CENTER FOR DRUG EVALUATION AND RESEARCH

Approval Package for:

APPLICATION NUMBER:

NDA 20-333/S-013

Name: Agrylin® Capsules

Sponsor: Shire Development, Inc.

Approval Date: June 17, 2008

This "Changes Being Effected" supplemental new drug application provides for revisions to the Carcinogenesis, Mutagenesis, Impairment of Fertility subsection of the PRECAUTIONS section.

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 20-333/S-013

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CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 20-333/S-013

APPROVAL LETTER

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

NDA 20-333/S-013

Shire Development, Inc.
Attention: Zohra Lomri
Associate Director, Global Regulatory Affairs
725 Chesterbrook Blvd.
Wayne, PA 19087

Dear Ms. Lomri:

Please refer to your supplemental new drug application dated June 13, 2007, received June 13, 2007, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Agrylin® (anagrelide hydrochloride) Capsules.

We acknowledge receipt of your submissions dated December 4, 2007 and June 13, 2008.

This "Changes Being Effected" supplemental new drug application provides for revisions to the **Carcinogenesis, Mutagenesis, Impairment of Fertility** subsection of the **PRECAUTIONS** section.

We completed our review of this application, as amended. This application is approved, effective on the date of this letter, for use as recommended in the agreed-upon labeling text.

The final printed labeling (FPL) must be identical to the enclosed labeling (text for the package insert) and/or submitted labeling (package insert submitted June 13, 2008).

Please submit an electronic version of the FPL according to the guidance for industry titled *Providing Regulatory Submissions in Electronic Format - NDA*. Alternatively, you may submit 20 paper copies of the FPL as soon as it is available but no more than 30 days after it is printed. Individually mount 15 of the copies on heavy-weight paper or similar material. For administrative purposes, designate this submission "**FPL for approved supplement NDA 20-333/S-013**." Approval of this submission by FDA is not required before the labeling is used.

If you issue a letter communicating important information about this drug product (i.e., a "Dear Health Care Professional" letter), we request that you submit a copy of the letter to this NDA and a copy to the following address:

MEDWATCH
Food and Drug Administration
Suite 12B05
5600 Fishers Lane
Rockville, MD 20857

Please submit one market package of the drug product when it is available.

We remind you that you must comply with reporting requirements for an approved NDA (21 CFR 314.80 and 314.81).

If you have any questions, call Hyon-Zu Lee, Pharm.D., Regulatory Project Manager, at 301-796-2050.

Sincerely,

{See appended electronic signature page}

Rafel Dwaine Rieves, M.D.
Director
Division of Medical Imaging and Hematology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research

Enclosure

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Rafel Rieves

6/17/2008 05:36:57 PM

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

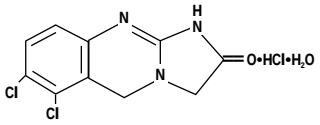
NDA 20-333/S-013

LABELING

AGRYLIN®
(anagrelide hydrochloride)
Capsules
Rx only

DESCRIPTION

Name: AGRYLIN® (anagrelide hydrochloride)
Dosage Form: 0.5 mg capsules for oral administration
Active Ingredient: AGRYLIN® Capsules contain 0.5 mg of anagrelide base (as anagrelide hydrochloride).
Inactive Ingredients: Anhydrous Lactose NF, Crospovidone NF, Lactose Monohydrate NF, Magnesium stearate NF, Microcrystalline cellulose NF, Povidone USP.
Pharmacological Classification: Platelet-reducing agent.
Chemical Name: 6,7-dichloro-1,5-dihydroimidazo[2,1-b]quinazolin-2(3H)-one monohydrochloride monohydrate.
Molecular formula: $C_{10}H_7Cl_2N_3O \cdot HCl \cdot H_2O$
Molecular weight: 310.55
Structural formula:



Appearance: Off-white powder.

Solubility:		
	Water	Very slightly soluble
	Dimethyl Sulfoxide	Sparingly soluble
	Dimethylformamide	Sparingly soluble

CLINICAL PHARMACOLOGY

The mechanism by which anagrelide reduces blood platelet count is still under investigation. Studies in patients support a hypothesis of dose-related reduction in platelet production resulting from a decrease in megakaryocyte hypermaturation. In blood withdrawn from normal volunteers treated with anagrelide, a disruption was found in the postmitotic phase of megakaryocyte development and a reduction in megakaryocyte size and ploidy. At therapeutic doses, anagrelide does not produce significant changes in white cell counts or coagulation parameters, and may have a small, but clinically insignificant effect on red cell parameters. Anagrelide inhibits cyclic AMP phosphodiesterase III (PDEIII). PDEIII inhibitors can also inhibit platelet aggregation. However, significant inhibition of platelet aggregation is observed only at doses of anagrelide higher than those required to reduce platelet count.
Following oral administration of ^{14}C-anagrelide in people, more than 70% of radioactivity was recovered in urine. Based on limited data, there appears to be a trend toward dose linearity between doses of 0.5 mg and 2.0 mg. At fasting and at a dose of 0.5 mg of anagrelide, the plasma half-life is 1.3 hours. The available plasma concentration time data at steady state in patients showed that anagrelide does not accumulate in plasma after repeated administration.
Two major metabolites have been identified (RL603 and 3-hydroxy anagrelide).

There were no apparent differences between patient groups (pediatric versus adult patients) for t_{max} and $t_{1/2}$ for anagrelide, 3-hydroxy anagrelide, or RL603.

Pharmacokinetic data obtained from healthy volunteers comparing the pharmacokinetics of anagrelide in the fed and fasted states showed that administration of a 1 mg dose of anagrelide with food decreased the C_{max} by 14%, but increased the AUC by 20%.
Pharmacokinetic (PK) data from pediatric (age range 7-14 years) and adult (age range 16-86 years) patients with thrombocythemia secondary to a myeloproliferative disorder (MPD), indicate that dose- and body weight-normalized exposure, C_{max} and AUC_{τ}, of anagrelide were lower in the pediatric patients compared to the adult patients (C_{max} 48%, AUC_{τ} 55%).
A pharmacokinetic study at a single dose of 1 mg anagrelide in subjects with severe renal impairment (creatinine clearance <30ml/min) showed no significant effects on the pharmacokinetics of anagrelide.
A pharmacokinetic study at a single dose of 1 mg anagrelide in subjects with moderate hepatic impairment showed an 8-fold increase in total exposure (AUC) to anagrelide.

CLINICAL STUDIES

A total of 942 patients with myeloproliferative disorders including 551 patients with Essential Thrombocythemia (ET), 117 patients with Polycythemia Vera (PV), 178 patients with Chronic Myelogenous Leukemia (CML), and 96 patients with other myeloproliferative disorders (OMPD), were treated with anagrelide in three clinical trials. Patients with OMPD included 87 patients who had Myeloid Metaplasia with Myelofibrosis (MMM), and 9 patients who had unknown myeloproliferative disorders.

Clinical Studies

Patients with ET, PV, CML, or MMM were diagnosed based on the following criteria:

ET
- Platelet count ≥ 900,000/μL on two determinations
- Profound megakaryocytic hyperplasia in bone marrow
- Absence of Philadelphia chromosome
- Normal red cell mass
- Normal serum iron and ferritin and normal marrow iron stores

CML
- Persistent granulocyte count ≥ 50,000/μL without evidence of infection
- Absolute basophil count ≥ 100/μL
- Evidence for hyperplasia of the granulocytic line in the bone marrow
- Philadelphia chromosome is present
- Leukocyte alkaline phosphatase ≤ lower limit of the laboratory normal range

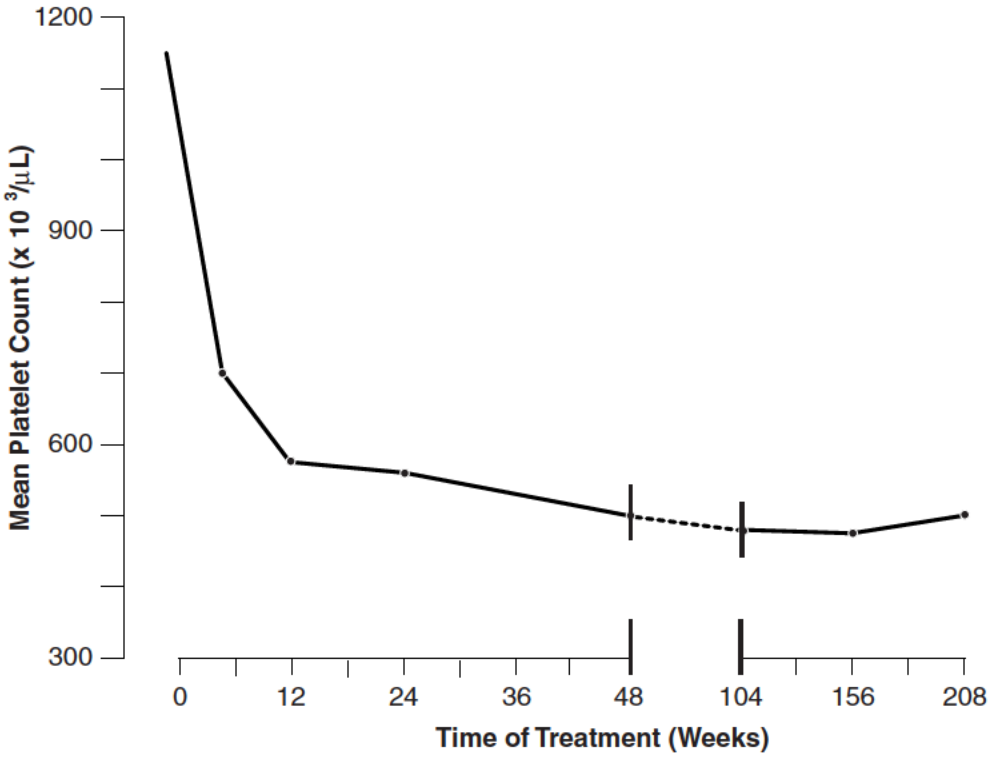
PV†
- A1 Increased red cell mass
- A2 Normal arterial oxygen saturation
- A3 Splenomegaly
- B1 Platelet count ≥ 400,000/μL, in absence of iron deficiency or bleeding
- B2 Leukocytosis (≥ 12,000/μL, in the absence of infection)
- B3 Elevated leukocyte alkaline phosphatase
- B4 Elevated serum B_{12}

