Expert Opinion

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Anagrelide: a decade of clinical experience with its use for the treatment of primary thrombocythaemia

Petro E Petrides
Hematology Oncology Center, Zweibrückenstr. 2, 80331 Munich, Germany

Primary thrombocythaemia (PT) is the most frequent among the rare chronic myeloproliferative disorders. Life expectancy is determined by thromboembolic and haemorrhagic complications, which can be prevented by cytoreductive therapy. For a long time, hydroxyurea has been considered as the therapeutic gold standard. However, hydroxyurea treatment is not lineage-specific, may not be tolerated because of adverse effects (skin, gastrointestinal tract) and is leukaemogenic when sequentially used with other DNA-targeting drugs. Hence, anagrelide was welcomed in 1988 when it was first described as being efficient at normalising elevated platelet counts, specific for megakaryocytes and non-mutagenic. Since then, anagrelide has been approved in the US and Canada (Agrylin®, Shire Pharmaceuticals) as well as in Austria and other countries of the EU (Thromboreductin®, AOP Orphan Pharmaceuticals). Clinical Phase III trials (PT1 and ANAHYDRET) are underway to directly compare the efficacy and safety of anagrelide and hydroxyurea.

Keywords: anagrelide, drug metabolism, hydroxyurea, leukaemia, megakaryocyte inhibition, phosphodiesterase 3, primary (essential) thrombocythaemia

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1. Introduction

Primary thrombocythaemia (PT) is a rare haematological disease characterised by platelets, which are elevated and functionally disturbed [1]. As the diagnosis of PT is one of exclusion, there is an intensive search for molecular markers of this disease [2].

Anagrelide (originally developed by Bristol-Myers Squibb and designated as BL-4162A) is a platelet-reducing drug, which can be administered orally and belongs to the group of imidazole (2,1-b) chinazoline-2-compounds (**Figure 1**). The substance has a relatively poor solubility in water.

Initially, on the basis of *in vitro* investigations, an anti-aggregating activity on platelets was attributed to anagrelide. Thereafter, experiments on humans showed, however, that anagrelide under *in vivo* conditions, had only little influence on platelet function but rapidly lowered platelet counts. This caused the initiation of a series of clinical studies which led to the approval of the substance in the US and Canada under the name Agrylin® (initially by Roberts Pharmaceuticals, then sold to Shire Pharmaceuticals), Switzerland under the name Xagrid®, and in Austria and other countries of the EU under the trade name Thromboreductin® (AOP Orphan Pharmaceuticals). The different drugs are pharmacodynamically equivalent [3].

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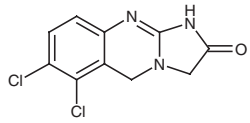

Figure 1. Structural formula of anagrelide hydrochloride.

2. Pharmacology of anagrelide

2.1 Mechanism of action of anagrelide

2.1.1 Platelet anti-aggregating properties in experimental animals and humans

For the first time in 1979 in experimental animals (Rhesus monkeys, rats, rabbits and dogs), the anti-aggregating activity of anagrelide was observed: under *in vitro* conditions, the substance inhibited in a dose-dependent manner (in mM concentrations), the ADP- or collagen-induced aggregation of platelets in platelet rich plasma [4]. The same activity was also present in humans, which was confirmed by other authors [5-8]. Under these experimental conditions, the activity of anagrelide, as well as that of its metabolites, which can be generated under the influence of enzymes in plasma, platelets and other blood cells, can be tested. Biologically-active metabolites, which are possibly produced in intestinal or hepatic metabolic pathways, are not recognised in this assay.

Platelet function is regulated through the intracellular turnover of cyclic nucleotides, which is determined by adenylcyclases and phosphodiesterases (PDEs). Physiological platelet antagonists, as well as various antiplatelet drugs, inhibit platelet function by activating adenyl- and guanyl cyclases, thereby increasing intraplatelet cAMP and cGMP levels. This elevation interferes with all known platelet activating signal pathways and blocks intracellular signalling networks, cytoskeletal rearrangements or fibrinogen receptor activation. Target molecules of cyclic nucleotides are cAMP- or cGMP-dependent protein kinases, which mediate their effects through the phosphorylation of specific substrates.

Several studies have revealed that anagrelide inhibits a Type 3 PDE [9,10] with a subsequent increase of cAMP [11], which results in unresponsiveness of the platelet to various stimuli.

The effects of anagrelide on the aggregation of platelets were also observed with aggregometric measurements after the intake of a total dosage of 6 or 8 mg/day p.o., respectively, in human volunteers [12]. Anagrelide also inhibited the release of arachidonic acid metabolites from human platelets after stimulation with thrombin [13].

When tested on whole blood or platelet-rich plasma of patients with myeloproliferative disorders, anagrelide did, however, not change the spontaneous or induced platelet aggregation [14]. Moreover, the *in vivo* platelet function was also not influenced as the bleeding time measured after 4 – 10 days treatment with anagrelide 4 – 6 mg/day, remained unchanged [14]. One has to keep in mind, however, that the bleeding time may not be a reliable test as, for example, it is normal in ~ 50% of patients with von Willebrand disease [15].

In rats, the application of anagrelide in a coronary stenosis model leads to the prevention of myocardial infarctions (MIs) [16].

2.1.2 Platelet lowering effects in humans

In humans (tested on nearly 100 healthy male volunteers), but not in experimental animals, anagrelide causes thrombocytopenia: when given at a dosage of 3 – 9 mg/day for 6 – 9 days, rapid reductions in platelet counts to values between 60,000 and 20,000/µl occur within 10 – 12 days, which reverse within 4 – 8 days after drug discontinuation with a rebound thrombocytosis [17-19].

In a double-blind study with 15 healthy volunteers (5 on placebo and 5 on anagrelide 1 and 2 mg, respectively), decrease in platelet count, as well as a slight shortening of the platelet survival time was observed (8.4 – 7.8 days). Influences on prothrombin time, activated partial thromboplastin time, bleeding time, haemoglobin, reticulocytes, leukocytes or differentials) were not seen [20].

In human cell culture, the action is also species specific: the substance inhibits the maturation, and thereby, the size and ploidy of megakaryocytes [21-23]. This activity is not mediated through the PDE3 system, although the underlying molecular mechanism is not yet known (see below).

2.1.3 In vivo activities of anagrelide in patients with thrombocythaemias

Bellucci *et al.* [24] observed in three patients with PT, an increase of the platelet volume. Such an effect cannot be detected upon treatment with hydroxyurea or IFN-α. Membrane protein glycoprotein IV (CD36) which was elevated in the platelets of the patients, remained elevated after normalisation of the platelet count (to 327,000, 492,000 or 334,000/µl, respectively). In addition, the absence of aggregation induced by adrenaline or the presence of an abnormal thrombospondin band were not eliminated upon anagrelide treatment. From these observations the authors concluded that anagrelide effectively lowers platelet counts but functional or biochemical alterations of the platelets are not influenced. This could explain a residual risk for thromboembolic complications upon anagrelide therapy (see below).

Lev *et al.* [25] investigated in 16 patients with PT, the plasma concentration of various cytokines (transforming growth factor [TGF]-β, platelet-derived growth factor [PDGF], basic fibroblast growth factor [bFGF]) before therapy and after normalisation of the platelet counts. The plasma levels of all three cytokines were elevated in all patients. After normalisation of the platelet count, the plasma levels of TGF-β and bFGF remained elevated, which may imply a participation of these cytokines in the development of PT. The concentrations of these mediators within the platelets were either normal (TGF-β), elevated (bFGF) or diminished (PDGF).

