Australian Government
Department of Health and Ageing
Therapeutic Goods Administration

Australian Public Assessment Report for Anagrelide hydrochloride

Proprietary Product Name: Thromboreductin

Sponsor: Orphan Australia Pty Ltd

May 2012

TGA Health Safety Regulation

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- An Australian Public Assessment Record (AusPAR) provides information about the evaluation of a prescription medicine and the considerations that led the TGA to approve or not approve a prescription medicine submission.
- AusPARs are prepared and published by the TGA.
- An AusPAR is prepared for submissions that relate to new chemical entities, generic medicines, major variations, and extensions of indications.
- An AusPAR is a static document, in that it will provide information that relates to a submission at a particular point in time.
- A new AusPAR will be developed to reflect changes to indications and/or major variations to a prescription medicine subject to evaluation by the TGA.

Contents

I. Introduction to Product Submission

Submission Details

Type of Submission	Orphan drug
Decision:	Rejected
Date of Decision:	12 February 2011
Active ingredient(s):	Anagrelide hydrochloride
Product Name(s):	Thromboreductin
Sponsor's Name and Address:	Orphan Australia Pty Ltd, 300 Frankston-Dandenong Road Dandenong VIC 3175
Dose form(s):	Hard gelatin capsules
Strength(s):	0.5 mg and 1.0 mg[1]
Container(s):	Plastic (HDPE[2]) bottle
Pack size(s):	100 capsules
Approved Therapeutic use:	Not applicable
Route(s) of administration:	Oral
Dosage:	Starting dose is 0.5-1 mg/day.
ARTG Number (s)	Not applicable

Published references cited in this AusPAR are listed at the end of this document under *References*.

Product Background

This AusPAR describes the application by Orphan Australia Pty Ltd to register Thromboreductin (anagrelide) for the treatment of essential thrombocythaemia (ET) in patients with high or intermediate risk of thrombosis or bleeding.

ET is an acquired myeloproliferative disorder characterised by an elevation of the platelet count above the normal range which in most laboratories is considered to be between 150.000 and 450.000/µl (Briere 2007, Petrides *et al*. 2003, Beykirch *et al*. 1997). The median age at diagnosis is 65 to 70 years, but the disease can occur at any age. The female to male ratio is about 2:1 (Briere 2007). ET is a very rare disease with an annual incidence

[1] The application for this strength was withdrawn by the sponsor prior to the Advisory Committee on Prescription Medicines (ACPM) meeting.

[2] High-density polyethylene

of about 2.5 cases/100.000/year. In general, increasing platelet counts, increasing age and additional risk factors such as hypercholesterinaemia and/or diabetes mellitus which lead to vascular alterations are associated with an increased risk for thromboembolic complications.

In principle the aim of a ET treatment should be (a) curing the underlying disease, or (b) a long-term decrease of the platelet number to normal or near to normal values to reduce clinical complications (for example, <450.000 – 600.000/µl). The underlying cause of ET is currently unknown and it can therefore not be cured with current treatment modalities. The therapeutic approach is therefore to find drugs to decrease elevated platelet counts and in doing so reduce the incidence or prevent clinical complications associated with elevated platelet counts.

Currently, one of the agents available for treatment of ET is hydroxyurea (HU). It is considered by European Authorities (European Medicines Agency (EMA)) as a standard therapy for the treatment of ET. In Australia the current standard for ET therapy is off-label use of HU. Cytoreductive therapy with HU leads to a statistically significant reduction of thrombotic events by lowering the platelet counts as shown in an open non-comparative prospective trial in patients with ET (Cortelazzo *et al*. 1995). HU is an oral drug which affects non-specifically all cell lines of the haematopoietic system. Therefore, it causes side effects like granulocytopenia and anaemia. In addition serious skin and gastrointestinal adverse effects (oral ulcers, leg ulcers, diarrhoea) are observed. The main concern associated with HU however is its long-term (>5 years) adverse event (AE) profile. Several studies have suggested that treatment with HU only is associated with an increased leukaemic conversion rate: figures range from 5 to 10% (Nand *et al*. 1996; Weinfeld *et al*. 1994).

An alternative therapy for ET is interferon-α. However, like HU, interferon-α treatment exhibits undesirable side effects. Alkylating agents such as busulfan have also been used but long term use with this drug is associated with severe leucopoenia and anaemia.

Anagrelide has several features which makes it a therapeutic option for treatment of ET. Anagrelide

- lowers platelet counts,
- reduces ET related clinical events
- and has not given evidence for long-term leukaemogenicity or induction of other neoplasias.

The current formulation of Thromboreductin has been licensed in several countries in the EU since 2001.

Currently Agrylin (currently marketed by Shire Australia Pty Ltd as 0.5 mg capsules) is the only anagrelide approved for marketing in Australia. Agrylin was initially sponsored in Australia by Orphan Australia but in 2008 sponsorship of the product was transferred to Shire Australia (Shire Pharmaceuticals markets the product internationally). Orphan Australia is now seeking registration of a different anagrelide product, Thromboreductin. Thromboreductin is not bioequivalent to the marketed Agrylin product and the sponsor has therefore provided new clinical data to demonstrate its efficacy and safety to support the registration of Thromboreductin as a new chemical entity in Australia.

A comparison of the new product with the Agrylin product is shown below (Table 1). The proposed product has a more restricted indication and a more conservative recommended dosage regimen.

Table 1: Thromboreductin-Agrylin comparison

	Thromboreductin	Agrylin
Presentations	0.5 and 1.0 mg	0.5 mg
Indication	Treatment of essential thrombocythaemia in patients with high or intermediate risk of thrombosis or haemorrhage.	Treatment of essential thrombocythaemia.
Starting dose	0.5 to 1.0 mg per day	2.0 mg per day
Maximum dose	5.0 mg per day	10.0 mg per day

Regulatory Status

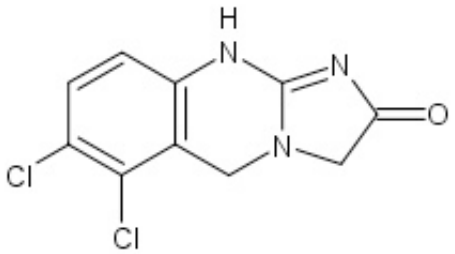
Thromboreductin was first registered in Austria in 2001 and has since gained marketing authorisations in many countries across Asia and Europe, including Switzerland.

II. Quality Findings

Drug Substance (active ingredient)

Anagrelide is achiral and synthetic. The capsules are formulated with anagrelide hydrochloride monohydrate.

Figure 1: Chemical structure



anagrelide

hydrochloride. monhydrate $C_{10}H_7N_3OCl_2.HCl.H_2O$ FW 310.57

There are no pharmacopoeial monographs. The proposed limits for particle size, controlled by microscopy, are not considered to provide good control of the particle size distribution. The proposed limits could pass a very fine drug batch which may provide capsules giving rapid drug release and, potentially, high maximum plasma concentrations in patients. It is not clear whether polymorphic variability could affect dissolution and bioavailability.

Drug Product

The proposed Thromboreductin 0.5 product is a hard gelatin capsule. Capsules have no printed markings. High-density polyethylene (HDPE) bottle packs of 100 capsules are proposed.

The capsule fills are made with conventional excipients (lactose, povidone, crospovidone, microcrystalline cellulose, magnesium stearate).

The formulation of the currently registered Agrylin capsules in Australia is very similar and it is not clear to the quality evaluator why the bioavailability differs between the two products.

The sponsor's Clinical Overview asserts that:

> Emphasis was put on the development of a formulation, **which would moderately delay the release of Anagrelide in order to favour drug tolerability**. In fact, the number of AEs* occurring briefly after ingestion seems to correlate with peak plasma levels (Petrides 2007).
>
> *=adverse events

However quality data, which details pharmaceutical development, did not describe either formulations with different release profiles, nor variation of parameters which are liable to affect bioavailability (such as anagrelide particle size or perhaps polymorphism).

Capsule dissolution is routinely tested and samples are analysed. The chosen medium has a high surfactant concentration which gives rapid dissolution. Staged limits are proposed, allowing wide individual capsule dissolution performance.

The discriminatory ability of the dissolution test method has not been established, and available data indicate that both Thromboreductin and Agrylin capsule batches would be likely to pass the proposed test. Agrylin capsules dissolve faster than the proposed Thromboreductin capsules.

Following TGA comments, the sponsor suggested setting a dissolution limit that might distinguish between Thromboreductin and Agrylin. This requires further work. The chosen dissolution test was not considered ideal (for example, it is susceptible to timing errors).

Given the claimed clinical advantage of delayed release from Thromboreductin, both the manufacturing method and batch release testing should ensure that this is maintained. Submitted controls do not appear to achieve this.

Only limited stability data were submitted and showed some samples failing the proposed specifications within the proposed shelf life. The proposed shelf lives are not considered to be supported.

Bioavailability

Study AOP-03-01

The current Orphan Australia application to register Thromboreductin capsules is supported by a bioequivalence study done in the USA in 2003 (Study AOP-03-01). This was a fasting crossover comparison of 4 x 0.5 mg Thromboreductin and Agrylin capsules followed by a crossover comparison of 2 x 1 mg Thromboreductin and Agrylin capsules in the same volunteers (22 subjects completed the study). Thromboreductin 0.5 and 1 mg capsules are apparently bioequivalent at the same dose (statistics not shown here). However, the study shows that Thromboreductin and Agrylin are not bioequivalent.

Table 2: Anagrelide pharmacokinetic data.

Anagrelide	Mean (SD)	T_{max} (h)	C_{max}	AUC_t	AUC_∞
0.5 mg	Thromboreductin	1.54 (0.62)	7.78 (3.7)	21.41 (9.4)	222.57 (9.91)
(4 x 0.5 mg)	Agrylin	0.96 (0.33)	11.7 (7.6)	27.5 (16.7)	28.99 (17.1)
	96% CI *		59–82%	73–92%	76–97%
1 mg	Thromboreductin	1.52 (0.79)	7.37 (3.9)	21.89 (11.6)	22.98 (12.0)
(2 x 0.5 mg)	Agrylin	1.59 (0.83)	8.6 (3.8)	25.16 (12.11)	26.58 (12.0)
	96% CI *		70–99%	74–97%	75–99%

* 96% confidence intervals were used because of an add-on study design. SD=standard deviation.

Thus, the time to maximum plasma concentration (T_{max}) is longer and the maximum plasma concentration (C_{max}) is notably lower following 0.5 mg Thromboreductin doses (C_{max} point estimate 0.69 [0.5 mg] or 0.83 [1 mg]); exposure is also lower (point estimate 0.86 [both]). Neither 0.5 nor 1 mg[3] Thromboreductin capsules are bioequivalent with the corresponding Agrylin capsules. Metabolite data are also outside bioequivalence limits.

Other studies submitted with the current application compared platelet counts with Agrylin and Thromboreductin dosing (see *Clinical Findings* below).

Literature

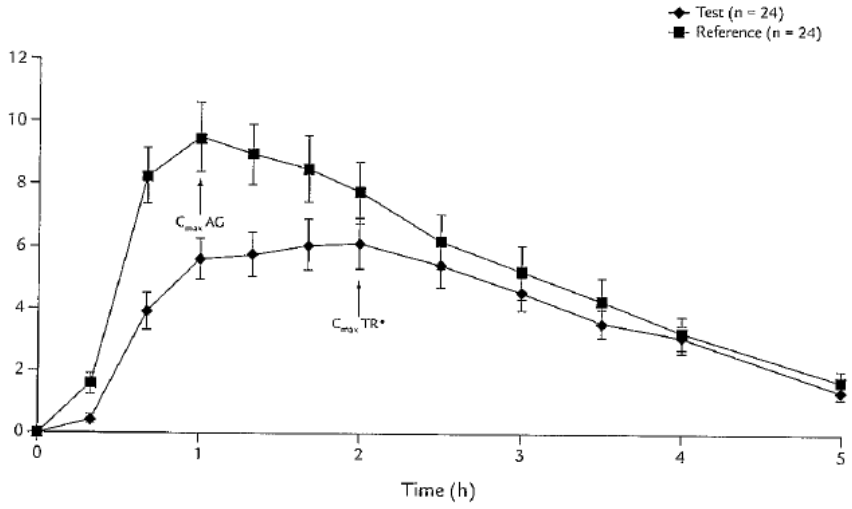
Not included in the current application is the recently published paper by Petrides *et al* (sponsored by AOP Orphan Pharmaceuticals AG)[4]. This paper summarises multiple studies, including a pilot, single dose pharmacokinetic study (Study SC03302) and *in vitro* and *in vivo* bioequivalence comparisons of Agrylin and Thromboreductin. Also reported are clinical switching studies.

The bioequivalence study which was included compared fasting 4 x 0.5 mg doses of Thromboreductin and Agrylin capsules in 24 healthy volunteers. Full pharmacokinetic details were not included but are sufficient to show that this is not the same as Study AOP-03-01 (see above). The paper reports that Thromboreductin capsules gave later T_{max} values for anagrelide and 3-hydroxyanagrelide ("1 hour longer"). While pharmacokinetic results are not detailed in the paper, there is a plot of the mean profiles (error bars show standard error of the mean (SEM) and are not an estimate of variability [that is, not SD]):

[3] The application for the 1 mg strength was withdrawn by the sponsor prior to the Advisory Committee on Prescription Medicines (ACPM) meeting.

[4] Pharmacokinetics, bioequivalence, tolerability, and effects on platelet counts of two formulations of anagrelide in healthy volunteers and patients with thrombocythemia associated with chronic myeloproliferation.

Figure 2: Plot of the mean profiles. Plasma anagrelide concentration (ng/mL) versus Time (h)



Mean (SE) plasma anagrelide concentrations after administration of the test formulation (Trademark: Thromboreductin® [AOP Orphan Pharmaceuticals AG, Vienna, Austria]) or reference formulation (Trademark: Agrylin®/Xagrid® [Shire Specialty Pharmaceuticals, Wayne, Pennsylvania]) of anagrelide in healthy volunteers. *$P < 0.001$ versus reference.

[from Petrides 2009[5]. test = *Thromboreductin*]

Thromboreductin capsules gave a significantly lower C_{max} (point estimate 66%; 90% CI 58%-76%) and area under the plasma concentration time curve from time zero to infinity ($AUC_{0-\infty}$) (point estimate 77%; 90% CI 68%-86%). These results are perhaps consistent with the slower *in vitro* dissolution of Thromboreductin also reported in the paper. Thus the 0.5 mg Thromboreductin capsules are **not** bioequivalent with the corresponding Agrylin capsules. This is consistent with the conclusion from Study AOP-03-01 (see above).

Quality Summary and Conclusions

The Pharmaceutical Sub-committee (PSC) considered the application at its 135th (2010/6) meeting and recommended:

1. The PSC was unable to recommend approval for registration on pharmaceutic and biopharmaceutic grounds of the application by Orphan Australia Pty Ltd to register Thromboreductin hard capsules containing 0.5 mg and 1.0 mg of anagrelide (as hydrochloride) due to the large number and nature of the identified deficiencies in the data provided in support of this application. The sponsor withdrew the application for the 1 mg product prior to the Advisory Committee on Prescription Medicines' (ACPM's) meeting.
2. The PSC endorsed all the questions raised by the TGA in relation to pharmaceutic and biopharmaceutic issues. In particular, the PSC supported the quality evaluator's questions and concerns in relation to the proposed overage, impurity limits and stability of the products.

[5] Petrides PE. Gisslinger H. Steurer M. Linkesch W. Krumpl G. Schuller A. Widmann R. *Clinical Therapeutics*. 31(2): 386-98, 2009.

3. The PSC considered that the sponsor should be asked to provide batch analysis data on three recent consecutive commercial scale validation batches for the drug product.
4. The PSC agreed that the sponsor should justify the proposed dissolution test method. The PSC considered that the proposed test method do not instil confidence that the process would ensure consistent future performance.
5. The PSC noted that Thromboreductin capsules were not bioequivalent to the currently registered Agrylin capsules. The reasons for the difference were not clear.
6. The PSC agreed that as modified release properties were claimed for the proposed products, the application should meet the guidances relating to such products. These include the provision of a food-effect bioavailability study.
7. In the Product Information (PI) the "Description" section should be amended to include the solubility as a function of pH, pKa and log p values.
8. The PSC therefore concluded that this application should be rejected in view of the very significant deficiencies in the pharmaceutic data provided in support of the application.

Recommendation

Some difference in the drug substance or capsule formulation or manufacture of the proposed Thromboreductin capsules causes them to have clearly lower bioavailability than the registered Agrylin capsules. The difference has not been identified and thus cannot be controlled.

The manufacturing controls (given lack of information on the critical parameters) and dissolution testing are not considered adequate to ensure supply of Thromboreductin capsules with drug release properties matching those used in clinical trials.

Registration is not recommended on chemistry and biopharmaceutical grounds.

III. Nonclinical Findings

Introduction

The nonclinical submission for Thromboreductin consisted of literature articles on anagrelide and RL603 (an active metabolite), two summary expert reports from the FDA for Agrylin (non-bioequivalent anagrelide hydrochloride) and Good Laboratory practice (GLP) compliant toxicological studies conducted with Thromboreductin examining cardiovascular effects in dogs and *in vitro*, pharmacokinetics, repeat dose toxicity in rats, genotoxicity *in vitro* and *in vivo*, reproductive toxicity in rats and toxicity of an active metabolite 2-amino-5,6-dichlor-3,4-dihydroquinazolin aceturat.

By conventional standards the Australian submission was limited, particularly with respect to safety pharmacology (primarily cardiovascular system only assessed) and toxicology assessment (repeat-dose toxicity studies in a single rodent species ≤3 months, no fertility study or embryofetal study in a second non-rodent species, no carcinogenicity study). However, it is important to note that anagrelide hydrochloride (as Agrylin) has previously been evaluated by the TGA with respect to its pharmacological and toxicological profile and has been approved for the same indication in Australia for over a decade.

Pharmacology

Primary pharmacodynamics

No primary pharmacology studies were conducted with Thromboreductin. However, published literature articles supplied for anagrelide hydrochloride and RL603, FDA expert evaluations for Agrylin and previous evaluations of anagrelide hydrochloride (as Agrylin) have demonstrated:

- inhibition of megakaryocyte (MK) formation and maturation at therapeutic concentrations *in vitro* (≥5/10 ng/mL)
- inhibition of platelet aggregation *in vitro* (50% effective concentration (EC_{50}) ≤1 µg/mL from several species) and *ex vivo* (50% effective dose (ED_{50}) 2-3 mg/kg orally (PO) for rats and dogs, <4 mg/kg PO monkeys).
- antithrombotic activity in animal models of experimental thrombosis *in vivo* (rat ≥1 mg/kg IV, ≥5 mg/kg PO; rabbit ≥10 mg/kg PO; dog ≥0.2/0.5 mg/kg PO, ED_{50} = 0.08 mg/kg intra dermally (ID))
- potentiated bleeding time *in vivo* (≥10 mg/kg PO rats and guinea pigs)
- inhibition of cyclic AMP and cyclic GMP phosphodiesterase activity in human and rabbit platelets *in vitro*, with 50% inhibitory concentration (IC_{50}) values in the nanomolar range
- activation of cyclic adenosine-monophosphate (cAMP)-dependent protein kinases in human platelets *in vitro* (0.1-1 µg/mL)

Anagrelide hydrochloride is proposed for the treatment of essential thrombocythaemia (ET) in patients with high or intermediate risk of thrombosis or bleeding. The thrombocytopenic effects of anagrelide appear to be species-specific to humans, a finding that has limited the ability of animal models to adequately assess this target pharmacological effect *in vivo*. This may be explained by either an intrinsic difference in reactivity of platelets from different species or by a difference in the metabolism of anagrelide.

The metabolic pathways of anagrelide have been shown to be qualitatively similar between animals and humans. However, while the major metabolites present in human urine have been previously observed in rats, dogs and monkeys following PO administration of radioactive carbon belled [^{14}C]-anagrelide, the major human metabolite, RL603 has been reported at much higher levels in humans (24% dose) compared to those in animal species (1-3% dose). Similarly, in the current application, RL603 was barely detectable in rats following PO administration. Nonetheless, the quantitative differences (lower % dose) are insufficient to account for species differences in primary pharmacological effects given the higher doses employed in animal models.

However, it should be noted that in the current application, RL603 was shown to cause a dose-dependent decrease in platelet levels in mice (ED_{50} = 100 µg/day intraperitoneally (IP) after 5 days). Mouse bone marrow also displayed a 45% decrease in the total number of MK cells. This metabolite blocked MK migration (≥10 ng/mL) *in vitro*. At 100 ng/mL it selectively blocked MK maturation, reduced cell number and ploidy, with greater potency than anagrelide. These studies suggest that RL603 selectively inhibits MK maturation and migration, but unlike anagrelide it lowers platelet levels without influencing platelet aggregation. Interestingly, these findings are in contrast to previous observations for studies conducted with Agrylin which showed no effect of RL603 on megakaryocytopoiesis, while the 3-hydroxyanagrelide (BCH24426) was found to have a

comparable IC_{50} to anagrelide (13 ng/mL compared to 7 ng/mL) for growth and differentiation of CD34+ stem cells into MKs *in vitro*.

Overall, the mechanism by which anagrelide hydrochloride decreases platelet production in humans appears to be through postmitotic inhibition of MK maturation and ploidy. Anagrelide metabolites (RL603 and/or 3-hydroxyanagrelide) may also partly contribute to these thrombocytopenic effects.