† Diagnosis positive if A1, A2, and A3 present; or, if no splenomegaly, diagnosis is positive if A1 and A2 are present with any two of B1, B2, or B3.

MMM
• Myelofibrotic (hypocellular, fibrotic) bone marrow
• Prominent megakaryocytic metaplasia in bone marrow
• Splenomegaly
• Moderate to severe normo-chromic normocytic anemia
• White cell count may be variable; (80,000-100,000/μL)
• Increased platelet count
• Variable red cell mass; teardrop poikilocytes
• Normal to high leukocyte alkaline phosphatase
• Absence of Philadelphia chromosome

Patients were enrolled in clinical trials if their platelet count was ≥ 900,000/μL on two occasions or ≥ 650,000/μL on two occasions with documentation of symptoms associated with thrombocythemia. The mean duration of anagrelide therapy for ET, PV, CML, and OMPD patients was 65, 67, 40, and 44 weeks, respectively; 23% of patients received treatment for 2 years. Patients were treated with anagrelide starting at doses of 0.5-2.0 mg every 6 hours. The dose was increased if the platelet count was still high, but to no more than 12 mg each day. Efficacy was defined as reduction of platelet count to or near physiologic levels (150,000-400,000/μL). The criteria for defining subjects as "responders" were reduction in platelets for at least 4 weeks to ≤600,000/μL, or by at least 50% from baseline value. Subjects treated for less than 4 weeks were not considered evaluable. The results are depicted graphically below:

Patients with Thrombocytosis Secondary to Myeloproliferative Disorders: Mean Platelet Count During Anagrelide Therapy



	Time on Treatment							
		Weeks				Years		
	Baseline	4	12	24	48	2	3	4
Mean*	1131	683	575	526	484	460	437	457
N	923†	868	814	662	530	407	207	55

*x $10^3/\mu L$

† Nine hundred and forty-two subjects with myeloproliferative disorders were enrolled in three research studies. Of these, 923 had platelet counts over the duration of the studies.

AGRYLIN® was effective in phlebotomized patients as well as in patients treated with other concomitant therapies including hydroxyurea, aspirin, interferon, radioactive phosphorus, and alkylating agents.

INDICATIONS AND USAGE

AGRYLIN® Capsules are indicated for the treatment of patients with thrombocythemia, secondary to myeloproliferative disorders, to reduce the elevated platelet count and the risk of thrombosis and to ameliorate associated symptoms including thrombo-hemorrhagic events (see **CLINICAL STUDIES**, **DOSAGE AND ADMINISTRATION**).

CONTRAINDICATIONS

Anagrelide is contraindicated in patients with severe hepatic impairment. Exposure to anagrelide is increased 8-fold in patients with moderate hepatic impairment (see **CLINICAL PHARMACOLOGY**). Use of anagrelide in patients with severe hepatic impairment has not been studied (see also **WARNINGS: Hepatic**).

WARNINGS

Cardiovascular

Anagrelide should be used with caution in patients with known or suspected heart disease, and only if the potential benefits of therapy outweigh the potential risks. Because of the positive inotropic effects and side-effects of anagrelide, a pre-treatment cardiovascular examination is recommended along with careful monitoring during treatment. In humans, therapeutic doses of anagrelide may cause cardiovascular effects, including vasodilation, tachycardia, palpitations, and congestive heart failure.

Hepatic

Exposure to anagrelide is increased 8-fold in patients with moderate hepatic impairment (see CLINICAL PHARMACOLOGY). Use of anagrelide in patients with severe hepatic impairment has not been studied. The potential risks and benefits of anagrelide therapy in a patient with mild and moderate impairment of hepatic function should be assessed before treatment is commenced. In patients with moderate hepatic impairment, dose reduction is required and patients should be carefully monitored for cardiovascular effects (see DOSAGE AND ADMINISTRATION for specific dosing recommendations).

Interstitial Lung Diseases

Interstitial lung diseases (including allergic alveolitis, eosinophilic pneumonia and interstitial pneumonitis) have been reported to be associated with the use of anagrelide in post-marketing reports. Most cases presented with progressive dyspnea with lung infiltrations. The time of onset ranged from 1 week to several years after initiating anagrelide. In most cases, the symptoms improved after discontinuation of anagrelide (See ADVERSE REACTIONS).

PRECAUTIONS

Laboratory Tests: Anagrelide therapy requires close clinical supervision of the patient. While the platelet count is being lowered (usually during the first two weeks of treatment), blood counts (hemoglobin, white blood cells), liver function (SGOT, SGPT) and renal function (serum creatinine, BUN) should be monitored.
In 9 subjects receiving a single 5 mg dose of anagrelide, standing blood pressure fell an average of 22/15 mm Hg, usually accompanied by dizziness. Only minimal changes in blood pressure were observed following a dose of 2 mg.
Cessation of AGRYLIN® Treatment: In general, interruption of anagrelide treatment is followed by an increase in platelet count. After sudden stoppage of anagrelide therapy, the increase in platelet count can be observed within four days.
Drug Interactions: Limited PK and/or PD studies investigating possible interactions between anagrelide and other medicinal products have been conducted. *In vivo* interaction studies in humans

have demonstrated that digoxin and warfarin do not affect the PK properties of anagrelide, nor does anagrelide affect the PK properties of digoxin or warfarin.

Although additional drug interaction studies have not been conducted, the most common medications used concomitantly with anagrelide in clinical trials were aspirin, acetaminophen, furosemide, iron, ranitidine, hydroxyurea, and allopurinol. There is no clinical evidence to suggest that anagrelide interacts with any of these compounds.

An *in vivo* interaction study in humans demonstrated that a single 1mg dose of anagrelide administered concomitantly with a single 900 mg dose of aspirin was generally well tolerated. There was no effect on bleeding time, PT or aPTT. No clinically relevant pharmacokinetic interactions between anagrelide and acetylsalicylic acid were observed. In that same study, aspirin alone produced a marked inhibition in platelet aggregation *ex vivo*. Anagrelide alone had no effect on platelet aggregation, but did slightly enhance the inhibition of platelet aggregation by aspirin.

Anagrelide is metabolized at least in part by CYP1A2. It is known that CYP1A2 is inhibited by several medicinal products, including fluvoxamine, and such medicinal products could theoretically adversely influence the clearance of anagrelide. Anagrelide demonstrates some limited inhibitory activity towards CYP1A2 which may present a theoretical potential for interaction with other co-administered medicinal products sharing that clearance mechanism e.g. theophylline.

Anagrelide is an inhibitor of cyclic AMP PDE III. The effects of medicinal products with similar properties such as inotropes milrinone, enoximone, amrinone, olprinone and cilostazol may be exacerbated by anagrelide.

There is a single case report which suggests that sucralfate may interfere with anagrelide absorption.

Food has no clinically significant effect on the bioavailability of anagrelide.

Carcinogenesis, Mutagenesis, Impairment of Fertility: In a two year rat carcinogenicity study a higher incidence of uterine adenocarcinoma, relative to controls, was observed in females receiving 30mg/kg/day (at least 174 times human AUC exposure after a 1mg twice daily dose). Adrenal phaeochromocytomas were increased relative to controls in males receiving 3mg/kg/day and above, and in females receiving 10mg/kg/day and above (at least 10 and 18 times respectively human AUC exposure after a 1mg twice daily dose). Anagrelide hydrochloride was not genotoxic in the Ames test, the mouse lymphoma cell (L5178Y, $TK^{+/-}$) forward mutation test, the human lymphocyte chromosome aberration test, or the mouse micronucleus test. Anagrelide hydrochloride at oral doses up to 240 mg/kg/day (1,440 mg/m^2/day, 195 times the recommended maximum human dose based on body surface area) was found to have no effect on fertility and reproductive performance of male rats. However, in female rats, at oral doses of 60 mg/kg/day (360 mg/m^2/day, 49 times the recommended maximum human dose based on body surface area) or higher, it disrupted implantation when administered in early pregnancy and retarded or blocked parturition when administered in late pregnancy.

