandomized studies demonstrating effective symptom reduction in ET by anagrelide [26,35]. In one study, a successful platelet count reduction was associated with normalization of platelet coagulant and endothelial function and disappearance of erythromelalgia [26]. In a prospective study of 17 ET patients, microvascular thrombotic and hemorrhagic symptoms also disappeared with the normalization of platelet count in all cases, and persisted with a long-term continuous anagrelide treatment with a follow-up period of between 2 and 6 years. However, ET-related symptoms reappeared in three patients, coinciding with an increased platelet count up to 600 × 10^9/l associated with anagrelide dose reduction [35].

Reduction of thrombohemorrhagic complications

In a multicenter, open-label, prospective study, 97 thrombocytosis patients (69 females, 28 males; median age: 59 years), 79 with ET, 16 with PV and two with PMF received anagrelide at a starting dose of 0.5 mg twice daily followed by individual dose adjustments [40]. After 6 months of treatment, the overall rate of major thrombotic complications (i.e., stroke, myocardial infarction, peripheral arterial disease, ileofemoral venous thrombosis, pulmonary infarction, thrombosis of the portal vein and Budd–Chiari syndrome) decreased from 5 to 2% (p = 0.26). However, for ET patients, the reduction of major thromboembolic complications was significant (p = 0.046). The rate of minor thromboembolic complications (e.g., transitory ischemic attacks [TIAs], angina pectoris, erythromelalgia or other microcirculatory disturbances and superficial thrombophlebitis) decreased from 25 to 14% (p = 0.03). Despite platelet count control, minor bleeding events were observed in 13% of patients who took anagrelide in combination with low-dose aspirin, compared with 2% of patients who took anagrelide alone.

PT1 study

To date, there is only one randomized, controlled, prospective clinical trial involving anagrelide, the PT1 trial [48]. PT1 was an open-label study in high risk ET patients that compared hydroxyurea (initial dose of 0.5–1.0 g/day; n = 404) with anagrelide (initial dose of 0.5 mg twice daily; n = 405) for a median of 39 months. Patients were considered high risk if they met one or more of the following criteria: aged over 60 years; platelet counts greater than 1000 × 10^9/l; a history of ischemia, thrombosis, embolism or hemorrhage; or hypertension or diabetes requiring therapy.

The dose of both drugs was adjusted to maintain the platelet count at less than 400 × 10^9/l. Treatment was considered to have failed in patients whose platelet count was not less than 600 × 10^9/l after having received the assigned therapy for at least 3 months; these patients were removed from the study. All patients received aspirin at a daily dose of 75–100 mg daily (n = 792) or, if aspirin was contraindicated, dipyridamole (n = 13) or clopidogrel (n = 4).

Although the diagnosis of ET was made using Polycythemia Vera Study Group ET criteria [49], red-cell-mass studies were not performed in all patients. This is not a trivial issue since a recent study demonstrated that, when a red-cell-mass study was performed, 44% of ET patients actually had PV and when the ET patients were *JAK2* V617F-positive, 64% had PV [50]. Newly diagnosed and previously treated patients were eligible for the trial. Approximately 30% had previously been treated with hydroxyurea and 18% had received no prior treatment. In the anagrelide group 23 (6%) patients had received busulfan compared with 13 (3%) in hydroxyurea group. The median disease duration was also longer in the anagrelide group.

Table 1. Response to anagrelide by diagnosis.

Myeloproliferative disorder	Patients (n)	Complete response* (%)	Partial response‡ (%)
ET	546	398 (73)	47 (9)
PV	113	76 (66)	9 (8)
CML	179	112 (63)	18 (10)
Others	108	79 (73)	11 (10)

*Reduction in platelet count to less than 600 × 10^9/l, or to 50%, or less of pretreatment level for at least 4 weeks.
‡20–50% reduction in platelet count from pretreatment level for at least 4 weeks.
CML: Chronic myelogenous leukemia; ET: Essential thrombocytosis; PV: Polycythemia vera.
Data from [45].

The composite primary end point was death from thrombosis or hemorrhage; or occurrence of arterial or venous thrombosis or serious hemorrhage. Secondary end points were the time to the first arterial or venous thrombosis, or to the first serious hemorrhage, the time to death, the incidence of transformation to myelofibrosis, acute myeloid leukemia (AML), myelodysplasia or PV and control of the platelet count.

Anagrelide and hydroxyurea, administered concomitantly with aspirin (or other antiplatelet-aggregating agents) to high-risk patients, reduced the platelet count similarly by 9 months after trial entry and afterwards. However, at 3 and 6 months, platelet counts were significantly higher in the anagrelide group. Since there was no significant heterogeneity between the odds ratio for events in the first 9 months, the authors concluded that differences in the platelet count between the two arms during the first 9 months were unlikely to be of clinical significance.

The median leukocyte count was also significantly and persistently lower in the hydroxyurea group starting at 3 months after trial entry [48]. An increased leukocyte count in ET at diagnosis has recently been associated with an increased risk for thrombosis during follow-up [51,52].

In the PT1 study, the primary end point (i.e., death, arterial or venous thrombosis or serious hemorrhage) occurred more often in the anagrelide group than in the hydroxyurea group (55 vs 36; odds ratio 1.57; 95% confidence interval [CI]: 1.04–2.37; p = 0.03) [48]. The estimated risk of the primary end point at 5 years was 16% in the anagrelide group and 11% in the hydroxyurea group but with overlapping CIs (95% CI: 12–21 vs 7–14).

Overall, arterial thrombosis occurred significantly more often in the anagrelide group than the hydroxyurea group (37 [9%] vs 17 [4%]). However, among the individual end points of the various types of arterial thrombosis, including unstable angina, myocardial infarction, stroke or arterial embolus of the lower or upper limbs, there was no significant difference between the two groups. Only TIAs occurred significantly more frequently in the anagrelide plus aspirin group than in the hydroxyurea plus aspirin group (14 [3.5%] vs 1 [0.25%]; p < 0.001) [48]. This can be explained by the nitric oxide donor effects of hydroxyurea [53–56]. It has also been speculated by others that the higher TIA incidence in the anagrelide group was caused by microbleeds and not by thrombotic events [57].

Venous thromboembolism, in general, occurred significantly less often in the anagrelide group compared with the hydroxyurea group (3 [1%] vs 14 [4%]; p = 0.006). Among the individual end points making up the total venous thromboses, there was no significant difference between the two groups in pulmonary embolism and hepatic vein thrombosis. Deep vein thrombosis happened significantly less often in the anagrelide group (1 [0.25%] vs 9 [2.2%]; p = 0.009) [48]. The overall logical evidence-based message from the aforementioned data for clinical practice would be to use anagrelide in ET patients with history of deep vein thrombosis, or aspirin with hydroxyurea in the high-risk ET patients with a previous TIA. However, given the relatively low event rates and uncertainty with respect to disease diagnosis since venous thrombosis is not a prominent feature of ET, absolute therapeutic recommendations are difficult to make.

Serious hemorrhage (particularly GI tract bleeding (13 [3.2%] vs 3 [0.7%]) occurred significantly more often in the anagrelide plus aspirin group. The rate of death due to the thrombohemorrhagic or malignant hematological events was similar between the two groups. In addition, it was reported that anagrelide accelerated myelofibrosis as a composite clinical diagnosis in comparison to hydroxyurea, which formed the basis for early study closure. However, the published supplemental data showed an imbalance in disease duration between the two groups that favored the hydroxyurea arm.

In a subsequent publication, the authors of the PT1 study determined the presence of the *JAK2* V617F mutation in 806 ET patients, including 776 from the PT1 study. Mutation-positive patients had multiple features resembling PV, including a significantly increased hemoglobin level, neutrophil count, bone marrow erythropoiesis and granulopoiesis, and more venous thromboses. *JAK2* V617F-positive individuals were also more sensitive to hydroxyurea therapy compared with anagrelide than those without the *JAK2* mutation [58].

Safety & tolerability

The most common adverse reactions of anagrelide based on the extended Anagrelide Study Group (942 patient cohort) [45], which was used by the manufacturer in the prescribing information, were headache (37% in all patients and 45% in ET), palpitation (26%), diarrhea (25%), fluid retention (22%), asthenia (22%), nausea (17%), abdominal pain (16%) and dizziness (15%). Headache was generalized and usually controlled by acetaminophen. It has been speculated that most anagrelide side effects are due to its positive inotropic and vasodilatory effects.