Secondary pharmacodynamics and safety pharmacology

Safety pharmacology studies conducted with Thromboreductin were limited to a single GLP compliant telemetry study in dogs (examining cardiovascular, respiratory and limited central nervous system (CNS) effects) and an *in vitro* Human-Ether-à-go-go Related Gene (HERG) study.

At PO doses ≥5 mg/kg, anagrelide hydrochloride caused reductions in blood pressure, increased heart rate and altered electrocardiogram (ECG) parameters (P wave amplitude/duration, P-Q interval and Q-T interval) at the time of the maximum anagrelide exposure. These doses were associated with maximum plasma concentration (C_{max}) values ≥167 ng/mL (6-fold extrapolated clinical C_{max} of 26 ng/mL for a 5 mg single daily dose (2.5 x 10.3 ng/mL for 2 mg dose)). The No Observable Effect Level (NOEL) for these changes was established at 0.5 mg/kg, which was not associated with any detectable plasma anagrelide. Anagrelide hydrochloride was not associated with remarkable effects on HERG tail currents *in vitro* at concentrations up to 100 µM. No effects on respiratory parameters or locomotor activity were observed at PO doses up to 50 mg/kg/day (C_{max} value 388 ng/mL; 38-fold extrapolated clinical C_{max} of 26 ng/mL).

While limited, these study results are consistent with previous cardiovascular investigations of anagrelide hydrochloride demonstrating positive inotropic and vasodilator effects *in vitro* and/or *in vivo* (Ahmad, 1996[6]). Dose-related decreases in blood pressure and reflexogenic increases in heart rate have been observed in several animal models including rats (conscious normotensive at ≥10 mg/kg, PO and spontaneously hypertensive ≥1 mg/kg, PO), ferrets (anaesthetised ≥0.3 mg/kg, ID) and dogs (conscious at ≥1 mg/kg, PO and anaesthetised with/without cardiac depression ≥0.1 mg/kg, ID). ECG effects (decreased PR and QT interval) were also observed at doses ≥10 mg/kg, PO in dogs. Similarly, no effects on gross behaviour, motor co-ordination or the autonomic nervous system were observed at 10 mg/kg PO in dogs.

No studies on renal function were provided in the current submission. However, anagrelide has been previously reported to alter renal function, including decreased urinary volume and sodium and chloride excretion in rats at PO doses ≥10 mg/kg (Ahmad, 1996).

Anagrelide was shown to concentration-dependently reduce rabbit ileum (EC_{50} = 163 µg/mL) and antagonise serotonin-induced rat fundic strip (EC_{50} = 2.5 µg/mL) contractions *ex vivo*. Anagrelides direct inhibitory and antiserotonergic effects on smooth muscle contractions *ex vivo* occurred at concentrations well in excess of those anticipated clinically.

Pharmacokinetics

Pharmacokinetic studies of Thromboreductin were limited to single dose and repeated dose (toxicokinetic) absorption studies in rats and metabolism studies *in vitro*.

[6] Ahmad T. (1996). Expert Report. Food and Drug Administration

Previous PK studies of anagrelide hydrochloride (Agrylin) in rats, dogs, monkeys and humans have shown it is rapidly absorbed after oral administration, extensively distributed and metabolised. The profile of urinary metabolites in animals and humans was qualitatively similar with all human metabolites detected in all species and therefore validating their use as animal models. However, quantitative differences were noted, particularly with respect to the major active human metabolite, 2-amino-5,6-dichloro-3,4-dihydroquinazoline (RL603).

In the current Australian submission for Thromboreductin, single dose studies in rats and humans showed rapid oral absorption of anagrelide and exposure to its hydroxy metabolite BCH24426 with a similar rapid elimination profile. However, as expected, RL603 was barely detectable in rat plasma while significant exposure was detected in human plasma. Repeat dose (toxicokinetic) studies in rats demonstrated non proportional increases in anagrelide exposure with dose, increased exposure with repeated dosing and no apparent gender differences in exposure.

In the current submission, Thromboreductin did not significantly inhibit any of the six cytochrome P450 (CYP450) isozymes tested in human liver microsomes *in vitro*. However, it is important to note that the microsomal CYP activity of the batch of microsomes tested in that study was low for CYP isozymes 1A2, 2C19 and 2D6. Therefore, these results are not considered reliable. Previous *in vitro* studies conducted with Agrylin have shown that anagrelide is metabolised by and is also inhibitory to CYP1A2 at high (≥462 ng/mL) concentrations *in vitro* and although limited, given the supratherapeutic concentrations required for this effect *in vitro*, has the potential for interactions with other drugs metabolised by CYP1A2. In a previous submission, anagrelide binding to plasma proteins *in vitro* was shown to be similar in humans (91%) and rats (86%), suggesting no significant need for correction of plasma exposure values for free anagrelide.

It is also important to note that Thromboreductin is a moderately delayed release formulation compared to Agrylin anagrelide and is also not bioequivalent with respect to classical pharmacokinetic parameters (AUC and C_{max}). Thus, Thromboreductin and Agrylin must be considered as different products with respect to both efficacy and safety.

Toxicology

Toxicology studies conducted with Thromboreductin were limited to assessment of repeat dose toxicity in rats (≤3 months), genotoxicity *in vitro* and *in vivo*, embryofetal and peri- and post-natal development in rats and acute toxicity and genotoxicity of the active metabolite 2-amino-5,6-dichlor-3,4-dihydroquinazolin aceturat. All pivotal studies were conducted according to GLP, employed adequate animal numbers, appropriate doses/concentrations, with one exception and animal models. However, no acute toxicity, repeat dose toxicity studies in a non-rodent species or rodents over 3 months, carcinogenicity, fertility or embryofetal development studies in a second species were conducted.

Nonetheless, it is important to note that the toxicological profile of anagrelide hydrochloride has been previously characterised for Agrylin. While Thromboreductin and Agrylin are not bioequivalent in terms of conventional pharmacokinetic parameters, a comparable toxicity profile is anticipated for the same active substance with an unremarkable excipient formulation. Therefore recent GLP studies conducted with Thromboreductin have been provided to support these earlier findings. Therefore, the absence of these core toxicology studies may be acceptable on the basis of the well-characterised toxicity profile of Agrylin and recently performed supportive studies for Thromboreductin.

Single dose toxicity

No conventional acute toxicity studies have been conducted with Thromboreductin. However, it was noted that an IV bolus injection or slow IV infusion of 10 mg/kg anagrelide hydrochloride was lethal to male rats in a single dose pharmacokinetic study. Low acute toxicity of the anagrelide metabolite 2-amino-5,6-dichlor-3,4-dihydroquinazolin aceturat was demonstrated in mice and rats with nonlethal doses of 22 mg/kg and 46 mg/kg IV, respectively.

Studies conducted with Agrylin in several animal species indicate anagrelide has very low acute toxicity. Oral doses up to 2500 mg/kg in mice, 1500 mg/kg in rats, 800 mg/kg in dogs and 200 mg/kg in monkeys, and IP doses of 250 mg/kg in (male only) mice were nonlethal and associated with transiently decreased activity in rodents, and signs of gastrointestinal tolerance in monkeys and dogs. An IP dose of 500 mg/kg to male mice caused muscle tremors and 100% lethality.

Repeat dose toxicity

Repeat dose toxicity studies in rats given Thromboreductin for up to three months identified the gastrointestinal system, heart, liver, spleen, kidney and adrenal glands as potential target organs for toxicity. Gastrointestinal irritation/ulceration associated with mortality and myocardial fibrosis/degenerative changes were observed at high (≥600 mg/kg/day) PO doses. Adaptive changes were observed in the adrenal glands (hypertrophy), liver (hypertrophy), spleen (haemopoiesis) and kidneys (vacuolation) at doses ≥200 mg/kg/day. Additional findings of atrophy in the thymus, spleen and reproductive organs in the 3 month study at 600/1000 mg/kg/day PO are potentially due to chronic stress and starvation at the high dose level. The No Observable Adverse Effect Level (NOAEL) for both the one and three month study was determined as 50 mg/kg/day. Based on a conservative estimate of Day 1 only values for anagrelide exposure (area under the plasma concentration time curve from time zero to infinity ($AUC_{0-\infty}$) = 20715-25729 ng.h/mL) in rats at the NOAEL, a greater than a 300-fold clinical safety margin (the extrapolated clinical $AUC_{0-\infty}$ of 71 ng.h/mL for a 5 mg single daily dose (2.5 x 28.4 ng.h/mL for 2 mg dose assuming dose linearity) was demonstrated.

These findings are consistent with previous repeat dose toxicity studies of Agrylin conducted in rats and dogs which also identified the gastrointestinal system, heart, liver, kidney and adrenals as potential target organs of toxicity. In rats, clinical signs and disturbances in electrolyte excretion (decreased chloride) were seen at PO doses ≥50 mg/kg/day for 17 days, and an increased incidence of intestinal lesions was observed at 15 mg/kg/day in the 3-month dietary study, with a NOEL of 10 mg/kg/day. In the 12 month dietary study in rats, doses ≥120 mg/kg/day resulted in mortality. Most of the observed changes in this study were indicative of liver and kidney injury: increased weights of kidneys and liver, changes in serum chemistry, diuresis, protein excretion and histopathological changes (nephropathy, hydronephrosis, hyperplasia of bile duct, liver congestion and necrosis). A NOEL was not established, and most of the changes were not dose-related, potentially due to saturated absorption at 120-1200 mg/kg/day. Increased adrenal weights and adrenal hyperplasia were also observed at ≥360 mg/kg/day. In the 23 month dietary carcinogenicity study in rats, kidney, liver and cardiac lesions were observed as with previous studies, in addition to bone abnormalities, at doses from 3 mg/kg/day. In dogs, a NOEL of 250 mg/kg/day PO was established for a 4-week study, but was not established in the 1-year study with renal effects (diuresis, increased protein excretion, slight and transient clinical chemistry changes indicative of renal or hepatic injury) and increased incidence of cardiac inflammatory lesions observed at all doses (10, 300 and 600 mg/kg/day).

Thus, no novel toxicities were observed following daily PO administration of Thromboreductin to rats for up to 3 months, with an adequate (≥300-fold) clinical safety margin demonstrated for potential gastrointestinal, cardiovascular, renal, hepatic or adrenal effects.

Genotoxicity

Thromboreductin was tested for potential genotoxic effects in a standard GLP compliant battery of *in vitro* (bacterial reverse mutation and forward gene mutation) and *in vivo* (rat micronucleus) test systems. Anagrelide hydrochloride was not mutagenic in bacterial or mammalian cells *in vitro* or clastogenic in rats *in vivo*. With the exception of the forward mutation assay, where higher concentrations could have been employed, all studies were generally conducted at adequate concentrations/dose levels.

Anagrelide metabolite 2-amino-5,6-dichlor-3,4-dihydroquinazolin aceturat which was anticipated to be pharmacologically active, was tested for potential genotoxic effects in GLP compliant *in vitro* bacterial reverse mutation and forward gene mutation test systems. The metabolite was not mutagenic in bacterial or mammalian cells *in vitro* at adequate concentrations.

No genotoxic effects were also reported for Agrylin in a similar GLP compliant battery of *in vitro* (bacterial reverse mutation and forward gene mutation) and *in vivo* (mouse micronucleus) test systems.

Carcinogenicity

No carcinogenicity studies have been conducted with Thromboreductin. However, proliferative lesions in the adrenal medulla, including hyperplasia and benign phaeochromocytomas were also observed in rats given ≥360 mg/kg/day Agrylin in the diet for one year. These findings were also reproduced in a 23 month dietary rat carcinogenicity study with an increase in hyperplasia (both focal and diffuse) and in benign and malignant adrenal phaeochromocytomas observed at all doses in males (≥3 mg/kg/day) and at ≥10 mg/kg/day in females given Agrylin. No NOEL was seen for males, and a NOEL of 3 mg/kg/day was observed for females. Given there is currently no evidence to suggest that these findings are species-specific and an apparent class effect, the clinical relevance of these tumours could not be dismissed.

Uterine adenocarcinomas were also increased at 30 mg/kg/day for females in this study, with a NOEL of 10 mg/kg/day. An epigenetic mechanism was considered likely. It was suggested that anagrelide may induce CYP1 enzymes, which might increase 4-hydroxy-oestradiol concentrations, which would increase endometrial adenocarcinoma in the rat. However, no measurements of plasma 4-hydroxy-oestradiol have been made, so there is no experimental evidence to support this mechanism. Given that it is possible that this mechanism could also operate in humans, the clinical relevance of these tumours could not be discounted.

Reproductive toxicity

Reproductive studies conducted with Thromboreductin were limited to assessment of embryofetal development and peri- and post-natal development in rats. Both pivotal studies were conducted according to GLP, employed adequate animal numbers and appropriate doses, based on dose range-finding studies. No fertility or embryofetal development studies in a second nonclinical species were conducted.

Maternotoxicity was observed at PO Thromboreductin doses from 45-100 mg/kg/day when given to rats during organogenesis and/or during post-partum periods. Maternotoxicity manifested as clinical signs, weight loss/reduced body weight gain,

reduced food consumption and/or necropsy findings. Adverse fetal effects (including decreased weights, increased resorptions and increased post-implantation loss) and increases in visceral (liver additional lobe with median cleft and liver additional cleft) and skeletal (wavy ribs, pelvic girdle displacement, misshapen humerus, zygomatic arch fusion and non- or incomplete bone ossification) abnormalities were observed at maternotoxic doses from 60 mg/kg/day given during embryogenesis. A NOEL could not be determined for embryofetal effects. Adverse fetal (increased post-implantation loss) and post-natal effects (including post-natal pup loss, reduced number of live pups and/or pup weight at birth) were observed in rats and the progeny of rats (increased mating time, reduced food consumption, body weight gain, gravid uterine weight, implantation number and live foetus number) given maternotoxic PO doses from 45-60 mg/kg/day during organogenesis through post-partum Day 14. A NOEL of 5 mg/kg/day was determined for pre- and postnatal effects. Based on gestation Day 6-20 values, anagrelide exposure ($AUC_{0-\infty}$ = 1436-1992 ng.h/mL) in rats represented a 20-28-fold safety margin (the extrapolated clinical $AUC_{0-\infty}$ of 71 ng.h/mL for a 5 mg single daily dose) over the maximum recommended clinical dose at the NOEL.

The findings are generally consistent with previous reproductive toxicity studies of Agrylin conducted in rats. Agrylin at doses up to 240 mg/kg/day PO had no effect on fertility and reproductive performance of male rats. However, fertility and reproductive performance of females was impaired at all dose levels (≥60 mg/kg/day) given prior to mating through gestation. Decreased numbers of pregnancies, implantation sites, live fetuses, fetal body weights, number of live-born pups and post-partum pup survival, as well as prolonged gestation time, increased number of resorptions and number of stillborn pups was observed at all doses. At doses ≥120 mg/kg/day, viability index was decreased, and at 240 mg/kg/day, all pups were dead before post-partum Day 2. In the embryofetal development study in rats, anagrelide administered at PO doses ≥300 mg/kg/day during organogenesis slightly increased numbers of resorptions and decreased fetal body weights. Delays in ossification (mainly in hyoid, caudal vertebrae, sternebral centres and metacarpals) were also seen in fetuses from all dose groups (12%-18% compared to 2% in controls). However, no embryofetal effects were observed in rabbits administered anagrelide at doses up to 20 mg/kg/day during organogenesis. In a pre- and postnatal development study in rats, anagrelide doses ≥60 mg/kg/day given during gestation through weaning caused prolongation of gestation and delivery, parturition difficulties and maternal mortality. The number of dams delivering litters and pup body weights were reduced, while the number of stillborn pups and pup mortality were also increased.

In the absence of placental or milk transfer studies, it is unclear whether the peri- and post natal effects observed in rats are due to *in utero* and/or lactational exposure. Given the maternotoxic and fetotoxic effects observed and potential risks to the mother during parturition, Thromboreductin should not be given to pregnant or in lactating women.

Nonclinical Summary and Conclusions

- The maximum recommended human dose (MRHD) of Thromboreductin is 5 mg/day PO. It should be noted that the MRHD for Agrylin is 10 mg/day. Therefore calculations of clinical exposure margins for Thromboreductin are based on a lower anticipated maximum daily dose.
- The thrombocytopenic effects of anagrelide appear to be species-specific to humans, an effect which has limited the ability of animal models to assess this pharmacological effect *in vivo*.

- The mechanism by which anagrelide decreases platelet production in humans appears to be through postmitotic inhibition of megakaryocyte maturation and ploidy. The relative contribution of its major metabolites to these effects *in vivo* is unknown.
- A single *in vivo* safety pharmacology study of Thromboreductin in dogs showed that PO doses ≥5 mg/kg reduced blood pressure, increased heart rate and altered ECG parameters. This dose was 6-fold the MRHD (based on C_{max}). The NOEL for these effects was established at 0.5 mg/kg, which was not associated with any detectable plasma anagrelide. No remarkable effects on *in vitro* HERG tail currents were observed at ≤100 µM. This was consistent with positive inotropic, vasodilator and ECG effects observed in dogs given Agrylin at similar PO doses (1-10 mg/kg) and in other species *in vivo* and *in vitro*.
- Pharmacokinetic studies of Thromboreductin were limited. Single dose kinetic studies in rats and humans demonstrated rapid oral absorption and metabolism to metabolites RL603 and BCH24426. Previous pharmacokinetic studies of Agrylin in rats, dogs, monkeys and humans showed a qualitatively similar metabolite profile in humans and animals species examined, validating their use as nonclinical models.
- Thromboreductin did not significantly inhibit any of the six CYP450 isozymes tested in human liver microsomes *in vitro*. However, these results were not considered reliable. Agrylin was shown to be metabolised by and is also inhibitory to CYP1A2 *in vitro*. Therefore anagrelide has the potential for interactions with other drugs metabolised by CYP1A2.
- Toxicology studies conducted with Thromboreductin were limited. However, the toxicological profile of anagrelide has been well-characterised in previous submissions for Agrylin. While Thromboreductin and Agrylin are not bioequivalent, a qualitatively comparable toxicity profile is anticipated for the same active ingredient with a similar, unremarkable excipient formulation.
- No acute toxicity studies were conducted with Thromboreductin. However, low acute IV toxicity of the anagrelide metabolite 2-amino-5,6-dichlor-3,4-dihydroquinazolin aceturat was demonstrated in rodents, and studies conducted with Agrylin in several animal species indicate that anagrelide has a very low acute toxicity.
- Repeat dose toxicity studies in rats given oral Thromboreductin for up to three months identified the gastrointestinal system, heart, liver, spleen, kidney and adrenal glands as potential target organs for toxicity. The NOAEL in both a one and three month study was determined as 50 mg/kg/day PO, which represented a greater than 300-fold clinical safety margin over the MRHD (based on AUC).
- Thromboreductin was not mutagenic in bacterial or mammalian cells *in vitro* or clastogenic in rats *in vivo*. The anagrelide metabolite 2-amino-5,6-dichlor-3,4-dihydroquinazolin aceturat was also not mutagenic in bacterial or mammalian cells *in vitro*.
- No carcinogenicity studies were conducted with Thromboreductin. However, an increase in benign and malignant adrenal phaeochromocytomas was observed at all doses in males (≥3 mg/kg/day) and at ≥10 mg/kg/day in females given Agrylin in the diet for 23 months. No NOEL was seen for males, while a NOEL of 3 mg/kg/day was observed for females. Uterine adenocarcinomas were also increased at 30 mg/kg/day for females in this study, with a NOEL of 10 mg/kg/day. The clinical relevance of these tumours could not be dismissed.

- Limited reproductive toxicity studies of Thromboreductin in rats showed adverse fetal and post-natal effects at maternotoxic doses (≥45-60 mg/kg/day PO) when administered during organogenesis and/or through weaning. A NOEL could not be determined for embryofetal effects, while the NOEL for post-natal effects of 5 mg/kg/day represented at least a 20-fold safety margin over the MRHD (based on AUC). In female rats given ≥60 mg/kg/day PO Agrylin prior to mating through gestation, impaired fertility and reproductive performance were also observed. In addition, Agrylin doses ≥60 mg/kg/day PO given to female rats during gestation and through to weaning caused prolongation of gestation and delivery, parturition difficulties and maternal mortality. No embryofetal effects were observed in rabbits given up to 20 mg/kg/day PO Agrylin during organogenesis. Given both the maternotoxic and fetotoxic effects observed and potential risks to the mother during parturition, the use of Thromboreductin in pregnancy or during lactation should be avoided.

Conclusions and Recommendations

The nonclinical studies provided for Thromboreductin were limited. Safety pharmacology studies were primarily confined to the cardiovascular system and toxicology studies were limited to a single rodent species for a maximum daily dosing duration of 3 months, with no fertility study, no embryofetal study in a second non-rodent species or carcinogenicity study conducted.

However, the toxicological profile of anagrelide has been well-characterised for the currently approved anagrelide product Agrylin. While Thromboreductin and Agrylin are not bioequivalent in terms of classical pharmacokinetic parameters, a qualitatively comparable toxicity profile is anticipated for the same active ingredient with a similar, unremarkable excipient formulation. Therefore, the nonclinical submission for Thromboreductin supplemented existing knowledge of anagrelide hydrochloride.