Pregnancy: Pregnancy Category C.

(i) Teratogenic Effects

Teratology studies have been performed in pregnant rats at oral doses up to 900 mg/kg/day (5,400 mg/m^2/day, 730 times the recommended maximum human dose based on body surface area) and in pregnant rabbits at oral doses up to 20 mg/kg/day (240 mg/m^2/day, 32 times the recommended maximum human dose based on body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to anagrelide hydrochloride.

(ii) Nonteratogenic Effects

A fertility and reproductive performance study performed in female rats revealed that anagrelide hydrochloride at oral doses of 60 mg/kg/day (360 mg/m^2/day, 49 times the recommended maximum human dose based on body surface area) or higher disrupted implantation and exerted adverse effect on embryo/fetal survival.

A perinatal and postnatal study performed in female rats revealed that anagrelide hydrochloride at oral doses of 60 mg/kg/day (360 mg/m^2/day, 49 times the recommended maximum human dose based on body surface area) or higher produced delay or blockage of parturition, deaths of nondelivering pregnant dams and their fully developed fetuses, and increased mortality in the pups born.

There are however, no adequate and well controlled studies with anagrelide hydrochloride in pregnant women. Because animal reproduction studies are not always predictive of human response, anagrelide hydrochloride should be used during pregnancy only if clearly needed.

Nonclinical toxicology:
In the 2-year rat study, a significant increase in non-neoplastic lesions were observed in anagrelide treated males and females in the adrenal (medullary hyperplasia), heart (myocardial hypertrophy and chamber distension), kidney (hydronephrosis, tubular dilation and urothelial hyperplasia) and bone (femur enostosis). Vascular effects were observed in tissues of the pancreas (arteritis/periarteritis, intimal proliferation and medial hypertrophy), kidney (arteritis/periarteritis, intimal proliferation and medial hypertrophy), sciatic nerve (vascular mineralization), and testes (tubular atrophy and vascular infarct) in anagrelide treated males.

Five women became pregnant while on anagrelide treatment at doses of 1 to 4 mg/day. Treatment was stopped as soon as it was realized that they were pregnant. All delivered normal, healthy babies. There are no adequate and well-controlled studies in pregnant women. Anagrelide hydrochloride should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.
Anagrelide is not recommended in women who are or may become pregnant. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential harm to the fetus. Women of child-bearing potential should be instructed that they must not be pregnant and that they should use contraception while taking anagrelide. Anagrelide may cause fetal harm when administered to a pregnant woman.
Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reaction in nursing infants from anagrelide hydrochloride, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.
Pediatric Use: Myeloproliferative disorders are uncommon in pediatric patients and limited data are available in this population. An open label safety and PK/PD study (see **CLINICAL PHARMACOLOGY**) was conducted in 17 pediatric patients 7-14 years of age (8 patients 7-11 years of age and 9 patients 11-14 years of age, mean age of 11 years; 8 males and 9 females) with thrombocythemia secondary to ET as compared to 18 adult patients (mean age of 63 years, 9 males and 9 females). Prior to entry on to the study, 16 of 17 pediatric patients and 13 of 18 adult patients had received anagrelide treatment for an average of 2 years. The median starting total daily dose, determined by retrospective chart review, for pediatric and adult ET patients who had received anagrelide prior to study entry was 1mg for each of the three age groups (7-11 and 11-14 year old patients and adults). The starting dose for 6 anagrelide-naive patients at study entry was 0.5 mg once daily. At study completion, the median total daily maintenance doses were similar across age groups, median of 1.75 mg for patients of 7-11 years of age, 2 mg in patients 11-14 years of age, and 1.5 mg for adults.
The study evaluated the pharmacokinetic (PK) and pharmacodynamic (PD) profile of anagrelide, including platelet counts (see **CLINICAL PHARMACOLOGY**).
The frequency of adverse events observed in pediatric patients was similar to adult patients. The most common adverse events observed in pediatric patients were fever, epistaxis, headache, and fatigue during a 3-months treatment of anagrelide in the study. Adverse events that had been reported in these

pediatric patients prior to the study and were considered to be related to anagrelide treatment based on retrospective review were palpitation, headache, nausea, vomiting, abdominal pain, back pain, anorexia, fatigue, and muscle cramps. Episodes of increased pulse rate and decreased systolic or diastolic blood pressure beyond the normal ranges in the absence of clinical symptoms were observed in some patients. Reported AEs were consistent with the known pharmacological profile of anagrelide and the underlying disease. There were no apparent trends or differences in the types of adverse events observed between the pediatric patients compared with those of the adult patients. No overall difference in dosing and safety were observed between pediatric and adult patients**.**

In another open-label study, anagrelide had been used successfully in 12 pediatric patients (age range 6.8 to 17.4 years; 6 male and 6 female), including 8 patients with ET, 2 patients with CML, 1 patient with PV, and 1 patient with OMPD. Patients were started on therapy with 0.5 mg qid up to a maximum daily dose of 10 mg. The median duration of treatment was 18.1 months with a range of 3.1 to 92 months. Three patients received treatment for greater than three years. Other adverse events reported in spontaneous reports and literature reviews include anemia, cutaneous photosensitivity and elevated leukocyte count.

Geriatric Use: Of the total number of subjects in clinical studies of **AGRYLIN**®, 42.1% were 65 years and over, while 14.9% were 75 years and over. No overall differences in safety or effectiveness were observed between these subjects and younger subjects, and other reported clinical experience has not identified differences in response between the elderly and younger patients, but greater sensitivity of some older individuals cannot be ruled out.

ADVERSE REACTIONS

Analysis of the adverse events in a population consisting of 942 patients in 3 clinical studies diagnosed with myeloproliferative diseases of varying etiology (ET: 551; PV: 117; OMPD: 274) has shown that all disease groups have the same adverse event profile. While most reported adverse events during anagrelide therapy have been mild in intensity and have decreased in frequency with continued therapy, serious adverse events were reported in these patients. These include the following: congestive heart failure, myocardial infarction, cardiomyopathy, cardiomegaly, complete heart block, atrial fibrillation, cerebrovascular accident, pericarditis, pericardial effusion, pleural effusion, pulmonary infiltrates, pulmonary fibrosis, pulmonary hypertension, pancreatitis, gastric/duodenal ulceration, and seizure.

Of the 942 patients treated with anagrelide for a mean duration of approximately 65 weeks, 161 (17%) were discontinued from the study because of adverse events or abnormal laboratory test results. The most common adverse events for treatment discontinuation were headache, diarrhea, edema, palpitations, and abdominal pain. Overall, the occurrence rate of all adverse events was 17.9 per 1,000 treatment days. The occurrence rate of adverse events increased at higher dosages of anagrelide.

The most frequently reported adverse reactions to anagrelide (in 5% or greater of 942 patients with myeloproliferative disease) in clinical trials were:

Headache.....43.5%
Palpitations 26.1%
Diarrhea 25.7%
Asthenia 23.1%
Edema, other 20.6%
Nausea.....17.1%
Abdominal Pain 16.4%
Dizziness.....15.4%
Pain, other 15.0%
Dyspnea 11.9%

Flatulence.....10.2%
Vomiting.....9.7%
Fever.....8.9%
Peripheral Edema.....8.5%
Rash, including urticaria.....8.3%
Chest Pain.....7.8%
Anorexia.....7.7%
Tachycardia.....7.5%
Pharyngitis.....6.8%
Malaise.....6.4%
Cough.....6.3%
Paresthesia.....5.9%
Back Pain.....5.9%
Pruritus.....5.5%
Dyspepsia.....5.2%

Adverse events with an incidence of 1% to < 5% included:

Body as a Whole System: Flu symptoms, chills, photosensitivity.

Cardiovascular System: Arrhythmia, hemorrhage, hypertension, cardiovascular disease, angina pectoris, heart failure, postural hypotension, thrombosis, vasodilatation, migraine, syncope.

Digestive System: Constipation, GI distress, GI hemorrhage, gastritis, melena, aphthous stomatitis, eructation.

Hemic & Lymphatic System: Anemia, thrombocytopenia, ecchymosis, lymphadenopathy.
Platelet counts below 100,000/μL occurred in 84 patients (ET: 35; PV: 9; OMPD: 40), reduction below 50,000/μL occurred in 44 patients (ET: 7; PV: 6; OMPD: 31) while on anagrelide therapy. Thrombocytopenia promptly recovered upon discontinuation of anagrelide.

Hepatic System: Elevated liver enzymes were observed in 3 patients (ET: 2; OMPD: 1) during anagrelide therapy.

Musculoskeletal System: Arthralgia, myalgia, leg cramps.

Nervous System: Depression, somnolence, confusion, insomnia, nervousness, amnesia.

Nutritional Disorders: Dehydration.

Respiratory System: Rhinitis, epistaxis, respiratory disease, sinusitis, pneumonia, bronchitis, asthma.