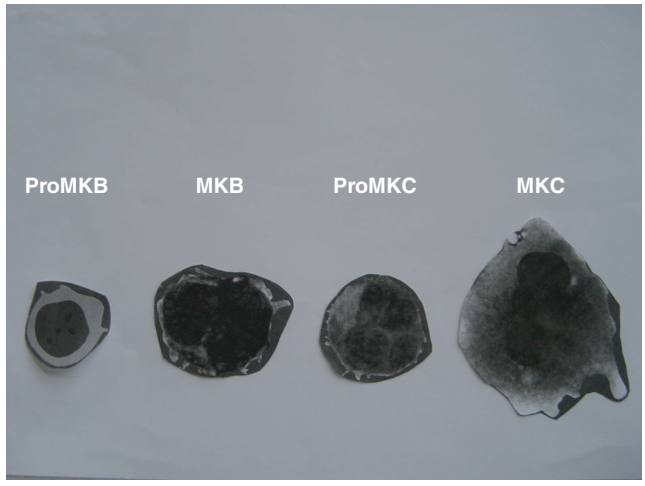

Figure 2. Development of megakaryocytes. The promegakaryoblast (ProMKB) is the first morphologically recognisable megakaryocyte precursor in the bone marrow. Megakaryoblasts (MKB) are 15 – 50 µm in diameter, with large oval nuclei (containing two sets of chromosomes = 4N) and a basophil cytoplasm without granules. Promegakaryocytes (ProMKC) are 20 – 80 µm in diameter and possess developing granules. Megakaryocytes (MKC) are up to 150 µm in diameter, undergo endomitosis and become polyploid through repetitive DNA replications without cell division. Most megakaryocytes have eight sets of chromosomes (16N). Cytokines, such as IL-3 and thrombopoietin, participate in the development of the megakaryocytes.

Laguna *et al.* [26] analysed the plasma levels of thromboxane B2 and PDGF in 17 patients with PT; the levels of thromboxane were elevated and normalised under therapy. PGDF levels, however, were diminished but not influenced by therapy with anagrelide.

Tomer [27,28] investigated the *in vivo* megakaryocytopoiesis in patients with PT under therapy with anagrelide; by utilising flow cytometry megakaryocytopoiesis in the bone marrow was quantified. During therapy, the number of megakaryocytes (identified through the lineage marker CD41 [glycoprotein (GP) IIb]) diminished in the bone marrow, their diameter and volume decreased from 46 to 40 µm and 48 to 34 × 10^3 µm, respectively, and their ploidy from 32N (with some 64 and 128 forms) to the normal 16N ploidy. Therefore, the total megakaryocyte mass was reduced by 66%, which correlated with the decrease in platelet count. These results confirmed the observations in cell culture (see above) in which anagrelide decreases the platelet level by inhibition of the hyperproliferation and differentiation of megakaryocytes.

Thiele *et al.* [29,30] investigated megakaryocytopoiesis in 10 idiopathic myelofibrosis (IMF) and 5 PT patients with immunohistochemical and morphometrical methods (using the differentiation marker CD61a). According to Thiele *et al.*, the reduction of the megakaryopoiesis by anagrelide was due to a specific arrest in the development of megakaryocytes with the production of more mature platelet secreting cellular forms. This led to a significant left shift with an increased presence of diploid 2N-promegakaryoblasts and megakaryoblasts (**Figure 2**). The total number of all CD61a-positive megakaryocytes was not altered. An effect of anagrelide on the formation of connective tissue fibres in PT or IMF was not found.

Yoon *et al.* [31] conducted a prospective study in patients with IMF who experienced possible alterations of the bone marrow under treatment with anagrelide through sequential biopsies; they observed an increase of the megakaryocyte number (identified through the determination of the so-called UEA-1-antigens) and a left shift. The concentration of PDGF and TGF-β was not influenced, which possibly also explained the missing (inhibiting) influence on the fibrosis of the bone marrow (duration of observation up to 4 years).

2.1.4 *Non-haematological effects*

In experimental animals, anagrelide has also a positive inotropic and vasodilatatory effect [32]. This effect may be due to the presence of a PDE3 in vascular smooth muscle cells. In humans (e.g., patients with myeloproliferative syndromes), the initiation of a therapy with anagrelide can cause a decrease of the systolic and diastolic blood pressures, which under maintenance therapy, increases to the initial value [33]. Some of the adverse effects of anagrelide are due to these inotropic/vasodilatatory effects (see below).

2.1.5 *Pharmacokinetics*

2.1.5.1 *Bioavailability and duration of elimination*

Oral intake of a radioactive (100 µCi of the ^{14}C-labelled substance) test dosage of 1 mg causes, in fasted healthy volunteers, a rapid increase in the anagrelide plasma level with a maximum after 1 h (3 ng/ml), a relative rapid decrease within 6 – 8 h and a slow decrease to ~ 10% of the maximum value within 24 h [34,35]. This observation was the basis of the recommendation to take anagrelide three times daily (every 8 h). The estimated terminal elimination half-life time was ~ 3 days.

The intake of food slows the resorption of anagrelide with a subsequent prolongation of the plasma half-life time. Clinical experience with anagrelide in therapeutic dosages in patients shows, however, that its efficacy is not influenced by food intake.

2.1.5.2 *Metabolism and excretion*

The excretion of the radioactivity occurs primarily through the kidneys (i.e., within 6 days, ~ 75% of the radioactivity are secreted in the urine and ~ 10% from the liver through the bile into the faeces). At least four different metabolites (two 'major' metabolites with 44 and 24%, respectively and two 'minor' with 7 and 2%, respectively) are detectable in urine with reverse-phase HPLC (in the original publication, however, no chromatograms are shown), anagrelide itself represents only 1% of the whole activity excreted in urine [34].

If one compares this distribution of urine metabolites with that found in dogs, monkeys and rats, there are differences: whereas the total activity in monkeys is similar (64%), it is much lower in dogs and rats (35 and 42%, respectively).

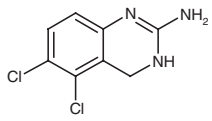

Figure 3 Structure of the anagrelide metabolite, FL603.

Moreover, the quantitative distribution is quite different from species to species, which could explain the missing platelet reducing activity of anagrelide in animals [FDA, data on file]. It is unknown which of the metabolites still possesses biological activity.

Lane *et al.* [36] identified a water soluble metabolite designated as FL603, which does not possess the imidazole side chain and is very soluble in water when compared to anagrelide (**Figure 3**). The publication, however, does not contain any information as to whether the metabolite is one of the substances in urine (see above) and how this metabolite was isolated.

The intraperitoneal injection of FL603 in Balbc mice causes a dose-dependent decrease of the circulating platelet counts. If the metabolite is active in mice, it should normally not be produced in these animals. Under *in vitro* conditions the metabolite inhibits the replication and maturation of megakaryocytes (with 50 times higher potency than anagrelide) without influencing the ADP-induced aggregation of platelets.

Erusalimski *et al.* [37,38] questioned the mechanism of action of anagrelide through a human-specific metabolite. They were not able to confirm the activity of the substance on megakaryocytes or in mice, and showed that FL603 is also present in rats, dogs and rabbits in significant amounts. Hence, they favour the hypothesis developed by McCarty *et al.* [39], which relates the species specificity of anagrelide to the c-mpl receptor, which is only 10% homologous in both men and mice. In *in vitro* experiments, anagrelide inhibits the thrombopoietin (TPO)-stimulated proliferation of megakaryocytes, which had been transfected with the human c-mpl receptor, but not in those who were transfected with the mouse gene. Rafil and Lane [40], however, do not accept this argumentation.

As anagrelide and its metabolites can be identified with HPLC methods [34] and characterised with chromatography/mass spectrometry analyses [41], it is unsatisfactory that to this day, anagrelide and its derivatives have not been determined in order to gain a better understanding of important questions, including primary resistance towards the drug, individual dosage requirements, the metabolism of the drug and also the question of which adverse effects are due to which metabolites?