Toxicity studies in rats given Thromboreductin for up to three months identified no novel toxicities. The gastrointestinal system, heart, liver, spleen, kidney and adrenal glands were identified as potential target organs for toxicity, with the NOAEL determined at 50 mg/kg/day, representing a 300-fold clinical safety margin over the MRHD (based on AUC). Pretreatment examination and/or regular monitoring of patients with known or suspected heart conditions, renal or hepatic impairment may be appropriate to mitigate any clinical safety concerns. Significant reproductive effects of Thromboreductin observed in animal species exposed *in utero* and/or through lactation suggest its use in pregnancy and during lactation should be avoided.

Given the fully defined profile of anagrelide hydrochloride in Agrylin, the rare occurrence and seriousness of the disease, prior clinical experience with anagrelide and absence of any novel target organ toxicities, no additional safety concerns are noted for Thromboreductin.

IV. Clinical Findings

Introduction

The clinical development plan consisted of the performance of clinical trials in volunteers as well as patients over a period of 6 years as follows:

- **Phase I**: Two studies in volunteers to study pharmacokinetics (n=16) (*Study AOP 06-007)* and metabolism (n=2) (*Study AOP 10-007*)

- **Phase II**: A total of 131 patients to study (a) short-term (4 weeks) platelet reduction (n=34) (*Studies AOP 01-007 and 04-007*) and (b) efficacy and safety over six months in an exploratory study design (n=97) (*Study AOP 02-007*)
- **Phase III**: A confirmatory study of Anagrelide versus Hydroxyurea (n=258)
- **Phase IV**: Patient registry with treatment duration of up to 5 years (n=722)

  (*Study AOP 05-007*).

Thus, despite ET being a rare disease, a total of 1111 patients were included in this development program to make a benefit/risk assessment of anagrelide hydrochloride in treating ET.

The sponsor stated that the clinical trials were performed in accordance with GCP.

Pharmacokinetics

Study AOP 06-007. Single dose in healthy volunteers

The pharmacokinetic Study AOP 06-007 in 16 volunteers aged between 18 and 50 years was designed to clarify the key PK parameters for anagrelide and the two main metabolites emerging in plasma, that is, 3-hydroxyanagrelide and 2-amino-5,6-dichloro-3,4- dihydroquinazoline, following a single oral dose of 2 mg.

Results

The data showed that peak plasma concentrations for anagrelide were reached within 1.38 hours. The peak was followed by a rapid decline with an elimination half-life of 1.38 hours. Peak plasma values for 3-Hydroxyanagrelide were slightly delayed emerging at 2.14 hours and the elimination half-life for this metabolite was 1.79 hours, slightly longer than anagrelide. Peak plasma levels of 2-amino-5,6-dichloro-3,4-dihydroquinazoline were reached after 2.22 hours. It had an elimination half-life of 6.35 h or 3 to 4 times longer than anagrelide and 3- hydroxyanagrelide (Table 3).

The data show that anagrelide and the two main metabolites are unlikely to accumulate in plasma. However, the data as assessed by the standard deviation show a high inter-subject variability of plasma levels for all three compounds.

Table 3: Descriptive statistics for computed pharmacokinetic parameters for Anagrelide, 3-OH-Anagrelide and 2- amino-5,6-dichloro-3,4-dihydrochinazoline after administration of Anagrelide (n=16)

Parameter	Anagrelide	3-OH Anagrelide	2-amino-5,6-dichloro-3,4-dihydrochinazoline
	Mean (± SD)	Mean (± SD)	Mean (± SD)
C_{max} (ng/mL)	10.28 (± 4.82)	5.03 (± 1.75)	4.82 (± 1.40)
$AUC_{0-\infty}$ (ng/mL.h)	28.39 (± 15.52)	18.40 (± 7.81)	31.21 (± 8.55)
t_{max} (h)	1.38 (± 0.40)	2.14 (± 1.04)	2.22 (± 0.91)
$t_{½}$ (h)	1.38 (± 0.54)	1.79 (± 0.52)	6.35 (± 1.53)
K_{el} (h^{-1})	0.550 (± 0.144)	0.42 (± 0.11)	0,115 (± 0.026)

Study AOP 02-007. Multiple dosing

Design and objectives

This was an open-label, non-controlled, multicentre study to assess the efficacy (effects on platelet numbers) and tolerability of anagrelide in patients with myeloproliferative disorders. The average study duration was six months. The primary endpoint was mean platelet numbers. The study also evaluated the response rate, clinical complications before and after anagrelide, and the number of patients discontinuing treatment as secondary endpoints. Pharmacokinetic data were measured in a subset of 30 patients.

Study treatment

Thromboreductin 0.5 mg capsules were used. The starting dose of anagrelide was 0.5 mg twice a day (bid), increased to 1 mg bid after 14 days and then adjusted individually for each patient.

Study population

Some 97 subjects were enrolled and evaluated for safety and efficacy analysis: 97 (ITT population). Inclusion criteria were: patients with essential thrombocythaemia, polycythemia vera, chronic myelogenous leukaemia or idiopathic myelofibrosis, age 18 to 75 years, platelet count >1.500.000/µl, clinical symptoms associated with thrombocythaemia and platelet counts between 600.000/µl and 1.000.000/µl, asymptomatic patients with a platelet count <1.500.000/µl and an increase in platelet count of >300.000/µl within the last 3 months, asymptomatic patients with platelet count above 600.000/µl and a previous history of thrombotic/haemorrhagic complications.

Results

Table 4 summarises the plasma levels of anagrelide following Thromboreductin administration for one week and three months.

Table 4: Corresponding dose and plasma levels of Thromboreductin after dosing for 7 days and 3 months.

	Day 7	3 Months
Dose (mg/d; mean - SD)	1.8+/-0.4	2.5+/-1.2
Plasma level (ng/ml; mean - SD)	2.3+/-1.3	5.1+/-3.3

It was not clear from the clinical study report whether the anagrelide plasma levels were peak, trough or randomly sampled levels. The data showed that the plasma levels were similar to those found in volunteers after single dosing. They also confirmed the high variability after single dose administration, since plasma levels in Study AOP 02-007 ranged from undetectable to 12.45 ng/mL. Concentrations in the same range were found after 4 weeks in steady state in patients with ET (Study AOP 01-007) and also in patients with ET, Polycythemia vera (PV) or Idiopathic myelofibrosis (IMF) (Study AOP 04-007). The mean steady state concentrations for the inactive metabolite 2-amino-5,6- dichloro-3,4-dihydrochinazoline were also in the same range ruling out accumulation. All these data have to be interpreted with caution since variability is very high.

Bioavailability. Study AOP 07-007

Study AOP 07-007 was an open-label, pharmacokinetic bioequivalence study comparing the

- Bioavailability of Thromboreductin to that of Agrylin and
- Dose Proportionality of anagrelide (Thromboreductin) 0.5mg versus 1.0mg

The dosage administered was 2 mg of Thromboreductin or Agrylin using either four 0.5 mg or two 1.0 mg capsules.

Results

The data show that Thromboreductin and anagrelide (Agrylin) after dosing at 0.5 mg are not bioequivalent with respect to the classical parameters AUC and C_{max} (see Table 5). Thus, Thromboreductin and anagrelide (Agrylin) have to be considered as different products with potentially different efficacy and tolerability. The data in Table 5 are results for analyses of anagrelide parent compounds.

Mean area under the plasma concentration time curve over a dosing interval (AUC_{0-t}) for the 2-amino-5,6- dichloro-3,4-dihydrochinazoline metabolite was 18.46 ng.h/ml following administration of the 0.5 mg capsule strength of Thromboreductin. This can be compared to 24.23 ng.h/ml for the same capsule strength of Agrylin. Mean AUC_{0-t} values for the 1.0 mg capsule strength of Thromboreductin and Agrylin were 14.24 ng.h/ml and 16.28 ng.h/ml, respectively. Mean area under the plasma concentration time curve from time zero to infinity ($AUC_{0-\infty}$) for anagrelide metabolite for the 0.5 mg capsule strength of Thromboreductin was 20.79 ng.h/ml which can be compared to 26.54 ng.h/ml for the same capsule strength of Agrylin. Mean $AUC_{0-\infty}$ for the 1.0 mg capsule strength of Thromboreductin and Agrylin were 16.60 ng.h/ml and 18.82 ng.h/ml, respectively. Mean maximum plasma concentration (C_{max}) for the anagrelide metabolite following 0.5 mg of Thromboreductin was 2.98 ng/mL. This can be compared to 4.73 ng/mL following the same capsule strength of Agrylin. Mean C_{max} values for the 1.0 mg capsule strength of Thromboreductin and Agrylin were 2.56 ng/mL and 2.84 ng/ml, respectively.

These data show differences in the plasma levels of the inactive metabolite following treatment with Thromboreductin and Agrylin. If differences also occur for the active metabolite, this may have implications for the efficacy and safety of the products.

Table 5: Thromboreductin versus Agrylin; AUC_{0-t}, $AUC_{0-\infty}$, C_{max} and corresponding Point Estimators at 96% confidence interval (CI).

	Thromboreductin®		Agrylin®		Thromboreductin® / Agrylin®	
0.5 mg formulation	Mean	SD	Mean	SD	Point Estimator	CI (96%)
AUC_{0-t}	21.41	9.37	27.50	16.65	0.82	0.73-0.92
$AUC_{0-\infty}$	22.57	9.91	28.99	17.08	0.86	0.76-0.97
C_{max}	7.78	3.72	11.73	7.64	0.69	0.59-0.82
1.0 mg formulation						
AUC_{0-t}	21.89	11.61	25.16	12.11	0.85	0.74-0.97
$AUC_{0-\infty}$	22.98	12.02	26.58	12.05	0.86	0.75-0.99
C_{max}	7.37	3.85	8.62	3.83	0.83	0.70-0.99

Dose proportionality and time dependency

For assessment of dose proportionality of the 0.5 mg or 1.0 mg formulation of Thromboreductin AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for anagrelide and the metabolite 2-amino-5,6-dichloro-3,4-dihydrochinazoline were determined. Results are shown in Table 6.

Table 6: Thromboreductin 0.5 mg versus 1.0 mg formulation; AUC_{0-t}, $AUC_{0-\infty}$, C_{max} of parent compound and metabolite and corresponding Point Estimators at 96% CI.

	Thromboreductin ®					
	0.5mg formulation		1.0mg formulation		0.5 mg vs. 1.0 mg formulation	
Anagrelide	Mean	SD	Mean	SD	Point Estimator	CI (96%)
AUC_{0-t}	21.41	9.37	21.89	11.61	0,99	0,87-1,12
$AUC_{0-\infty}$	22.57	9.91	22.98	12.02	1,00	0,87-1,14
C_{max}	7.78	3.72	7.37	3.85	1,04	0,88-1,23
Metabolite						
AUC_{0-t}	18.46	7.12	14.24	4.56	1,27	1,04-1,55
$AUC_{0-\infty}$	20.79	7.54	16.60	4.60	1,20	1,03-1,39
C_{max}	2.98	1.43	2.56	0.89	1,13	0,92-1,38

The 90% t-confidence interval of the C_{max} data for anagrelide was 88% to 123%, which is well within the acceptance limits of 80% to 125%. The same is true for AUC_{0-t} (range of 87% to 112%) and for $AUC_{0-\infty}$ (range of 87% to 114%). Dose proportionality was shown for anagrelide but not for the metabolite.

These data show that the two formulations tested (Thromboreductin and Agrylin) are two different products, which may result in differences in platelet reducing efficacy and tolerability, and that the two formulations of Thromboreductin (0.5 mg and 1.0 mg) are dose proportional.

Absorption

After oral administration, anagrelide is rapidly absorbed and the drug is metabolised mainly during first pass to two main metabolites; the active 3-hydroxy anagrelide and the inactive 5,6-dichloro-3,4-dihydroquinazol-2-yl-amine. Peak plasma concentrations of anagrelide and the active metabolite are reached in about 2 hours. Systemic exposure to 3-

hydroxy anagrelide is about twice that of the parent compound in patients with essential thrombocythaemia (Wagstaff and Keating, 2006).

Influence of food

Bioavailability of anagrelide seems to differ slightly when administered postprandially or to fasting patients. Postprandial administration of 0.5 mg anagrelide resulted in a modestly reduced bioavailability (based on AUC values) by an average of 13.8% and slightly increased plasma half-life (up to 1.8 h) when compared to administration under fasting conditions to the same subject (Petrides *et al*., 1997). In addition the peak plasma level was lowered by an average of 45% and delayed by 2 hours. These kinetic changes do not translate into changes of efficacy of the drug and therefore an additional clinical efficacy study for the current formulation was not considered necessary. Furthermore plasma levels of anagrelide after chronic use in patients with myeloproliferative disorders and thrombocytosis were shown to be highly variable and no correlation with dose taken could be found (Study AOP 02-007). Taking these findings together and noting that bio-equivalence could not be shown, it was recommended by the FDA that to prove therapeutic equivalence is sufficient[7].

Metabolism

Study AOP 10-007

Anagrelide is extensively metabolised. This was originally shown in initial experiments when anagrelide was being developed as an anti-aggregating drug. The first pharmacokinetic study was performed twenty years ago using radio-labelled substance (Gaver *et al*., 1981). After administration of radio-labelled anagrelide to volunteers a total of four to five peaks could be separated by high-performance liquid chromatography (H PLC) indicating emerging metabolites in plasma. About 75% of the radioactivity was shown to be excreted via the kidneys into the urine and the remainder through the liver into the faeces. Less than 1% was recovered in the urine as anagrelide.

The structures of the metabolites were not identified at that time. Therefore the applicant has performed an experimental study in two volunteers to clarify the structure of the metabolites emerging in plasma and urine using HPLC with mass spectroscopy (Study AOP 10-007).

Plasma and urine samples were investigated for the presence of further anagrelide metabolites in addition to the one known metabolite 2-Amino-5,6-dichlor-3,4-dihydro-quinazolin.

Results

The data show that anagrelide is hydroxylated at position 3 of the imidazochinazoline structure, and several other metabolites emerge after opening of the imidazol ring in plasma. Two metabolites showed very strong signals, that is, 3-Hydroxyanagrelide and 2-amino-5,6-dichloro-3,4-dihydroquinazoline. In urine, anagrelide was found either unchanged or as the glucuronidated conjugate. Moreover, several other metabolites were identified in glucuronidated and oxo-glucuronidated forms.

[7]www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugsgen/ documents/document/ucm071436.pdf - visited October, 12 2009.

Special populations

Pharmacokinetics after chronic intake in elderly patients and patients with renal and hepatic dysfunction

The applicant elected not to perform a formal PK steady state trial in elderly healthy subjects after multiple dosing because of ethical reasons. Instead plasma levels of anagrelide and 2-amino-5,6-dichloro-3,4-dihydrochinazoline were determined in samples collected during a Phase II program from 34 patients with myeloproliferative diseases (MPDs) (age 18 to 80 years) following treatment for four weeks (Studies AOP 01-007 and AOP 04-007; the primary endpoint in these studies was platelet reduction). The aim was to determine, if anagrelide and 2-amino-5,6-dichloro-3,4-dihydrochinazoline accumulate and if differences after multiple dosing occur in the subsets >65 and <65 years.

Results

Results of the PK analyses from Studies AOP 01-007 and 04-007 are shown in Table 7. These data show that plasma levels of anagrelide and its metabolite are comparable in the two subsets of patients and do not vary significantly with age.

Because of the rarity of the disease a study in ET patients with renal or liver disease was not considered feasible. Careful risk/benefit assessment and control of liver/kidney function is therefore recommended in the Product Information (PI) when Thromboreductin is used in this patient group. Long-term data generated in a patient registry do not suggest a long-term effect on renal/hepatic parameters as assessed by serum creatinine and alanine aminotransferase (ALT) (discussed later in this evaluation report).

It has been reported that total drug exposure (AUC) increases 8-fold when anagrelide is given to patients with moderate hepatic impairment (FDA Medical Watch, 2005). Possibly, this accumulation was caused by the inability of the liver to metabolise the drug.

Table 7: Plasma levels of anagrelide and 2-amino-5,6-dichloro-3,4-dihydrochinazoline in patients younger or older than 65 years.

Total patients (n=34)	Anagrelide	Metabolite*
mean plasma levels (ng/ml)	2.05	2.36
SD (ng/ml)	1.85	1.40
n (samples analysed)	29	32
Age <65 years (n=16)		
mean plasma levels (ng/ml)	1.84	2.26
SD (ng/ml)	1.13	0.95
n (samples analysed)	14	15
Age >/=65 years (n=18)		
mean plasma levels (ng/ml)	2.25	2.44
SD (ng/ml)	2.36	1.73
n (samples analysed)	15	17

Metabolite* = 2-amino-5,6-dichloro-3,4-dihydrochinazoline

Interactions

Anagrelide is mainly metabolised via cytochrome P450 enzyme subtype 1A2, so interactions with drugs such as fluvoxamine are possible. No evidence of an interaction with hydroxycarbamide, digoxin or warfarin has been reported so far. Individual titration of dosages allows for the effects of age or hepatic or renal impairment (Wagstaff and Keating, 2006).

In vitro studies using human liver microsomal preparations and model substrates for the major cytochromes showed that anagrelide (but not the inactive metabolite) appeared to inhibit the actions of CYP1A2 on its model substrate ethoxyresorufin. This suggests that anagrelide could elicit an inhibitory effect on its own metabolism, thereby decreasing its own clearance after repeated dose and consequently increasing plasma concentration. No effects were seen in dogs on the kinetics of either anagrelide or hydroxyurea in the presence of the other when administered in the ratio of the clinically used doses (total doses of 2 mg anagrelide and 500 mg hydroxyurea) (EMEA – scientific discussion, October 10, 2009).

Evaluator's overall conclusions on pharmacokinetics

The PK data showed that peak plasma concentrations for anagrelide were reached within 1.38 hours followed by a rapid decline with an elimination half-life of 1.38 hours. Peak plasma values for 3-Hydroxyanagrelide were slightly delayed emerging at 2.14 hours. Its elimination half-life was 1.79 hours being slightly longer compared to anagrelide. In addition, peak plasma levels of 2-amino-5,6-dichloro-3,4-dihydroquinazoline were reached after 2.22 hours with an elimination half-life 3 to 4 times longer than anagrelide and 3-Hydroxyanagrelide (6.35 h).

The data showed that anagrelide and the two emerging main metabolites are unlikely to accumulate in plasma. However, the data showed a high inter-subject variability of plasma levels for all three compounds (as assessed by the standard deviation).

Data from Studies AOP 01-007 and 04-007 showed that plasma levels of anagrelide and its metabolite are comparable in patients aged >65 years and < 65 years. PK results for the two subsets of patients did not seem to vary significantly with age.

The bioequivalence study showed that the two formulations tested (Thromboreductin and Agrylin) are different products that may have differences in platelet reducing efficacy and tolerability. The two formulations of Thromboreductin (0.5 mg and 1.0 mg) showed dose proportional PK profiles**.**

Pharmacodynamics

Introduction

No pharmacodynamic studies have been conducted with Thromboreductin to support this application. Instead the sponsor provided an overview on pharmacodynamics of anagrelide based on published literature.

Primary pharmacology

Anagrelide and its active metabolite 3-hydroxy anagrelide specifically prevent megakaryocyte differentiation *in vitro*. Hydroxy anagrelide, like the parent compound, has a marked effect on megakaryocyte growth and is similar in potency and specificity to anagrelide in this respect. The development of megakaryocytes was inhibited by 50% at concentrations of 26 and 44 nmol/L by anagrelide and 3-hydroxy anagrelide, respectively. It is likely that 3-hydroxy anagrelide contributes substantially to the clinical platelet lowering effects of the formulation, considering the higher systemic exposure to the metabolite than to the parent compound.

Effects on Bone Marrow/Platelets

The mechanism of action of anagrelide has not yet been fully characterised. The lack of animal models of the thrombocytopenic effects of anagrelide (which are more evident in humans than in laboratory animals) has limited study of the pharmacodynamic effects of the drug. Essential thrombocythaemia is associated with hyperproliferation of megakaryocytes, with increased cell size and ploidy (higher proportions of 32N or higher ploidy cells versus healthy controls), and a mean platelet turnover rate about six times higher than that in healthy controls.

Relationship between plasma concentration and effect

Anagrelide dose-dependently and reversibly prevents number and maturation of megakaryocytes in non-mitotic late stages of development (size, surface irregularity, optical density and ploidy of the megakaryocytes are reduced) which results in increased numbers of precursor cells (promegakaryoblasts and megakaryoblasts).

Anagrelide does not affect haematopoietic stern cells and does not damage DNA. Oral treatment with either anagrelide or hydroxycarbamide (hydroxyurea) resulted in increased incidence of promegakaryoblasts and microforms in 20 patients with initial to early stage chronic idiopathic myelofibrosis and thrombocythaemia in a bone marrow immunohistochemical and morphometric study. However, while no such changes were seen with anagrelide, hydroxycarbamide was associated with significant abnormalities of megakaryocytic differentiation indicative of dysplastic changes and therefore of leukaemogenic potential.

The platelet turnover rate is reduced to close to normal rates but platelet survival is unaffected in patients with essential thrombocythaemia receiving anagrelide. Thus, oral anagrelide caused reversible reductions in platelet counts in healthy volunteers and platelet counts were normalised in patients with essential thrombocythaemia. This thrombocytopenic effect does however not occur in all patients.