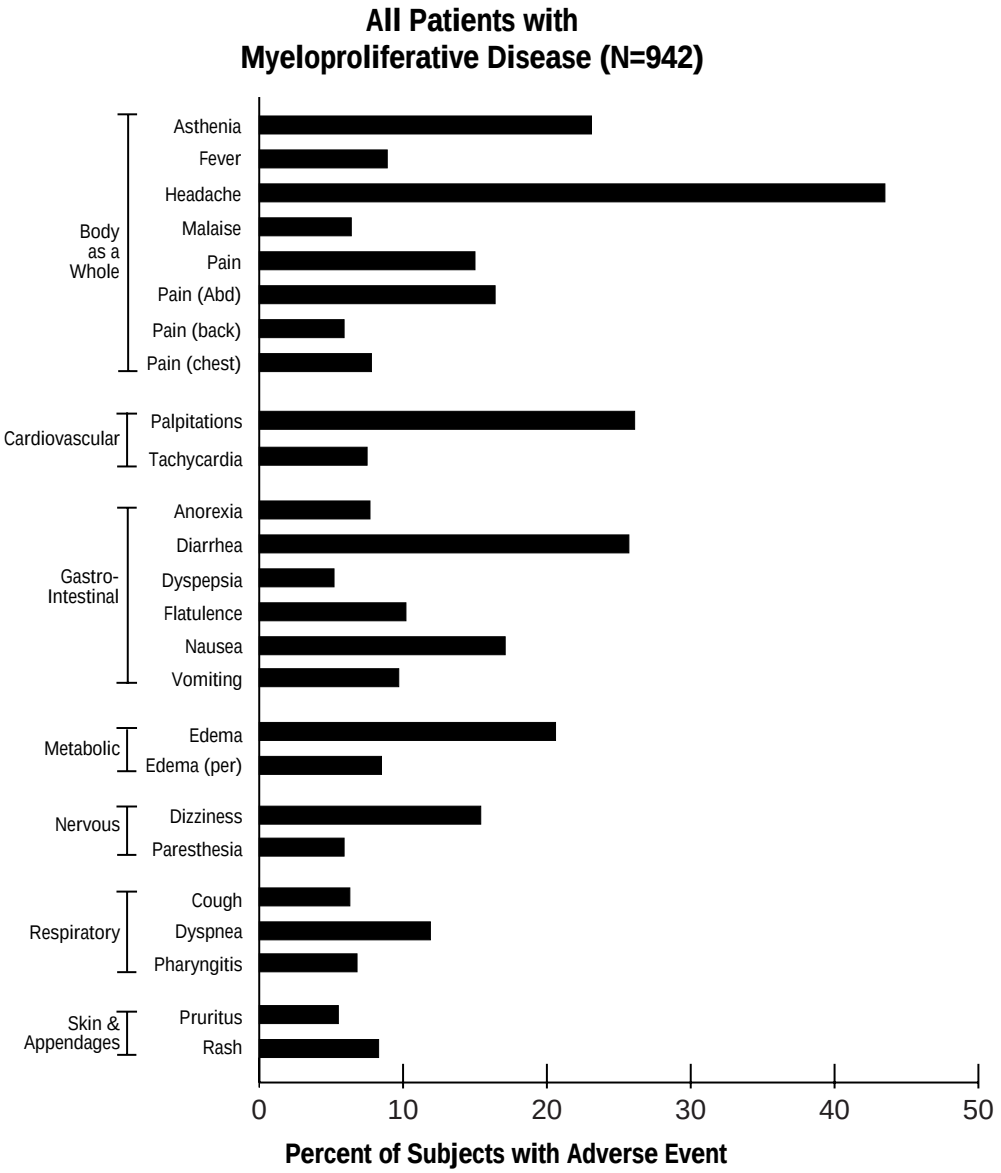
Skin and Appendages System: Skin disease, alopecia.

Special Senses: Amblyopia, abnormal vision, tinnitus, visual field abnormality, diplopia.

Urogenital System: Dysuria, hematuria.

Renal abnormalities occurred in 15 patients (ET: 10; PV: 4; OMPD: 1). Six ET, 4 PV and 1 with OMPD experienced renal failure (approximately 1%) while on anagrelide treatment; in 4 cases, the renal failure was considered to be possibly related to anagrelide treatment. The remaining 11 were found to have pre-existing renal impairment. Doses ranged from 1.5-6.0 mg/day, with exposure periods of 2 to 12 months. No dose adjustment was required because of renal insufficiency.

The adverse event profile for patients in three clinical trials on anagrelide therapy (in 5% or greater of 942 patients with myeloproliferative diseases) is shown in the following bar graph:



Postmarketing Reports

Interstitial lung diseases (including allergic alveolitis, eosinophilic pneumonia and interstitial pneumonitis) have been reported in patients who have taken anagrelide treatment in post-marketing reports (See WARNINGS, Interstitial Lung Diseases).

OVERDOSAGE

Acute Toxicity and Symptoms

Single oral doses of anagrelide hydrochloride at 2,500, 1,500 and 200 mg/kg in mice, rats and monkeys, respectively, were not lethal. Symptoms of acute toxicity were: decreased motor activity in mice and rats and softened stools and decreased appetite in monkeys.

There have been postmarketing case reports of intentional overdose with anagrelide hydrochloride. Reported symptoms include sinus tachycardia and vomiting. Symptoms resolved with conservative management. Platelet reduction from anagrelide therapy is dose-related; therefore, thrombocytopenia,

which can potentially cause bleeding, is expected from overdosage. Should overdosage occur, cardiac and central nervous system toxicity can also be expected.

Management and Treatment

In case of overdosage, close clinical supervision of the patient is required; this especially includes monitoring of the platelet count for thrombocytopenia. Dosage should be decreased or stopped, as appropriate, until the platelet count returns to within the normal range.

DOSAGE AND ADMINISTRATION

Treatment with **AGRYLIN**® Capsules should be initiated under close medical supervision. The recommended starting dosage of **AGRYLIN**® for adult patients is 0.5 mg qid or 1 mg bid (2 capsules of 0.5 mg twice a day), which should be maintained for at least one week. Starting doses in pediatric patients have ranged from 0.5 mg per day to 0.5 mg qid. As there are limited data on the appropriate starting dose for pediatric patients, an initial dose of 0.5 mg per day is recommended. In both adult and pediatric patients, dosage should then be adjusted to the lowest effective dosage required to reduce and maintain platelet count below 600,000/µL, and ideally to the normal range. The dosage should be increased by not more than 0.5 mg/day in any one week. Maintenance dosing is not expected to be different between adult and pediatric patients. Dosage should not exceed 10 mg/day or 2.5 mg in a single dose (see **PRECAUTIONS**).

There are no special requirements for dosing the geriatric population.

It is recommended that patients with moderate hepatic impairment start anagrelide therapy at a dose of 0.5 mg/day and be maintained for a minimum of one week with careful monitoring of cardiovascular effects. The dosage increment must not exceed more than 0.5 mg/day in any one-week. The potential risks and benefits of anagrelide therapy in a patient with mild or moderate impairment of hepatic function should be assessed before treatment is commenced. Use of anagrelide in patients with severe hepatic impairment has not been studied. Use of anagrelide in patients with severe hepatic impairment is contraindicated (see **CONTRAINDICATIONS**).

To monitor the effect of anagrelide and prevent the occurrence of thrombocytopenia, platelet counts should be performed every two days during the first week of treatment and at least weekly thereafter until the maintenance dosage is reached.

Typically, platelet count begins to respond within 7 to 14 days at the proper dosage. The time to complete response, defined as platelet count ≤ 600,000/µL, ranged from 4 to 12 weeks. Most patients will experience an adequate response at a dose of 1.5 to 3.0 mg/day. Patients with known or suspected heart disease, renal insufficiency, or hepatic dysfunction should be monitored closely.

HOW SUPPLIED

AGRYLIN® is available as:

0.5 mg, opaque, white capsules imprinted "S 063" in black ink:
NDC 54092-063-01 = bottle of 100
Store at 25°C (77°F) excursions permitted to 15-30°C (59-86°F), [See USP Controlled Room Temperature]. Store in a light resistant container.

Manufactured for
Shire US Inc.
725 Chesterbrook Blvd.
Wayne, PA 19087, USA

1-800-828-2088
By MALLINCKRODT INC.
Hobart, NY 13788

Rev. 11/07 063 0117 016 Printed in USA

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 20-333/S-013

LABELING REVIEWS

Division of Medical Imaging and Hematology Products

Regulatory Project Manager Review

Application Number: NDA 20-333/SLR-013

Name of the Drug: Agrylin® (anagrelide hydrochloride) Capsules

Sponsor: Shire Development, Inc.

Material Reviewed:

Submission date: June 13, 2007

Stamp date: June 13, 2007

Background and Summary:

Agrylin® Capsules are indicated for the treatment of patients with thrombocythemia, secondary to myeloproliferative disorders, to reduce the elevated platelet count and the risk of thrombosis and to ameliorate associated symptoms including thrombo-hemorrhagic events.

The sponsor submitted a Safety Report on March 6, 2007 that described the occurrence of adenocarcinoma among female animals exposed to anagrelide hydrochloride in the carcinogenicity study. The Division requested a labeling supplement to reflect these findings in the package insert.

The submitted draft package insert, identified as "Rev. 6/07" was compared to the agreed upon package insert text that was approved with NDA 20-333/S-010 and included in the December 17, 2004 Approval letter.