In the PT1 study, headache (51 [12.5%] vs 8 [2%] in the anagrelide and hydroxyurea groups, respectively), palpitation (63 [16%] vs 7 [2%]), diarrhea (18 [4.5%] vs 6 [1.5%]), edema (25 [6%] vs 5 [1%]), asthenia (41 [10%] vs 12 [3%]) and abdominal pain (9 [2%] vs 1 [0.25%]) were significantly more common with anagrelide than hydroxyurea. Ulcers, leg (9 [2%] vs 20 [5%]) or mouth (1 [0.25%] vs 8 [2%]), not surprisingly, were significantly more common with hydroxyurea [48].

Dropout rates

There are conflicting reports on whether anagrelide's side effects abate with time. In a retrospective study of younger patients (aged <50 years) with a median of a 10-year follow-up, the incidence of the common side effects subsided in the long term [37]. By contrast, in a prospective study, half of the study population dropped out within 2 years for a variety of reasons including adverse events.

The reported dropout rates also varied among different trials, which may reflect study size. A large retrospective study reported a dropout of 47% [46]. In the initial Anagrelide Study Group, 34% of the patients discontinued the treatment, whereas in a small prospective study, only five out of 48 patients (10%) stopped the treatment due to the intolerable side effects [32].

The number of patients who withdrew from the PT1 study was significantly higher in the anagrelide group than the hydroxyurea group (148 [37%] vs 79 [20%]). The main reasons for withdrawal

were side effects (88 [22%] for anagrelide vs 43 [11%] for hydroxyurea), followed by serious adverse or end point events (22 [5.5%] vs 4 [1%], respectively) [48].

Serious side effects

Significant adverse events that have been associated with anagrelide include cardiac failure, serious arrhythmias, pericardial and pleural effusions and pulmonary infiltrates, fibrosis and hypertension [302]. There are also case reports of anagrelide-associated hypersensitivity pneumonitis [59] and renal tubular injury [60]. Recently, a successful rechallenge with anagrelide in a patient with anagrelide-associated cardiomyopathy was reported [61].

In a multicenter, open-label, Phase II study of anagrelide treatment in 60 chronic MPD patients during 2 years follow-up by the Swedish Myeloproliferative Disorder Study Group, significantly higher bone marrow reticulin and hyaluronan scores were found in the chronic MPD patients than in the normal controls and AML patients at the study entry. The study had a 50% dropout rate. In 19 out of 30 available patients, at the end of the study, pretreatment bone marrow biopsies were compared with follow-up samples. After 2 years of anagrelide therapy, the reticulin and hyaluronan scores were significantly higher than before treatment, suggesting progression of fibrosis [62].

In the PT1 study, myelofibrosis was reported to be significantly higher in the anagrelide group than the hydroxyurea group (16 [4%] vs 5 [1.2%]). The myelofibrotic transformation of ET was defined as at least grade 3 reticulin fibrosis in a bone marrow biopsy (plus an increase by at least one grade from presentation or trial entry biopsy) in addition to at least two of: increase in spleen size by at least 3 cm; unexplained decrease in hemoglobin by at least 2 g/dl; immature myeloid or erythroid cells or teardrop poikilocytes in the blood smear; or B symptoms (i.e., night sweats, bone pain or weight loss of more than 10% in 6 months). However, it is essential to note that, in this study, a bone marrow biopsy was not performed in all patients at entry and follow-up biopsies were only performed based on clinical suspicion. Thus, pretreatment bone marrow biopsy results were only available in 12 of the 21 reported cases of defined myelofibrosis. The interpretation becomes even more difficult because those 12 patients were unevenly distributed in the study groups, ten and two patients in the anagrelide and hydroxyurea groups, respectively.

To date, there is no evidence showing that anagrelide is leukemogenic. By using the WHO criteria [63], there was no significant transformation to AML or myelodysplasia in the PT1 study, although the study duration was too short to reach any definitive conclusions.

Thrombopoiesis

Thrombopoiesis is the orderly continuous process by which 10^{11} platelets per day are produced and released into the circulation [64]. In contrast to erythropoiesis and granulopoiesis, in which each red cell or white cell derives from a single differentiated progenitor, a single MK gives rise to 1000–3000 platelets [65]. The series of events that occur as a MK transitions from a megakaryoblast to a mature cell capable of releasing platelets from its cytoplasm is highly regulated and unique among hematopoietic progenitor cells [66]. In contrast to other hematopoietic progenitor cells, which undergo mitotic division, MK progenitors undergo nuclear endomitosis, a process that mediates cellular enlargement and amplifies their DNA content by as much as 64-fold [67]. The result is a cell with a polyploid, multilobated nucleus with a DNA content ranging from 4 to 128 N within each MK [68].

Megakaryocyte maturation is associated with remarkable cytoplasmic expansion and establishment of a centrosomal microtubular array. The formation of this expansive and interconnected membranous network of cisternae and tubules, known as the demarcation membrane system, is thought to function primarily as the reservoir for proplatelets, the immediate precursors of the platelets [66]. Sliding of overlapping microtubules drives proplatelet elongation [69]. Organelles follow the trail into the proplatelet ends where they are trapped [70]. Eventually, the entire MK cytoplasm is converted into a mass of proplatelets, which are released from the cell, and individual platelets are released from proplatelet ends into the circulation.

Thrombopoietin, the primary regulator of platelet production, is an acidic glycoprotein produced primarily in the liver and is currently the only known cytokine required for the maintenance of a constant MK mass [71]. In contrast to erythropoietin, whose production is both constitutive and inducible according to tissue oxygen demands, TPO production is constitutive but not normally inducible, and its plasma concentration varies according to its rate of consumption by platelets and MKs [72]. Thus, a reduction in marrow MKs and circulating platelets results in an increase in circulating TPO, while an increase in the marrow MK mass or platelet count reduces the plasma TPO level. The inflammatory cytokine IL-6 can independently induce hepatic TPO production, resulting in the thrombocytosis associated with inflammation, infection and malignancy [73,74].

Thrombocytosis

Elevated platelet counts can be grouped according to their etiology into two categories: reactive and clonal. Reactive causes are responsible for approximately 85–95% of thrombocytosis encountered clinically [75–77]. **Table 2** demonstrates the etiology of thrombocytosis in 732 patients (in-patients and out-patients) with a platelet count greater than 500×10^9/l (range: 500–1346×10^9 l^{-1}) seen over a 4-year period [77]. Transient reactive thrombocytosis, in another report, occurred with acute hemorrhage, trauma, major surgical procedures and severe physical exertion, with major surgery responsible for approximately 85% of cases [78]. In approximately a third of the cases, surgery was performed because of a malignant tumor, but thrombocytosis was first seen postoperatively. Infection, malignancy and chronic inflammatory disorders accounted for the majority of more prolonged reactive thrombocytosis. Iron deficiency anemia was responsible for less than 0.5% of the cases.

Clonal hematopoietic stem cell disorders are responsible for the remaining 5–15% of cases. These include PV, PMF, ET, CML and the myelodysplastic syndrome. In one study, ET was the most common cause of clonal thrombocytosis [77].

In reactive thrombocytosis, an elevated platelet count, *per se*, does not carry clinically important consequences [79], nor has the degree of thrombocytosis been significantly correlated with thrombosis risk in either PV or ET [80–84]. On the other hand, a significant association between MPD and a high rate of arterial, venous and microcirculatory thromboembolic complications is well documented [77,85]. At initial presentation, the reported incidence of thrombosis and bleeding in ET varied from 11–25% to 3.5–37%, respectively. The corresponding figures for PV were 12–39% and 1.5–20%. Despite the relatively wide range of values, the frequency of thrombosis has been consistently greater than that of bleeding in both ET and PV [86]. In a retrospective study, the overall risk of thrombosis and bleeding in ET was 6.6 and 0.33% per patient-year, respectively. In an historical control group, no severe bleeding episodes were recorded and the incidence of thrombotic events was 1.2% per patient-year [87].