In contrast to HU and interferon-α, anagrelide does not appear to be associated with bone marrow angiogenesis in patients with essential thrombocythaemia. Levels of platelet count-corrected vascular endothelial growth factor (VEGF) and platelet factor 4 (PF4), markers of angiogenesis, were increased at baseline compared to those of healthy controls. VEGF and PF4 levels were normalised with anagrelide therapy. However, platelet levels were increased further in patients receiving HU or interferon-α (n= 13). Similarly, bone marrow vessel-related CD34+ progenitor cell levels which are also used to mark angiogenesis, were not increased by anagrelide (n = 15).

Many abnormal aspects of platelet function associated with essential thrombocythaemia are improved with anagrelide treatment. Platelet count-corrected thromboxane B2 values tended toward normalisation in 17 patients with essential thrombocythaemia during anagrelide-induced remission. Similarly, platelet coagulant activity and endothelial function (PF4, prothrombin fragment 1+2, plasminogen activator inhibitor- 1, tissue factor pathway inhibitor) were normalised in 17 patients with essential thrombocythaemia who responded to anagrelide. In this and other studies, normalisation of endothelial function was associated with disappearance of erythromelagic symptoms.

Both anagrelide and its active metabolite inhibit platelet cyclic adenosine monophosphate phosphodiesterase (PDE)-3 and phospholipase A2 (PLA2), an effect that is independent of the effects on megakaryocyte differentiation. 3-Hydroxy anagrelide is nearly 40 times more potent than anagrelide in inhibiting PDE-3, an effect with possible cardiovascular consequences. While significant inhibition of platelet aggregation appears only to occur at plasma anagrelide concentrations bigger than those required for the thrombocytopenic effect, 3-hydroxy anagrelide has some antiaggregatory activity at therapeutic concentrations as suggested by its greater anti-PDE-3 potency. The effects of anagrelide on the abnormal platelet aggregation seen in some patients with essential thrombocythaemia are as yet unresolved.

Anagrelide had little effect on the increased plasma levels of other growth factors involved in myelofibrosis (transforming growth factor-ß and basic fibroblast growth factor) in these patients. In line with these results, after a median duration of about 2 years there was no evidence of a stimulating effect of anagrelide on the progression of myelofibrosis as assessed using bone marrow biopsies in two small studies (one prospective: n = 17; one retrospective: n = 15).

Pharmacodynamic interactions with other medicinal products or substances

While there are no reports of increased bleeding associated with anagrelide monotherapy, there are indications that the addition of aspirin to anagrelide treatment can synergistically increase the incidence of bleeding in patients with essential thrombocythaemia possibly as a result of the known effects of aspirin being aggravated by the vasodilating effect of anagrelide. In a 6-month study in patients with myeloproliferative disorders (n = 97), bleeding events occurred in nine patients receiving anagrelide plus concomitant low-dose aspirin compared to two receiving anagrelide alone.

Cardiovascular Effects

Anagrelide-associated decreased peripheral vascular resistance and blood pressure and increased heart rate and positive inotropic effects (increased ventricular contractility) noted in nonclinical studies have since been confirmed in humans, although blood pressure appears to return to baseline levels during maintenance therapy. Vasodilation, as shown by increases in microvascular area in anagrelide recipients as a consequence of the PDE-3- inhibiting effects of the drug, can cause headache, fluid retention, dizziness and postural hypotension, and occasionally serious cardiovascular disease such as congestive heart failure. The main contributor to the cardiovascular effects of anagrelide is likely to

be the active metabolite 3-hydroxy anagrelide. Because of the inhibitory effect of anagrelide and its metabolite on PDE-3, concomitant administration of drugs such as milrinone, enoximone, amrinone, olprinone or cilostazol may result in exacerbation of their effects.

Evaluator's overall conclusions on pharmacodynamics

The main pharmacological actions of anagrelide are platelet reduction, which is the therapeutic target, and PDE-3 inhibition. Dosages required for the platelet lowering activity in humans were lower than those calculated for the anti-aggregating effect (Silverstein *et al*. 1988). Twenty years later, the molecular basis of the platelet reducing effect is still unknown. This is at least partly due to the fact that the direct platelet lowering effect can only be studied in humans. Studies on human megakaryocytes grown in culture show that anagrelide interferes with the maturation of these platelet precursors (Mazur *et al*. 1992, Solberg *et al*. 1997).

Since relatively high *in vitro* dosages are required it was postulated that a metabolite not present in *in vitro* environment is required for the *in vivo* activity of anagrelide. This would be in accordance with the clinical observation that about 15% of the patients with primary thrombocythaemia are primarily refractory against the drug. It has been demonstrated that anagrelide reduces thrombopoietin-mediated megakaryocyte proliferation through inhibition of intracellular signalling events (McCarty *et al*. 2006). These events take some days until they manifest themselves in a platelet response and therefore platelet reduction can only be observed after several days of exposure to anagrelide.

Both anagrelide and 3-Hydroxyanagrelide inhibit PDE-3 activity (Wang *et al*. 2005) an enzyme which is contained in cardiovascular tissues such as cardiomyocytes. PDE-3 mediates inactivation of cyclic adenosine monophosphate (cAMP). As a consequence it protects the human organism from excessive adrenergic stimuli which may be harmful long-term. Indeed, in laboratory animals anagrelide was shown to be a potent inotropic and vasodilatory agent (Kastner *et al*. 1985). Recently it was found that 3-Hydroxyanagrelide has a 40-fold higher potency for PDE-3 inhibition than anagrelide and therefore is the main contributor in mediating cardiovascular effects (Wang *et al*. 2005). PDE-3 associated AEs observed in humans, like headache, fluid retention, dizziness and postural hypotension are also likely to be a consequence of 3-hydroxyanagrelide emerging from anagrelide.

Efficacy

Introduction

The clinical development plan was conducted in the context of the following circumstances which influenced the design of studies to show efficacy.

- ET is a rare disease with a prevalence of less than 2.5 cases per 10.000 and therefore is considered as an Orphan disease.
- Current knowledge indicates that only patients with a high risk profile should receive platelet reducing treatment. Therefore only high risk patients were considered to be eligible for recruitment.
- Because ET is an Orphan disease it is extremely difficult to recruit large patient numbers for clinical trials. In particular it is hard to find treatment naïve patients who can produce data free of pretreatment bias.
- HU is considered as standard therapy for ET by regulatory authorities in the EU (although not approved for marketing in all member states).

- The main clinical characteristics of ET, which lead to significant morbidity and even mortality in patients, are thrombohaemorrhagic events. The rate of thrombohaemorrhagic complications was 4.2% in anagrelide treated patients (Study AOP 02-007). Thus, any trial to show superiority to HU would need a sample size of about 1600 patients to demonstrate clinical superiority (that is, a reduction in thrombohaemorrhagic events by 50% after 1 year, based on a study of 4 years duration, alpha = 0.025 one-sided, beta = 0.100).
- Different diagnostic standards for ET exist and these include diagnosing patients according to Polycythemia Vera Study Group (PCVSG) or World Health Organization (WHO) criteria, and this makes comparability of studies difficult.

It was decided to perform a classical development program including Phase I to IV clinical studies taking the above mentioned constraints into account. Particular emphasis was given to include patients who were expected to receive treatment after marketing authorisation, that is, patients aged over 18 years including elderly and patients at high risk for ET-associated clinical complications. Children were not included in formal clinical studies since ET is very rare in this patient subgroup. However, data were collected in a small number of children after the first marketing approval of Thromboreductin in 2001.

The clinical documentation for demonstration of efficacy includes four studies sponsored by the applicant as well as data from a patient registry (see Table 8).

Table 8: Overview of clinical trials

Trial	Phase	Aim	No. patients	Diagnosis
AOP 01-007	II	Short-term efficacy (platelet reduction)	15	ET
AOP 04-007	II	Short-term efficacy (platelet reduction)	19	ET, PV, IMF
AOP 02-007	II/III	Efficacy/adverse effects (platelet reduction/ET related clinical events)	97	ET/PV/IMF
AOP 03-007	III	Efficacy and safety in comparison to HU	258	ET
AOP 05-007	IV	Long-term efficacy and safety with particular emphasis on renal/hepatic and cardiac safety	722	ET

Dose-response studies

Study "AOP 01-007"

This study was conducted from 8 June 2000 to 9 December 2000.

Objectives

The primary objective was to prove the pharmacodynamic bioequivalence of the new formulation of anagrelide (Thromboreductin 0.5 mg capsules) with the existing formulation of anagrelide (Agrylin 0.5 mg capsules) with respect to maintaining the platelet counts from the end of Thromboreductin therapy to the end of Agrylin therapy.

Secondary objectives included:

- Determination of steady state anagrelide plasma levels after therapy with Agrylin or Thromboreductin.

- Number of patients achieving a response at the end of the treatment (platelet count <600.000 or a reduction by 50% compared to pretreatment values).
- Number of patients discontinuing therapy because of adverse events, lack of compliance or specific reasons.
- Documentation of adverse events.

Methodology

This was an open, controlled, single-centre, pharmacodynamic bioequivalence study in patients with thrombocythaemia associated with myeloproliferative disease. Patients on a stable dose of Agrylin were switched to the same dose of Thromboreductin and maintained at this level for four weeks.

Treatment

Thromboreductin capsules were administered at the pretreatment dose for four weeks. At the end of the trial (Day 28) Thromboreductin was offered on a compassionate use basis for another six months.

Criteria for evaluation

The primary objective of the trial was to prove pharmacodynamic bioequivalence of a new formulation of anagrelide (Thromboreductin 0.5 mg capsules) with the existing formulation of anagrelide (Agrylin 0.5 mg capsules) with respect to lowering elevated platelet counts. The target parameter was the change in mean number of platelets following a switch from one anagrelide formulation (Agrylin 0.5 mg capsules) to the new anagrelide formulation (Thromboreductin 0. 5 mg capsules).

Statistical methods

The Type I error was set to 5% and is based on two-sided tests. The two-sided 5% level was defined for statistical significance of the primary endpoint. The primary endpoint (steady state platelet counts) was analysed by a paired t-test methodology and intra-individual comparison between treatments. The power was set to 80%.

In addition to this, analysis changes of thrombocytes were analysed with 90% and 95% confidence limits as a secondary statistical endpoint using a predefined delta (Â) of +/- 150 G/l (range 300 G/l).

Patient population

Fifteen patients between 18 and 80 years of age with essential thrombocythaemia and on therapy with anagrelide (Agrylin) for at least three months were included in the study. All patients had given their informed consent. There were eight female and seven male patients with a mean and median age of 49.1 and 49.0 years, respectively (range 31-66 years).

All patients had essential thrombocythaemia. All patients had a platelet count >900.000/µl on two determinations or >600.000/µl with clinical symptoms or a history of thrombotic/haemorrhagic complications before treatment with Agrylin.

All patients had profound megakaryocytic hyperplasia in bone marrow. In eight patients the absence of Philadelphia chromosome had been diagnosed (there were no data from 7 patients). Thirteen patients had normal serum iron and ferritin and normal marrow iron stores, (one patient abnormal and one patient with no data).

Results

The mean platelet count, as assessed from historical records, was more than 2-fold above the normal value at diagnosis or before initiation of cytoreductive therapy either with HU or alpha interferon (see Table 9 - Note: the column for mean platelet count before cytoreductive therapy is composed from values at diagnosis or before cytoreductive therapy with HU, interferon or anagrelide). Therapy with anagrelide (Agrylin) decreased the mean platelet count by 49%. Following a switch to the novel formulation, the maximum change of the mean platelet count was 9% within 28 days.

The change in platelets from Day 0 to the end of study Day 28 did not achieve the nominal 5% level of statistical significance: the average platelet change from Day 0 to Day 28 is 35 G/l with a calculated p value of the paired t-test of $p<0.27$ (not statistically significant).

Under stable disease the conclusion is that bioequivalence has been shown with a critical limit of 260 G/l (+- 130) even with a slightly higher number of patients.

The calculated 90% confidence interval of the average change is from -20 G/l to +88 G/l and those limits for the 95% level is from -31 G/l to +100 G/l lying within the predefined margins for clinical bioequivalence. In this study the margin of clinical equivalence (Â) defining the largest difference, that is clinically acceptable, was a range of 300 G/l (delta +/- 150 G/l). The confidence limits for the 90% and 95% level contain this limit fully.

The steady-state anagrelide plasma levels after therapy with Agrylin and Thromboreductin are shown in Table 10 below. It was not clear in the clinical study report whether these were peak, trough or randomly sampled levels.

Table 9: Mean number of platelets following switch from Agrylin to Thromboreductin.

			Platelet Counts (G/L)					
Study number	Centre	Pat Id	Diagnosis / BCT 1)	Day 0 2)	Day 7	Day 14	Day 21	Day 28
1007	1	1001	2200	316	348	262	345	196
1007	1	1002	1000	463	401	250	365	430
1007	1	1003	916	477	522	421	420	344
1007	1	1004	1180	634	613	558	685	600
1007	1	1005	1200	690	719	603	756	708
1007	1	1006	874	544	530	617	573	607
1007	1	1007	1136	579	492	554	530	561
1007	1	1008	859	876	793	760	715	894
1007	1	1009	835	664	448	450	398	480
1007	1	1010	619	456	314	388	453	421
1007	1	1011	919	394		637	273	292
1007	1	1012	948	380	304	242	217	233
1007	1	1013	844	421	252		272	328
1007	1	1014	1113	689	624	654	666	659
1007	1	1015	904	361	636	709	599	672
Mean			1036	530	500	508	484	495
SDEV			357	156	165	173	175	198
N			15	15	14	14	15	15

Table 10: Study AOP 01-007 - Descriptive statistics of steady-state plasma levels of anagrelide and one of its metabolites after therapy with Agrylin (Day 0) or Thromboreductin (Day 28)

	Agrylin® plasma level day 0	Thromboreductin® plasma level day 28
mean	0.95 ng/ml	1.31 ng/ml
median	0.78 ng/ml	0.94 ng/ml
std.dev.	1.11 ng/ml	1.60 ng/ml
minimum	0.001 ng/ml	0.001 ng/ml
maximum	3.97 ng/ml	5.13 ng/ml
number of observations	15	15

The steady-state plasma levels of one of the anagrelide metabolites after therapy with Agrylin and Thromboreductin are shown in Table 11 below. The clinical study report did not identify which metabolite was measured. It was also not clear whether these were peak, trough or randomly sampled levels.

Table 11: Study AOP 01-007 - Descriptive statistics of steady-state plasma levels of one of anagrelide metabolites after therapy with Agrylin (Day 0) or Thromboreductin (Day 28).

	anagrelide metabolite after therapy with Agrylin® (day 0)	anagrelide metabolite after therapy with Thromboreductin® (day 28)
mean	4.49 ng/ml	2.02 ng/ml
median	2.38 ng/ml	1.54 ng/ml
std.dev.	5.55 ng/ml	1.64 ng/ml
minimum	0.36 ng/ml	0.37 ng/ml
maximum	16.94 ng/ml	6.32 ng/ml
number of observations	15	15

The data show that both Agrylin and Thromboreductin are absorbed and that the same metabolite emerges following administration of each compound.

Number of patients achieving a response

Response was defined as platelet count <600.000/µl or a reduction by 50%. Table 12 summarises the platelet counts that were documented.

Ten out of 15 patients responded to Agrylin and 9 out of 15 patients responded to Thromboreductin. Two patients with Thromboreductin were borderline cases for response. The response rates of Agrylin and Thromboreductin were calculated against historical data of the platelet count at diagnosis or before cytoreductive therapy and are therefore only a rough estimate of the efficacy of both compounds.

Table 12: Study AOP 01-007. Platelet counts for individual patients.

Pretreatment (G/l)	end of Agrylin® treatment (day 0) G/l	Response Y/N	end of Thromboreductin® treatment (day 28) G/l	Response Y/N
2200.0	316.0	Y	196.0	Y
1000.0	463.0	Y	430.0	Y
916.0	477.0	Y	344.0	Y
1180.0	634.0	N	600.0	N
1200.0	690.0	N	708.0	N
874.0	544.0	Y	607.0	N
1136.0	579.0	Y	561.0	Y
859.0	876.0	N	894.0	N
835.0	664.0	N	480.0	Y
619.0	456.0	Y	421.0	Y
919.0	394.0	Y	292.0	Y
948.0	380.0	Y	233.0	Y
844.0	421.0	Y	328.0	Y
1113.0	689.0	N	659.0	N
904.0	361.0	Y	672.0	N

Dosing during 4 weeks of treatment with Thromboreductin

The dose was maintained during the four week treatment period; the changes in 1.5 mg and 2.0 mg dose on Days 7 and 14 were due to missing values.

Table 13: Study AOP 01-007. Dosing during 4 week treatment with Thromboreductin

	number of patients				
dose per day	day 0	day 7	day 14	day 21	day 28
missing value	--	1	1	--	--
0.5 mg	3	3	3	3	3
1.0 mg	4	4	4	4	4
1.5 mg	2	1	2	2	2
2.0 mg	3	3	2	3	3
2.5 mg	1	1	1	1	1
3.0 mg	1	1	1	1	1
4.0 mg	1	1	1	1	1

Study AOP 04-007

This was an open, pharmacodynamic bioequivalence study of anagrelide in patients with thrombocythaemia. The 4-week study in 19 patients compared anagrelide (Agrylin) to the novel formulation of Thromboreductin. Enrolment started in on the 8 August 2000 and the study was completed on the 26 January 2001.

Objectives

The primary objective was to prove pharmacodynamic bioequivalence of a new formulation of anagrelide (Thromboreductin 0.5 mg capsules) to the existing formulation of anagrelide (Agrylin 0.5 mg capsules) with respect to maintaining the platelet counts from the end of Thromboreductin therapy to the end of Agrylin therapy.

Secondary objectives were the same as for Study AOP 01-007 (see above).

Design and methodology

This was an open, controlled, multicentre, pharmacodynamic bioequivalence study on patients with thrombocythaemia associated with myeloproliferative disease. Patients on a stable dose of Agrylin were switched to the same dose of Thromboreductin and maintained at this dose level for four weeks. It was planned that a total 36 patients would participate, but 19 patients were ultimately included and analysed.

Main criteria for inclusion

- Patients with essential thrombocythaemia, polycythemia vera chronic myelogenous leukaemia or idiopathic myelofibrosis.
- Age 18 to 80 years.
- Diagnosis of thrombocytosis associated with myeloproliferative diseases
- Therapy with Agrylin for a minimum of three months.

Patients on a stable dose of Agrylin capsules were included in the trial. On Day 0 they were switched to the test product Thromboreductin capsules at the pretreatment dose.

Duration of treatment

Thromboreductin capsules were administered at the pretreatment dose for four weeks. At the end of the trial (Day 28), Thromboreductin was offered on a compassionate use basis and the patients are seen every three months for as long as feasible.

Statistical methods

Similar methods were used as described for Study AOP 01-007.

Patient population

Patients had a mean and median age of 62.6 and 64.0 years, respectively (range 40-82 years).

From the total of 19 patients, three patients had the following significant protocol violations:

- Two were older than 80 years (both 82), and the investigators included the patients into the study despite the protocol violation. One of these patients withdrew the informed consent after Day 7.
- One patient was on Agrylin for 44 days only instead of the full months.

Some 16/19 patients had essential thrombocythaemia. One patient had polycythaemia vera, another had idiopathic myelofibrosis and a third patient was recorded as having chronic myelogenous leukaemia.

Results

According to the sample size calculation it was planned to include 36 patients in the trial. However, only 19 patients were included in the study. Nine patients had not completed the study at Day 28 but completed at later time points (between 29 and 55 days). These patients were treated in the statistical analysis as minor protocol violators and treated as if they had a normal study termination on Day 28. One patient withdrew the informed consent after Day 7. There were three patients with major protocol violation (see above).

The efficacy analysis was an intention-to-treat (ITT) analysis and was based on the data of 18 patients (all patients who completed the study). The patient who terminated the study on Day 7 was excluded from analysis. Data on the follow up period (control every three months as long as feasible) were not submitted.

Primary efficacy analysis

The primary efficacy variable was the mean number of platelets following the switch from Agrylin to Thromboreductin. Results are shown in Table 14. The mean of platelet counts, as assessed from historical records, was more than 2-fold above the normal value at diagnosis or before initiation of cytoreductive therapy either with hydroxyurea or alpha interferon. Therapy with anagrelide (Agrylin) decreased the mean platelet count by 5l%. Following the switch to the novel formulation, the maximum change of the mean platelet count within 28 days was 10%.

Steady state platelet counts were analysed for difference from Day 0 to Day 7, Day 0 to Day 14, Day 0 to Day 21 and Day 0 to Day 28. Results are shown in Table 15 below.

Table 14: Study 04-007. Mean number of platelets following switch from Agrylin to Thromboreductin.