2.1.5.3 Elimination in impaired liver or kidney function

As the metabolism of anagrelide occurs primarily in the liver and the excretion mainly through the kidneys, it is possible that diseases of these organs impair the excretion of the drug. Patients with impaired renal or liver function have therefore, been carefully checked for overdosage of the drug, and the anagrelide dose to be decreased if necessary (see below).

2.1.5.4 Elimination in elderly patients

The excretion of anagrelide in elderly patients is possibly decreased and the half-life time extended. Elderly patients should therefore be carefully checked for any evidence of an overdosage of the drug.

2.1.6 Toxicological properties

2.1.6.1 Toxicity in experimental animals

In experimental animals (mice, rats, monkeys), maximal doses were not lethal; in rodents, they caused increased motor activity, increased heart frequency and decreased blood pressure, and in monkeys, soft stool and reduced food intake [36].

2.1.6.2 Mutagenic potential/reproductive toxicity

Anagrelide is not mutagenic in the Ames Test, the human lymphocyte chromosome aberration test [FDA, data on file] or mouse lymphoma cells [3]. A leukaemogenic effect of anagrelide has not been observed until now. Teratological studies have been performed in rats and rabbits. In female rats, anagrelide impaired the implantation and the development of pregnancy.

2.6.1.3 Placental transfer

Because of its low-molecular weight, anagrelide can cross the placental membrane. Premenopausal women should therefore use contraceptive measures during the intake of anagrelide as a teratogenic effect in humans has not been ruled out. The drug should also not been taken by breastfeeding mothers.

3. Clinical applications

There are nine clinical studies with anagrelide which have been published in peer-reviewed journals from 1988 until 2001 [14,33,43-46,50-54]. All are uncontrolled, non-comparison studies, which have been carried out in the US, Germany, Italy, Switzerland, Australia, Norway and Argentina [42]. The most relevant results of these studies are discussed below. As PT is a rare (orphan) disease, the number of patients in most of these studies is low. For this reason, detailed information about each study will be provided.

3.1 Evaluation of the individual published clinical studies

3.1.1 The initial Silverstein report

At the end of the 1980s, Murray Silverstein and his group from the Mayo Clinic in Rochester reported the first results of the first Phase II studies in 20 patients with myeloproliferative disorders with platelet counts of > 900,000/µl (**Table 1**) [33]. In this study (started in October 1985), a high induction dosage of anagrelide was given (8 mg/day). A haematological response was defined as a decrease in platelet counts of < 50% of the initial value: only 2 of 17 (11%) patients with PT did not respond (i.e., even with dosages of 8 – 10 mg/day, no normalisation of the platelet count was reached) (**Table 2**). Before therapy, the medium platelet count was 1.381×10^6/µl

Table 1. An overview of the clinical studies.

Study	Ref.	Total N	Female:male ratio	Median age (years)	PT	PV	CML	Others
US-I	[33]	20	13:7	55 (25 – 83)	17	2	1	0
US-II (I+II)	[43]	577	339:238	61	335	68	114	60
US-III (II+III)	[44]	942	556:386	58	546	113	179	108
Italy I	[14]	8	4:4	60	5	0	2	1
Italy II	[45]	20	12:8	33	20	0	0	0
Germany I	[46]	48	27:21	54	48	0	0	0
Germany II	[50]	12	5:7	58	0	0	12	0
Switzerland	[51]	6	3:3	48	2	3	1	0
Australia	[52]	16	12:4	58	16	0	0	0
Argentina	[53]	17	12:5	34	17	0	0	0
Norway	[54]	10	6:4	< 60	10	0	0	0
Total		**1079**	**637:442**		**664**	**116**	**194**	**109**

CML: Chronic myeloid leukaemia; N: Number of patients; PT: Primary thrombocythaemia; PV: Polycythaemia vera.

Table 2. Haematological response rates in the published clinical studies (note: different response criteria were used).

Study	Ref.	Total N	Female:male ratio	Type of chronic myeloproliferative disorder	CR	PR	NR
US-I	[33]	20	13:7	20 (17 ET, 2 PV, 1 CML)	18		2
US-II	[43]	577	339:238	424	396		28
US-III (I+II included)	[44]	942	556:386	942 (113 PV, 546 ET, 179 CML, 108 non-classified)	665	85	192
Italy I	[14]	8	4:4	8 (5 ET, 2 CML, 1O MF)	5		3
Italy II	[45]	20	12:8	19 (all ET)	13	3	3
Germany I	[46]	48	27:21	48 (all ET)	42	3	3
Germany II	[50]	12	5:7	12 (CML)	12		
Switzerland	[51]	6	3:3	6 (2 ET, 3 PV, 1 CML)	5	1	
Australia	[52]	17	2:4	16 (all ET)	14		2
Argentina	[53]	17	12:5	17 (all ET)	17		
Norway	[54]	10	6:4	10 (all ET)	7	1	2*
Total		**1079**	**637:442**	**1078**	**780**	**93**	**203**

*Discontinuation because of intolerability.

CML: Chronic myeloid leukaemia; CR: Complete responders; ET: Essential thrombocythaemia; MF: Myelofibrosis; N: Number of patients; NR: Non-responders; PR: Partial responders; PV: Polycythaemia vera.

(from 947,000 to 2.8 × 10^6/µl). Initially an induction dosage of 2 mg was given every 6 h (corresponding to a total dosage of 8 mg/day). Shortly after initiation of the study, it became clear that the chosen dose was too high and therefore, the induction dosage was reduced to 1 – 1.5 mg every 6h (corresponding to 4 – 6 mg/day). Typically, the platelet count decreased from day 5 and became normal within 2 weeks when the patients responded. The maintenance dosage was 1 – 4 mg/day.

Adverse effects were observed in six responders (i.e., nausea, bloating or headache) during the induction phase. These symptoms disappeared when the anagrelide dosage was reduced to the maintenance dosage. As anagrelide 20 mg/day causes a drop of the blood pressure in normal volunteers [32], the blood pressure was carefully observed: during the induction phase, the diastolic and systolic blood pressures fell by an average value of 5 mmHg. At a maintenance dosage of up to 4 mg/day, the blood pressure resumed in all patients. A morphological analysis of the bone marrow prior to initiation of the therapy with anagrelide and normalisation of the platelet counts was performed in eight patients: bone marrow cellularity and morphology remained unaltered. In three patients, the level of colony formation from bone marrow stem cells before and after intake of anagrelide was investigated: the

megakaryocyte colony forming unit and erythroid burst-forming unit, did not change during therapy.

This small study also showed that some individuals primarily do not respond to anagrelide.

3.1.2 The results of the Anagrelide Study Group

Based on these encouraging data, a large study group was formed and a study initiated. A total of 333 patients were included in this study, and a further 244 patients were treated on so-called compassionate-use basis. The data for both patient groups were pooled and published together [43]. The first patient in the total cohort of 577 patients was treated in October 1985 and the last included on December 28, 1990. A total of 90% of the patients had been pretreated with cytoreductive drugs. In this larger cohort, the efficacy of anagrelide was also confirmed. Cardiac side effects in potential risk patients were identified as a major problem. As an induction dose, anagrelide 2 – 4 mg/day were given. Of the 577 patients, 335, 114, 68 and 60 had PT, chronic myeloid leukaemia (CML), polycythaemia vera (PV) and a non-classifiable myeloproliferative disorder with thrombocythaemia, respectively (**Table 1**). Of these, 504 (87%) had been previously treated with other modalities: the median age at the initiation of therapy was 57 – 66 years. Of the 577 patients, only 424 could be evaluated. At a dosage of 2 – 4 mg/day, anagrelide caused a decrease of the platelet count to at least 50% or < 600,000 µl for at least 28 days in 396 of 424 (93%) evaluable patients. The time required to reach a 50% reduction in platelet count after initiation of the anagrelide treatment was 11 days in the total group of patients. After 6 – 10 weeks of therapy, the platelet counts had to be reduced to < 500,000/µl in the responder group and had to remain at this level for up to 2 years. The longitudinal evaluation of the platelet counts showed for all responders a decrease when compared to the levels at the beginning of the therapy. At the same time, an increase of the white blood cell counts and a decrease in haemoglobin values were observed. The median dosage for a response was 2.57 mg/day with a value from 2.52 to 2.88 mg/day depending upon the type of the disease. A total of 95% of patients responded to a dose of ≤ 4 mg/day. The median duration of therapy was 5.6 months to a maximum of 61 months. The maintenance dosage was 1.7 – 2.8 mg/day.