Review:

Deletions are shown as ~~strikeouts~~ and additions as double underlines. The following revisions were noted.

Package insert:

1. The sponsor revised the Dosage Form and the Active Ingredient under the DESCRIPTION section as follows:

   "**Dosage Form:** 0.5 mg ~~and 1 mg~~ capsules for oral administration

Active Ingredient: AGRYLIN® Capsules contain ~~either~~ 0.5 mg ~~or 1 mg~~ of anagrelide base (as anagrelide hydrochloride)."

Comment: These revisions are appropriate and acceptable since the 1 mg capsules are no longer manufactured as stated in the cover letter.

2. The sponsor revised the Carcinogenesis, Mutagenesis, Impairment of Fertility subsection of the PRECAUTIONS section as follows:

"~~No long-term studies in animals have been performed to evaluate carcinogenic potential of anagrelide hydrochloride.~~ In a two year rat carcinogenicity study a higher incidence of uterine adenocarcinoma, relative to controls, was observed in females receiving 30mg/kg/day (at least 174 times human AUC exposure after a 1mg twice daily dose). Adrenal phaeochromocytomas were increased relative to controls in males receiving 3mg/kg/day and above, and in females receiving (b) (4) /kg/day and above (at least 10 and 18 times respectively human AUC exposure after a 1mg twice daily dose). Anagrelide hydrochloride was not genotoxic in the Ames test, the mouse lymphoma cell (L5178Y, TK+/-) forward mutation test, the human lymphocyte chromosome aberration test, or the mouse micronucleus test. Anagrelide hydrochloride at oral doses up to 240 mg/kg/day (1,440 mg/m2/day, 195 times the recommended maximum human dose based on body surface area) was found to have no effect on fertility and reproductive performance of male rats. However, in female rats, at oral doses of 60 mg/kg/day (360 mg/m2/day, 49 times the recommended maximum human dose based on body surface area) or higher, it disrupted implantation when administered in early pregnancy and retarded or blocked parturition when administered in late pregnancy."

Comment: This revision requires a Pharm/Tox Review.

3. The sponsor added a POSTMARKETING REPORTS section before the OVERDOSAGE section that reads as follows:

"POSTMARKETING REPORTS

(b) (4)

Comment: This revision requires a Medical Officer (MO) review and a review from the Office of Surveillance and Epidemiology (OSE).

4. The sponsor added the following sentences in the Acute Toxicity and Symptoms subsection under the OVERDOSAGE section as follows:

"OVERDOSAGE

Acute Toxicity and Symptoms

Single oral doses of anagrelide hydrochloride at 2,500, 1,500 and 200 mg/kg in mice, rats and monkeys, respectively, were not lethal. Symptoms of acute toxicity were: decreased motor activity in mice and rats and softened stools and decreased appetite in monkeys.
There have been (b) (4) postmarketing case reports of intentional overdose with anagrelide hydrochloride. Reported symptoms include sinus tachycardia and vomiting. Symptoms resolved with conservative management.
Platelet reduction from anagrelide therapy is dose-related; therefore, thrombocytopenia, which can potentially cause bleeding, is expected from overdosage. Should overdosage occur, cardiac and central nervous system toxicity can also be expected."

Comment: This revision requires a MO and OSE Review.

5. The sponsor revised the second sentence in the DOSAGE AND ADMINISTRATION section as follows:

   "The recommended starting dosage of **AGRYLIN**® for adult patients is 0.5 mg qid or 1 mg bid (2 capsules of 0.5 mg twice a day), which should be maintained for at least one week."

   Comment: This revision is appropriate and acceptable.

6. The sponsor revised the HOW SUPPLIED section as follows:

   "**AGRYLIN**® is available as:
   0.5 mg, opaque, white capsules imprinted "S 063" in black ink:
   NDC 54092-063-01 = bottle of 100
   ~~1 mg, opaque, gray capsules imprinted "S 064" in black ink:~~
   ~~NDC 54092-064-01 = bottle of 100~~

   Comment: This revision is appropriate and acceptable.

7. The sponsor revised the manufacturing section as follows:

   "Manufactured for
   Shire US Inc.
   725 Chesterbrook Blvd.
   Wayne, PA 19087~~-5637~~, USA
   1-800-828-2088
   By MALLINCKRODT INC.
   Hobart, NY 13788
   © ~~2004~~ 2007 Shire US Inc.
   Rev. ~~12/04~~ 6/07 063 0117 013~~5~~ Printed in USA

   ~~MG #12610~~

   Comment: These revisions are editorial and are acceptable.

Conclusions:

1. Revision #2 requires a Pham/Tox Review. That review is pending.
2. Revisions #3 and #4 require MO and OSE Review. Those reviews are pending.
3. Revisions #1, #5, #6 and #7 are acceptable.

_____
Hyon-Zu Lee, Pharm.D.
Regulatory Project Manager

_____
Alice Kacuba, R.N., R.A.C.
RPM Team Leader, Hematology

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/s/

Hyon Z Lee
8/15/2007 11:28:29 AM
CSO

Alice Kacuba
8/20/2007 02:28:22 PM
CSO

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 20-333/S-013

MEDICAL REVIEWS

DIVISION OF MEDICAL IMAGING AND HEMATOLOGY PRODUCTS

MEDICAL OFFICER'S REVIEW

NDA	20-333/S-013
Sponsor:	Shire Development Inc.
Drug name:	Agrylin
Indication:	Treatment of thrombocythemia, secondary to myeloproliferative disorders, to reduce the elevated platelet count and the risk of thrombosis and to ameliorate associated symptoms including thrombo-hemorrhagic events.
Route of administration:	Oral
Submission:	Labeling Supplement, CBE
Date submitted:	June 13, 2007
Review completed:	June 5, 2008
Reviewer:	Min Lu, M.D., M.P.H.

Background and Rationale

Agrylin was approved on March 14, 1997 for the indication for the treatment of patients with thrombocythemia, secondary to myeloproliferative disorders, to reduce the elevated platelet count and the risk of thrombosis and to ameliorate associated symptoms including thrombo-hemorrhagic events.

The sponsor submitted a labeling supplement (S-013) on June 13, 2007 in response to the Agency request (dated April 13, 2007) to add important safety findings of occurrence of adenocarcinoma in female animals from a carcinogenicity study. The Agency also requested the sponsor to provide a summary and detailed description of post-marketing and pre-marketing reports of the occurrence of cancer among patients exposed to anagrelide hydrochloride.

In this labeling supplement, the sponsor proposed labeling changes to add safety information under PRECAUTIONS regarding a higher incidence of uterine adenocarcinoma and adrenal pheochromocytomas relative to controls observed in carcinogenicity study with requested information. The sponsor also proposed to add allergic alveolitis as an adverse event under ADVERSE REACTIONS and to add intentional overdose under OVERDOSAGE based on post-marketing reports.

Proposed Labeling Changes

1. Under Carcinogenesis, Mutagenesis, Impairment of Fertility subsection of the PRECAUTIONS section, the proposed changes are as follows:

   "~~No long-term studies in animals have been performed to evaluate carcinogenic potential of anagrelide hydrochloride.~~ In a two year rat carcinogenicity study a higher incidence of uterine adenocarcinoma, relative to controls, was observed in females receiving 30mg/kg/day (at least 174 times human AUC exposure after a 1mg twice daily dose). Adrenal phaeochromocytomas were increased relative to controls in males receiving 3mg/kg/day and above, and in females receiving 10mg/kg/day and above (at least 10 and 18 times respectively human AUC exposure after a 1mg twice daily dose). (b) (4) Anagrelide hydrochloride was not genotoxic in the Ames test, the mouse lymphoma cell (L5178Y, TK+/-) forward mutation test, the human lymphocyte chromosome aberration test, or the mouse micronucleus test. Anagrelide hydrochloride at oral doses up to 240 mg/kg/day (1,440 mg/m2/day, 195 times the recommended maximum human dose based on body surface area) was found to have no effect on fertility and reproductive performance of male rats. However, in female rats, at oral doses of 60 mg/kg/day (360 mg/m2/day, 49 times the recommended maximum human dose based on body surface area) or higher, it disrupted implantation when administered in early pregnancy and retarded or blocked parturition when administered in late pregnancy."

   No addition was proposed with regard to cancer in humans associated with anagrelide.

2. The sponsor proposed addition of POSTMARKETING REPORTS section in regard to allergic alveolitis at the end of ADVERSE REACTIONS section:

   "POSTMARKETING REPORTS

   (b) (4)

3. Under Acute Toxicity and Symptoms subsection of the OVERDOSAGE section, the proposed changes are as follows:

   "OVERDOSAGE

   Acute Toxicity and Symptoms
   Single oral doses of anagrelide hydrochloride at 2,500, 1,500 and 200 mg/kg in mice, rats and monkeys, respectively, were not lethal. Symptoms of acute toxicity were: decreased motor activity in mice and rats and softened stools and decreased appetite

in monkeys.

There have been [redacted] (b) (4) postmarketing case reports of intentional overdose with anagrelide hydrochloride. Reported symptoms include sinus tachycardia and vomiting. Symptoms resolved with conservative management. Platelet reduction from anagrelide therapy is dose-related; therefore, thrombocytopenia, which can potentially cause bleeding, is expected from overdosage. Should overdosage occur, cardiac and central nervous system toxicity can also be expected."