Extreme thrombocytosis

A platelet count greater than a million per microliter is not specific for clonal thrombocytosis. Although the mean platelet count is significantly higher in clonal thrombocytosis than reactive thrombocytosis [77,79,88], in a retrospective investigation of 535 cases with extreme thrombocytosis – defined as a platelet count greater than or equal to 1000×10^9/l – reactive thrombocytosis was the underlying etiology in two-thirds of the cases [89]. Of the remaining third with clonal thrombocytosis, 93.8% had a MPD other than ET. In this study, approximately 8% of patients with reactive thrombocytosis experienced bleeding and vasoocclusive complications, compared with 17% of the clonal thrombocytosis patients [89].

In another study of 280 patients with extreme thrombocytosis, 231 (82%) had reactive thrombocytosis, 38 (14%) had a MPD and 11 (4%) were of uncertain etiology [90]. Symptoms of bleeding and/or vasoocclusive phenomena were noted in 21 (56%) of the MPD patients in this study but in only ten (4%) of the reactive thrombocytosis patients. The authors concluded that extreme thrombocytosis was not a rare event in an acute-care hospital population, and usually represented a reactive phenomenon.

Essential thrombocytosis

Essential thrombocytosis occurs in approximately 0.5–2.5 per 100,000 population per year [91]. According to some currently proposed definitions, diagnosis of ET can be established with a threshold platelet count of 600×10^9/l on two occasions, separated by at least 1 month with no evidence of reactive thrombocytosis and after elimination of PV and PMF [49,92]. However, a defined lower limit for thrombocytosis has not been established and the diagnosis becomes more likely the higher the platelet count. Persistence of thrombocytosis for more than 8 months also improves diagnostic accuracy. In an epidemiologic study of 99 patients with platelet counts greater than 400×10^9/l, only eight still had thrombocytosis 8 months later [93].

Unfortunately, ET is the only MPD without a specific phenotype. Since isolated thrombocytosis can be the initial clinical manifestation of PV, PMF or CML, ET is not only a diagnosis of exclusion, it should also not be considered a single disease entity [94]. The

Table 2. Etiology of thrombocythemia in 732 patients.

Diagnosis	Number (%)
Primary thrombocytosis	**89 (12.3)**
Essential thrombocythemia	40 (5.5)
Chronic myelogenous leukemia	24 (3.3)
Polycythemia vera	18 (2.5)
Myelofibrosis	4 (0.6)
Unclassified myeloproliferative disease	3 (0.4)
Secondary thrombocytosis	**643 (87.7)**
Tissue damage • Major abdominal surgery • Cardiovascular surgery • Orthopedic surgery • Thoracic surgery • Acute pancreatitis • Extensive myocardial infarction • Burns • Miscellaneous operations	269 (36.7)
Infection • Pneumonia • Gastrointestinal infections • Hepatobilliary infections • Soft-tissue infections • Osteoarthritis and osteomyelitis • Septicemia • Urinary tract infections • Tuberculosis • Nervous system infections • Miscellaneous infections	154 (21.0)
Malignancy • Gastrointestinal carcinomas • Lymphoma • Lung cancers • Carcinoma of liver, gallbladder and pancrease • Ovarian or breast cancer • Soft-tissue or bone sarcomas	85 (11.6)
Chronic inflammatory disorders • Crohn's disease • Ulcerative colitis • Rheumatoid arthritis • Chronic pancreatitis	65 (8.9)
Multiple causes • Post-surgical infection • Post-chemotherapy rebound • Thrombocytosis and acute infection	14 (1.9)
Renal disorders	13 (1.8)
Post-splenectomy	12 (1.6)
Upper gastrointestinal tract bleeding	5 (0.7)
Iron deficiency anemia	2 (0.3)
Autoimmune hemolytic anemia	1 (0.1)
Uncertain etiology	23 (3.1)

Adapted from [77].

degree of female predominance in ET is also unique among the MPDs as is its natural history, which is compatible with a normal lifespan [95,96]. Evaluation for *JAK2* V617F provides an opportunity to identify approximately 50% of patients with isolated thrombocytosis as possibly having ET and, importantly, also further distinguishes them as possibly having PV [50,58,97]. Unfortunately, the bone marrow histological criteria for distinguishing true ET from prefibrotic myelofibrosis with thrombocytosis recommended by the WHO have not been reproducible and there are currently no means of identifying prefibrotic primary myelofibrosis [98]. Finally, context is also important. While ET patients can have leukocytosis and splenomegaly, if these are not extremely modest and if extramedullary hematopoiesis is present in the form of a leukoerythroblastic reaction, another MPD should be considered because these findings suggest a clonal burden larger than usually encountered in ET.

Considering all of the above, we offer the following ET diagnostic guideline (**Box 1**) from the perspective that the proposed new WHO diagnostic criteria are unsatisfactory [99].

The majority of ET patients are asymptomatic or suffer from non-life-threatening microvascular complications, such as ocular migraine, visual disturbances, light-headedness, atypical chest pain, acral dysesthesia or erythromelalgia [3,88]. Life expectancy does not seem to be affected in patients with ET [96]. Hence, the important questions are which ET patients require platelet reduction therapy and with what medication?

Management of thrombocytosis

Nowhere in clinical medicine is the dictum 'first do no harm' more applicable than in the management of thrombocytosis. Thrombocytosis is a laboratory abnormality not a disease and is either a benign, reversible and reactive condition or due to a clonal disorder and with respect to these, ET is compatible with a normal life span. Therefore, doing no harm demands accuracy in diagnosis and the administration of therapy that is safe as well as effective. Safety is not a trivial issue because of the chronicity of most clonal disorders associated with thrombocytosis and, accordingly, the treatment cannot be worse than the disease.

Since ET has no phenotype and isolated thrombocytosis can be the presenting manifestation of any of the MPDs, diagnostic accuracy can be a formidable challenge. The *JAK2* V617F mutation has been a valuable advance in this regard, since over 92% of PV patients and approximately 50% of ET and PMF patients express the mutation. Therefore, the presence of *JAK2* V617F establishes that the thrombocytosis is due to an MPD, with the exception of the small group of patients with sideroblastic anemia associated with thrombocytosis [100]. At the same time, expression of the mutation does not identify which MPD is present [101,102]. of importance, recently, approximately 65% of a series of *JAK2* V617F-positive ET patients were found to have an elevated red cell mass when this was measured directly. Equally important is the fact that if the new WHO ET diagnostic guidelines had been used, all of these patients would have been misclassified as having ET [50], because there is no correlation between the hematocrit and the red cell mass. None of this should be surprising when one considers that *JAK2* is the cognate kinase for the erythropoietin and thrombopoietin receptors and, thus, PV is actually the ultimate expression of the *JAK2* V617F mutation, while erythrocytosis is an abnormality unique to PV amongst the MPD. As a corollary, a red cell mass determination is more important than a bone marrow examination in determining the etiology of isolated thrombocytosis [50,103]. The ultimate importance of this diagnostic exercise is to define those patients at risk for thrombosis due to an elevated red cell mass, since there is a direct correlation between the red cell mass and blood viscosity and the treatment for erythrocytosis is phlebotomy not chemotherapy. Indeed, it is well established that cytotoxic chemotherapy is ineffective in preventing macrovascular arterial or venous thrombosis in MPD patients [96,104,105].

It is generally agreed that ET patients above 60 years of age, those with a history of thrombotic or hemorrhagic events, patients with cardiovascular disease risk factors (e.g., obesity, smoking, diabetes and dyslipidemia) and those with a platelet count greater than 1500×10^9/l are considered at high risk for a thrombotic event and eligible for cytoreductive treatment [106].

Major thrombotic events (i.e., stroke, myocardial infarction, digital arterial occlusion, deep vein thrombosis and pulmonary embolism) have been estimated to occur at a rate of 10.7% per patient-year in high-risk ET patients [107]. Major bleeding (resulting in a substantial drop in hemoglobin or occurring in a critical organ) occurred in less than 10% of patients at or after diagnosis [84].

Box 1. Diagnostic criteria for essential thrombocytosis.