3.1.3 The 942 patient analysis of the Mayo Clinic

In 1997, an actualisation of the study was published by the Mayo Group, which included 942 patients with myeloproliferative thrombocythaemia (**Table 1**). These patients had been treated for at least 4 years [44]. The median age was 58 years (10 – 94). The median platelet count before therapy was 1,131,600/µl. Two-thirds of the patients had obtained other platelet reducing drugs prior to treatment with anagrelide. The response rates were lower than in the previous analysis, which was explained by the authors by the fact that > 200 patients were treated by physicians with less experience with this drug. The response rates in the Mayo Clinic patients were higher (85, 94 and 95% in PV, PT and in the remaining group, respectively). Some patients with extremely high platelet counts and practically all symptomatic patients started with a daily induction dosage of 4 mg. The time required to the haematological response inversely correlated with the induction dosage; in practically all responders, the platelet reduction occurred within 1 week. The average maintenance dosage in patients with thrombocythaemia in PV was 2.4 mg/day; however, in all remaining myeloproliferative disorders, it was 2.0 mg/day.

3.1.4 The first Italian study (Pavia)

In 1992, Balduini's group from Pavia [14] reported on eight patients who had been treated with anagrelide (**Table 1**). Their ages ranged from 41 to 72 years. With the exception of two patients, all had received prior treatment with pipoproman, busulfan, hydroxyurea, IFN-α or a combination of these drugs. The mean duration of therapy was 26 weeks (2 – 89 weeks). In five of the eight patients, anagrelide caused a persisting reduction of the platelet level to < 500,000/µl (three PT, one CML, one IMF). A further one CML and one PT patient did not reach platelet counts of < 1×10^6/µl. The mean platelet value of all responders prior to therapy was > 1×10^6/µl; after 7 days, this value fell to counts of < 400,000/µl. A total of four patients showed a decrease in their haemoglobin of > 1 g/dl within 45 days of therapy, and in one patient, a decrease of 4.4 g/dl after 4 months of therapy was observed. After discontinuation of anagrelide, the haemoglobin value became normal again (causes unclear). Other side effects were headache, palpitations/tachycardias, nausea and diarrhoea (in five, four, two and three patients, respectively). This was the first study in Europe but with a very small and heterogeneous patient group.

3.1.5 The second Italian study (Rome)

Also in 1992, Mazzuconi *et al.* [45] reported on their experiences in the treatment of 20 patients with PT (**Table 1**). In this group, anagrelide was given at a low induction dosage of 1 mg/day and the dosage increased by 0.5 mg/week until a haematological response was obtained. The platelet counts were determined during the induction and maintenance phase. The aim of the study was to identify the individual tolerability of anagrelide and the minimal dosage with therapeutic efficacy. Between June 1989 and July 1991, 20 patients were treated, their mean age was 33 years (26 – 51 years). A total of 6 patients had been treated with pipoproman without sufficient normalisation of their platelet levels. A toral of 14 patients had not been pretreated. At the beginning of the study, 14 of the 20 patients were asymptomatic. Five patients showed PT associated symptoms such as headache, scotoma, paraesthesia, or Raynaud symptoms (two, one, one and one patient, respectively). At the beginning of therapy, the median platelet counts was 1.16×10^6/µl (610,000 – 1.86×10^6/µl). Only 19 of 20 patients could be evaluated as 1 patient was taken off study because of gastrointestinal (GI) side effects. Anagrelide led to a reduction and maintenance of reduced platelet counts to < 500,000/µl in 13 of 19 (68%) patients.

The mean duration to haematological response was 5 months (1 – 24 months). A total of 3 (16%) patients showed a stable platelet count decrease to < 600,000/µl with a mean dosage of 2.3 mg/day (1.5 – 3 mg/day) and were considered to be partial responders. The other 3 (16%) patients were classified as non-responders. The mean follow-up was 16 months (4 – 30 months), the mean maintenance dosage was 2.4 mg/day. Adverse effects such as tachycardia (4), GI complaints (3) and ankle oedema (1), were observed in 8 of 20 patients. The adverse effects occurred relatively late during the therapy, which was attributed to the escalating dosage scheme. In 6 patients, the adverse effects were considered Grades III-IV according to the World Health Organization, so that the therapy was discontinued, for example, in a patient after 1 month because of the severe GI intolerability (symptoms, however, were not described).

3.1.6 The German data

3.1.6.1 The German primary thrombocythaemia series

In 1998, Petrides *et al.* [46] reported on 48 patients with PT who had been treated with anagrelide. This is still the largest European study with a homogeneous cohort of patients with PT. Patients 18 – 80 years of age were offered anagrelide when their platelet count was ~ 900,000/µl or between 600,000 and 900,000 µl and disease symptoms were present. The induction dose was 2 mg/day. The age of the patients (women:men ratio 27:21) at initiation of therapy with anagrelide was between 19 and 79 years: one-third of the patients was either not pretreated or had a pretreatment with hydroxyurea alone or sequential treatments with various drugs such as hydroxyurea, busulphan, IFN-α, melphalan or radiophosphor. In addition to this, ~ 50% (26 of 48) of the patients received low-dose aspirin therapy (100 mg/day). A total of 12, 19 and 17 patients were < 40, > 40 but < 60 and ≥ 60 years, respectively. Of the 48 patients, 41 had symptoms such as microvascular (paraesthesia, erythromelalgia, transient ischaemic attack), thromboembolic (pulmonary embolism, spleen infarction, portal venous thrombosis, deep venous thrombosis, central venous thrombosis, angina pectoris or MI) or haemorrhagic complications (mouth, intracerebral, GI or skin bleeding). On the basis of their history of thrombosis and age (> or < 60 years of age), 50% of the PT patients belonged to the high-risk group and the remaining 50% to the low-risk group. The platelet counts prior to therapy were between 600,000 and 899,000/µl, 900,000 and 1.499,000/µl and > 1.5×10^6/µl in 2, 27 and 11 patients, respectively. The remaining 8 patients had platelet values between 150,000 and 810,000/µl being under treatment with hydroxyurea.

Patients in whom the platelet counts fell to < 600,000/µl or < 50% of the initial value and in whom these counts remained stable for at least 1 month, were considered to be complete haematological responders. A decrease of 20 – 50% was considered to be a partial response, and lack of response a decrease of < 20%. According to these criteria, 42 of 48 (87%) PT patients were complete haematological responders, the remaining 6 either partial responders (in 2 patients the platelet counts increased further after reaching a nadir of 750,000/µl) or primarily refractory. This indicates a heterogeneity of the disease on the molecular level or a patient-specific metabolism of the drug. Of the responding patients, 23 had platelet counts of < 450,000/µl whereas the other 19 had platelet counts of > 450,000/µl.