Sponsor's Supporting Documents

The following is the summary of sponsor's submission to support the proposed labeling changes.

Occurrence of cancer cases from clinical data

Leukemia and pre-leukemic conditions including myelofibrosis, lymphomyeloproliferative disorder, myelodysplastic disorder

Data from clinical trials:

Of 2654 ET patients from studies conducted by Roberts pharmaceuticals (700-012, 700-014, 700-999 and 13970-301), 54 patients (2.0%) experienced a transformation of the underlying condition to acute leukemia/myelodysplasia. However, the vast majority of these patients had received other cyto-reductive therapy before anagrelide. Of the 390 treatment-naïve essential thrombocythemia (ET) patients, who had not received other prior cyto-reductive therapy, one patient (0.3%) developed a transformation of the underlying disease to acute leukemia. These incidences fall within the published rates (1.5-2%) for leukemic transformation in ET (Tefferi, 2005).

No case of leukemia was reported among the 1245 patients exposed to anagrelide in Shire sponsored studies. All patients in Shire sponsored studies were treated for ET.

The sponsor indicated that none of clinical trials were randomized, controlled trials.

Data from post-marketing spontaneous reports, literature reports and reports from authorities

Since the initiation of marketing, the following three literature reports were received on transformation to leukemia.

Case SGB1-2004-00394 reports on a 43 year old, male patient with a 14 years history of ET. The patient was treated with hydroxyurea since 1997 and with busulfan (therapy dates unknown). In 1998, he suffered from a blast crisis and was switched to anagrelide

therapy one year later. Three months into therapy, progression to acute myelogenous leukemia was diagnosed.

Case SUS1-2006-00513 reports on a 71 year old female patient who had been treated with hydroxyurea for ET for six years. Three weeks after anagrelide therapy was commenced, the patient was diagnosed with CML.

Case SPV1-2006-00390 reports on a 60 year old female patient who was treated with hydroxyurea for ET since 2000. Between 2001 and 2005 she was treated with different combinations of hydroxyurea, anagrelide and interferon. Transformation of the disease to biphenotypic leukemia was diagnosed in 2005.

The sponsor indicated that for cases SGB1-2004-00394 and SUS1-2006-00513, the events are not likely related to anagrelide because of the implausible timeframe, and in case SPV1-2006-00390, prior and concomitant use of hydroxyurea posed an additional risk.

All other cancer types

The sponsor performed a search of the Shire Global Safety System database for all tumor cases reported from all data sources. Seventy-eight tumor cases other than leukemia/myeloproliferative disorders were identified of which five patients had benign conditions of different origin and were excluded from further analysis. One patient, because of a coding error, initially was reported to suffer from an unspecified malignancy, and died due to the underlying malignancy (myelogenous leukemia) three weeks into therapy. The case was also excluded from the sponsor's analysis.

Data from clinical trials

Seventy-one cases including 74 cancer events were reported from clinical trials where a total of 5845 patients have been exposed to anagrelide during clinical trials as of 13 March 2007 (1245 of these 5845 patients were included in Shire sponsored trials). Approximately two thirds of the clinical trial populations were patients with ET. In 49 of the 71 clinical trial cases of diagnosed malignancies, the underlying condition was ET, and in 11 patients the underlying condition was polycythemia vera (PV). Five patients were suffering from CML and six from other myeloproliferative disorders. The sponsor indicated that none of clinical trials were randomized, controlled clinical trials.

Three of the 71 cases report on patients suffering from two different cancer types (**SUS2-2000-00255**: ovarian cancer and malignant lymphoma; **SUS2-2000-01181**: lung cancer and colon cancer; **SUS2-2000-00276**: breast carcinoma and lung cancer), so that in total, 74 cancer events are analyzed that occurred in 71 patients.

None of the 71 clinical trial cases reporting on 74 cancer events was assessed as related to anagrelide by the investigator. However, for 11 cancer events, the assessment of the investigator was not provided; these cases were previously handled as related (in doubt

reporting) by the sponsor.

Analysis of primary organs affected by the malignancy

The following table summarizes the frequency distribution of reported malignancies by affected organ. Lung, prostate, breast, skin, colon, and bladder cancer were the most common types of cancer reported in the clinical trials population, reflecting the distribution of frequent cancer types in the normal population. Two cases of uterine cancer were reported.

Table 1: Number of Cancer Events by Affected Organ

Primary malignancy	Number of cancer events
Lung cancer (including 2 reports of metastases due to lung cancer)	14
Prostate cancer (including one case of liver metastasis)	10
Breast carcinoma	10
Skin cancer	7
Colon Cancer (including 2 reports of liver metastases)	5
Bladder cancer	4
Ovarian Cancer	3
Gastric cancer	3
Renal cancer	2
Splenic cancer	1
Pancreatic cancer	2
Esophageal cancer	2
Uterine cancer	2
Pleural cancer	1
Tongue cancer	1
Brain Cancer	1
Vocal cord carcinoma	1
Plasmacytoma	1
Unspecified cancer	2
Lymphoma	2
TOTAL	74

Cancer incidence rates from the Surveillance Epidemiology and End Results Program (SEER Stat Database: Incidence - SEER 17 Regs Public-Use, Nov 2005 Sub (2000-2003), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2006) were compiled for comparison to rates from patients enrolled in anagrelide clinical trials. SEER is a compilation of state-based and population-based cancer registry statistics combined to be comparable to the US general

population. Based on the organ groupings of the tumor sites reported in the anagrelide studies, the SEER data set was searched for similar tumor sites and grouped by organ and system. Once the groupings were prepared, the 2000-2003 SEER incidence rates for these tumor types were adjusted to the age distribution of the standard US population and prepared for comparison to the incidence rates in the clinical trial population (N = 5,845). Given the age distribution in the anagrelide clinical trials, SEER data were then limited to individuals 60 years of age and older to more closely approximate the age distribution in the clinical trial groups.

Incidence rates for the anagrelide clinical trial patients were calculated assuming that patients had only one year of follow-up.

The incidence rates observed in the anagrelide clinical trials group as compared to SEER data is shown in the table below. The sponsor indicated that for the six most frequently reported cancer types the incidence rates were lower than the rates in the age-comparable United States population, with the exception of skin cancer. However, SEER does not report on the incidence of basal cell (BCC) or squamous cell carcinoma (SCC) of the skin. When these subtypes are removed from the skin cancer cases in the clinical trials group, the US SEER population had a higher incidence rate of skin cancer. Regarding the specific interest in uterine cancer, the incidence rate from the clinical trials group was only slightly more than half the incidence rate reported in the United States SEER population.

Table 2: Cancer Incidence Rates (per year) in SEER and in Anagrelide Clinical Trial Patients, by Cancer Site

SEER Data		Anagrelide Clinical Trials Patients (N = 5,845)		
Cancer Site	Incidence Rate (per 100,000)	Cancer Site	Number of Cancer Events	Incidence Rate (per 100,000)
Colon excluding rectum	187	Colon cancer	5	86
Lung and bronchus	320	Lung cancer	14	240
Breast	239	Breast carcinoma	10	171
Cervix uteri & corpus and uterus, NOS	55	Uterine cancer	2	34
Prostate	368	Prostate cancer	10	171
Urinary bladder	106	Bladder cancer	4	68
Skin (excluding BCC and SCC	64	Skin cancer	7	120*

*This number is 51 when BCC and SCC cases are excluded. BCC=basal cell carcinoma; SCC=squamous cell carcinoma.

The sponsor indicated that nine cancer events that were known to have occurred prior to initiation of anagrelide therapy are included in the assessment.

Reviewer's Comments:
The overall cancer incidence rate, gender-specific cancer incidence rates, and the incidence rate in the various cancer sites also should be compared between anagrelide patients in clinical trials and SEER data. In addition, SEER data were from the general population aged 60 years or greater. The age distribution of patients in anagrelide in clinical trials is not clear and the sponsor was asked to provide this information and additional comparison data (4/11/2008). The sponsor responded on 4/25/08 that only 56.8% of anagrelide patients in the clinical trials were 60 years or above. The comparison of cancer incidence rates between anagrelide patients for all ages and SEER data for general population 60 years or over may not be justified. Additional comparisons on age-adjusted cancer rates for age <60 years and ≥ 60 years between anagrelide patients and SEER data were requested from the sponsor on 4/29/2008. In an email response on 5/14/2008, the sponsor plans to compare cancer rates of anagrelide patients with SEER data for entire adult population and also plans to revise the analysis plan to calculate the actual person-years of follow-up instead of using assumed 1 year follow-up for anagrelide patients in clinical trials. The sponsor plans to send the information by June 20, 2008 and the response is pending currently.

Histological cancer type
The histological type of the malignancy was reported for only 17 of the 74 cancer events (2 breast cancers, 7 lung tumors, 2 prostate, and 6 skin tumors). Among the 7 lung cancer events, the diagnosis was squamous cell carcinoma in 3, adenocarcinoma in another 3, and oat cell carcinoma in 1 case. The skin cancer group presented 3 squamous cell carcinomas, 2 melanomas and 1 basal cell carcinoma.