- Persistent thrombocytosis greater than 400,000/μl in the absence of a reactive cause
- Absence of iron deficiency (normal serum ferritin for gender)
- *JAK2* V617F assay (peripheral blood): expression establishes the presence of an myeloproliferative disorder but not its type; the absence of *JAK2* V617F does not exclude an myeloproliferative disorder
- Hemoglobin less than 16 g/dl in a man or less than 14 g/dl in woman (hematocrit < 47% in a man or < 44% in a woman) in the absence of splenomegaly
- Otherwise, red cell mass and plasma volume determinations are mandatory if a *JAK2* V617F assay is positive
- Negative *Bcr-Abl* FISH (peripheral blood) if a *JAK2* V617F assay is negative
- If there is anemia or macrocytosis or leukopenia, or evidence of extramedullary hematopoiesis (i.e., circulating nucleated erythrocytes, immature myelocytes or splenomegaly), a bone marrow examination (including flow cytometry and cytogenetics) is mandatory regardless of *JAK2* V617F expression status

The incidence of major thrombotic or hemorrhagic complications in patients below 60 years of age, with no history of thrombohemorrhagic events and a platelet count less than 1500 × 10^9/l was addressed by a prospective, controlled study [108]. Interestingly after a median follow-up of 4 years, 7.7% (1.9% per patient-year) of patients with ET had thrombotic episodes versus 6.2% (1.5% per patient-year) of age- and sex-matched controls. The difference was not statistically significant (p = 0.36). Recently, however, data have been presented demonstrating leukocyte count, even within the normal range, may be a risk factor for thrombosis in low-risk ET patients [51], but prospective studies to establish this have not yet been performed. The rare ET patients who are homozygous for *JAK2* V617F may also be at higher risk for thrombotic events than their heterozygous or nonmutated counterparts [109] but, overall, the presence of the *JAK2* V617F mutation and ET does not indicate an increased tendency for thrombosis [110].

In a retrospective multicenter study, the rate of recurrent thrombosis in a cohort of 494 patients with PV (n = 235) and ET (n = 259) was estimated. The patients had history of previous arterial (67.6%) or venous thrombosis (31%) or both (1.4%). Thrombosis recurred in 166 patients (33.6%), with an incidence of 7.6% per patient-years. Gender, diagnosis (PV versus ET) and the presence of cardiovascular risk factors did not predict recurrence, whereas an age of over 60 years did (multivariable hazard ratio [HR]: 1.67; 95% CI: 1.19–2.32). In this retrospective study, cytoreduction therapy decreased the overall risk of thrombosis by half (HR: 0.53; 95% CI: 0.38–0.73), and combination therapy with antiplatelet agents or oral anticoagulants was more effective than administration of a single drug. The overall incidence of major bleeding was 0.9% patient-years, which increased to 2.8% in patients receiving both antiplatelet and anticoagulant agents [111].

It is not clear which ET patients are at higher risk for transformation to myelofibrosis or acute leukemia [112]. Cytoreductive therapy in ET has not been shown to influence overall survival [113] or the clinical course in young asymptomatic patient without extreme thrombocytosis [114].

The management of ET with the purpose of decreasing the risk of thrombohemorrhagic complications and alleviating vasomotor symptoms, has included a different variety of chemotherapeutic agents: alkylating agents (e.g., busulfan), hydroxyurea, IFN-α and anagrelide alone or in combination with platelet anti-aggregants have been tried for treating ET patients [115]. However, not only is busulfan a known mutagen but there is also accumulating evidence that hydroxyurea, which is a known tumor promoter for skin cancers [116,117], is also a mutagen [118–120], especially in patients who have been exposed to busulfan [121], and this needs to be factored into the management of ET patients projected to have a normal life span but who require control of platelet production [122,123]. In a prospective, randomized controlled clinical trial comparing hydroxyurea to pipobroman in PV patients, the incidence of acute leukemia was approximately 10% in both arms of the trial after 10 years [122].

Thus, in MPD patients with thrombocytosis and microvascular symptoms, except for TIA (for which no other cause can be found) we recommend the use of aspirin. In those patients with acquired von Willebrand's disease due to extreme thrombocytosis in whom aspirin cannot be administered, anagrelide is the initial drug of choice to lower the platelet count. Patients experiencing TIA should be treated with hydroxyurea and aspirin. In patients not responsive to aspirin or anagrelide or who cannot tolerate anagrelide, consideration should be given to IFN-α.

IFN-α antagonizes the action of PDGF, TGF-β and, possibly, other cytokines that are involved in the development of myelofibrosis. It also suppresses the proliferation of hematopoietic progenitors and has a direct inhibitory effect on bone marrow fibroblast progenitor cells. IFN-α, with an average dose of 3 million IU daily, reduced the platelet count to less than 600 × 10^9/l in approximately 90% of patients after 3 months [124]. INF-α has not been reported to be teratogenic and does not cross the placenta. Hence, it has been used successfully in some ET patients during pregnancy [125].

Thus far, no leukemogenic effect has been reported for interferon [124]. A decrease of *JAK2* V617F expression in 89% of patients and an undetectable level in one patient, after 12 months of therapy, was reported in a multicenter Phase II trial of pegylated INF-α in 27 PV patients [126]. A high incidence of side effects is the main problem with INF-α therapy. Fever and influenza-like symptoms occur almost universally and weakness, myalgia, weight loss, alopecia, depression, neurologic, gastrointestinal and cardiovascular symptoms result in discontinuation of the drug in approximately a third to over a half of patients [124,127]. INF-α should be considered for pregnant women and in patients younger than 60 years of age who cannot tolerate first-line therapies.

The goal of platelet count reduction is not normalization since no drug currently available can eradicate the abnormal clone but, rather, reduction to a level at which symptoms are no longer present. If hydroxyurea must be used, an attempt should be made not to use it chronically or to use it in combination with anagrelide or interferon. Given the potential for gastrointestinal hemorrhage, the combination of aspirin and anagrelide should be avoided. Bleeding due to thrombocytosis and acquired von Willebrand's disease can be treated effectively with ε-amino caproic acid [128,129].

Conclusion

Thrombocytosis is a common feature of the chronic MPDs that may be asymptomatic or associated with transient microvascular vasoocclusive symptoms or large vessel arterial or venous occlusion and, paradoxically, if the platelet exceeds 1 million, a hemorrhagic diathesis due to the absorption of high-molecular-weight von Willebrand multimers by the large platelet mass. Failure of either the hematocrit or the platelet count to correlate with thrombotic events is a peculiar conundrum of the chronic MPDs. Failure of the hematocrit to correlate with thrombotic events is easily explained by the fact that the peripheral blood hematocrit is not representative of the hematocrit in the smaller vessels. Failure of the platelet count to correlate with thrombotic events in ET relates to patients actually have unrecognized PV with its attendant elevated red cell mass, particularly when they express *JAK2* V617F [50]. Cardiovascular risk factors, age and comorbidities also contribute, as do other causes, to a hypercoagulable state, to which MPD patients are not immune. It is also certainly the case that once a thrombosis has occurred, patients are at higher risk for another.

It is undisputed that lowering the platelet count reduces the incidence of microvascular events as well as the acquired von Willebrand syndrome associated with extreme thrombocytosis. At the same time, to date, no study has demonstrated that platelet count reduction prolongs survival in ET and drugs, such as busulfan and hydroxyurea, have mutagenic potential. Therefore, the best treatment strategy relies on the correct diagnosis and the use of the safest form of therapy when treatment is needed.

Expert commentary

Myeloproliferative disorders are uncommon and chronic disorders that many physicians, including hematologists, are rarely able to follow during the full scope of the disorder. Although thrombocytosis is a common feature among the MPDs, clonal disorders are responsible for only 10% of thrombocytosis cases. ET patients have a normal life span. Therefore, when treatment is indicated, choosing the most nontoxic and effective agent among the platelet reducing drugs is logical. We suggest the following approach to thrombocytosis.