A total of 21 patients had been treated at the time of publication for a period of ≥ 12 months. The longest duration of treatment was 72 months in a young woman who requested a discontinuation of therapy after this time period, and 84 months in an elderly lady who was 80 years of age at the time of publication. The median anagrelide maintenance dosage was 2.5 mg/day in the patients who were complete responders and treated for > 12 months. Of the 48 patients, 20 (40%) developed side effects, which were often caused by the vasodilatatory properties of anagrelide. In this regard, all symptoms documented in the study were considered totally independent whether a cause or relationship with the intake of anagrelide could be proven or not. Adverse effects included headache (most frequent), tachycardia, palpitations, fluid retention (only in these patients who had take 12 or 10 mg/day, respectively), abdominal pain, haemoglobin decrease, nausea and cardiac insufficiency or diarrhoea. The latter symptom was possibly due to the presence of lactose in the drug formulation. The overwhelming number of adverse effects was of mild nature and disappeared within 4 weeks. Only in 5 of 48 patients did the adverse events persist so that the therapy had to be discontinued. Of the 48 patients, 14 patients discontinued therapy for various other reasons: lack of response or only partial response, transformation into acute myeloid leukaemia, development of a myelofibrosis, personal wish to discontinue the study, elevation of pancreatic enzyme of unknown origin or non-therapy-related death (4, 1, 1, 4, 1 and 3 patients, respectively).

Transformation into acute myeloid leukaemia occurred in one patient who had obtained five different cytoreductive drugs prior to anagrelide therapy. Death in three patients was due to surgery, cardiac insufficiency or apoplectic insult.

An advantage of this study was that very detailed information for each individual patient who had been treated with anagrelide with regard to symptoms anti-aggregating additional drugs platelet response, adverse effects, were available. A total of 21 patients were treated at least for 12 months, an actualisation of the data were published in abstract from in 1999 [47] and an additional abstract was completed in 2004 and is due for publication as a full paper [48]. A detailed analysis of the patients is also available in the doctoral thesis of Trapp [49].

3.1.6.2 The German chronic myeloid leukaemia data

In 1998, Trapp *et al.* [50] reported on 12 patients with CML who had developed thrombocythaemia. None of patients responded to hydroxyurea but responded to anagrelide. Inclusion criteria were an age of > 18 years and the diagnosis of CML with platelet counts of > 900,000/µl, which were not

treatable with other drugs. Treatment was initiated with a dosage of 1 to 2 mg/day. The median age was 58 years (women:men ratio 5:7) (**Table 1**). All patients had been treated with hydroxyurea directly prior to the commencement of anagrelide therapy. Some of them had been administered IFN-α, melphalan of busulphan prior to hydroxyurea. The mean platelet count before initiation of the treatment with anagrelide was 2×10^6/µl. Of the 12 patients, 7 were symptomatic because of their elevated platelet counts and either had microcirculatory disturbances, thrombosis or bleeding. A reduction of platelet count was observed in all patients. The mean dosage, which was required for the maintenance of the platelet count for at least 4 weeks, was 1.9 mg/day.

Adverse effects were observed only in 3 patients (i.e., headache, tachycardia, palpitation or fluid retention). After controlling the platelet counts with anagrelide, the hydroxyurea dosage in 9 of 12 patients could be reduced. At the time of publication, 6 of 12 patients were treated with anagrelide (mean treatment duration: 15.7 months). Causes for the discontinuation of therapy were bone marrow transplantation, osteomyelofibrosis, blast crisis or patient wish.

3.1.7 Report of the Swiss group

In 1998, a Swiss group from Zürich reported their long-term experience in six patients with myeloproliferative disorders [51]. All patients had been treated between 1991 and 1997. The mean duration of therapy was 54 months with a total response of 100%. Inclusion criteria were platelet counts of > 600,000 or 650,000/µl with PT-associated symptoms. Of the study group, three were men and three women with a median age of 44 years (19 – 80 years) at the time of diagnosis and a median age of 48 years (39 – 80 years) at the time of initiation of therapy with anagrelide. A total of three, two and one patient had PV, PT and CML, respectively. Of the six patients, four had pretreatments and five of the six patients were symptomatic (**Table 1**). The mean duration of therapy was 54 months (17 – 75 months) with a mean induction dosage of 2 mg/day. The mean maintenance dosage of anagrelide was 2.75 mg/day (2 – 4 mg). Of the six patients, five were complete responders. Prior to treatment, the mean platelet count was 1.211 mio/µl, which was reduced to a mean value of 570,000/µl (216,000 – 667,000/µl). Neither changes of the haemoglobin nor white blood cell counts were observed. In 4 out of 5 symptomatic patients, the symptoms disappeared.

3.1.8 The Australian data

Mills *et al.* [52] in Brisbane, reported on 17 patients with PT who had been treated with anagrelide; 1 patient was excluded because of non-compliance. The remaining 16 (4 men, 12 women) had a mean age of 58 years (14 – 74 years). Of these, 14 (88%) were either symptomatic or high risk patients because of pre-existing venous or arterial diseases. All patients were pretreated with hydroxyurea, IFN-α, warfarin, radiophosporus, aspirin and/or busulphan (15, 7, 5, 3, 2 and 1 patient, respectively). A total of 10 (63%) patients were either pretreated with two or more cytoreductive drugs. A total of 4 started with anagrelide because of their concern of a possible leukaemogenic action of hydroxyurea, the remaining 12 (75%) patients were non-responders or had not tolerated other therapies. A further 2 patients did not show a response to IFN-α, 5 to hydroxyurea or phosphorous 32 and 5 others suffered from hydroxyurea intolerability (myelosuppression, skin changes). The other 16 patients were observed for a mean duration of 7 months (15 days to 36 months). The average dosage for a platelet reduction in the first 3 months was 1.9 mg/day (1 – 3 mg). Despite the initial reduction of platelets which was due to other therapies, a further significant reduction of the platelet count within the first three months from 728,000 to 425,000/µl occurred. A total of 7 patients showed a complete haematological remission in 3 months, developing a platelet count of < 400,000/µl and 14 (88%) patients showed a partial response as they reached a platelet count of > 400,000/µl). There were no major alterations of haemoglobin or white blood cell counts.

3.1.9 The Argentinean data

In 2000, a group from Argentina [53] reported on 17 patients with newly diagnosed PT. The mean age at diagnosis was 34 years (21 – 68; female:male ratio 12:5). The diagnosis was between 3 months and 8 years prior to the initiation of treatment with anagrelide with a follow-up between 2 and 6 years. A total of 10 patients had symptoms; 8 were asymptomatic at the beginning of the anagrelide therapy. The platelet values before and after treatment upon anagrelide-induced remission were 980,000 or 378,000/µl, respectively. Clinical manifestations, such as circulatory disturbances or bleeding complications, disappeared during the anagrelide-reduced remission in all 10 symptomatic patients. The authors also investigated the spontaneous platelet aggregation. During remission, a spontaneous platelet aggregation was only observed in 1 patient. The difference between the percentage of spontaneous platelet aggregation of 35% before and 5% during anagrelide treatment was significant ($p = 0.02$). In 3 patients with PT, symptoms reappeared when the platelet count increased to > 600,000/µl.

3.1.10 The Norwegian data

Knutsen *et al.* [54] reported on 10 patients < 60 years of age with PT of whom, 9 had been pretreated. All in all, 7, 1 and 2 patients developed a complete remission, partial remission or discontinued therapy, respectively. In all complete responders (< 500,000/µl), no vascular complications occurred.

3.2 Unpublished clinical studies

3.2.1 AOP 02-007 study (multinational)

In the largest Phase II Good Clinical Practice (GCP) study with anagrelide in Austria, Poland and the Czech Republic, 97 patients (21 – 80years of age; female:male ratio 69:28) with thrombocythaemia in myeloproliferative disorders (79 PT, 16 PV, 2 IMF) were treated according to GCP guidelines. Patients with a high-risk profile were included (platelet counts > 1×10^6/µl, increase of the platelets by > 300,000/µl within 3 months, previous thrombotic haemorrhagic complications or

lack of response to or intolerability of prior cytoreductive therapies). The patients were given anagrelide during weeks 1 and 2 at a dosage of 1 and 2 mg/day, respectively. After that the dosage was increased weekly for ≥ 1 month, according to the therapeutical response, the dose was either increased or reduced in increments of 0.5 mg, and the therapeutic response checked every month (duration of study 6 months). Response was defined as a reduction of the platelet count to < 600,000/µl (complete responders) or a reduction by 50% (partial responders) over a 4-week period. The intent-to-treat analysis revealed a rate of response (CR + PR) of 87%, in ~ 90% of the patients, a dose of 0.5 – 3.0 mg/day was sufficient. The percentage of patients with a platelet count of < 600,000/µl increased during therapy with anagrelide from 30% at the initiation of therapy to 77% after 6 months. In this clinical study, a reduction of clinical complications caused by a decrease of the platelet count with anagrelide could be documented: the percentage of patients with severe clinical complications (e.g., MI) decreased from 5.2% 6 months prior to therapy to 2.1% 6 months after therapy with anagrelide ($p < 0.001$). The most frequent side effects were headache, diarrhoea and palpitations. A total of nine patients discontinued the study due to death (no causal relationship with study medication), not wishing to continue within the study, side effects, protocol violation and because of insufficient compliance (three, two, two, one and one patient, respectively) [55,56].