Age
The mean age of the 71 patients with reports of malignancies during clinical trials was 66.7 years. The mean age in the subgroup of breast carcinoma patients was 63.7 years, of lung carcinoma-64.5 years, of prostate cancer-69.9 years, of skin cancer-70 years, of colon cancer-73.8 years, and of bladder cancer-71 years. The low number of patients for each of the other reported organ class tumors does not allow calculating a mean age; the ages of individual patients are presented in the table below.

Table 3: Individual Patients' Age by Affected Organ

Cancer type	Age in years of individual patients
Ovarian cancer	49,79,69
Gastric cancer	74,73,50
Pancreatic cancer	77,68
Esophageal cancer	65,79
Uterine cancer	68,49
Renal cancer	68,71
Lymphoma	62,79
Splenic cancer	66
Pleural cancer	68
Tongue cancer	68
Brain cancer	69
Plasmacytoma	56
Vocal cord carcinoma	58
Unspecified cancer	34,63

Most of the patients were over 60 years of age. Fourteen of all 71 patients were under 60 years old; one of these patients was 34 years, 4 patients were between 40-50 years, and 9 patients were between 50-60 years old. The 34-year-old patient with an unspecified "carcinomatosis of abdomen" had a medical history of Wilms tumor, thyroid carcinoma and radiotherapy prior to anagrelide therapy.

Gender

Of the 71 patients with reported malignancies during clinical trials, 39 were male, and 31 female. The gender of one patient was not reported. The 39 male patients experienced 39 cancer events, and the 31 female patients reported 34 cancer events. The following table provides the frequency distribution of gender by organ class affected.

Table 4: Gender Distribution by Affected Organ

Cancer type	Cancer events in males, n=39	Cancer events in females, n=34	Gender unknown, n=1
Lung cancer	7	6	1
Prostate cancer	10	-	-
Breast carcinoma	-	10	-
Skin cancer	3	4	-
Colon cancer	3	2	-
Bladder cancer	3	1	-
Ovarian cancer	-	3	-
Gastric cancer	3	-	-
Pancreatic cancer	2	-	-
Esophageal cancer	2	-	-
Uterine cancer	-	2	-
Renal cancer	-	2	-
Lymphoma	1	1	-
Splenic cancer	1	-	-
Pleural cancer	1	-	-
Tongue cancer	-	1	-
Brain cancer	1	-	-
Plasmacytoma	1	-	-
Vocal cord carcinoma	1	-	-
Unspecified cancer	-	2	-

Time to onset
Systematic screening for tumors was not undertaken as part of the clinical study protocols.

Of the total 74 cancer events reported in 71 patients in clinical trials, nine were already known prior to the onset of anagrelide therapy. Twenty four of the remaining 65 cancer events occurred within one year after onset of anagrelide therapy; 8 within the first month; 7 within 2 to 6 months; and 9 within 7 to 12 months.

For the remaining 41 cancer events, no specific time pattern was observed between onset of anagrelide therapy and cancer occurrence to suspect a tumor induction effect of anagrelide. The time to onset is unknown for 7 events, 22 events were reported within 1-3 years into anagrelide therapy, 12 events were reported after 3 years, and for the remainder of the events, there was a broad spectrum of time to reported event, up to 6 years.

Confounders

Within the clinical trials program, information on risk factors like genetic predisposition/ family history, environmental or occupational exposure to carcinogens, and previous long-term exposure to drugs was not systematically collected. The sponsor indicated that overall, the investigated patient group was at higher risk to develop a malignancy compared to the normal population due to the following reasons:

- All patients treated with angrelide were suffering from an advanced stage of myeloproliferative disorder, which itself is caused by an uncontrolled proliferation of cell lines
- Severe chronic diseases have a negative impact on the immune system
- The majority of the patients (53 patients experiencing 56 cancer events) were previously or concomitantly treated with known carcinogenic agents (i.e. hydroxyurea/carbamide or alkylating agents), some of them for years
- The patients were elderly with a mean age of 66.7 years.

In addition, the following confounding factors were identified by the sponsor for 19 of the 71 cases based on review of the narratives:

Table 5: Confounding Factors by Affected Organ

Cancer type	Risk factor
Breast carcinoma	Two patients had a history of hormone replacement therapy, 3 had a history of conjugated estrogen treatment, 1 patient had a history of benign breast tumor
Lung cancer	Five patients were smokers, 1 patient had a history of radioactive phosphate therapy, 1 patient had a history of chronic obstructive lung disease, 2 patients had a medical history of cancer affecting other organs and it is unclear whether the lung cancer was another primary tumor or a metastasis
Skin cancer	In 4 cases, the cancer occurred at constantly light exposed skin areas (face)
Esophageal cancer	One patient had a history of Barrett esophagus
Vocal cord cancer	One patient was a smoker and had a history of vocal cord keratosis
Bladder cancer	One patient had a history of breast and skin cancer
Ovarian cancer	One patient had a history of pancreatic cancer
Unspecified cancer	One patient had a medical history of Wilms tumor and thyroid cancer

Data from spontaneous reports, literature reports and reports from authorities

One case of other cancer types was reported spontaneously.

Case STX1-1999-00257 was reported by a 72 year old male patient who was treated with anagrelide "for elevated platelets" (not further specified) from 4-May-1999 to 18-May-1999. The patient had a history of "elevated PSA" (not further specified). Two weeks after anagrelide was started, the PSA values were found "higher" than usual (not further specified). Anagrelide was stopped, but the PSA value further increased during the next month. A biopsy revealed prostate cancer. Due to the brief duration of dosing and implausible timeframe, anagrelide induced prostate carcinoma is excluded.

No cases of uterine carcinoma have been reported in the 10 years of postmarketing experience worldwide.

The sponsor concluded that despite the higher risk profile of patients treated for myeloproliferative disorders, a detailed analysis of all 75 solid tumor cancer events reported in 72 patients (including 1 spontaneous report and 71 cases from clinical trials) did not reveal any specific pattern with regard to organ class manifestation, age, gender, or time to onset that would raise a signal of cancer risk associated with anagrelide treatment. Although there are no available published data sources providing rates of different solid tumor malignancies among patients with myeloproliferative disorders, the incidence rates reported for the clinical trial population for the most frequently seen tumors as well as uterine cancer are well under the incidence rates of those reported historically for the general United States SEER population, age 60 and older.

AERS Search/OSE Consult

The submitted cancer data in anagrelide clinical trials were not reviewed by OSE. AERS search conducted by OSE (see Consult Review by Betsy Scroggs, Pharm. D. and Corrinne Kulick, Pharm. D. dated 1/2/2007) identified 10 unduplicated reports of cancer among a total of 592 adverse event reports are contained in AERS for anagrelide as November 5, 2007.

There were six reports of solid tumors and four reports of leukemia. These reported 10 cases included acute leukemia (1), acute myeloid leukemia (1), central nervous system neoplasm (1), chronic myeloid leukemia (2), Hodgkin's disease (1), squamous cell carcinoma (larynx) (1), non-Hodgkin's lymphoma (1), prostate cancer (1), and squamous cell carcinoma (skin) (1). Three cases were reported from US and 7 were reported from foreign countries. The mean age of these cases was 61 years with range of 43 to 72 years. The median time of onset was 67 weeks with a range of 2 weeks to 4 years.

There were no reports of uterine adenocarcinoma based on AERS search.

All ten cases of cancer either contained insufficient information to assess the drug-event relationship or were complicated by underlying disease state/risk factors or use of concomitant medications.

OSE concluded that although a possible relationship cannot be excluded, the current case series does not support a clear drug event relationship between anagrelide and the development of cancer. The proposed labeling on cancer is acceptable since the sponsor did not propose any additions to the product label with regard to their analyses of reported cases of cancer in humans associated with anagrelide.

Occurrence of Allergic Alveolitis

There were no case reports of allergic alveolitis or hypersensitivity pneumonitis occurring during clinical trials, postmarketing studies, or compassionate use programs.

Spontaneous reports for the period prior to September 12, 2006 (submitted on 6/13/2007)

As of the cut-off date of 12-Sep-2006, the sponsor's safety database for anagrelide identified two reports of interstitial pneumonitis, four reports of hypersensitivity pneumonitis/allergic alveolitis and one report of eosinophilic pneumonia.

Interstitial pneumonia

There have been two spontaneous cases of interstitial pneumonia reported to the sponsor since 1997. The following are case narratives.

SGB1-2003-00323 (serious, unlisted): This report from the UK describes the occurrence of the reported event acute interstitial pneumonia in a male patient of unspecified age prescribed anagrelide for essential thrombocythemia. No information was provided regarding the patient's past medical history, concurrent medical conditions, or concomitant medications. The patient commenced treatment with anagrelide on an unspecified date and after an unspecified time interval on the drug was reported to have developed interstitial pneumonia. He was treated with antibiotics for an unspecified time period. He was reported to have improved after starting steroid therapy and stopping anagrelide. The report notes that the reporting hematologist suspected that the patient had experienced interstitial pneumonia. However, he did not provide information that would indicate that the diagnosis had been confirmed by thoracoscopic or open lung biopsy, either of which is necessary for confirmation of this event. There were also no reported diagnostic studies (for example, chest x-rays or CT scans of the chest) that, although not definitive, could lend support to this diagnosis.