First, reactive causes (Table 2) must be ruled out. Second, to distinguish PV from ET, a red cell mass and plasma volume determination may be necessary, particularly if *JAK2* V617F is present. Rarely, fluorescent *in situ* hybridization or reverse trascription-PCR for *BCR-ABL* may be needed to distinguish ET from CML. Bone marrow examination (including flow cytometry and cytogenetics) is indicated only if there is anemia, macrocytosis, leukopenia or evidence of extramedullary hematopoiesis in association with thrombocytosis. Third, asymptomatic thrombocytosis in young ET patients with no cardiovascular risk factors does not require treatment. Fourth, when treatment is required, it makes sense to use drugs that are not myelotoxic. Based on the PT1 study, hydroxyurea is the treatment of choice for TIAs that fail to respond to aspirin. Hydroxyurea does not prevent venous thrombosis or arterial thrombosis and its long-term safety has not been established. Fifth, although no leukemogenesis or teratogenesis has been shown for IFN-α, the common and significant side-effect profile makes it less desirable as a first-line agent for most patients. IFN-α should, however, be considered for use during pregnancy. Finally, since no curative therapy for ET exists currently, clinicians should choose the least myelotoxic drug to effectively reduce the platelet count. In this regard, anagrelide can be considered a first-line agent.

Five-year view

Recruitment for a single-blind, multicenter, randomized multinational Phase III study to compare the Efficacy and Tolerability of Anagrelide vs Hydroxyurea in Patients with Essential Thrombocythaemia (ANAHYDRET) has been closed as the goal of 258 patients was reached at the end of 2005. The final analysis is pending.

The effect of the *JAK2* V617F mutation on thrombotic risk in ET patients is an ongoing area of research. A recently published meta-analysis [130] showed an association but not a direct causality between *JAK2* V617F expression and thrombosis in ET. Whether this association is independent of other confounding factors is unknown, since PV was not excluded in the *JAK2* V617F-positive patients. In this regard, clinical trials employing *JAK2* inhibitors are also being conducted currently in ET patients.

Financial & competing interests disclosure

Jerry L Spivak is a consultant for Shire, Novartis, Johnson and Johnson and Roche. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Anagrelide is indicated for the reduction of elevated platelet counts in nonpregnant essential thrombocytosis patients with one or more of the following:
  - Age less than 60 years
  - A high platelet count associated with bleeding and reduced ristocetin cofactor activity
  - A high platelet count associated with microvascular symptoms and reduced ristocetin cofactor activity
  - A history of thrombohemorrhagic events (except for transitory ischemic attack patients who fail to respond to aspirin)
  - A history of intolerance to the current therapy
  - Failure of the current therapy to reduce platelet count
- The recommended starting dosage of anagrelide is 0.5 mg every 6–12 h for 7 days but experience has suggested that drug tolerance is improved if the drug is started at a lower dose then is slowly titrated upward. Subsequently, the daily dose should be adjusted to the lowest dose required to maintain platelet count below 600–900 × 10^9/l or whatever platelet count is associated with relief of symptoms. The dosage should not be increased by more than 0.5 mg/day in any given week. Dosage should not exceed 10 mg/day or 2 mg/dose. Daily dosages exceeding 4–5 mg are associated with higher incidence of side effects.
- The platelet count usually begins to respond within 1–2 weeks at the effective dose. The time to a platelet count no more than 600 × 10^9/l ranges from 1 to 3 months.
- In responding patients, maintenance therapy is required to keep the platelet count at the desired level. The blood count should be checked weekly during the first month of therapy, every 2 weeks during the second and third month, every month for 6 months, then 3–4-times a year afterwards, since there will be fall in hematocrit in some patients.
- We recommend echocardiography and an ECG before starting treatment, followed by periodic cardiac evaluation.

References

1 Budde U, van Genderen PJ. Acquired von Willebrand disease in patients with high platelet counts. *Semin. Thromb. Hemost.* 23(5), 425–431 (1997).

2 Michiels JJ, Berneman Z, Schroyens W, Finazzi G, Budde U, van Vliet HH. The paradox of platelet activation and impaired function: platelet–von Willebrand factor interactions, and the etiology of thrombotic and hemorrhagic manifestations in essential thrombocythemia and polycythemia vera. *Semin. Thromb. Hemost.* 32(6), 589–604 (2006).

3 Michiels JJ, Abels J, Steketee J, van Vliet HH, Vuzevski VD. Erythromelalgia caused by platelet-mediated arteriolar inflammation and thrombosis in thrombocythemia. *Ann. Intern. Med.* 102(4), 466–471 (1985).

4 Michiels JJ, Koudstaal PJ, Mulder AH, van Vliet HH. Transient neurologic and ocular manifestations in primary thrombocythemia. *Neurology*, 43(6), 1107–1110 (1993).

5 Jabaily J, Iland HJ, Laszlo J *et al.* Neurologic manifestations of essential thrombocythemia. *Ann. Intern. Med.* 99(4), 513–518 (1983).

6 Michiels JJ, van Genderen PJ, Jansen PH, Koudstaal PJ. Atypical transient ischemic attacks in thrombocythemia of various myeloproliferative disorders. *Leuk. Lymphoma* 22(Suppl. 1), 65–70 (1996).

7 Fleming JS, Buyniski JP. A potent new inhibitor of platelet aggregation and experimental thrombosis, anagrelide (BL-4162A). *Thromb. Res.* 15(3–4), 373–388 (1979).

8 Baryla UM, Fleming JS, Stanton HC. The anesthetized ferret, an *in vivo* model for evaluating inotropic activity: effects of milrinone and anagrelide. *J. Pharmacol. Methods* 20(4), 299–306 (1988).

9 Wang G, Franklin R, Hong Y, Erusalimsky JD. Comparison of the biological activities of anagrelide and its major metabolites in haematopoietic cell cultures. *Br. J. Pharmacol.* 146(3), 324–332 (2005).

10 Gaver RC, Deeb G, Pittman KA, Smyth RD. Disposition of anagrelide, an inhibitor of platelet aggregation. *Clin. Pharmacol. Ther.* 29(3), 381–386 (1981).

11 Oertel MD. Anagrelide, a selective thrombocytopenic agent. *Am. J. Health Syst. Pharm.* 55(19), 1979–1986 (1998).

12 Wagstaff AJ, Keating GM. Anagrelide: a review of its use in the management of essential thrombocythaemia. *Drugs* 66(1), 111–131 (2006).

13 Tomer A. Effects of anagrelide on *in vivo* megakaryocyte proliferation and maturation in essential thrombocythemia. *Blood* 99(5), 1602–1609 (2002).

14 Mazur EM, Rosmarin AG, Sohl PA, Newton JL, Narendran A. Analysis of the mechanism of anagrelide-induced thrombocytopenia in humans. *Blood* 79(8), 1931–1937 (1992).

15 Tefferi A, Silverstein MN, Petitt RM, Mesa RA, Solberg LA Jr. Anagrelide as a new platelet-lowering agent in essential thrombocythemia: mechanism of actin, efficacy, toxicity, current indications. *Semin. Thromb. Hemost.* 23(4), 379–383 (1997).

16 Hong Y, Wang G, Del Arroyo AG, Hernandez J, Skene C, Erusalimsky JD. Comparison between anagrelide and hydroxycarbamide in their activities against haematopoietic progenitor cell growth and differentiation: selectivity of anagrelide for the megakaryocytic lineage. *Leukemia* 20(6), 1117–1122 (2006).

17 Lane WJ, Hattori K, Dias S *et al.* Anagrelide metabolite induces thrombocytopenia in mice by inhibiting megakaryocyte maturation without inducing platelet aggregation. *Exp. Hematol.* 29(12), 1417–1424 (2001).

18 Erusalimsky JD, Hong Y, Franklin R. Is the platelet lowering activity of anagrelide mediated by its major metabolite 2-amino-5,6-dichloro-3,4-dihydroquinazoline (RL603)? *Exp. Hematol.* 30(7), 625–626; author reply: 626–627 (2002).

19 McCarty JM, Melone PD, Simanis JP, Kanamori D, Dessypris EN, Warshamana-Greene GS. A preliminary investigation into the action of anagrelide: thrombopoietin-c-Mpl receptor interactions. *Exp. Hematol.* 34(1), 87–96 (2006).

20 Birgegard G, Bjorkholm M, Kutti J *et al.* Adverse effects and benefits of two years of anagrelide treatment for thrombocythemia in chronic myeloproliferative disorders. *Haematologica* 89(5), 520–527 (2004).

21 Solberg LA Jr, Tefferi A, Oles KJ *et al.* The effects of anagrelide on human megakaryocytopoiesis. *Br. J. Haematol.* 99(1), 174–180 (1997).