3.2.2 *The MRC-PT1-Study (Great Britain)*

This study, organised by the Medical Research Council (MRC), aims to recruit 300 – 500 patients to either: aspirin 75 mg/day for low-risk patients (< 40 years of age with a platelet count of 600,000 – 999,000/µl plus neither thrombohaemorrhagic complications nor erythromelalgia); either aspirin 75 mg/day or hydroxyurea plus aspirin for intermediate-risk patients (40 – 59 years of age with a platelet count of 600,000 – 999,000/µl and neither thrombohaemorrhagic complications nor erythromelalgia); and either aspirin plus hydroxyurea or aspirin plus anagrelide (Agrylin®) for high-risk patients (either > 60 years of age or a platelet count of $> 1 \times 10^6$/µl or thrombohaemorrhagic complications or erythromelalgia) [57]. Pretreatment with another cytoreductive treatments was no exclusion criterion; a bone marrow biopsy at the time of diagnosis was not mandatory. Recently, the high risk arm was prematurely closed (for details see [201]). Results of this study are expected shortly.

3.2.3 *The ANAHYDRET study (International)*

In this multinational (10 EU countries), placebo-controlled, randomised, Phase III study (AOP 03-007), only untreated patients can be included (aim of recruitment 230 patients). In this study, ANAgrelide (Thromboreductin®) is being compared with HYDRoxyurea in high-risk patients with Essential Thrombocythaemia (≥ 60 years or platelets $> 1 \times 10^6$/µl or increase of the platelets by > 300,000/µl within 3 months or vascular risk factors). The drugs are provided for 12 months; since fall 2002, > 120 patients have been recruited, an interim analysis is expected at the end of 2004 (for details see [202]).

4. Efficacy

4.1 Clinical, haematological and haemastaseological efficacy

When the therapeutic efficacy of a drug for the therapy of thrombocythaemia is evaluated, three aspects are important:

- Clinical efficacy, that is, the reduction of thrombocythaemia-associated symptoms such as erythromelalgia, paraesthesia, bleeding tendency or transitory ischaemic attacks, such as visual symptoms (clinical response).
- The reduction of the platelet counts whenever possible into the normal range (haematological response).
- The primary or secondary prevention of thromboembolic complications (which could be called a haemastaseological response).

Not all of the studies refer to the reduction of clinical symptoms. In the US study [60], the time-dependent reduction (in intervals of 3 months) of the symptoms was analysed; their number decreased continuously from 0.66/patient prior to initiation of therapy to 0.07 after 30 months of continuous therapy. In the German CML study, all patients became asymptomatic. The Swiss haematologists reported an amelioration of thrombocythaemia-associated symptoms in four of five patients. In the Australian study, 50% of the symptomatic patients showed a decrease of their symptoms. In the Argentinean study, microvascular disturbances and bleeding disappeared in all patients by the anagrelide-induced remission, although they reappeared in three patients at a reduction of the dosage and an increase of the platelet count.

Table 2 shows the haematological response rates in the published studies; however, in the various studies, different response criteria (i.e., platelet counts) were used. Moreover, in the individual studies, patients with different myeloproliferative disorders were treated. Nevertheless, ~ 80% of the treated patients responded to the therapy with anagrelide, the remainder were refractory, which indicates an individual metabolism of anagrelide and/or different subtypes of PT.

With regard to the haemostaseological response, several studies indicate that a decrease of the platelet count is accompanied by a protection against thromboembolic complications. The US study shows a decrease of thromboembolic complications with anagrelide therapy [60], as well as the German and Norwegian data [46,54]. On the other hand, there is also evidence that a reduction of the platelet count does not fully protect against thromboembolic complications [59,60]. This may be due to the fact that the extent of the reduction of the platelet counts is critical (600,000 or 400,000/µl) and that also at normalised platelet counts, the aberrant functionally disturbed platelet clone is still present. Reports from Israel have shown that already at slightly elevated platelet counts, thromboembolic complications can occur [61]. Moreover, it is

Table 3. Clinical adverse effects in the Non-US studies.

Study	Ref.	Patient number	Adverse effects
Italy I	[14]	8	Headache (5), palpitations (4), nausea (2), diarrhoea (3)
Italy II	[45]	20	8/20: tachycardia, GI tract, oedema
Germany I	[46]	48	12/48: short duration, 8/48: long duration
Germany II	[50]	12	3/12: headache, palpitations, tachycardias, oedema
Switzerland	[51]	6	4/6: headache, palpitations, diarrhoea
Australia	[52]	16	Headache, palpitations, abdominal pain, cough
Argentina	[53]	17	Some (no precise information)
Norway	[54]	10	Diarrhoea and palpitations

GI: Gastrointestinal.

Table 4. Most frequent adverse effects in patients treated at the Mayo Clinic [44].

Adverse effect	Percentage
Headache	27
Diarrhoea	20
Oedema	16
Palpitations	14

possible that simultaneously non-thrombocythaemic thrombophilic factors do exist (activated protein C-resistance, prothrombin gene-polymorphism) and contribute to the development of thromboembolic complications [62].

4.2 Primary and secondary resistance

Approximately 15% of all patients are primary refractory to the treatment with anagrelide (i.e., they do not respond to a continuous increase of the dosage with a platelet decrease). This indicates that the development of thrombocythaemias is caused by different molecular mechanisms [63] or the metabolism of anagrelide is individually different. A secondary resistance has not been observed yet.

5. Adverse effects

5.1 Non-mutagenic clinical adverse effects

All clinical studies addressed the question of safety of the drug. Side effects were mainly due to the positive inotropic and vasodilatatory effects of the substance. Shorter side effects disappeared normally with 4 weeks, long-term side effects persisted, and included headache, palpitations, tachycardia, dizziness or oedema (**Table 3** and 4).

The ingestion of the lactose containing drug can in lactase deficient individuals lead to diarrhoea, abdominal pain, dyspepsia and flatulescence.

In a few patients skin reactions, pulmonary hypersensitivity (with biopsy proven fibrosis, see also below) or elevation of hepatocellular enzymes have been described [43].

Clinical observations of more than one decade have shown that the rate of adverse effects (such as palpitations or headache) decreases with increasing experience of the treating physicians with the drug. Thus, the initiation of therapy with a lower starting dosage (1 mg/day) is associated with a better clinical tolerability.

Apart from the adverse effects which have been documented within clinical studies, there are few case reports regarding side effects. Ruiz *et al.* [64] reported a skin reaction, although the patient had been pretreated with hydroxyurea. Wirth *et al.* [65] observed the disappearance of an ulceration in a patient with PT upon treatment with anagrelide. Moreover, in other case reports, a cardiomyopathy in a patient with PV [66], erectile dysfunction [67], a hypersensitivity pneumonia or pneumonitis [68] and hallucinations [69] have been causally linked to the ingestion of anagrelide. Normally, the question of anagrelide-induced side effects can only be proven by discontinuation of the drug and if feasible, by reexposition.