	Platelet Counts (G/L)				
Diagnosis / BCT *	Day 0 1)	Day 7	Day 14	Day 21	Day 28
958	405	364	344	421	399
1080	525	485	414	437	515
973	297		255	258	274
877	159	90	194	223	98
2211	830	942	796	715	798
1054	326	460	381	300	400
726	422	493			
1651	531	687	411	660	700
832	481	484	470	423	550
1300	317	294	289	321	241
1509	859	916	743	760	656
1527	412	376	484	339	398
741	442	459	365	479	446
826	435	721	749	664	666
782	607	743	575	562	449
885	569	667	600	442	532
600	460	504	431	368	437
461	777	764	641	680	714
590	797	652	647	629	502

*BCT Before Cytoreductive Therapy with Agrylin, ** Patient excluded from ITT analysis

Mean			1048	513	565	488	482	488
SDEV			441	198	225	178	169	179
N			18	18	18	18	18	18

Comments:

1) last day of Anagrelide (Agrylin), next day switch to Anagrelide new formulation

Table 15: Study AOP 04-007. Steady state platelet counts for difference from Day 0 to Day 7, Day 0 to Day 14, Day 0 to Day 21 and Day 0 to Day 28.

	day 0 vs.			
	day 7	day 14	day 21	day 28
t-Test				
P Exact	0,136	0,346	0,206	0,391
P classic	n.s	n.s	n.s	n.s

Abbreviations: p> 0.10 n.s
p<0,10>0,05 (+)
p<0,05 *

The change in platelets from Day 0 to the end of study Day 28 (18 patients) did not achieve the nominal 5% level of statistical significance: the average platelet change from Day 0 to Day 28 is 25 G/l and the standard deviation is 122 G/l leading to a calculated p value of the paired t-test of p<0.391 and therefore not statistically significant.

Under stable disease the conclusion is that bioequivalence was shown with a critical limit of 120 G/l (+- 60).

In the present study 19 patients were recruited and 18 of these were analysed in the ITT. Using the significance test approach and requiring the power level to be stable at 80% then the reduction from 26 to 18 patients leads to a widening of the critical difference

from a level of 120 G/l (+/- 60 G/l) to approximately 170 G/l (+/- 85 G/l). Amending the power calculations formulae to the actually observed standard deviations and the paired t-test methodology one gets a statistical power in excess of 95% to detect the prespecified critical limit of +/- 60 G/l (or 120 G/l) as the observed standard deviation of the differences is about half of the value of the study protocol.

The calculated 90% confidence interval of the average change is from -25 G/l +75 G/l and those limits for the 95% level is from -35 Gn to +86 Gil and must lie within the predefined margins for clinical bioequivalence. In this trial the margin of clinical equivalence defining the largest difference that is clinically acceptable was a range of 300 G/l (delta +/- 150 G/l). The confidence limits for the 90% and 95% level contain this limit fully.

Results for the steady-state anagrelide plasma levels after therapy with Agrylin and Thromboreductin are shown in Table 16 below. It was also not clear whether these were peak, trough or randomly sampled levels.

Table 16: Study 04-007. Steady-state anagrelide plasma levels after therapy with Agrylin and Thromboreductin.

	Agrylin® plasma level day 0 (ng/ml)	Thromboreductin® plasma level day 28 (ng/ml)
mean	1,49	2,34
median	0,90	1,59
std.dev.	1,86	1,99
minimum	0,001	0,330
maximum	6,99	7,67
number of observations	18	17

The steady-state plasma levels of one of the anagrelide metabolites after therapy with Agrylin and Thromboreductin are shown in Table 17 below.

Table 17: Study AOP 04-007. Descriptive statistics of steady-state plasma levels of one of anagrelide metabolites after therapy with Agrylin (Day 0) or Thromboreductin (Day 28).

	anagrelide metabolite after therapy with Agrylin® (day 0)	anagrelide metabolite after therapy with Thromboreductin® (day 28)
mean	2,91	2,52
median	2,72	2,33
std.dev.	1,99	1,23
minimum	0,001	1,00
maximum	8,72	5,61
number of observations	18	17

The data showed that both Agrylin and Thromboreductin are absorbed and that the same metabolite emerges in plasma from the both compounds.

Number of patients achieving a response

Response was defined as platelet count <600.000/µl or a reduction by 50%. Values at the end of treatment are compared against values at diagnosis, before cytoreductive therapy or before Agrylin (newly diagnosed patients).

ITT Analyses

Fourteen out of 18 patients responded to Agrylin and 17 out of 18 patients responded to Thromboreductin, making the response rates comparable. The response rates to Agrylin

and Thromboreductin were calculated against historical data of the platelet count at diagnosis or before cytoreductive therapy and thus are only a rough estimate of the efficacy of both compounds. Data on patient platelet counts during the follow up period were not available. Table 18 shows platelet counts that have been documented.

Table 18: Study AOP 04-007. Platelet counts (G/l).

Diagnosis / BCT *	end of Agrylin® treatment (day 0)	Response Y/N	end of Thromboreductin® treatment (day 28)	Response Y/N
958	405	Y	399	Y
1080	525	Y	515	Y
973	297	Y	274	Y
877	159	Y	98	Y
2211	830	Y	798	Y
1054	326	Y	400	Y
726	422			
1651	531	Y	700	Y
832	481	Y	550	Y
1300	317	Y	241	Y
1509	859	N	656	Y
1527	412	Y	398	Y
741	442	Y	446	Y
826	435	Y	666	Y
782	607	N	449	Y
885	569	Y	532	Y
600	460	Y	437	Y
461	777	N	714	N
590	797	N	502	Y

Dosing during 4 weeks of treatment with Thromboreductin

Table 19 shows dosing over the four week period. In this study the dose could not been maintained in all patients during the four week treatment period. Medical reasons necessitated the dose adjustments. In the table the changes in daily dose have been marked in bold letters.

Table 19: Study AOP 04-007 - Dosing during 4 week treatment with Thromboreductin

day 0	day 7	day 14	day 21	day 28
6.0 mg/week	6.0 mg/week	6.0 mg/week	6.0 mg/week	6.0 mg/week
2.0	2.0	2.0	2.0	2.0
1.5	--	1.5	1.5	1.5
1.5	**0.5**	**1.0**	**1.0**	**0.5**
1.5	1.5	1.5	1.5	1.5
1.5	1.5	1.5	1.5	1.5
1.5	1.5	1.5	1.5	1.5
2.0	2.0	2.0	2.0	2.0
1.5	1.5	1.5	1.5	1.5
1.5	1.5	1.5	1.5	1.5
2.0	2.0	2.0	2.0	2.0
2.5	2.5	2.5	2.5	2.5
1.0/1.5	1.0/1.5	1.0/1.5	1.0/1.5	1.0/1.5
1.5	**1.5**	**2.0**	**2.5**	**2.5**
2.5	**1.0**	**1.0**	**3.0**	**3.0**
3.0	3.0	3.0	3.0	3.0
1.5	1.5	1.5	1.5	1.5
2.0	2.0	2.0	2.0	2.0
2.0	2.0	2.0	2.0	2.0

Study AOP 02-007

Study AOP 02-007 was an open, prospective, international multicentre study that evaluated the efficacy and safety of Thromboreductin in thrombocytosis associated with myeloproliferative disorders. Newly diagnosed patients and patients pretreated with cytoreductive agents were enrolled in the trial.

Study design and treatments

This was an open-label study of six months duration. After end of the study patient were offered compassionate use program with follow up for up to 5 years if feasible. Twelve centres participated in the study and although it was planned to enrol 100 subjects, 97 subjects participated and were analysed for safety and efficacy.

Patients with ET, PV, CML and IMF (newly diagnosed patients) were started or switched to anagrelide. Screening assessments were done within one month before start of treatment. The starting dose of anagrelide was 0.5 mg bid, and increased to 1 mg bid after 14 days and then was adjusted individually for each patient.

Platelet counts were measured at Day 7, 14, 21, 28 and at Months 2, 3, 4, 5 and 6. Patients were then followed for up to 5 years.

The open and non comparative design was chosen because

(i) performing a placebo controlled trial is not feasible in these patients, and treatment carries an inherent risk of clinical complications, and

(ii) there was no registered drug available (busulphan has been abandoned because of its leukaemogenicity) serving as a generally accepted comparator in countries where the study was conducted.

In this study newly diagnosed patients and patients with a pre existing therapy, for example, α interferon or hydroxyurea, were switched to anagrelide without defining a washout period. The reasons were two-fold:

(i) interferon-α or hydroxyurea are commonly used off label as non specific cytoreductive drugs and data for safely switching patients to anagrelide are missing for interferon-α and limited for hydroxyurea, and

(ii) a washout period until platelets rise again before initiating anagrelide therapy would pose an unacceptable risk to patients who are already on therapy.

Study endpoints

Platelet reduction was used as the primary surrogate endpoint. This endpoint was considered justified by several studies performed in ET patients demonstrating that reduction of elevated platelet counts leads to a decrease in the rate of thrombohaemorrhagic events (Cortelazzo *et al*. 1995, Beykirch *et al*. 1997). However, careful attention was paid in Study AOP 02-007 to evaluate thrombohaemorrhagic events to support data for the primary surrogate endpoint and to optimise hypothesis generation in the confirmatory Phase III study.

Secondary efficacy endpoints included response rate and the rate of clinical complications (ET related events) before start of the therapy and 3 and six months after start of Thromboreductin therapy.

Response is defined as the number of subjects with:

(i) Complete response: Normalisation of platelet counts (<400.000/µl) or close to normalisation (<600.000/µl) for a period of four weeks.

(ii) Partial response: Decrease of platelets by more than 50%, but not complete responder for a period of four weeks.

(iii) Failure: No decrease in platelet counts or a decrease of 50% or less.

The definition of "thrombohaemorrhagic" was done according to a previous study in ET, where a list of more than 20 minor (such as a transient ischaemic attack) and major thrombotic (such as stroke) and haemorrhagic (such as a major bleeding) events were defined based on related clinical symptoms (Gisslinger *et al*. 1989).

Statistical methods

The main statistical analysis strategy was exploratory, since this was a Phase II study with emphasis on estimators of effect sizes with two-sided 95% confidence intervals according to the guidance document[8].

Missing values of thrombocytes were replaced by means of Last Value Carried Forward technique (LVCF technique). Nonparametric procedures were used for all test statistical analyses. In addition to P-values, all results were presented by means of Mann-Whitney (MW) estimators as nonparametric effect sizes together with their two-sided (95% confidence intervals in order to evaluate the size of the effects according to ICH E9 Guidelines. Within-group comparisons of the thrombocytes effect sizes and confidence intervals were available for KxK ordered categories. The test procedure was the generalised McNemar's test for KxK ordered categories[9].

[8] ICH Topic E9: Note for guidance on statistical principles for clinical trials. CPMP/ICH/362/96. www.ema.europa.eu/pdfs/human/ich/036396en.pdf

[9] McNemar's test is a non-parametric method used on nominal data. It is applied to 2 × 2 contingency tables with a dichotomous trait, with matched pairs of subjects, to determine whether the row and column marginal frequencies are equal ("marginal homogeneity").

Study population

Patients were diagnosed according to Polycythemia Vera Study Group (PVSG) criteria[10] and had to be between 18 and 75 years old. Only patients with a predefined high risk status were included, that is,

- platelet count >1.500.000/mL,
- clinical symptoms associated with essential thrombocythaemia and a platelet count between 600.000/mL and 1.500.000/mL,
- platelet count <1.500.000/mL and an increase of 300.000/mL within the last 3 months,
- or a platelet count above 600.000 and a history of thrombohaemorrhagic events.

Patients previously treated for ET had to be refractory in terms of inadequate reduction of platelet counts or intolerant to HU or interferon-α.

Some 97 patients were recruited into the study and data for all these patients were available. Some 88 patients terminated the study according to the protocol and nine patients discontinued the study prematurely.

Diagnoses of patients recruited into the study were as follows:

- Essential thrombocythaemia (79 patients).
- Polycythaemia vera (16 patients).
- Idiopathic myelofibrosis/Myelodysplastic Syndrome (2 patients).
- No patient was recruited with Chronic myelogenous leukaemia.

Patients were 'recruited into the study for following reasons (multiple reasons were admissible):

(i) newly diagnosed

(ii) intolerant to current treatment

(iii) refractory to current treatment

(iv) ineligible to hydroxyurea

(v) ineligible to alpha interferon.

[10] All of the following criteria must be fulfilled to make a diagnosis of ET: 1. Platelet count greater than 600 x 10^9/L. 2. Hematocrit less than 40 or normal red blood cell mass. 3. Stainable iron in the marrow or normal RBC mean corpuscular volume (If these measurements suggest iron deficiency, polycythemia vera cannot be excluded unless a trial of iron therapy fails to increase the red blood cell mass into the polycythemic range.) 4. No Philadelphia chromosome or *bcr/abl* gene rearrangement. 5. Collagen fibrosis of the bone marrow absent or less than one third of the biopsy area without both marked splenomegaly and a leukoerythroblastic blood film. 6. No cytogenetic or morphologic evidence for a myelodysplastic syndrome. 7. No cause for a reactive thrombocytosis.

Results

Some 88 out of 97 patients completed the study according to protocol. The daily dose of Thromboreductin increased during the six months of the study from a median of 1.0 mg (range 0.5 mg to 2.0 mg) to a median of 2.0 mg (range 0.5 to 4.5 mg). In about 90% of the patients a daily dosage of anagrelide between 0.5 mg and 3.0 mg was sufficient to achieve the observed results. At the beginning of the study, 19 patients obtained concomitant therapy with interferon-α (n=9) or HU (n=10). This number decreased to seven patients (four and three patients, respectively) after six months of treatment.

In the reporting of results in the tables to follow the abbreviations shown in Table 20 below will be used.

Table 20: List of abbreviations used in tables summarising platelet counts

PL-AM3DOLV:	thrombocytes, absolute values at start of therapy (Day 0)
PL-D7LV:	thrombocytes, absolute values at Day 7
PL-D14LV:	thrombocytes, absolute values at Day 14
PL-D21LV:	thrombocytes, absolute values at Day 21
PL-D28LV:	thrombocytes, absolute values at Day 28
PL-M2LV:	thrombocytes, absolute values at Month 2
PL-M3LV:	thrombocytes, absolute values at Month 3
PL-M4LV:	thrombocytes, absolute value at Month 4
PL-M5LV:	thrombocytes, absolute value at Month 5
PL-M6LV:	thrombocytes, absolute value at Month 6
Mean:	arithmetic mean
Std Dev:	standard deviation
Min:	minimum
Lo Quar:	lower quartile
Med:	median
Up Quar:	upper quarter
Max:	maximum
Valid N:	valid number

Table 21 below shows the results of the number of thrombocytes (G/l) at baseline and at the various follow-up visits with classical and robust statistics for the Intention-to-treat population (lTT).

The results of the study showed, that in patients with myeloproliferative disorders and thrombocytosis, treatment with Thromboreductin statistically significantly decreases platelet numbers. The above table shows a thrombocyte decrease during of the six months study therapy from a baseline mean of 802.6 (Median 743.0, Minimum 335, Maximum 1912) to a final mean of 486.1 (Median 441.0, Minimum 153, Maximum 1141). Within three weeks the platelet counts were reduced to 68% of the diagnosis level. Thereafter the mean platelet number was maintained in a narrow range. The effect size indicates superiority of anagrelide at Month 6 compared to prestudy results (MW=0.7629, 95% CI from 0.6866 to 0.8392).

Table 21: Thrombocytes, Absolute values, Basic statistics, lTT

	PL-AM3DOLV	PL-D7LV	PL-D14LV	PL-D21LV	PL-D28LV
Mean	802.6	697.5	552.3	541.6	522.5
Std Dev	339.38	276.79	239.39	207.13	200.20
Min	335	237	63	79	173
Lo Quar	563.0	513.0	385.0	405.0	385.0
Median	743.0	598.0	529.0	514.0	491.0
Up Quar	950.0	873.0	695.0	673.0	626.0
Max	1912	1533	1210	1116	1158
Valid N	97	95	97	97	97
	PL-M2LV	**PL-M3LV**	**PL-M4LV**	**PL-M5LV**	**PL-M6LV**
Mean	515.1	502.4	473.3	484.8	486.1
Std Dev	178.28	185.91	176.37	192.50	193.25
Min	170	180	190	122	153
Lo Quar	384.0	387.0	351.0	350.0	361.0
Median	514.0	498.0	448.0	463.0	441.0
Up Quar	617.0	595.0	553.0	563.0	569.0
Max	924	1141	1141	1141	1141
Valid N	97	97	97	97	97

The total response rate in all treated patients was 77.3%. By means of the lower bound of the two-sided 95% CI (worst case) a response rate of 67.7% is “proven”. Some 79.4% of the patients showed partial response, which was defined as a final percent change from baseline of the thrombocytes of more than 50% in combination with a final level of the thrombocytes below 600.000/µL.

Table 22 below shows the results of the number of thrombocytes (G/l) at baseline and the various follow-up visits with for the ITT population in the subgroup of patients with polycythaemia and essential thrombocytosis (ET).

Table 22: Thrombocytes, Absolute values, Basic statistics, ITT, subgroup 'Polycythemia' versus subgroup' Essential Thrombocytosis.

	PL-AM3DOLV	PL-D7LV	PL-D14LV	PL-D21LV	PL-D28LV
PT					
Mean	755.3	687.7	632.4	635.7	553.4
Std Dev	156.49	201.07	204.24	190.75	172.68
Min	562	402	254	238	193
Lo Quar	641.0	537.0	504.0	561.5	449.0
Median	735.5	656.5	623.5	668.0	557.0
Up Quar	817.5	836.5	781.0	728.5	635.5
Max	1071	1122	1024	1061	925
Valid N	16	16	16	16	16
ET					
Mean	803.5	687.0	528.4	516.5	507.9
Std Dev	364.36	283.04	234.62	199.16	194.91
Min	335	237	63	79	173
Lo Quar	523.0	495.0	373.0	377.0	377.0
Median	729.0	593.0	526.0	479.0	474.0
Up Quar	950.0	853.0	654.0	623.0	626.0
Max	1912	1533	1210	1116	982
Valid N	79	77	79	79	79

In patients newly diagnosed with ET and polycythaemia vera the total response rate was 75%. In patients with previous cytoreductive therapy the baseline level was not only stabilised but could be further reduced: 59.4% of the patients in this subgroup showed further reduction of the thrombocytes by more than 20% and some 29% of the patients showed further reduction by more than 50% after six months of treatment with anagrelide.

Previous cytoreductive therapy had failed in 16 out of 69 patients (23.2%). After switching to treatment with Thromboreductin only 9 out of 69 (13.0%) failed. This indicates that anagrelide may be effective in patients who previously did not respond adequately to other cytostatic agents.

ET related complications when compared pre- to post-treatment were reduced from 25.0% to 13.5% (mean=0.5573, 95% CI from 0.5063 to 0.6083, p=0.0278), and from 5.2%

to 2.1% for major complications (mean=0.5156, 95% CI from 0.4886 to 0.5426, p=0.2568). For the subgroup, ET superiority could also be shown for major complications (mean=0.5256, 95% CI from 0.5005 to 0.5508, p=0.0455).

These data supported that Thromboreductin is an effective treatment for reducing platelet counts in newly diagnosed patients with myeloproliferative disorders and thrombocythaemia and that patients can be effectively and safely switched to Thromboreductin, if they are refractory, intolerant or not eligible for interferon-α or HU. Response rates for Thromboreductin are high, even if previous cytoreductive therapy has failed. Furthermore, it was found that Thromboreductin reduced the rate of ET-related clinical events in patients with ET. The rate of major events was very low, and comparable to the rate obtained with HU (Cortelazzo *et al*. 1995)

Comment: *The results of this exploratory Phase II Study AOP 02-007 showed that the rate of minor and major thrombohaemorrhagic events, following anagrelide (Thromboreductin) is very low. This indicated that anagrelide might have the same efficacy as HU with respect to reduction of thrombotic events. The low incidence of thrombohaemorrhagic events in the patients studied had major implications on the design of the Phase III trial. Since ET is an orphan disease it was decided to design a non-inferiority trial with platelet counts as the primary surrogate endpoint and to use additional criteria (selectivity and incidence of ET related minor and major events) as secondary endpoints to support the primary endpoint.*

ANAYHYDRET (AOP 03-007) Study

This Australian Public Assessment Report (AusPAR) does not include details of the ANAHYDRET study, a Phase III study comparing the efficacy and tolerability of anagrelide versus hydroxyurea monotherapies in patients with essential thrombocythaemia. This information was not included as the sponsor made the application to register Thromboreductin (anagrelide) before the Therapeutic Goods Information Specification 2009 (Information Specification) became law. For applications made after this date, the Information Specification gives the decision maker an express power to publish information about clinical studies such as ANAHYDRET study in an AusPAR.

Supportive studies

Patient Registry AOP 05-007

Efficacy and safety of Thromboreductin for long-term treatment of essential thrombocythaemia:

Treatment of ET requires long-term administration of a particular drug and therefore it was of particular importance to follow up patients to demonstrate long-term efficacy and safety. For this purpose a multinational patient registry was established (Study AOP 05-007) into which patients from short-term studies and patients treated with Thromboreductin after marketing authorisation were entered. The objective of the patient registry was to generate long-term efficacy and safety data. In particular, data on long-term platelet reduction and incidence of ET-related complications as well as long-term safety data in respect to organ function (renal, hepatic and cardiac safety) were gathered. As soon as a patient started a therapy with Thromboreductin the patient was entered into a data base. The patients were asked to contact their attending physician at three monthly intervals, but at least once a year for assessment. Thromboreductin was provided in containers of 100 capsules (0.5 mg each) as needed and patients were treated and assessed during routine clinical practice. In the submitted data included patients treated up to 5 years.

Endpoints

Patients were evaluated for long-term efficacy and safety by assessing the following parameters:

- Reduction of platelet counts
- Response rate
- Incidence of ET-related complications (for example, thromboembolic, ischaemic or haemorrhagic complications).
- Overall tolerability and safety of Thromboreductin as assessed by documentation of AEs
- Laboratory parameters for renal and liver function
- Treatment discontinuation
- Progression of the disease.