22 Tomer A, Harker LA. Measurements of *in vivo* megakaryocytopoiesis: studies in nonhuman primates and patients. *Stem Cells* 14(Suppl. 1), 18–30 (1996).

23 Thiele J, Kvasnicka HM, Ollig S, Schmitt-Graff A. Anagrelide does not exert a myelodysplastic effect on megakaryopoiesis: a comparative immunohistochemical and morphometric study with hydroxyurea. *Histol. Histopathol.* 20(4), 1071–1076 (2005).

24 Yoon SY, Li CY, Mesa RA, Tefferi A. Bone marrow effects of anagrelide therapy in patients with myelofibrosis with myeloid metaplasia. *Br. J. Haematol.* 106(3), 682–688 (1999).

25 Cacciola RR, Francesco ED, Giustolisi R, Cacciola E. Effects of anagrelide on platelet factor 4 and vascular endothelial growth factor levels in patients with essential thrombocythemia. *Br. J. Haematol.* 126(6), 885–886 (2004).

26 Cacciola RR, Cipolla A, Di Francesco E, Giustolisi R, Cacciola E. Treatment of symptomatic patients with essential thrombocythemia: effectiveness of anagrelide. *Am. J. Hematol.* 80(1), 81–83 (2005).

27 Lev PR, Salim JP, Kornblihtt LI *et al.* PDGF-A, PDGF-B, TGFβ, and bFGF mRNA levels in patients with essential thrombocythemia treated with anagrelide. *Am. J. Hematol.* 78(2), 155–157 (2005).

28 Thiele J, Kvasnicka HM, Fuchs N, Brunnbauer K, Volkwein N, Schmitt-Graeff A. Anagrelide-induced bone marrow changes during therapy of chronic myeloproliferative disorders with thrombocytosis. an immunohistochemical and morphometric study of sequential trephine biopsies. *Haematologica* 88(10), 1130–1138 (2003).

29 Silverstein MN, Petitt RM, Solberg LA Jr, Fleming JS, Knight RC, Schacter LP. Anagrelide: a new drug for treating thrombocytosis. *N. Engl. J. Med.* 318(20), 1292–1294 (1988).

30 Mazzucconi MG, De Sanctis V, Chistolini A, Dragoni F, Mandelli F. Therapy with anagrelide in patients affected by essential thrombocythemia: preliminary results. *Haematologica* 77(4), 315–317 (1992).

31 Balduini CL, Bertolino G, Noris P, Ascari E. Effect of anagrelide on platelet count and function in patients with thrombocytosis and myeloproliferative disorders. *Haematologica* 77(1), 40–43 (1992).

32 Petrides PE, Beykirch MK, Trapp OM. Anagrelide, a novel platelet lowering option in essential thrombocythaemia: treatment experience in 48 patients in Germany. *Eur. J. Haematol.* 61(2), 71–76 (1998).

33 Trapp OM, Beykirch MK, Petrides PE. Anagrelide for treatment of patients with chronic myelogenous leukemia and a high platelet count. *Blood Cells Mol. Dis.* 24(1), 9–13 (1998).

34 Mills AK, Taylor KM, Wright SJ *et al.* Efficacy, safety and tolerability of anagrelide in the treatment of essential thrombocythaemia. *Aust. NZ J. Med.* 29(1), 29–35 (1999).

35 Laguna MS, Kornblihtt LI, Marta RF, Michiels JJ, Molinas FC. Effectiveness of anagrelide in the treatment of symptomatic patients with essential thrombocythemia. *Clin. Appl. Thromb. Hemost.* 6(3), 157–161 (2000).

36 Knutsen H, Hysing J. [Anagrelide in primary thrombocythemia]. *Tidsskr. Nor. Laegeforen.* 121(12), 1478–1482 (2001).

37 Storen EC, Tefferi A. Long-term use of anagrelide in young patients with essential thrombocythemia. *Blood* 97(4), 863–866 (2001).

38 Kornblihtt LI, Vassallu PS, Heller P, Molinas FC. [Treatment of essential thrombocythemia with anagrelide: a ten-year experience]. *Medicina (B. Aires)* 62(3), 231–236 (2002).

39 Mazzucconi MG, Redi R, Bernasconi S *et al.* A long-term study of young patients with essential thrombocythemia treated with anagrelide. *Haematologica* 89(11), 1306–1313 (2004).

40 Steurer M, Gastl G, Jedrzejczak WW *et al.* Anagrelide for thrombocytosis in myeloproliferative disorders: a prospective study to assess efficacy and adverse event profile. *Cancer* 101(10), 2239–2246 (2004).

41 Penninga E, Jensen BA, Hansen PB *et al.* Anagrelide treatment in 52 patients with chronic myeloproliferative diseases. *Clin. Lab. Haematol.* 26(5), 335–340 (2004).

42 Silver RT. Anagrelide is effective in treating patients with hydroxyurea-resistant thrombocytosis in patients with chronic myeloid leukemia. *Leukemia* 19(1), 39–43 (2005).

43 Penka M, Schwarz J, Pavlik T *et al.* [Essential thrombocytemia and other myeloproliferations with thrombocytemia in the data of the register of patients treated with Thromboreductin till the end of 2006]. *Vnitr Lek.* 53(6), 653–661 (2007).

44 Anagrelide Study Group. Anagrelide, a therapy for thrombocythemic states: experience in 577 patients. *Am. J. Med.* 92(1), 69–76 (1992).

45 Petitt RM, Silverstein MN, Petrone ME. Anagrelide for control of thrombocythemia in polycythemia and other myeloproliferative disorders. *Semin. Hematol.* 34(1), 51–54 (1997).

46 Fruchtman SM, Petitt RM, Gilbert HS, Fiddler G, Lyne A. Anagrelide: analysis of long-term efficacy, safety and leukemogenic potential in myeloproliferative disorders. *Leuk. Res.* 29(5), 481–491 (2005).

47 Lackner H, Urban C, Benesch M *et al.* Long-term use of anagrelide in the treatment of children with essential thrombocythemia. *Eur. J. Haematol.* 77(4), 358–359 (2006).

48 Harrison CN, Campbell PJ, Buck G *et al.* Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. *N. Engl. J. Med.* 353(1), 33–45 (2005).

49 Murphy S, Peterson P, Iland H, Laszlo J. Experience of the Polycythemia Vera Study Group with essential thrombocythemia: a final report on diagnostic criteria, survival, and leukemic transition by treatment. *Semin. Hematol.* 34(1), 29–39 (1997).

50 Cassinat B, Laguillier C, Gardin C *et al.* Classification of myeloproliferative disorders in the *JAK2* era: is there a role for red cell mass? *Leukemia* 22(2), 452–453 (2008).

51 Carobbio A, Finazzi G, Guerini V *et al.* Leukocytosis is a risk factor for thrombosis in essential thrombocythemia: interaction with treatment, standard risk factors, and *Jak2* mutation status. *Blood* 109(6), 2310–2313 (2007).

52 Falanga A, Marchetti M, Evangelista V *et al.* Polymorphonuclear leukocyte activation and hemostasis in patients with essential thrombocythemia and polycythemia vera. *Blood* 96(13), 4261–4266 (2000).

53 Cokic VP, Beleslin-Cokic BB, Tomic M, Stojilkovic SS, Noguchi CT, Schechter AN. Hydroxyurea induces the eNOS-cGMP pathway in endothelial cells. *Blood* 108(1), 184–191 (2006).

54 Cokic VP, Andric SA, Stojilkovic SS, Noguchi CT, Schechter AN. Hydroxyurea nitrosylates and activates soluble guanylyl cyclase in human erythroid cells. *Blood* 111(3), 1117–1123 (2008).

55 Gambero S, Canalli AA, Traina F *et al.* Therapy with hydroxyurea is associated with reduced adhesion molecule gene and protein expression in sickle red cells with a concomitant reduction in adhesive properties. *Eur. J. Haematol.* 78(2), 144–151 (2007).

56 Canalli AA, Franco-Penteado CF, Saad ST, Conran N, Costa FF. Increased adhesive properties of neutrophils in sickle cell disease may be reversed by pharmacological nitric oxide donation. *Haematologica* 93(4), 605–609 (2008).