5.2 Cardiac toxicity

Whereas tachycardias and signs of cardiac dysfunction (congestive heart failure, AV-block or atrial fibrillation) have been observed in 942 patients with and without cardiac diseases no increased cardiovascular morbidity and mortality has been observed [58,70,71]. However, in individual situations it is difficult to decide whether congestive heart failure or a MI is due to anagrelide or not. MI under treatment with anagrelide could be due to the inotropic activity of the drug in sensitive individuals; alternatively, there is increasing evidence that even the normalisation of platelet counts is not necessarily associated with an elimination of the aberrant clone. Laguna *et al.* [53] observed that in one patient despite platelet count normalisation by anagrelide the spontaneous aggregation activity persisted.

A cardiac examination and the careful follow-up of the patient is therefore necessary in all patients with suspected heart problems or an age of > 60 years [46]. One should also measure in risk patients in whom anagrelide therapy is taken up the concentration of BNP (b type natriuretic peptide) which is a good indicator of a left ventricular dysfunction. If

there is any evidence for an anagrelide-induced cardiac dysfunction, the drug has to be immediately discontinued. In patients with known cardiovascular risk factors anagrelide should not be given.

5.3 Mutagenic side effects

None of the studies published thus far shows, in individuals who have been exclusively been treated with anagrelide, an increased rate of leukaemia or solid tumours. Moreover, no increased myelofibrotic transformation has been documented. Pregnant women should not take the drug as anagrelide can cross the placenta.

6. Long-term observations

As thrombocythaemias require, especially in young individuals, long-term treatment, it is mandatory to accumulate long-term experience with the drug.

Storen and Tefferi [73], in the US, have reported on 35 young female patients (17 – 48 years of age) who were given dosages of anagrelide from 1 to 10 mg/day. The mean maintenance dosage was 2.5 mg/day. Of the patients, 94% responded to the drug (74% CR, 20% PR). Of the responders, 27 (82%) were administered anagrelide for an average of 10.8 years (7 – 15.5 years). Of these, 66 and 34% were CRs and PRs, respectively, with 8 (24%) patients developing a drop in haemoglobin level of > 3 g/dl as a long-term side effect. The cause of the anaemia remained unknown. Of the patients, 20% suffered both thromboembolic, and as many haemorrhagic complications. All complications occurred at platelet levels of > 400,000/µl. This indicates that a complete normalisation of the platelet count is required for the maximal prevention of complications.

Petrides and Beykirch [47] in Germany, reported in 1999 that data of 22 of 48 study patients could be obtained, of whom 17 are still under therapy with anagrelide. One female patient had used anagrelide for > 8. 5years, the others between 23 and 67 months (mean: 33 months). None of these patients reported adverse effects. Reasons for the discontinuation of therapy were arrhythmias (after 34 months), bleeding tendency, pulmonary embolism (after 19 months) or MI (after 11 months of therapy) inspite of normalised platelet counts. None of the patients developed leukaemia. Siegel and Petrides [48] continued this analysis over 5 additional years; the patient who had been treated for the longest period of time has undergone anagrelide therapy for almost 14 years.

Kornblihtt *et al.* [74] in Argentina, reported on the long-term experiences in 54 patients (median: 34 months; range: 2 – 100). The age at diagnosis was 39 years (11 – 83 years). Complete remission was observed in 96.3% of patients (77 and 18.5% with platelet counts of < 400,000 and < 600,000/µl, respectively). The maintenance dosage was 1.5 mg/day, in 40% of the patients, mild-to-moderate anaemia was seen. A total of eight patients had microvascular complications with platelet levels of > 400,000/µl and seven had normal values. One patient developed myelofibrosis and five patients died of reasons not associated with PT.

Birgegard *et al.* [75] of the Swedish Myeloproliferative Disease (MPD) group reported on 60 patients (43 PT, 17 PV, 1 IMF; female:male ratio 35:25; median age: 25 – 75 years; period of observation 2 years): indication for therapy were either platelet counts of > 600,000/µl and the presence of symptoms, or value > 1×10^6/µl. A total of 21 patients had been pretreated with hydroxyurea, 1 with hydroxyurea and busulphan, 4 with IFN-α, 1 with hydroxyurea in combination with IFN-α; 33 patients had not been pretreated. The aim of the therapy were platelet counts of < 400,000/µl in symptomatic and < 600,000/µl in asymptomatic patients. Of the patients enrolled, 40 (67%) had a CR (PT 76, PV 41%), 4 a PR and 16 were refractory. In addition, 14 other patients discontinued therapy (10 because of side effects despite very good efficacy). By using a questionnaire (10-point scale) patients and treating physicians were asked how content they were with the drug. The points were in patients as well as physicians between 7.6 after 3 months and 9 points after 24 months.

Rosenbaum *et al.* [76] from Israel reported on 60 patients (42 PT, 6 PV, 5 IMF, 5 non-classifiable, 1 myelodysplastic syndrome (MDS), 1 CML; age 58 years [20 – 93]) over a median time of observation of 88 weeks (5 – 272 weeks). Of the patients, 76% were pretreated, with 38% of the patients reporting side effects; in 12 patients, the therapy was discontinued (6 because of cardiac arrhythmias). Neither thromboembolic complications nor leukaemias were observed.

Fruchtman *et al.* [77] from the US, presented an analysis of data of the US approval study: 3660 patients (PT 2251 = 61.5%, non-PT 1409 = 32.3%) of whom, 81 had been pretreated and 45% with symptoms were analysed. Of this patient group (1618, of which, 934 PT), the efficacy could be evaluated: 79% of the PT patients responded (CR and PR). In 47 of the 2251 PT patients, a transformation occurred (2.1%), nearly all had been pretreated; the median time from diagnosis of PT until the manifestation of a MDS or acute leukaemia was 3.6 years (median duration of prior therapy 2.9 years; median duration of anagrelide therapy 0.6 years). The maximum follow-up was 7.1 years. Of the PV patients, 2.8% developed a transformation (median time from the diagnosis to the transformation 12.9 years; median duration of pretreatment 10.7 years, maximum duration of anagrelide therapy 3.9 years). The maximum follow-up in the PV patients was 7 years. From these results the authors concluded that long-term treatment with anagrelide is not associated with an elevated risk of leukaemia.

7. Practical recommendations

7.1 Dosage recommendations

Generally, therapy should be initiated with a dosage of 0.5 mg b.i.d. and increased every 7 days by 0.5 mg if the platelet count has not sufficiently dropped. Experiences over a period of > 10 years have shown that with this lower initial dosage, a

better adaptation of the organism to the inotropic and vasodilatatory effects and therefore, resulting in better tolerability. The aim of therapy is the constant adjustment of the platelet counts to values between 150,000 and 450,000/µl with the lowest possible anagrelide dose.

In some individuals, a better efficacy and/or tolerability can also be obtained with three times daily dosing. In the author's experience, a long-term dosage of > 5 mg is not beneficial as the adverse effects (but not necessarily the efficacy) increase under therapy with anagrelide with higher dosage. If patients with coronary heart diseases are treated with anagrelide (if no other drugs can be used) an even more careful dosing regime is required (initiate with 0.5 mg/day).

McCune has reported on a rapid drop of platelets under the therapy with anagrelide [78,79]; however, this has been questioned by Petitt [80]. If, for example, hydroxyurea has to be switched to anagrelide the pretreatment has to be continued until the platelets show a falling tendency. Only then one should start with a stepwise reduction of the drug used prior to treatment with anagrelide. In patients who develop GI side effects which are due to lactose intolerance lactase (Laluk®) can be tried.