Statistical analyses

For statistical analyses the Wilcoxon-Mann-Whitney test was performed to compare groups. Results of proportion of patients with response, sustained response and complete response of thrombocytes were given. Evaluation of AEs was done in a descriptive way.

Study population

A total of 722 patients diagnosed with ET were entered into a patient registry and followed up for up to 5 years. Patients had a median age of 58 years (range 6 to 91 years). About 66% of the patients were female, 42.7% of patients were older than 60 year and 52.4% had previously been treated with cytoreductive agents. The main inclusion criteria were those defined for qualifying a patient to be at high risk; age >60 years, previous thrombohaemorrhagic events or cardiovascular risk factors.

Efficacy results

Thromboreductin reduced platelet counts effectively in previously untreated patients from a median baseline of 920.000/µL to 581.000/µL (after 3 months) and 382.000/µL (after 60 months). In pretreated patients median platelet counts were reduced from 608.000/µL to 502.000/µL (after three months) and 436.000/µL (after 60 months). The response rate (at least ≤ 600.000/µL on two occasions) was 67% (pretreated patients) and 64% (not pretreated patients). During the 5 year observation period, 65 – 100% of the patients had a platelet count of less than 600.000/µL in response to Thromboreductin treatment. The rate of complete response (at least <450.000/µL on one occasion per year) ranged between 58% and 71% (observation period Year 1 and Year 1-5, respectively). In pretreated patients the complete response rate was 71% which can be compared to 66% in patients not pretreated. The constancy of the response to Thromboreductin over the 5 year period was further confirmed by the analysis of all pretreated and not pretreated patients. Group comparisons revealed a significant response to Thromboreductin in patients below and above an age of 60 years, irrespective of prior treatment. These data also confirm that treatment response persists after treatment phases of up to 60 months.

ET- related complications

The rate of ET-related complications including bleeding was very low. Results showed a rate of 2.0% per 100 patients years for major ET-related events, providing evidence that long-term treatment of high risk patients with Thromboreductin decreases the risk status

of these patients from a high rate of thrombohaemorrhagic complications (> 15% per 100 patient years) to a low rate (2.0% per 100 patients years) (Cortelazzo *et al*. 1990).

Analysis of clinical information relevant to dosing recommendations

Daily medication with Thromboreductin as an orphan drug has been extensively investigated in a large number of patients receiving long term treatment up to 5 years (Study AOP 05-007). Earlier studies have revealed that an upper dose limit of 5 mg per day is sufficient and safe over six months (Studies AOP 02-007, AOP 03-007 (ANAHYDRET)). In Study AOP 02-007, the administered daily dose range never exceeded 4.5 mg.

In the patient registry (Study AOP 05-007), the median dose in pretreated patients was predominantly 2.0 mg/day but 1.5 mg/day in non pretreated patients (see Table 23 below).

Table 23: Median daily oral dose of Thromboreductin in patients with ET and a high risk profile over 5 years.

Study number	patients / volunteers	Number subjects	Median daily oral dose				
AOP 05-007	patients	722	Month 12	Month 24	Month 36	Month 48	Month 60
Patient registry	pre-treated patients	Median dose	1,5	2,0	2,0	2,3	2,0
March 19, 2007		n	187	106	66	32	21
	non pre-treated patients	Median dose	1,5	1,5	1,5	1,5	1,5
		n	187	75	31	17	6

For anagrelide, a starting oral dosage for adults of 0.5 mg to 1mg daily (US) is recommended. This dosage should be maintained for approximately one week and thereafter titrated individually to achieve a platelet count <600 x 10^9/L and ideally 150-400 x 10^9/L. The dose titration is recommended due to the blood pressure lowering effects of the drug and blood pressure returns to base line levels during maintenance therapy.

Evaluator's overall conclusions on clinical efficacy

The pivotal study was performed in accordance with relevant regulatory guidelines and clinically relevant outcome measures were examined.

Study AOP 03-007 failed to show confirmatory proof for non-inferiority of anagrelide versus HU with regard to the primary efficacy criteria of platelet count reduction. In Stage I, non-inferiority of anagrelide to hydroxyurea was not demonstrated. In relation to haemoglobin, neutrophils and ET-related complications the two treatments were shown to yield similar results.

In Stage II non-inferiority of anagrelide to hydroxyurea was also not demonstrated. In Stage II and with respect to haemoglobin, hydroxyurea was shown to be superior to anagrelide.

In the long term phase of the study, results for platelet reduction showed that anagrelide was inferior to HU. In addition anagrelide was shown to be inferior with respect to effects on haemoglobin levels.

Response rates in the short and long term phases were similar for anagrelide and HU.

With regard to an undesired reduction of neutrophils, the lower bounds of the conventional two-sided 95.0% confidence intervals were above the lower equivalence margin of -10.0% and also above the benchmark for equality, demonstrating superiority of the anagrelide group in Stage II with regard to neutrophil counts.

Based on the overall efficacy results it appears that treatment with anagrelide is less efficacious than treatment with HU. The data do not provide convincing support that Thromboreductin is an appropriate treatment for patients with ET.

Safety

Introduction

Data from 1111 patients treated with Thromboreductin were available to assess the AE and safety profile of this drug. The data comprise three Phase II studies (n=131), one Phase III study (n=258) and data from a patient registry (n=722) (Study AOP 05-007)).

Patient exposure

The exposure to Thromboreductin is presented in Tables 24 and 25.

Table 24: Exposure to Thromboreductin

Study number	Patients / Volunteers	Number subjects	Daily oral dose / subjects										Duration of treatment
			0,5 mg/d	1 mg/d	1,5 mg/d	2,0 mg/d	2,5 mg/d	3,0 mg/d	3,5 mg/d	4,0 mg/d	4,5 mg/d	5,0 mg/d	
AOP 01-007	patients	15	3	4	2	3	1	1	0	1			4 weeks
AOP 04-007	patients	18	0	0	8	5	2	3					4 weeks
AOP 03-01	volunteers	24				2							2 single doses
AOP 06-007	volunteers	16				16							single dose
SC03302	volunteers	16				16							single dose
AOP10-007	volunteers	2				2							single dose
AOP 02-007	patients	97		median at study begin Range 0.5–2.0 mg/d		median after 6 months Range 0.5–4.5 mg							6 months
AOP 03-007	patients	111		first week of treatment		Max. dose week 2						Max. dose	6 months

Table 25: Exposure to Thromboreductin in Study AOP 05-007

Study number	Patients / Volunteers	Number subjects	Median daily oral dose				
AOP 05-007	patients	722	Month 12	Month 24	Month 36	Month 48	Month 60
Patient registry	Pretreated patients	Median dose	1,5	2,0	2,0	2,3	2,0
March 19, 2007		n	187	106	66	32	21
	non pre-treated patients	Median dose	1,5	1,5	1,5	1,5	1,5
		n	187	75	31	17	6

Adverse events

Studies AOP 01-007 and AOP 04-007 (n=34)

In these short-term studies patients were switched from the anagrelide formulation Agrylin to Thromboreductin. Patients who had a therapy with Agrylin for at least 3 month

continued treatment at the same dose level with Thromboreductin for an additional 4 weeks.

In Study AOP 01-007 all patients showed AEs with headache being most common (four out of 15 patients). However, no serious or unexpected AEs were reported. In Study AOP 04-007 seven patients reported AEs. Again no serious or unexpected AEs were reported and headache was the most common AE (four out of 19 patients).

No serious or unexpected AEs occurred (during the four week period) after switching patients from the anagrelide formulation Agrylin to Thromboreductin at the same dose.

Study AOP 02-007 (n=97)

In total 329 AEs (serious and non-serious) were documented for 69 patients, including 28 serious adverse events (SAEs) in 21 patients. The most common recorded events were headache (10.9%), diarrhoea (5.6%) and palpitations (4.1%), mainly at the beginning of the therapy. Headache and palpitations may be due to the vasodilating and positive inotropic effects of Thromboreductin. Diarrhoea may be caused by the inactive ingredient lactose. Approximately 94% of the non-SAEs were rated as mild or moderate.

Patient Registry AOP 05-007 (n=722)

With regard to safety, as evaluated from data of 722 patients, there is no evidence that the AE profile changes when Thromboreductin is used long-term compared to short-term in clinical trials. There is no evidence that Thromboreductin increased rate of bleedings or disease progression. No substantial change of the safety laboratory parameters aspartate aminotransferase (AST), ALT and serum creatinine as indicators for liver and renal function were seen. During the 5 year observation period, spontaneous AEs were recorded for 24% of patients.

Serious adverse events and deaths

In total 13 deaths (three in Study AOP 02-007, one in Study AOP 03-007, and nine in Study AOP 05-007) were recorded.

Study AOP 02-007 (n=97)

A total of 28 SAEs were recorded. Among these, three deaths occurred in the study but none were related to the study drug. Four of the remaining 25 SAEs were possibly related and one was rated 'not judgeable': one congestive heart failure, one bleeding after bone marrow biopsy, one cerebral bleeding, one transient ischaemic attack (the latter three occurring in one patient), one severe transaminase increase (rated as non judgeable, because of a pre-existing hepatic vein thrombosis).

A dose modification was necessary after 13 serious/significant events (4%): in nine cases a temporary reduction of the dose was performed whereas in four cases the drug was permanently discontinued.

All three deaths in Study AOP 02-007 were rated as "not related" to the study drug. One patient died after a surgical embolectomy (aneurysm), one after a major cerebral haemorrhage/heart death (the patient had a history of significant bleeding tendency) and another because of an embolic event in the lung.

Patient Registry AOP 05-007 (n=722)

One SAE was reported (acute coronary syndrome). Treatment discontinuations as a result of AEs were low. About 5% of the patients discontinued treatment with Thromboreductin because of medical reasons which included no response to treatment or a negative risk/benefit judgement of the treating physician. Among these 12 patients (1.7%)

discontinued because of cardiac events/negative cardiac benefit risk analysis only four patients discontinued Thromboreductin due to a disease progression.

A small number of patients (12 out of 722, 1.7%) died during the five years of observation. Three deaths were already recorded in Study AOP 02-007 and were not related to the study drug. The remaining nine cases were also not causally linked to Thromboreductin. One patient died because of general bad status. One patient died after a fall and one after a brain stem insult with ischaemic changes/vascular encephalopathy. The causes for the other deaths were one due to bilateral pneumonia, one cerebral haemorrhage, one increase in anaemia with existing oedema/ulcus cruris and cardiac insufficiency, one cardiac decompensation, one bronchial carcinoma and one thrombosis of the spleen.

By way of comparison, in the literature a total of 50 deaths have been reported in a total of 942 patients (Petitt *et al*. 1997): 5 % of the patients died during the 10-year study. According to the authors "no deaths appeared directly related to anagrelide, but in seven instances limited reporting makes it impossible to completely exonerate the drug". In a German ET study the deaths of three patients out of 48 also were not therapy-related. One patient died as a consequence of a surgical procedure, one because of heart failure and one because of a stroke (Petrides *et al*. 1998). In the Australian study (Mills *et al*. 1999) there was one death due to cerebral haemorrhage. Here again, it is not possible to assign a causal relationship with anagrelide (Agrylin) therapy.

Laboratory findings

The key clinical laboratory data evaluation across all relevant studies is summarised in Table 26 below. There were three major findings:

- No clinically relevant or significant changes for any parameter were found in studies up to 4 weeks.
- The two long term studies (six months) showed significant changes which were categorised to be either of no clinical relevance (Study AOP 02-007) or of minor clinical relevance (Study AOP 03-007).
- In the largest patient cohort treated up to 5 years (Study AOP 05-007, patient registry) no changes in any relevant parameter could be detected.

Notable changes in laboratory parameters are discussed below and are reflected in the proposed PI for Thromboreductin; close monitoring of kidney and liver function, white and red blood cell counts and for haemoglobin are requested in the draft PI.

Table 26: Clinical laboratory data evaluation across studies

Study number	Patients / Volunteers	Number subjects	
AOP 01-007	Patients	15	No relevant or significant changes
AOP 04-007	Patients	18	No relevant or significant changes
AOP 03-01	Volunteers	24	two single doses
AOP 06-007	Volunteers	16	single dose
SC03302	Volunteers	16	single dose
AOP10-007	Volunteers	2	single dose
AOP 02-007	Patients	97	Significant but clinically not relevant changes in severall parameters (refer to discussion)
AOP 03-007	Patients	111	significant but clinically non relevant changes are given below; all other parameters remained unchanged
AOP 05-007 Patient registry	Patients	722	No substantial changes in safety laboratory parameters

Study AOP 02-007

All mean values, except those for leukocytes were within normal ranges at any time of the six month study period. Leukocyte levels were already increased at Day 0 and there was a further increase of up to 5% during the study period. During the study there was a significant decrease of haemoglobin levels and MCV%, as well as a significant decrease in erythrocyte levels at Day 14. This indicated a small effect on erythropoiesis. However, the decrease in haemoglobin and MCV% was less than 5% and was rated as clinically not relevant. Other statistically significant effects included increases in creatinine and alkaline phosphatase, decreases in ALT, total serum protein, albumin and total bilirubin.

The electrolyte levels showed a statistically significant decrease in potassium and calcium and a significant increase in chloride. Due to the design of the study (open-label, non-comparative) it is not possible to determine whether the observed effects are due to study medication, underlying disease or any concomitant therapy. All the observed effects were judged to be of no clinical relevance as the changes were less than 10% throughout the study period and remained in the normal range. The exception was leucocyte counts, which were elevated already at baseline.

Safety in special populations

Cardiac Safety

Anagrelide exhibits positive inotropic activity (refer to information about PDE-3 activity) and may therefore cause concern with respect to cardiac safety, in particular those of congestive heart failure, AV-block or atrial fibrillation. However, since ET is a disease which is *per se* associated with thrombotic events like myocardial infarction, it often may be difficult to differentiate a drug related event from a disease related event.

In Studies AOP 02-007 and AOP 03-007, patients with cardiac diseases Grade 4 (Common Toxicity Criteria, 1992[11]) were excluded. Patients suffering from cardiac diseases Grade 3 were included only after a careful risk/benefit analysis on behalf of the investigator.

A total of eight cases of cardiac events associated with SAEs/deaths have been observed in Studies AOP 02-007 and AOP 03-007. Three out of these eight had a "possible" causal relationship to drug intake (congestive heart failure, supraventricular tachycardia and heart infarction). Five cases in which the relationship was rated as "unlikely" included intracerebral bleeding with heart death (n=1), AV block with symptomatic bradycardia (n=1), subacute myocardial infarction (n=1), antero-lateral myocardial infarction (n=1) and coronary disease (n=1).

In the patient registry (AOP 05-007), one SAE with likely relationship to Thromboreductin intake was recorded (acute coronary syndrome). Furthermore a total of eight reported cardiac events led to discontinuation of Thromboreductin therapy, because of negative benefit/risk analyses (one case denoted in the patient registry as a cardiac event has already been included in the section about Stage II of Study AOP 03-007 (heart infarction)). The narrative of these events supports the fact, that treating physicians used a benefit/risk analysis according to the data sheet for their decision to switch therapy. In this context the switch to another cytoreductive therapy (namely HU) was documented for three patients. Another four patients with cardiac events leading to discontinuation were recorded in an updated discontinuation list. One of these patients was switched to Peg-Interferon, one to HU. In the other four discontinuations which were related to cardiac events (retrosternal pain (n=1), heart failure (n=1), right heart failure (n=1) and myocardial infarction (n=1)), therapy failure was recorded.

The recommended approach is not to give anagrelide to patients with known cardiovascular problems. Ideally, every patient should undergo thorough cardiac examination prior to therapy with anagrelide. Careful attention should be paid to inform doctors about this potential side effect, to enable and ensure a proper benefit/risk assessment. This approach has been successful in the past and is documented in the patient registry (Study AOP 05-007), where several patients were taken off the drug following negative cardiac risk assessment.

Renal/hepatic function

Limited data are available describing the use of anagrelide in patients with impaired renal function. According to the patient registry (Study AOP 05-007) Thromboreductin seems to be without concern for long-term use. One publication raised a single case with renal tubular injury after treatment with Agrylin (Rodwell *et al*. 2005): the marker for renal function, plasma creatinine, was already elevated before the administration of Agrylin, which renders the causal connection at least doubtful.

Data on patients with hepatic impairment are also limited. It is reported that anagrelide plasma levels increased 8-fold in patients with moderate liver impairment (FDA MedWatch, 2005). This accumulation was possibly caused by the inability of the liver to metabolise the drug.

[11]Common Toxicity Criteria (CTC) is a standardised classification of side effects used in assessing drugs. Specific conditions and symptoms may have values or descriptive comment for each level, but the general guideline is 1 – Mild, 2 – Moderate, 3 – Severe, 4 - Life threatening, 5 - Death.

Use in Children

There is only limited data on the safety of use in children. Only eleven children have been treated with Thromboreductin so far.

Concomitant use with ASA

ASA plays a role in preventing ET related symptoms like erythromelagia (Van Genderen 1997). No remarkable effects on platelet aggregation were observed in patients at anagrelide doses used in humans. Nevertheless an increased incidence of bleeding was observed in Study AOP 02-007 when Thromboreductin was administered concomitantly with ASA and compared to monotherapy. The use of ASA in combination with anagrelide is not recommended unless suggested by the occurrence of specific symptoms which are known to clearly respond to ASA, together with a positive benefit/risk assessment.

Transformation/leukaemogenic effects

In the current clinical program no patient in the Phase II and III studies showed transformation to leukaemia within six months. However, four patients in the patient registry transformed to myeloid metaplasia after a mean treatment time of 23.4 months.

It is unlikely that anagrelide has a comparative potential to HU and causes transformation, including development of acute leukaemia. Data have shown that anagrelide in contrast to HU does not cause dysplastic changes of megacaryocytopoiesis. In addition, anagrelide also does not stimulate fibrosis (Thiele *et al*. 2006).

In addition to the data presented in the current Australian submission, several publications regarding anagrelide are available and these include data on transformation and development of leukaemia. Following treatment up to 15 years, four out of 30 patients developed myelofibrosis (Petrides 2006), and two out of 30 patients when treated for 12.5 years (Mazzucconi *et al*. 2004). A large cohort of patients treated for up to 7 years was retrospectively analysed by Fruchtman *et al*. (2005); only 47 out of 2251 patients (2.1%) developed acute leukaemia but all had been pretreated with other drugs.

It is difficult to compare the submitted data with data reported in the publications above because of differences regarding diagnostic standards, patients (whether pretreated or not) and data collection (for example, prospective versus retrospective data).

Post marketing experience

Post marketing surveillance data are essentially provided from the patient registry (Study AOP 05-007) which has included 722 patients. Extended post marketing surveillance data were provided from the Periodic Safety Update Report (PSUR) from December 2005 to March 2009. Some 15 patients with adverse events were reported. No unknown adverse events emerged.

Evaluator's overall conclusions on clinical safety

The main short- and long term AEs associated with treatment with anagrelide include headache, palpitations, tachycardia, dizziness, oedema or chest pain. Lactose intolerance may cause diarrhoea, abdominal pain, dyspepsia or flatulence. Rare but serious adverse events in patients treated with Thromboreductin included myocardial infarction, congestive heart failure, complete heart block or atrial fibrillation. In patients with known cardiovascular risk factors Thromboreductin should not be used. Cardiovascular examination and careful monitoring is recommended in all individuals with presumed cardiac problems or an age over of 60 (Petrides *et al*. 2004). In patients with cardiac risk factors Thromboreductin is not recommended or should only be recommended when the risk/benefit-ratio favours its use.

A major issue with long-term treatment of patients with thrombocythaemia is a potential risk of induced leukaemogenesis. This is important since the spontaneous transformation of primary thrombocythaemia into acute leukaemia is very low. The potential benefit of a cytoreductive therapy has to be weighed against the risk of leukaemogenic transformation. The leukaemogenic potential of HU is considered to be in the range of 5 to 10 %. This has not been observed for anagrelide up to now in previously untreated patients. Some 1111 patients have been exposed to Thromboreductin for up to 5 years, and no unexpected new AEs have been reported in the literature. However, caution is advised in patients with pre-existing or emerging cardiac diseases and attention should be paid to patients with renal and hepatic impairment.

List of Questions

During 2010, the TGA began to change the way applications were evaluated. As part of this change, after an initial evaluation, a "list of questions" to the sponsor is generated.

The following question was posed by the clinical evaluator:

Pharmacokinetics

The sponsor should be asked to clarify whether levels of anagrelide and its metabolites measured in the clinical studies were peak, trough or random samples.

Clinical Summary and Conclusions

The bioequivalence study showed that the two formulations tested (Thromboreductin and Agrylin) are different products which may result in differences in platelet reducing efficacy and tolerability. Furthermore, this study showed that the two formulations of Thromboreductin (0.5 mg and 1.0 mg) are dose proportional.

The PK data showed that peak plasma concentrations for anagrelide were reached within 1.38 hours followed by a rapid decline with an elimination half-life of 1.38 hours. Peak plasma values for 3-Hydroxyanagrelide were slightly delayed emerging at 2.14 hours. Its elimination half-life was 1.79 hours being slightly longer compared to anagrelide. In addition, peak plasma levels of 2-amino-5,6-dichloro-3,4-dihydroquinazoline were reached after 2.22 hours with an elimination half-life 3 to 4 times longer than that of anagrelide and 3-Hydroxyanagrelide (6.35 hours).