57 Petrides PE. Anagrelide: what was new in 2004 and 2005? *Semin. Thromb. Hemost.* 32(4 Pt 2), 399–408 (2006).

58 Campbell PJ, Scott LM, Buck G *et al.* Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on *JAK2* V617F mutation status: a prospective study. *Lancet* 366(9501), 1945–1953 (2005).

59 Raghavan M, Mazer MA, Brink DJ. Severe hypersensitivity pneumonitis associated with anagrelide. *Ann. Pharmacother.* 37(9), 1228–1231 (2003).

60 Rodwell GE, Troxell ML, Lafayette RA. Renal tubular injury associated with anagrelide use. *Nephrol. Dial. Transplant.* 20(5), 988–990 (2005).

61 Wong RS, Lam LW, Cheng G. Successful rechallenge with anagrelide in a patient with anagrelide-associated cardiomyopathy. *Ann. Hematol.* DOI: 10.1007/s00277-008-0451-6 (2008) (Epub ahead of print).

62 Hultdin M, Sundstrom G, Wahlin A *et al.* Progression of bone marrow fibrosis in patients with essential thrombocythemia and polycythemia vera during anagrelide treatment. *Med. Oncol.* 24(1), 63–70 (2007).

63 Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood* 100(7), 2292–2302 (2002).

64 Kaushansky K. Historical review: megakaryopoiesis and thrombopoiesis. *Blood* 111(3), 981–986 (2008).

65 Stenberg PE, Levin J. Mechanisms of platelet production. *Blood Cells* 15(1), 23–47 (1989).

66 Patel SR, Hartwig JH, Italiano JE Jr. The biogenesis of platelets from megakaryocyte proplatelets. *J. Clin. Invest.* 115(12), 3348–3354 (2005).

67 Ravid K, Lu J, Zimmet JM, Jones MR. Roads to polyploidy: the megakaryocyte example. *J. Cell. Physiol.* 190(1), 7–20 (2002).

68 Odell TT Jr, Jackson CW, Friday TJ. Megakaryocytopoiesis in rats with special reference to polyploidy. *Blood* 35(6), 775–782 (1970).

69 Patel SR, Richardson JL, Schulze H *et al.* Differential roles of microtubule assembly and sliding in proplatelet formation by megakaryocytes. *Blood* 106(13), 4076–4085 (2005).

70 Richardson JL, Shivdasani RA, Boers C, Hartwig JH, Italiano JE Jr. Mechanisms of organelle transport and capture along proplatelets during platelet production. *Blood* 106(13), 4066–4075 (2005).

71 Kaushansky K. The molecular mechanisms that control thrombopoiesis. *J. Clin. Invest.* 115(12), 3339–3347 (2005).

72 Kuter DJ, Rosenberg RD. The reciprocal relationship of thrombopoietin (c-Mpl ligand) to changes in the platelet mass during busulfan-induced thrombocytopenia in the rabbit. *Blood* 85(10), 2720–2730 (1995).

73 Wolber EM, Fandrey J, Frackowski U, Jelkmann W. Hepatic thrombopoietin mRNA is increased in acute inflammation. *Thromb. Haemost.* 86(6), 1421–1424 (2001).

74 Kaser A, Brandacher G, Steurer W *et al.* Interleukin-6 stimulates thrombopoiesis through thrombopoietin: role in inflammatory thrombocytosis. *Blood* 98(9), 2720–2725 (2001).

75 Syed NN, Usman M, Khurshid M. Thrombocytosis: age dependent aetiology and analysis of platelet indices for differential diagnosis. *Indian J. Pathol. Microbiol.* 50(3), 628–633 (2007).

76 Aydogan T, Kanbay M, Alici O, Kosar A. Incidence and etiology of thrombocytosis in an adult Turkish population. *Platelets* 17(5), 328–331 (2006).

77 Griesshammer M, Bangerter M, Sauer T, Wennauer R, Bergmann L, Heimpel H. Aetiology and clinical significance of thrombocytosis: analysis of 732 patients with an elevated platelet count. *J. Intern. Med.* 245(3), 295–300 (1999).

78 Santhosh-Kumar CR, Yohannan MD, Higgy KE, al-Mashhadani SA. Thrombocytosis in adults: analysis of 777 patients. *J. Intern. Med.* 229(6), 493–495 (1991).

79 Tefferi A, Murphy S. Current opinion in essential thrombocythemia: pathogenesis, diagnosis, and management. *Blood Rev.* 15(3), 121–131 (2001).

80 Buss DH, Stuart JJ, Lipscomb GE. The incidence of thrombotic and hemorrhagic disorders in association with extreme thrombocytosis: an analysis of 129 cases. *Am. J. Hematol.* 20(4), 365–372 (1985).

81 Bellucci S, Janvier M, Tobelem G *et al.* Essential thrombocythemias. Clinical evolutionary and biological data. *Cancer* 58(11), 2440–2447 (1986).

82 Wehmeier A, Daum I, Jamin H, Schneider W. Incidence and clinical risk factors for bleeding and thrombotic complications in myeloproliferative disorders. A retrospective analysis of 260 patients. *Ann. Hematol.* 63(2), 101–106 (1991).

83 Lengfelder E, Hochhaus A, Kronawitter U *et al.* Should a platelet limit of 600 × 10^9/l be used as a diagnostic criterion in essential thrombocythaemia? An analysis of the natural course including early stages. *Br. J. Haematol.* 100(1), 15–23 (1998).

84 Besses C, Cervantes F, Pereira A *et al.* Major vascular complications in essential thrombocythemia: a study of the predictive factors in a series of 148 patients. *Leukemia* 13(2), 150–154 (1999).

85 Landolfi R, Rocca B, Patrono C. Bleeding and thrombosis in myeloproliferative disorders: mechanisms and treatment. *Crit. Rev. Oncol. Hematol.* 20(3), 203–222 (1995).

86 Elliott MA, Tefferi A. Thrombosis and haemorrhage in polycythaemia vera and essential thrombocythaemia. *Br. J. Haematol.* 128(3), 275–290 (2005).

87 Cortelazzo S, Viero P, Finazzi G, D'Emilio A, Rodeghiero F, Barbui T. Incidence and risk factors for thrombotic complications in a historical cohort of 100 patients with essential thrombocythemia. *J. Clin. Oncol.* 8(3), 556–562 (1990).

88 Tefferi A, Fonseca R, Pereira DL, Hoagland HC. A long-term retrospective study of young women with essential thrombocythemia. *Mayo Clin. Proc.* 76(1), 22–28 (2001).

89 Wiwanitkit V. Extreme thrombocytosis: what are the etiologies? *Clin. Appl. Thromb. Hemost.* 12(1), 85–87 (2006).

90 Buss DH, Cashell AW, O'Connor ML, Richards F 2nd, Case LD. Occurrence, etiology, and clinical significance of extreme thrombocytosis: a study of 280 cases. *Am. J. Med.* 96(3), 247–253 (1994).

91 Johansson P. Epidemiology of the myeloproliferative disorders polycythemia vera and essential thrombocythemia. *Semin. Thromb. Hemost.* 32(3), 171–173 (2006).

92 Green AR, Vassiliou GS, Curtin N, Campbell PJ. Management of the myeloproliferative disorders: distinguishing data from dogma. *Hematol. J.* 5(Suppl. 3), S126–S132 (2004).

93 Ruggeri M, Tosetto A, Frezzato M, Rodeghiero F. The rate of progression to polycythemia vera or essential thrombocythemia in patients with erythrocytosis or thrombocytosis. *Ann. Intern. Med.* 139(6), 470–475 (2003).

94 Campbell PJ, Green AR. The myeloproliferative disorders. *N. Engl. J. Med.* 355(23), 2452–2466 (2006).

95 Rozman C, Giralt M, Feliu E, Rubio D, Cortes MT. Life expectancy of patients with chronic nonleukemic myeloproliferative disorders. *Cancer* 67(10), 2658–2663 (1991).

96 Passamonti F, Rumi E, Pungolino E *et al.* Life expectancy and prognostic factors for survival in patients with polycythemia vera and essential thrombocythemia. *Am. J. Med.* 117(10), 755–761 (2004).

97 Moliterno AR, Williams DM, Rogers O, Spivak JL. Molecular mimicry in the chronic myeloproliferative disorders: reciprocity between quantitative *JAK2* V617F and Mpl expression. *Blood* 108(12), 3913–3915 (2006).