7.2 Combination with other platelet influencing drugs

Most patients with thrombocythaemias obtain acetylic salicylic acid (ASA) as primary therapy. The authors recommended the discontinuation of ASA therapy when patients have reached normal platelet counts upon anagrelide therapy [46]. Steurer *et al.* [56] observed more bleeding complications in patients who had taken ASA in combination with anagrelide (13 versus 2% in those taking anagrelide alone). However, for some patients, the continuation of ASA therapy may be beneficial, in case their aberrant hyperaggregating clone is not eliminated by a lowering or normalisation of the platelet count.

The reason as to why the combination of anagrelide and ASA may cause an increasing bleeding tendency is not known yet. ASA itself is associated with an increased rate of bleeding in patients with PT compared to the risk of bleeding in other patients treated with aspirin [81]. At concentrations of 6 – 8 mg (i.e., above the daily dosage used in most patients), an effect on the aggregation of platelets can be observed [12]. At lower daily doses, synergistic effects with other platelet modulating drugs such as ASA, may occur and explain the bleeding tendency observed in some individuals.

Therefore, if one recommends the concomitant use of anagrelide and aspirin, one has to instruct the patient and carefully look for early signs and symptoms of bleeding.

The same is probably true for the use of clopidrogel. In addition, also heparin (e.g., perioperatively) should be carefully used [FDA, data on file].

7.3 Interaction with other platelet-reducing drugs

Tsimberidou *et al.* [82] investigated the practicability of a combination therapy of imatinib mesylate (Glivec®) and anagrelide in patients with Philadelphia-positive CML (18), IMF (1), PT (2) and refractory anaemia with excess blasts (originating from cMPD) and persisting thrombocythaemia. The combination of both drugs was well-tolerated and led to a haematological response in 89% of the patients.

Voskaridou *et al.* [83] treated one patient with thalassaemia major and CML with hydroxyurea and anagrelide. The combination therapy was well-tolerated and led to a normalisation of granulocyte and platelet counts.

Yoon [84] reported on the successful treatment of one patient with a PT with a combination with IFN-α and anagrelide. Some haematologists also combine anagrelide with hydroxyurea.

7.4 Treatment of children and elderly patients with anagrelide

PT occurs rarely in children [85]. Several publications underline the successful use of this drug in children. Chintagumpala *et al.* [86,87] report on an 11-year-old with PT, Hermann *et al.* [88] on a 8.5-year-old girl and Lackner *et al.* [89] on three children. Scherer *et al.* [90] described the treatment of a 5-year-old with symptomatic PT; they observed the complete normalisation of the platelet counts, but at the same time, a relatively early development of anaemia. With regard to treatment with anagrelide, there is no limitation of age. Patients > 90 years of age have been treated [44].

8. Comparison of anagrelide with hydroxyurea and IFN-α

Anagrelide and hydroxyurea possess different mechanisms of action and profile tolerability [91]; whereas hydroxyurea acts directly on a haematopoietic stem cell and therefore inhibits not only the production of platelets, but also of granulocytes and erythrocytes, anagrelide acts primarily on megakaryocytes. Therefore, upon treatment with hydroxyurea also granulocytopenias and anaemias are observed. However, upon long-term treatment with anagrelide, anaemias are also reported in some patients, the mechanism of development is still unknown.

The mechanism of action of hydroxyurea is possibly important for the development of thromboses as interactions of platelet with granulocytes seem to be involved in the development of thrombosis [92]. The capability of hydroxyurea in lowering the rate of thromboembolic complications in patients with myeloproliferative disorders has been documented [93,94]. However, the interpretation of the results of this study was intensively discussed [95-97].

Hydroxyurea causes a number of different side effects, such as GI complications, skin alterations [98], pneumonitis [99] or skin tumours [100]. During therapy with hydroxyurea, fluctuations of the platelet counts are also observed [101]. There is controversy with regard to its possible or potential leukaemogenic action: whereas some authors dispute a leukaemogenic activity, others recommend a prudent use,

especially in younger patients [102-111]. With a high likelihood the sequential use of busulphan and hydroxyurea increases the risk of leukaemia; IFN-α (IntronA®, Roferon®) is effective in the treatment of myeloproliferative disorders [112]. However, because of its side effects it is not widely used, although the tolerability of PEG-IFN_{2b} seems to be better according to a pilot study [113].

9. Costs of therapy with anagrelide

In Europe, anagrelide is approved in Switzerland, Austria and six other countries of the EU. In Germany, the import price for a package with 100 Agrylin® 0.5 mg capsules and Thromboreductin® is €827 and 623, respectively. At a dose of 2 mg/day, the drug costs per month amount to €1000 or 750, respectively. On the other hand, the monthly costs for IFN-α 3 mU/day and hydroxyurea 2000 mg/day are €1350 and 240, respectively. For cost-effectiveness considerations, the reader is referred to Golub *et al.* [114] and Bennett *et al.* [115].

10. Conclusion

A number of clinical studies performed in various countries [14,33,43-46,50-54], as well as reports and reviews [116-131], indicate that anagrelide is a major contribution to the treatment of thrombocythaemias. It is efficacious, as in the majority of patients platelet counts are efficiently lowered. When incremental increases in dosages are used, it is well-tolerated and upon long-term use, no increased rates of leukaemias or solid tumours have been observed. Long-term observations show that the use of anagrelide also reduces clinical symptoms and thromboembolic complications, but can cause a drop of the haemoglobin level. The drug has to be used carefully in individuals with cardiac risk factors.

11. Expert opinion

In an accompanying editorial to the article by Silverstein *et al.* [33] in 1988, John W Adamson discussed the 'ideal drug for the treatment of PT: one selective for platelets and free of the potential to enhance malignant transformation' and asked the question as to whether 'anagrelide does really represent an advance'. He concluded that 'time will tell' [132]. What has time told us over the 16 years which have elapsed since Dr Adamson made this statement?

The original observations by Silverstein have been expanded by his own group and various centres worldwide, although none of these studies were GCP controlled or randomised and the size of the non-US studies was small (although detailed information was provided about individual patients), all studies have unanimously confirmed that anagrelide could be an alternative to hydroxyurea as it efficiently lowers platelets and prevents complications, is well-tolerated by most individuals and free of the potential to enhance malignant transformation. This has led to the widespread use of the drug also in countries where the drug is not yet registered (e.g., Germany). The only long-term analysis published thus far has shown that in some individuals, the drug may not be selective for platelets as its use can be associated with a drop of the haemoglobin level.

Too little progress has been made, however, with regard to its metabolism, the structural and functional characterisation of its metabolites and their molecular mechanism of action. A better understanding could have practical consequences as there may exist a metabolite with a more specific target and/or better tolerability profile. Moreover, the species-specific action of the drug on human megakaryocytes is remarkable. This has excluded the use of animal models, but should force us to perform more translational research on patients who take the drug [24,28].

From preclinical studies, it was known that anagrelide is less active on human, than on animal platelets. This activity may become, however, more pronounced when anagrelide is combined with aspirin, which by itself is more active in PT patients than in the general population. Again, more bedside research is necessary which may be facilitated by modern approaches such as the analysis of the platelet proteome [2].

To answer Dr Adamson´s question of whether anagrelide does really represent an advance, comparative trials with standard therapy (hydroxyurea) are required. As the anagrelide-containing high-risk arm of the PT1 trial has been prematurely closed, it is likely that the crucial data to answer this question will become available from the multinational ANAHYDRET trial. Results should facilitate the registration of the drug in the remainder of the countries in Europe where the drug is not approved yet.

PT is a haematological disorder, which is not only rare, but also heterogeneous [63]. This makes research on the disease and treatment of our patients difficult. Nevertheless, progress has been made since an additional drug has been shown useful for the individualised therapy of patients with this disorder.

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Affiliation

Petro E Petrides MD, PhD
†Author for correspondence
Adjunct Professor of Medicine (University of Munich Medical School),
Hematology Oncology Center,
Zweibrückenstr. 2, 80331 Munich, Germany
Tel: +49 89 229009;
Fax: +49 89 229448;
E-mail: Petrides@onkologiemuenchen.de