The data showed that anagrelide and the 2 emerging main metabolites are unlikely to accumulate in plasma. However, the data as assessed by the standard deviation showed a high inter-subject variability of plasma levels for all three compounds.

Data from Studies AOP 01-007 and 04-007 showed that plasma levels of anagrelide and its metabolite are comparable in patients aged >65 years and < 65 years; PK results for the two subsets of patients did not seem to vary significantly with age. The main pharmacological actions of anagrelide are platelet reduction, which is the therapeutic target, and PDE-3 inhibition. The precise molecular basis of the platelet reducing effect is still unknown. This is at least partly due to the fact that the direct platelet lowering effect can only be studied in humans. Studies on human megakaryocytes grown in culture show that anagrelide interferes with the maturation of these platelet precursors (Mazur *et al*. 1992, Solberg *et al*. 1997). Both anagrelide and 3-Hydroxyanagrelide inhibit PDE-3 activity (Wang *et al*. 2005) an enzyme which is contained in for example, cardiomyocytes and vascular tissue. PDE-3 mediates inactivation of c-AMP. As a consequence it protects the human organism from excessive adrenergic stimuli which may be harmful long-term. Indeed, in laboratory animals anagrelide was shown to be a potent inotropic and vasodilatory agent (Kastner *et al*. 1985). Recently it was found that 3-Hydroxyanagrelide has a 40-fold higher potency for PDE-3 inhibition than anagrelide and therefore is the main contributor in mediating cardiovascular effects (Wang *et al*. 2005).

The pivotal efficacy and safety study (Study AOP03-007) was performed in accordance with relevant regulatory guidelines and clinically relevant outcome measures were examined. Study AOP 03-007 failed to show confirmatory proof for non-inferiority of anagrelide versus HU with regard to the primary efficacy criteria platelet reduction. In Stage I, non-inferiority of anagrelide to hydroxyurea was not demonstrated. In relation to haemoglobin, neutrophils and ET-related complications the two treatments were shown to yield similar results.

In Stage II, non-inferiority of anagrelide to HU was also not demonstrated. In addition, in Stage II hydroxyurea was shown to be superior to anagrelide in relation to effects on haemoglobin.

In the long term phase of the study results for platelet reduction showed that anagrelide was inferior to HU. In addition anagrelide was shown to be inferior with respect to effect on haemoglobin.

Response rates in the short and long term phases were similar for anagrelide and HU.

With regard to an undesired reduction of neutrophils, the lower bounds of the conventional two-sided 95.0% confidence intervals were above the lower equivalence margin of -10.0% and also above the benchmark for equality demonstrating superiority of the anagrelide group in Stage II with regard to neutrophil count.

Based on the overall efficacy results, it appears treatment with Thromboreductin is less efficacious than treatment with HU. The data do not provide convincing support that anagrelide is appropriate treatment for patients with ET.

The main short- and long-term AEs associated with treatment with anagrelide include headache, palpitations, tachycardia, dizziness, oedema or chest pain. Lactose intolerance may cause diarrhoea, abdominal pain, dyspepsia or flatulence. Rare but serious adverse events in patients treated with Thromboreductin included myocardial infarction, congestive heart failure, complete heart block or atrial fibrillation. In patients with known cardiovascular risk factors Thromboreductin should not be used. Cardiovascular examination and careful monitoring is recommended in all individuals with presumed cardiac problems or an age over of 60 (Petrides *et al*. 2004). In patients with cardiac risk factors Thromboreductin is not recommended or should only be recommended when the risk/benefit-ratio favours its use.

Some 1111 patients have been exposed to Thromboreductin for up to 5 years, and no unexpected new AEs have been reported in the literature. However, caution is advised in patients with pre-existing or emerging cardiac diseases and attention should be paid to patients with renal and hepatic impairment.

Benefit risk assessment

Benefits

Thromboreductin has been shown to be efficacious in reducing platelet counts with a response rate in the range of 60 to 80%. Thromboreductin does reduce ET related clinical events in high risk patients suffering from thrombocythaemia. However anagrelide has not been shown to be non-inferior to HU with respect to platelet reduction. Results also did not consistently confirm noninferiority of anagrelide with respect to effects on haemoglobin. Results from the pivotal study are not highly persuasive of superior efficacy of anagrelide over HU which is used off label to treat patients with essential thrombocythaemia.

Risks

Adverse effects of anagrelide are mainly caused by its positive inotropic and vasodilatory effects and include headache, palpitations, tachycardia, dizziness, oedema or chest pain. Rare but observed SAEs were myocardial infarction, congestive heart failure, complete heart block or atrial fibrillation. Thus, in patients with known cardiovascular risk factors Thromboreductin should only be used after thorough benefit/risk evaluation. A cardiovascular examination and careful monitoring is recommended in all individuals with presumed cardiac problems or an age over 60. Limited data are available in patients with renal or hepatic impairments.

The concomitant use of Thromboreductin and ASA is not recommended unless specifically indicated (and carefully monitored) because of an increased bleeding tendency.

A Risk Management Plan was provided. There were no notable deficiencies.

Balance

The clinical evaluator considered that in high risk patients suffering from ET related complications the efficacy of Thromboreductin has not been adequately established. Non-inferiority to HU, the current standard therapy was not shown. It is true that Thromboreductin may have a more favourable safety profile overall than HU with respect to long-term leukaemogenicity and the occurrence of neutropaenia and its consequences. However, in the absence of convincing data supporting efficacy, concerns remain that the benefit/risk profile for Thromboreductin is not positive.

Conclusions

Thromboreductin is a novel formulation of anagrelide, which has been shown to reduce elevated platelet counts in individuals with ET, who require cytoreductive therapy. However, in a pivotal Phase III study the efficacy of anagrelide was shown to be inferior to HU which itself is not approved for treatment of essential thrombocythaemia. It is therefore considered that it is premature to approve anagrelide for treatment of ET based on the data currently submitted for evaluation.

Recommended Conditions for Registration

On the basis of the data presented for evaluation it is recommended that this application to register a new chemical entity, anagrelide hydrochloride, with the trade name Thromboreductin should be rejected.

V. Pharmacovigilance Findings

Risk Management Plan (RMP)

The RMP (dated 1 July 2010) provided for evaluation was considered unacceptable particularly relating to the provision of inadequate safety related data, a lack of information about a patient registry study and the absence of identification of important safety related concerns and appropriate pharmacovigilance and risk minimisation assessments.

The sponsor provided an updated RMP (that included the recommended safety concerns. This updated document was reviewed and considered acceptable for the current submission, however it was recommended that with the next RMP the sponsor be advised to adhere to the EU guidelines (volume 9A) regarding the content of the RMP. For example, in the sponsors assessment of important safety concerns, it is not considered adequate to just state that these are listed in the SmPC and CSP. It was also recommended that in the RMP the important potential risk stated as "*leukaemogenisis in treatment with*

cytoreductive agents, such as hydroxyuria" be changed to just "*leukaemogenesis*" as the issue is one of close monitoring of the potential for leukaemic transformations in patients treated with Thromboreductin.

In the sponsors responses reference is made to EU Product Safety Update Report (PSUR) assessment, 2 August 2010 regarding the safety related concerns. This assessment was reviewed and the following was highlighted.

In the revised Australian RMP the sponsor has committed to close monitoring with discussion and comments in the next PSUR for hepatic disorders, vasculitis, pulmonary fibrosis/interstitial lung disease, as well as areas of benign or malignant neoplasms, thrombohaemorrhagic events and increase in BNP (brain natriuric peptide) in cardiac adverse drug reactions (ADRs). The EU assessment of the PSUR has requested the following additional monitoring/points to be addressed in the next PSUR and it would be reasonable that the sponsor provide the same to the TGA in future PSURs:

- A follow-up analysis regarding assessment of causality/relationship between anagrelide and pulmonary congestion.
- Close monitoring and cumulative review including literature review for skin ulcers, severe allergic skin reaction sand progression to myelofibrosis.
- Cumulative reviews for lack of efficacy, use in special populations (elderly patients), drug interaction and overdose.

The OPR reviewer also made a number of recommendations concerning the proposed PI and Consumer Medicine Information documents but these are beyond the scope of this AusPAR

VI. Overall Conclusion and Risk/Benefit Assessment

The submission was summarised in the following Delegate's overview and recommendations:

Quality

Multiple deficiencies have been identified in the chemistry, manufacturing and quality control data. The deficiencies include:

- the proposed limits for drug substance particle size do not provide good control of particle size distribution;
- the proposed 1 mg capsule contains a 3% overage and this has not been adequately justified;
- the sponsor is claiming some modified release characteristics for the formulation (see 'Issues' below). However, the pharmaceutical chemistry characteristics that result in such modified release have not been identified;
- the dissolution test method proposed by the sponsor is inadequate to ensure consistent manufacture of the product;
- the proposed shelf lives for the product are not supported by the submitted data.

The quality evaluator did not support registration.

The Pharmaceutical Subcommittee (PSC) considered the application at its November 2010 meeting. The Subcommittee recommended rejection of the application due to the

numerous deficiencies in the quality data. The PSC also considered that a food effect study should be required.

Nonclinical

Limited nonclinical data were submitted. The nonclinical evaluator commented that the toxicological profile of anagrelide has been well-characterised through previous evaluations of the currently registered Agrylin product and that the excipients in the Thromboreductin formulation were unremarkable. Repeat dose toxicity studies in rats with the Thromboreductin formulation did not identify any novel toxicities.

The nonclinical evaluator had no objections to registration.

Clinical

The clinical evaluator has recommended rejection of the application on efficacy grounds. The clinical evaluator's recommendation is discussed further below.

Pharmacokinetics (PK)

Limited PK data were presented for the new product.

Anagrelide is metabolically cleared with two main metabolites produced:

- 2-amino-5,6-dichloro-3,4-dihydroquinazol-2-ylamine (RL603), which is generally considered to be inactive, although nonclinical data in the current submission suggested that it may have some platelet-lowering effect; and
- 3-hydroxy anagrelide, which is equipotent with anagrelide in terms of platelet-lowering effect, and 40-fold more potent than anagrelide in terms of PDE-3 inhibition.

Inhibition of PDE-3 is thought to be the mechanism of action for many of the adverse effects of anagrelide (such as vasodilatation, tachycardia).

Study AOP-06-007 examined the PK of anagrelide and its two main metabolites after a single 2 mg dose in 16 healthy volunteers.

Study AOP-02-007 examined the PK of anagrelide in 30 subjects with various myeloproliferative disorders. Samples were collected at 7 days and at 3 months. It was unclear from the study report whether the plasma levels obtained were peak, trough or random sample values.

Study AOP-07-007 compared the bioavailability of Thromboreductin with that of the marketed Agrylin product in healthy volunteers. The study compared the 0.5 and 1 mg strengths of both products in a 4-period crossover design. All subjects received a single 2 mg dose in each period. The study demonstrated that the Thromboreductin product has a lower bioavailability than the Agrylin product, with AUC values for anagrelide being approximately 15% lower.

The study also compared the two products with respect to plasma concentrations of the *inactive* metabolite. The Thromboreductin product produced lower levels of this metabolite. Concentrations of the active metabolite were not measured.

The study also examined dose proportionality of the proposed 0.5 and 1.0 mg strengths. Dose proportionality of the 0.5 and 1.0 mg formulations was demonstrated with respect to anagrelide but <u>not</u> with respect to the inactive metabolite.

Efficacy – Phase II studies

The submission included two small Phase II trials in which subjects who were on a stable dose of Agrylin were switched to the same dose of Thromboreductin and followed for 4 weeks. Subjects had one measurement of platelet count at baseline (Day 0) while on Agrylin and then at weekly intervals while on Thromboreductin.

- *Study AOP-01-007* enrolled 15 subjects with essential thrombocythaemia. Equivalence was to be concluded if the 95% CI for the change in mean platelet count over the 4 week period fell entirely within the interval of ± 150 $x10^9$. The observed change was a decrease of 35 x 10^9 (from 530 to 495 x 10^9). The 95% CI for the change was from a decrease of 100 $x10^9$ to an increase of 31 $x10^9$. Of note, one subject had a significant *increase* in platelet count after switching formulations (from 361 to 672 x 10^9).
- *Study AOP-04-007* enrolled 19 subjects with thrombocythaemia due to various causes. Equivalence was to be concluded if the 95% CI for the change in mean platelet count over the 4 week period fell entirely within the interval of ± 150 $x10^9$. The observed change was a decrease of 25 x 10^9 (from 513 to 488 x 10^9). The 95% CI for the change was from a decrease of 86 $x10^9$ to an increase of 35 $x10^9$. Again of note, one subject (patient ID 3003) had a significant *increase* in platelet count after switching formulations (from 435 to 749 x 10^9). Two subjects required dose increases after the switch.

Although these two studies are described as 'bioequivalence' studies, they cannot be considered adequate to demonstrate pharmacodynamic bioequivalence, as they only measured platelet count once while the patients were receiving Agrylin. A properly conducted PD bioequivalence trial would have compared AUC of the platelet count versus time curve with both treatments having been administered for an adequate time period.

One other open uncontrolled Phase II study (AOP-02-007) was included in the submission. The study enrolled patients with myeloproliferative disorders, considered to be at high risk due to their platelet counts. Subjects were treated with Thromboreductin and followed for six months. A total of 97 subjects were enrolled, of whom 79 had essential thrombocythaemia. Platelet counts decreased after initiation of Thromboreductin and the decreased levels were maintained with ongoing treatment.

Efficacy – Pivotal Phase III study

The submission included one pivotal randomised controlled trial (*AOP-03-007* aka the ANAHYDRET study). The study has not been published in a peer reviewed journal but has been presented as a conference abstract[12].

In this prospective randomised single blind international multicenter phase III study efficacy and tolerability of anagrelide and hydroxyurea monotherapies were compared in high risk ET patients diagnosed according to the WHO classification. The study was designed as a non-inferiority trial as the limited number of treatment naïve ET patients available and the expected low number of ET related events following treatment with a cytoreductive therapy would not have allowed the conduct of a conventional superiority trial. After informed consent 258 treatment naïve, high risk patients with ET were randomized to receive either anagrelide (n=122) or hydroxyurea (136). The patients characteristics were equally distributed within both arms with a median age of 58,1 years,

[12] Gisslinger H *et al* (2007). Non-Inferiority of Anagrelide Compared to Hydroxyurea in Newly Diagnosed Patients with Essential Thrombocythemia: The ANAHYDRET-Study. *Blood* (ASH Annual Meeting Abstracts) 2007 110: Abstract 3547

range 19-90 years; 46 male, 76 female in the anagrelide arm vs. a median age of 56,4 years, range 22-83 years, 47 male, 89 female in the hydroxyurea arm. Anagrelide (Thromboreductin) was started with a dose of 1mg/day and hydroxyurea was initiated using a dose of 1500mg/day.

A central blinded pathologic review of 236 bone marrow biopsies revealed a high reproducibility of the ET diagnosis by applying the WHO classification: 194 (82.2%) of patients were classified as ET, 16 patients (6,8%) were reclassified as PMF-0 and 16 patients (6,8%) were considered to be early PVs with an ET-like clinical phenotype. Confirmatory proof of non-inferiority was achieved after a mean observation time of 2,1 years (comprising 539 patient years) based on predefined equivalence criteria for platelet counts, course of haemoglobin levels and white cell count during therapy, and for the rate of ET related events. In the anagrelide arm 75,4% of the patients received a complete response of platelet counts (<450x109/l) compared to 81,7% in the hydroxyurea arm. Neutrophil counts remained unchanged in the anagrelide arm but were significantly reduced by hydroxyurea.

Safety

Approximately 253 subjects received Thromboreductin in the Phase II and III studies. The submission also included data from a Phase IV patient registry (Study AOP-05-007) which enrolled a further 722 subjects.

No significant differences were observed for the rates of major and minor clinical complications in the anagrelide group (4,29%, and 16,8%, respectively) compared to hydroxyurea (4,25%, and 12,8%, respectively). During the whole study period 11 major ET related complications occurred in the anagrelide group (5 arterial events, 2 venous thrombotic complications and 4 bleedings) and 12 major events were seen in the hydroxyurea arm (5 arterial events, 5 venous thrombotic events and 2 bleedings). 43 minor ET related events occurred in the anagrelide arm as compared to 36 such events in the hydroxyurea arm. Adverse drug reactions or poor response were reasons for discontinuations of the study drug in 19 patients treated with anagrelide and in 10 patients treated with hydroxyurea. Transformations to myelofibrosis were not reported during the whole study period. The JAK2 mutation status was evaluated in 189 patients with 101 JAK2 positive (53,4%) and 88 (46,6%) JAK2 negative patients.

The toxicities observed with Thromboreductin in the Phase II and Phase IV studies were consistent with those previously documented for anagrelide.

Risk Management Plan

The sponsor submitted an RMP for the product which was evaluated by the TGA's Office of Product Review (OPR). The deficiencies identified by the OPR evaluator have been addressed by the sponsor and the revised RMP was considered acceptable.

Risk-Benefit Analysis

Delegate Considerations

1. Efficacy

The clinical evaluator has recommended rejection of the application on the grounds that non-inferiority to hydroxyurea could not be concluded. Thromboreductin failed to meet the predefined non-inferiority criterion in each of the two stages of the pivotal trial. However, using the predefined statistical methods for combining the two stages, the sponsor concluded that non-inferiority was established. The clinical evaluator has also drawn attention to a slightly higher complete response rate with hydroxyurea. The

sponsor has provided a response to the clinical evaluation report, a copy of which is included in the agenda papers.

The Delegate considered that it is not essential to establish non-inferiority against hydroxyurea in order to demonstrate acceptable efficacy of the product, for the following reasons:

- The clinical data in the submission demonstrate that the use of Thromboreductin reduces platelet count compared to baseline to a clinically significant extent.
- A published randomised controlled trial comparing the other anagrelide product marketed in Australia (Agrylin) with hydroxyurea (the PT-1 trial) also found that hydroxyurea was more effective in reducing platelet count over the first six months of treatment.
- The choice of either anagrelide or hydroxyurea by a treating physician is likely to also depend on the safety profile of the two drugs (for example, anagrelide is more likely to be used in a younger patient because of concerns regarding mutagenicity with hydroxyurea). Even if the efficacy of Thromboreductin is less than that of hydroxyurea, an anagrelide product may still be preferred in some clinical settings.

The choice of hydroxyurea as the comparator agent in the Phase III trial is questionable. The trial began in 2003 and the Agrylin product has been registered in Australia, the USA and Canada since at least 1999. In the Australian context, this application is basically one for approval of a version of an already-marketed drug, supported by clinical rather than bioequivalence data. A more appropriate comparison for the Phase III trial would have been a comparison of Thromboreductin versus Agrylin. *In their pre-ACPM response, the sponsor is requested to justify the choice of hydroxyurea as the comparator agent, rather than the Agrylin formulation of anagrelide.*

2. Non-bioequivalence with Agrylin

The data submitted establish that Thromboreductin is not bioequivalent with Agrylin on PK criteria, with AUC for anagrelide being approximately 15% lower. As indicated above, the submitted data are also not adequate to establish that the two formulations are therapeutically equivalent. If both are marketed in Australia simultaneously, it is possible that patients will be switched between formulations. Such a transfer may require re-titration of dose. This issue could possibly be handled by inclusion in the PI of statements regarding non-interchangeability of formulations and the need to re-titrate dose on transfer. The sponsor agreed to the inclusion of the requested passage in the draft PI.

There is also a lack of data on how the two formulations compare with respect to the active metabolite, which is equipotent to anagrelide in terms of anti-platelet effect, and 40-fold more potent in terms of PDE-3 inhibition. If the Thromboreductin formulation were to produce higher systemic concentrations of the active metabolite, this could result in an inferior safety profile.

3. Effect of food

The submission **c**ontains the following statements:

> "*Emphasis was put on the development of a formulation, which would moderately delay the release of Anagrelide in order to favour drug tolerability. "*
>
> and
>
> *"Thromboreductin is a novel non-immediate release formulation of Anagrelide, which has been shown to effectively reduce elevated platelet counts in individuals with ET, who require cytoreductive therapy. "*

The sponsor is therefore claiming that the Thromboreductin formulation has some modified release characteristics. If this were true, it would be important to establish the effect of co-administration with food on the release of anagrelide from the product, as recommended by the PSC. No food effect study was included in the current Australian submission. A food effect study has been conducted for the Agrylin formulation and this is described in the draft PI submitted for Thromboreductin. However the findings of this study may not be applicable to Thromboreductin.

4. Dose proportionality of 0.5 and 1.0 mg strengths

The submitted data demonstrate that the two strengths of Thromboreductin are bioequivalent with respect to anagrelide concentrations. However, they are not bioequivalent with respect to the inactive metabolite, with the 0.5 mg strength producing an approximately 20% greater AUC. This raises the possibility that the two strengths may not be bioequivalent with respect to the active metabolite, which is equipotent with anagrelide in terms of platelet-lowering effect, and 40-fold more potent than anagrelide in terms of PDE-3 inhibition. Switching between the two strengths in clinical practice may be associated with unpredictability in terms of efficacy and side effects.

The Delegate proposed to reject the application on the following grounds:

- the multiple deficiencies identified in the chemistry, manufacturing and quality control data;
- inadequate comparative safety and efficacy data with the formulation of anagrelide already marketed in Australia;
- absence of a study on the effects of food on the purported modified release characteristics of the formulation;
- concerns regarding non-dose proportionality of the two strengths of the product with respect to the active metabolite.