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

99 Spivak JL, Silver RT. The revised World Health Organization diagnostic criteria for polycythemia vera, essential thrombocytosis and primary myelofibrosis: an alternative proposal. *Blood* 112(2), 231–239 (2008).

100 Schmitt-Graeff AH, Teo SS, Olschewski M *et al. JAK2* V617F mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. *Haematologica* 93(1), 34–40 (2008).

101 Spivak JL. Phenotype and genotype in the myeloproliferative disorders. *Eur. J. Haematol.* 79(Suppl. 68), 9–12 (2007).

102 Percy MJ, Jones FG, Green AR, Reilly JT, McMullin MF. The incidence of the JAK2 V617F mutation in patients with idiopathic erythrocytosis. *Haematologica* 91(3), 413–414 (2006).

103 Jantunen R, Juvonen E, Ikkala E, Oksanen K, Anttila P, Ruutu T. Development of erythrocytosis in the course of essential thrombocythemia. *Ann. Hematol.* 78(5), 219–222 (1999).

104 Landolfi R, Marchioli R, Kutti J *et al.* Efficacy and safety of low-dose aspirin in polycythemia vera. *N. Engl. J. Med.* 350(2), 114–124 (2004).

105 Finazzi G, Barbui T. How I treat patients with polycythemia vera. *Blood* 109(12), 5104–5111 (2007).

106 Barbui T, Barosi G, Grossi A *et al.* Practice guidelines for the therapy of essential thrombocythemia. A statement from the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation. *Haematologica* 89(2), 215–232 (2004).

107 Cortelazzo S, Finazzi G, Ruggeri M *et al.* Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis. *N. Engl. J. Med.* 332(17), 1132–1136 (1995).

108 Ruggeri M, Finazzi G, Tosetto A, Riva S, Rodeghiero F, Barbui T. No treatment for low-risk thrombocythaemia: results from a prospective study. *Br. J. Haematol.* 103(3), 772–777 (1998).

109 Vannucchi AM, Antonioli E, Guglielmelli P *et al.* Clinical profile of homozygous *JAK2* 617V>F mutation in patients with polycythemia vera or essential thrombocythemia. *Blood* 110(3), 840–846 (2007).

110 Wolanskyj AP, Lasho TL, Schwager SM *et al. JAK2* mutation in essential thrombocythaemia: clinical associations and long-term prognostic relevance. *Br. J. Haematol.* 131(2), 208–213 (2005).

111 De Stefano V, Za T, Rossi E *et al.* Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia: incidence, risk factors, and effect of treatments. *Haematologica* 93(3), 372–380 (2008).

112 Cervantes F, Tassies D, Salgado C, Rovira M, Pereira A, Rozman C. Acute transformation in nonleukemic chronic myeloproliferative disorders: actuarial probability and main characteristics in a series of 218 patients. *Acta Haematol.* 85(3), 124–127 (1991).

113 Finazzi G, Barbui T. Risk-adapted therapy in essential thrombocythemia and polycythemia vera. *Blood Rev.* 19(5), 243–252 (2005).

114 Finazzi G, Barbui T. Treatment of essential thrombocythemia with special emphasis on leukemogenic risk. *Ann. Hematol.* 78(9), 389–392 (1999).

115 Spivak JL, Barosi G, Tognoni G *et al.* Chronic myeloproliferative disorders. *Hematology* 200–224 (2003).

116 Best PJ, Petitt RM. Multiple skin cancers associated with hydroxyurea therapy. *Mayo Clin. Proc.* 73(10), 961–963 (1998).

117 De Benedittis M, Petruzzi M, Giardina C, Lo Muzio L, Favia G, Serpico R. Oral squamous cell carcinoma during long-term treatment with hydroxyurea. *Clin. Exp. Dermatol.* 29(6), 605–607 (2004).

118 Adams M, Warr JR. The mutagenic activity of hydroxyurea in *Chlamydomonas reinhardi*. *Mutat. Res.* 41(2–3), 217–224 (1976).

119 Ziegler-Skylakakis K, Schwarz LR, Andrae U. Microsome- and hepatocyte-mediated mutagenicity of hydroxyurea and related aliphatic hydroxamic acids in V79 Chinese hamster cells. *Mutat. Res.* 152(2–3), 225–231 (1985).

120 de Lima PD, Cardoso PC, Khayat AS, Bahia Mde O, Burbano RR. Evaluation of the mutagenic activity of hydroxyurea on the G_1–S–G_2 phases of the cell cycle: an *in vitro* study. *Genet. Mol. Res.* 2(3), 328–333 (2003).

121 Nielsen I, Hasselbalch HC. Acute leukemia and myelodysplasia in patients with a Philadelphia chromosome negative chronic myeloproliferative disorder treated with hydroxyurea alone or with hydroxyurea after busulphan. *Am. J. Hematol.* 74(1), 26–31 (2003).

122 Najean Y, Rain JD. Treatment of polycythemia vera: the use of hydroxyurea and pipobroman in 292 patients under the age of 65 years. *Blood* 90(9), 3370–3377 (1997).

123 Kiladjian JJ, Rain JD, Bernard JF, Briere J, Chomienne C, Fenaux P. Long-term incidence of hematological evolution in three French prospective studies of hydroxyurea and pipobroman in polycythemia vera and essential thrombocythemia. *Semin. Thromb. Hemost.* 32(4 Pt 2), 417–421 (2006).

124 Lengfelder E, Griesshammer M, Hehlmann R. Interferon-α in the treatment of essential thrombocythemia. *Leuk. Lymphoma* 22(Suppl. 1), 135–142 (1996).

125 Griesshammer M, Bergmann L, Pearson T. Fertility, pregnancy and the management of myeloproliferative disorders. *Baillieres Clin. Haematol.* 11(4), 859–874 (1998).

126 Kiladjian JJ, Cassinat B, Turlure P *et al.* High molecular response rate of polycythemia vera patients treated with pegylated interferon α_{2a}. *Blood* 108(6), 2037–2040 (2006).

127 Lengfelder E, Berger U, Hehlmann R. Interferon α in the treatment of polycythemia vera. *Ann. Hematol.* 79(3), 103–109 (2000).

128 Bartholomew JR, Salgia R, Bell WR. Control of bleeding in patients with immune and nonimmune thrombocytopenia with aminocaproic acid. *Arch. Intern. Med.* 149(9), 1959–1961 (1989).

129 Ogston D. Current status of antifibrinolytic drugs. *Blood Rev.* 3(1), 1–4 (1989).

130 Ziakas PD. Effect of *JAK2* V617F on thrombotic risk in patients with essential thrombocythemia: measuring the uncertain. *Haematologica* 93(9), 1412–1414 (2008).

Patents

201 Ishikawa F. Imidazoquinazolin-2-one compounds. US4610987 (1986).

202 Chodnekar MS, Kaiser A. Imidazo[2,1-b] quinazolin-2(3H)-ones and pharmaceutical compositions for treatment and prophylaxis of cardiac insufficiency and cardiac failure. US4256748 (1981).

203 Campbell SF, Roberts DA. Tetrahydroimidazoquinazolinone inotropic agents. US4783467 (1988).

204 Peet NP, Sunder S. Substituted imidazo(2,1-b)quinazolin-5(3H)-ones and related tricyclic compounds and use as bronchodilators. US4871732 (1989).

Websites

301 Evidence for presystemic metabolism of anagrelide www.fda.gov/OHRMS/DOCKETS/dailys/04/aug04/081604/04p-0365-cp00001-09-Tab-H-vol1.pdf

302 Shire Pharmaceuticals Limited UK. Xagrid 0.5 mg hard capsule prescribing information http://emc.medicines.org.uk

303 AGRYLIN® (anagrelide hydrochloride) capsules www.fda.gov/medwatch/safety/2005/Agrylin_PI_12_04.pdf

Affiliations

- Ashkan Emadi, MD, PhD
Department of Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD 21231, USA
- Jerry L Spivak, MD
Johns Hopkins University School of Medicine, Traylor 924, 720 Rutland Avenue, Baltimore, MD 21205, USA
Tel.: +1 410 955 5454
Fax: +1 410 614 0854
jlspivak@jhmi.edu