The advice of the ACPM was requested.

Response from Sponsor

Sponsor's response to the quality evaluation

Some difference in the drug substance or capsule formulation or manufacture of the proposed Thromboreductin capsules causes them to have clearly lower bioavailability than the registered Agrylin capsules. The difference has not been identified and thus cannot be controlled. The lack of bioequivalence between the two formulations of anagrelide may not be necessarily due to a specific difference in the drug substance or capsule. Various differences between the products may contribute to their different release profile, such as quantitative composition of non-active ingredients, ratio of active versus non active ingredients, and the manufacturing process per se. Since AOP Pharma which developed Thromboreductin neither know the quantitative composition nor the manufacturing process of Agrylin, the extent to which these factors contribute is unknown. However, the fact that Thromboreductin is not bioequivalent to Agrylin is unimportant because the safety and efficacy of Thromboreductin in the proposed indication have been independently demonstrated. There is no impact on the clinical use of Thromboreductin. Anagrelide treatment naïve patients have to be titrated to dose. Patients may also be switched between formulations. Upon switching, the dose may need to be titrated accordingly.

Taking the above into consideration, the manufacturing controls and dissolution testing are considered adequate to ensure supply of Thromboreductin capsules with drug release properties matching those used in clinical trials.

Sponsor's response to the Delegate's overview:

The proposed limits for drug substance particle size do not provide good control of particle size distribution. These limits have been tightened.

Comment: The proposed 1 mg capsule contains a 3% overage and this has not been adequately justified.

Answer: *The sponsor withdrew their application for this strength in the course of their Pre-ACPM response.*

C: The sponsor is claiming some modified release characteristics for the formulation (see "Issues" below). However, the pharmaceutical chemistry characteristics that result in such modified release have not been identified.

A*: In vitro experiments performed by AOP Pharma during development of Thromboreductin demonstrated that the release of anagrelide from Thromboreductin was slower than that from Agrylin. The sponsor does not claim modified release characteristics for Thromboreductin. Quality data of the submission provides for an immediate release capsule formulation. Therefore there are not particular pharmaceutical chemistry characteristics that result in a modified release formulation. See above.*

C: The dissolution test method proposed by the sponsor is inadequate to ensure consistent manufacture of the product.

A: *The quality evaluator believed that the dissolution method proposed is inadequate because the evaluator believes Thromboreductin to be a modified release product, and the difference in release profile between the two products may have a clinical impact. As Thromboreductin is an immediate released capsule, and the different release profile has no clinical impact due to the manner of administration of the product, the proposed dissolution method is believed to be adequate to ensure a product of consistent quality. There has been over 9 years of post marketing experience with Thromboreductin overseas.*

C: The proposed shelf lives for the product are not supported by the submitted data.

A: *The sponsor believed that the proposed shelf life for the product is supported by the submitted data. Nevertheless, the sponsor is willing to discuss a reduced shelf life with the TGA if necessary.*

Phase II studies

C: The submission included two small Phase II trials in which subjects who were on a stable dose of Agrylin were switched to the same dose of Thromboreductin and followed for 4 weeks. Subjects had one measurement of platelet count at baseline (Day 0) while on Agrylin and then at weekly intervals while on Thromboreductin…Although these two studies are described as 'bioequivalence' studies, they cannot be considered adequate to demonstrate pharmacodynamic bioequivalence…

A: *The sponsor agreed the term "bioequivalence studies" is inappropriate. Unfortunately this term was used in the protocol by the statistician who designed the study. These studies nevertheless demonstrate that patients can be safely switched*

from Agrylin® to Thromboreductin using short (weekly) follow up periods to assess the patients for platelet control.

One other open uncontrolled Phase II study (AOP-02-007 was included in the current Australian submission. The study enrolled patients with myeloproliferative disorders (MPD) that were considered to be at high risk due to their platelet counts. Subjects were treated with Thromboreductin and followed for six months. A total of 92 subjects were enrolled, of whom 79 had essential ET.

Safety

The Delegate commented that the toxicities observed with Thromboreductin in the Phase II and IV studies were consistent with those previously documented for anagrelide. In addition, since the Thromboreductin birth date of 20 November 2001, there has been over 9 years of post marketing experience. Adverse events (AEs) associated with Thromboreductin are well defined, predictable, and no different from those associated with Agrylin. There are no new AEs. Although there is lack of bioequivalent to Agrylin, both products have the same active ingredient and similar excipients commonly found in immediate release capsules.

Issues: Efficacy

C: The Delegate considered that it was not essential to establish non-inferiority against hydroxyurea (HU) in order to demonstrate acceptable efficacy of the product. A published randomised controlled trial comparing the other anagrelide product marketed in Australia (Agrylin) with HU (the PT-l trial) also found that HU was more effective in reducing platelet count over the first 6 months of treatment.

A: *The PT-1 trial[13] included pre-treated (including HU) patients with ET diagnosed according to the PVSG criteria, and which has been severely criticized in respect to design and conduct (see EPAR Xagrid/Agrylin, EMA 2005[14]). These diagnostic criteria are outdated since it makes it difficult to exclude patients with prefibrotic idiopathic myelofibrosis, polycythaemia vera and other forms of MPD[15]. The PT- 1 trial included 30 % of patients diagnosed with ET according to WHO criteria[16]; and 70 % with MPD. It is essential to use WHO criteria (2002) for diagnosis, since differentiation of "true" ET from other MPDs like idiopathic myelofibrosis is possible only by histopathology[17]. Hence all previous clinical studies (such as the MRC/PT-1*

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13 Harrison CN *et al*. (2005). Hydroxyurea Compared with Anagrelide in High-Risk Essential Thrombocythemia. *N Engl J Med* 353:33-45.

14http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000480/human_med_001153.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058001d124

15 Thiele J *et al.* (2009). Bone Marrow Fibrosis and Diagnosis of Essential Thrombocythemia. *Journal of Clinical Oncology* 27(34):e220-e221.

16 Imbert M, Pierre R, Thiele J, Vardiman JW, Brunning RD, Flandrin G. Essential thrombocythaemia. In: Jaffe ES, Harris NL, Stein H, et al, editors. WHO classification of tumours: tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2001. p. 39-41.

17 Thiele and Kvasnicka (2001). Clinicopathology and histochemistry on bone marrow biopsies in chronic myeloproliferative disorders – a clue to diagnosis and classification *Pathol Biol* 49:140-7.

17 Thiele and Kvasnicka (2001). Clinicopathology and histochemistry on bone marrow biopsies in chronic myeloproliferative disorders – a clue to diagnosis and classification *Pathol Biol* 49:140-7.

19 Petrides PE *et al.* (1997). Anagrelide (Agrylin) Pharmacological profile and clinical use. *Onkologe* 3:298-302 (German).

study) lacked proper diagnosis, because patients recruited might have suffered from other MPD entities; and results do not adequately reflect treatment effects in ET and must not be extrapolated to ET patients. The ANAHYDRET trial is the only prospective comparative trial in a study population diagnosed according to WHO criteria, and demonstrates non inferiority of Thromboreductin versus HU based on the statistical hypothesis in patients diagnosed according to WHO. Therefore the sponsor believed it was not appropriate to compare the results of these two trials in the context of this regulatory submission.

C: The choice of hydroxyurea as the comparator agent in the Phase III trial is questionable. The trial began in 2003 and the Agrylin product has been registered in Australia, the USA and Canada since at least 1999.

A: *The use of HU as the comparator agent in a pivotal trial is justified; it is considered standard therapy for ET in clinical practice (Clinical Evaluation). While Agrylin was registered in the USA/Canada without a pivotal comparator trial (despite HU was the state of the art and standard therapy based on a randomised trial published in 1995[18]), in Europe, the EMA had initially approved anagrelide only as a second line indication (Xagrid / Agrylin) where standard therapy with HU failed. The PT1 trial, despite its limitations, also reinforced the role of HU in patients diagnosed according to PSVG criteria. However, neither the role of HU nor Agrylin in patients diagnosed according to WHO was defined, so it was absolutely appropriate to study Thromboreductin versus HU in the ANAHYDRET trial, rather than compare Thromboreductin versus Agrylin. The sponsor agreed that long term use with HU may raise concerns in respect to mutagenicity, but there is an ongoing debate on this.*

Additionally, a direct comparison of Thromboreductin versus Agrylin would have required hundreds of patients to test for meaningful differences in efficacy and safety parameters, a task difficult to achieve in orphan diseases.

C: **The choice of either anagrelide or HU by a treating physician is likely to also depend on the safety profile of the two drugs … the efficacy of Thromboreductin is less than that of HU…**

A: The sponsor agreed that the choice of either anagrelide or HU by a treating physician is likely to depend on the safety profile of the two drugs. We do not support the assessor's statement that Thromboreductin's efficacy is less than that of HU, because non-inferiority has been demonstrated and the reduction of platelet count is similar to that of HU.

C: Non-bioequivalence with Agrylin. The safety and efficacy of Thromboreductin have been demonstrated by clinical studies; bioequivalence with Agrylin should not be a requirement for registration of the product.

A: *The sponsor agreed that it is possible that patients will be switched between formulations and upon switching, slight dose modifications may be necessary and that this information and the draft PI has been modified accordingly. The safety record of Thromboreductin, however, after 9 years of use does not give any evidence that the safety profile might be worse compared to Agrylin (see PSUR).*

[19] Petrides PE *et al.* (1997). Anagrelide (Agrylin) Pharmacological profile and clinical use. *Onkologe* 3:298-302 (German).

C: Effect of food on the PK of Thromboreductin.

> ***A:*** *Thromboreductin is an immediate release formulation with the normal excipients for products of its kind. Although Thromboreductin demonstrates a slight delayed onset of the active anagrelide when compared to Agrylin, it is not a modified release formulation. Food intake does not influence bioavailability of anagrelide[19], in Agrylin, as measured by AUC and it is believed these results are generalisable to Thromboreductin. Therefore there are no restrictions on the timing of food intake for the patients in a clinical trial.*

Advisory Committee Considerations

The ACPM, having considered the evaluations and the Delegate's overview, as well as the sponsor's response to these documents, agreed with the Delegate's proposal.

The ACPM recommended rejection of the submission from Orphan Australia Pty Ltd to register the new chemical entity anagrelide hydrochloride (Thromboreductin).
In making this recommendation, the ACPM considered that insufficient evidence of manufacturing and quality control were provided.
Multiple deficiencies were identified in this application, especially in the light of it being submitted as a new chemical entity. In addition, there were very limited safety and efficacy data submitted for this product. The committee noted that bioequivalence with the currently registered product was not being claimed, however, the committee was concerned that patients would be switched between products without due attention to titration. The absence of data on the effect of food was also considered a deficiency.

Outcome

Based on a review of quality, safety and efficacy, TGA decided to reject the registration of Thromboreductin (anagrelide HCl) for essential thrombocythaemia at 0.5-5 mg/day PO.

Final Outcome

Following the initial decision described above, the sponsor sought a review under the provisions of Section 60 of the Therapeutics Goods Act. The Delegate of the Minister for the review noted that paragraph 25(1)(d) of the Therapeutic Goods Act, which requires the goods to be evaluated with regard to whether the quality, safety and efficacy of the goods for the purposes for which they are to be used have been satisfactorily established, is of particular relevance.

The following is an excerpt from the Delegate of the Minister's report.

Safety

Orphan Australia claims:

> Thromboreductin has been demonstrated to have similar safety and efficacy to Agrylin by AOP 04-007. Thromboreductin has also been shown to have equivalent efficacy to hydroxyurea, the standard treatment of ET in many countries including Australia. The main difference is Thromboreductin demonstrates lower systemic availability (AUC) of anagrelide compared to Agrylin, the relevance of which is unknown as there is no clinical evidence that systemic availability (AUC) is directly correlated with reduction of platelet counts.

Study AOP 04-007 was a study designed to prove pharmacodynamic bioequivalance of a new formulation of anagrelide (Thromboreductin 0.5mg capsules to the existing

[19] Petrides PE *et al.* (1997). Anagrelide (Agrylin) Pharmacological profile and clinical use. *Onkologe* 3:298-302 (German).

formulation of anagrelide (Agrylin 0.5mg capsules) with respect to maintaining platelet counts from the end of Thromboreductin therapy to the end of Agrylin therapy. It included only 19 patients of a planned 35 patients and 9 patients had not completed the study on Day 28. Although the response rates for Agrylin and Thromboreductin were comparable -14 of 18 patients responded to Argylin and 17 of 18 to Thromboreductin- the response rates were calculated against historical data on the platelet count at diagnosis and were therefore only a rough estimate. Data on the platelet count during the follow up period were not undertaken following the study and therefore were not available to the Delegate. The design and implementation of study was assessed as deficient in meeting the claimed objectives.

Therefore, based on consideration of Study AOP 04-007, the Delegate was not satisfied that Orphan Australia had demonstrated that Thromboreductin was equivalent to Agrylin in terms of its safety profile. Accordingly, data in relation to the safety of Agrylin was not relevant to the Delegate's consideration of this matter. As a result, there was currently insufficient evidence to satisfy him of the safety profile of Thromboreductin because the clinical studies were designed to compare Thromboreductin to Agrylin, rather than demonstrate the safety of Thromboreductin in its own right, as a new chemical entity.

Further, based on the evidence available to the Delegate, Thromboreductin was not bioequivalent and had a different rate of absorption to Agrylin that is currently approved in Australia for the broader indication of the treatment of thrombocythaemia associated with a range of myeloproliferative disorders which include essential thrombocythaemia. As a result, the Delegate agreed with the Clinical Delegate and the ACPM that since bioequivalence had not been shown and there was a delay in the absorption of Thromboreductin compared to Agrylin, this presented a risk to intended therapeutic outcomes and toxicity in clinical practice should there be an interchange between two approved formulations of anagrelide with different bioavailability and absorption characteristics.

The Delegate concurred with the views of the Clinical Delegate and the ACPM that food does have clinical effect on the bioavailability in anagrelide compared to Thromboreductin and the difference in bioavailability between Agrylin and Thromboreductin, and therefore, the effect on the platelet count would be unpredictable. The Delegate also agreed with the Clinical Delegate and the ACPM that this may not be adequately compensated by supervision of the titration of the dose of anagrelide hydrochloride by the treating clinicians and the Delegate did not consider the risk acceptable.

For a period of up to five years it is reported that 1111 patients have been exposed to Thromboreductin. The rate of adverse events is reported to be 25% and the pattern did not include any unexpected new adverse events recognised with the administration of anagrelide hydrochloride. The data indicate that the more serious reported adverse reactions include cardiac events, 8 cases were observed in Studies AOP 02-007 and AOP 03-007 of which 3 were considered to have causal relationship. In the patient registry AOP 05-007 it is reported that there were a total of 8 cardiac events that led to the discontinuation of Thromboreductin. Another 4 patients with cardiac events leading to discontinuation of therapy were recorded in an updated discontinuation list. It is clear that careful assessment of a patient's cardiovascular status is required when therapy of essential thrombocythaemia with Thromboreductin contemplated.

On the available evidence the pattern of adverse events for Thromboreductin appear consistent with that of Agrylin but a valid direct comparison between the two formulations could not be made on the limited evidence made available. Therefore, the Delegate was not satisfied that data generated that was relevant to Agrylin also applied to Thromboreductin.

Since Orphan Australia had applied for Thromboreductin to be included in the ARTG as a new chemical entity, there was an expectation for a full safety (and efficacy) study in respect of Thromboreductin. Accordingly, the Delegate was not satisfied that Orphan Australia had established the safety of Thromboreductin for the purpose for which it is to be used.

Quality

In deciding whether the quality of Thromboreductin for the purposes for which it is to be used had been satisfactorily established, the following 2 matters are relevant:

- Compliance with the Code of Good Manufacturing Practice for prescription medicines; and
- A compilation of scientific evidence that validates the methods used to assure quality of the manufacturing process.

The Delegate was satisfied that Orphan Australia had complied with the Code of Good Manufacturing Practice for prescription medicines because Orphan Australia had provided evidence of a current licence of compliance issued by an overseas competent authority recognised by the TGA.

The Delegate, however, was not satisfied that Orphan Australia had provided sufficient scientific evidence to validate the methods used to assure quality of the manufacturing process.

The outcome of the evaluation of the methods used were detailed in the "Evaluation of Quality and Biochemical Data" by the Pharmaceutical Chemistry Evaluator dated 03 November 2010.

In that report, a number of deficiencies were identified so a request was made to Orphan Australia by the Pharmaceutical Chemistry Evaluator seeking further information under s31 of the Act.

The evaluation of Orphan Australia's responses to the section 31 request of 03 November 2010 by the Pharmaceutical Chemistry Evaluator dated 17 December 2010 shows acceptance of 7 of Orphan Australia's responses. However, after consideration of the remaining 10 responses from Orphan Australia, the Pharmaceutical Chemistry Evaluator summarised in the conclusion of the assessment report that bioequivalence with Agrylin had not been shown, the proposed testing method of the product was not considered ideal, dissolution performance was not well controlled and variable, there was not acceptable control of batch testing which was considered to be poor so that future consistency of the product could not be assured. As a result, the Pharmaceutical Chemistry Evaluator recommended rejection of the application.

The Delegate agreed that it would have been procedurally appropriate for Orphan Australia to have been provided with sufficient time to respond to the s31 prior to any recommendations going to the PSC. The PSC only considered the report of the Pharmaceutical Chemistry Evaluator at the meeting on the 22 November 2010. The PSC did not have the responses of Orphan Australia or the evaluation of those responses by Pharmaceutical Chemistry Evaluator. It was noted that Orphan Australia subsequently responded to the s 31 request on 29 November 2010 (within the appropriate timeframe).

However, the Delegate was satisfied that the conclusions of the Pharmaceutical Chemistry Evaluator arising from the assessment of Orphan Australia's response appeared consistent with the recommendation of the PSC of the 22 November 2010. This was despite the fact that the PSC not having Orphan Australia's response and the Pharmaceutical Chemistry Evaluator's assessment of these responses at the time of the meeting.

As a result, there was no evidence to suggest to the Delegate that the outcome would have been different if the PSC had before it the responses of Orphan Australia to the s31 request or the evaluation of those responses by Pharmaceutical Chemistry Evaluator.

Based on the above, the Delegate was not satisfied that Orphan Australia had established the quality of Thromboreductin for the purpose for which it is to be used. The Delegate was also not satisfied that the manufacturing and quality control procedures used in the manufacture of Thromboreductin were acceptable.

Efficacy

Orphan Australia has relied on Study AOP 03-007 as a pivotal study in support of demonstrating efficacy.

In the view the Delegate, Study AOP 03-007 should be viewed as supportive data rather than a pivotal contribution to the application. It confirmed that Thromboreductin does reduce the platelet count but the Delegate agreed with the assessment of the clinical evaluator that the study failed to show confirmatory proof of non-inferiority of anagrelide compared to hydroxyurea with regard to the primary efficacy criteria, that is, platelet reduction. The evaluator also noted that hydroxyurea is not approved for the treatment of essential thrombocythaemia. The evaluator concluded that Thromboreductin is a novel non-immediate release formulation of anagrelide (which is disputed by the Sponsor). There was evidence to demonstrate that Thromboreductin reduces platelet count, but there was insufficient evidence to demonstrate that it was more effective than hyroxyurea, which was what the trial was designed to do.

The Delegate also considered Study AOP 02-007. Study AOP 02-007 enrolled 97 newly diagnosed patients with myeloproliferative disorders that were started on or switched to Thromboreductin. The study showed that Thromboreductin was effective in lowering the platelet count in 75% of patients. However this trial included patients in a broader category (that is, other related myeloproliferative disorders) than merely the indication of the application for essential thrombocythaemia. The Delegate agreed with the clinical evaluator's conclusion that Thromboreductin may be effective in patients who previously did not respond adequately to other cytostatic agents.

The relevant evidence (clinical data) showed that Thromboreductin does reduce the platelet count; however, the level of evidence was compromised because of the design and implementation of the clinical trial. The Delegate considered that it was important that non-inferior efficacy be established for approval as a primary treatment.

It should be noted that essential thrombocythaemia is a slowly progressive disorder with long asymptomatic periods punctuated by thrombotic or hemorrhagic events. Treatment regimes can reduce and control the number of platelets, which reduces the risk of thrombotic or haemorrhagic events. The lifespan of a person with well controlled essential thrombocythaemia is well within the expected range for a person of similar age without essential thrombocythaemia. It is for this reason that treatment with anagrelide is usually considered as second line therapy in those patients who have failed or no longer show responses to primary cytoreductive therapies. As has been previously stated this is a rare clinical circumstance and there is already a well characterised anagrelide product in Australia available for the treatment of these patients.

Conclusion

For the reasons discussed above, the Delegate was of the view that Orphan Australia had not established to the Delegate's satisfaction the quality, safety and efficacy of the product, Thromboreductin (anagrelide hydrochloride) 0.5mg & 1.0mg Capsules for the purposes for which it to be used. Accordingly, the Delegate decided to confirm the initial decision to

reject the application to register Thromboreductin (anagrelide hydrochloride) 0.5mg & 1.0mg Capsules in the ARTG. The Delegate was also not satisfied that the manufacturing and quality control procedures used in the manufacture of Thromboreductin were acceptable for the reasons outlined above.

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