SGB1-2005-00467 (serious, unlisted): This report from the French Regulatory Agency describes the occurrence of the reported event interstitial pneumonia in a 72-year-old male prescribed anagrelide for an unspecified indication. The patient's medical history

included neurotic depression and essential thrombocythemia. After 28 days of therapy with anagrelide, the patient was reported to have experienced interstitial pneumonia, with diffuse bilateral pneumonia with fever and a regressive inflammatory syndrome. The patient was reported to have had a second chest x-ray, which revealed an improvement of the interstitial pneumonia. His serology was positive for Chlamydia. He was treated with ceftriaxone, clarithromycin, and intravenous corticosteroid therapy, anagrelide was discontinued, and he recovered. No information was provided regarding whether the patient underwent confirmatory diagnosis with a thoracoscopic or open lung biopsy, and thus the diagnosis, from the information provided, cannot be confirmed.

Hypersensitivity pneumonitis/allergic alveolitis

There has been one spontaneous case of allergic alveolitis and two or three spontaneous cases of hypersensitivity pneumonitis reported to the sponsor. The following are case narratives.

SUS2-2002-00108 (previously reported as STX1-2002-00772/SUS2-2002-00096) (serious, unlisted): This UK report describes the occurrence of allergic alveolitis and pulmonary infiltration in a 44 year-old female prescribed anagrelide for thrombocythemia associated with myelofibrosis. Past medical history included recurrent pulmonary emboli during treatment with hydroxyurea. Concomitant medication included long-term warfarin. For the first month of therapy with anagrelide, hydroxyurea treatment was continued and then stopped. Two months after commencement of anagrelide, the patient complained of increasing breathlessness on minimal exertion and occasionally at rest. A CT pulmonary angiogram confirmed the diagnosis of recurrent pulmonary emboli. She experienced progressive breathlessness, particularly on exertion, over the next several months (time interval not specified). Three months after her initial complaint of breathlessness, a perfusion scan was performed, with no new evidence of further defects. She was evaluated by a respiratory physician, who assessed her symptoms as potentially being due to anagrelide use. A high-resolution CT scan revealed subtle changes suggestive of early pulmonary parenchymal infiltration, “possibly” consistent with a drug reaction.

Pulmonary function tests showed a restrictive lung defect with reduced transfer factor on pulmonary testing. Anagrelide was stopped after 4 months of treatment and therapy with hydroxyurea was reinitiated. Six weeks after discontinuing anagrelide, the patient’s pulmonary function tests (PFTs) showed a 25% improvement. A repeat high resolution CT scan performed 5 months after stopping anagrelide showed further improvement, as did repeat PFTs. The attending physician’s assessment was that the patient had experienced allergic alveolitis secondary to anagrelide use.

SUS1-2003-00278 (serious, unlisted): This US literature report describes the occurrence of hypersensitivity pneumonitis in a 60 year-old female with chronic myeloid leukemia being treated with anagrelide for thrombocytosis. (This report is derived from an article entitled “Severe Hypersensitivity Pneumonitis Associated with Anagrelide” by Raghavan, Mazar, and Brink, published in the “Annals of Pharmacotherapy”, September,

2003, Vol. 37, pp. 1228 – 1231). Concurrent medical conditions included non-insulin-dependent diabetes mellitus, essential hypertension, chronic renal failure, and gout. Concomitant medications included allopurinol, glipizide, lisinopril, furosemide, and erythropoietin. The patient had been treated with hydroxyurea for 7 years prior to commencement of anagrelide. Anagrelide therapy was initiated on an unspecified date and treatment with hydroxyurea was continued. After an unspecified time interval on anagrelide (described as "soon after" initiation of anagrelide therapy), the patient developed severe hypersensitivity pneumonitis requiring intubation and mechanical ventilation. A high-resolution CT scan of the chest revealed extensive multifocal ground glass attenuation and patchy alveolar consolidation involving both lungs. Bronchoalveolar lavage revealed a preponderance of lymphocytes, suggestive of a hypersensitivity phenomenon. Discontinuance of anagrelide and hydroxyurea and institution of corticosteroid therapy resulted in significant improvement in the patient's clinical condition.

SGB1-2004-00295 (serious, unlisted): This UK report from the MHRA describes the occurrence of hypersensitivity and pneumonitis in a 45-year-old male prescribed anagrelide for essential thrombocythemia. After approximately 5 years of therapy with anagrelide, the patient experienced hypersensitivity and pneumonitis and was admitted to the intensive therapy unit. Blood cultures revealed the presence of Streptococcus pneumonia. Anagrelide was discontinued and treatment with co-amoxiclav, gentamicin, clarithromycin, levofloxacin, and highdose prednisone was initiated. The patient recovered.

SGB1-2006-00083 (serious, unlisted): This UK literature report describes the occurrence of both Streptococcal pneumonia and hypersensitivity pneumonitis in a 45-year-old male prescribed anagrelide for essential thrombocythemia. (This report is derived from an article entitled "Double Hit From Streptococcal Pneumonia and Hypersensitivity Pneumonitis associated with Anagrelide" by Spencer and Lawrence, published in the journal "Clinical Laboratory Hematology", Vol.28, 2006, pages 63-66). The patient had been diagnosed with this condition 6 years previously, was an ex-smoker, and was described as otherwise "fit and well". Concomitant medications were unspecified. After 3 ½ years of therapy with anagrelide, the patient experienced a 3-day period of lethargy, productive cough, dyspnea, pyrexia, and left-sided pleuritic chest pain. He was hospitalized. His chest x-ray (CXR) showed extensive consolidation at the left base and probable effusion. He developed leukocytosis, hypoxia, elevated C-reactive protein, and acute renal impairment. Therapy with intravenous co-amoxiclav and clarithromycin was initiated. A repeat CXR the next day showed worsening of his bilateral pneumonia. Blood cultures revealed the presence of Streptococcus pneumoniae sensitive to penicillin. Clarithromycin was discontinued. Six days later, the patient showed little improvement. His antibiotics were changed to benzylpenicillin and gentamicin. By day 11, he had still not made any progress either clinically or radiologically, with continued severe hypoxia. On day 13, anagrelide was stopped and prednisolone was started. Over the next 3 days, the patient improved significantly. The patient was discharged 22 days after hospitalization. Two months later, he was reported to be symptom free with a normal CXR. Hydroxyurea was initiated. Due to similarities in

patient demographics, the presence of Streptococcus pneumonia bacteremia and pneumonia, and the occurrence of hypersensitivity, Shire suspects that reports SGB1-2006-00083 and SGB1-2004-00295 are duplicates describing the same clinical event.

Spontaneous reports for the period between Sept. 12, 2006 and Feb. 20, 2008 (submitted on 3/4/2008)

For period from Sep. 12, 2006 to Feb. 20, 2008, three cases of lower respiratory tract disorders were reported from foreign countries. These included one case of pneumonitis in a 50-year-old male reported from Spain. The patient was positive for anti-glomerular basement membrane antibody. Other two cases included pulmonary fibrosis and pulmonary edema. This information was provided on March 4, 2008 upon the information request. Only line list with incomplete information was provided by the sponsor.

AERS Search/OSE Consult

A search of AERS conducted by OSE (see Consult Review by Betsy Scroggs, Pharm. D. and Corrinne Kulick, Pharm. D. dated 1/2/2007), using OSE case definition for interstitial lung diseases (ILD), identified 12 unduplicated cases among a total of 592 adverse event reports are contained in AERS for anagrelide as November 5, 2007. No additional cases were identified from the literature.

Of the 12 total cases, one case was excluded because the patient was diagnosed with pneumonia. Eleven cases were reviewed to analyze the potential association between anagrelide and ILD.

Of the 11 reports, 8 met the OSE case definition of ILD because of a clinical diagnosis of allergic alveolitis (3), eosinophilic pneumonia (1), pneumonitis (3), or interstitial lung disease (1) and are included in the case series. In all 8 cases the onset of the pulmonary event was consistent with the clinical profile for 2 ILD (*i.e.*, 1 week to 3.5 years) and manifest while patients were taking anagrelide.

The time of onset ranged from 1 week to 3.5 years with a median of 21 weeks for the 8 cases.

These 8 cases included the sponsor submitted 6 cases that mentioned above. The additional case of pneumonitis that was provided on March 4, 08 was not included. Overall, 9 cases of ILD have been reported from post-marketing/literature reports.

OSE concluded that although patients may have received previous or concomitant therapy with a pharmacologic agent that is labeled for this event (i.e., hydroxyurea, lisinopril, hydrochlorothiazide, or hydrochlorothiazide/triamterene), there is a temporal relationship of the event with the initiation of anagrelide for all reported cases. This

evidence, in conjunction with a definitive report of a positive dechallenge and rechallenge in one case, supports a plausible association between anagrelide and ILD.

OSE recommended that the sponsor's proposed addition of this information in the Adverse Reactions –Postmarketing Experience section of the product label is inadequate to describe the potential for this event following anagrelide exposure. Because of the rapid decline in respiratory function culminating in acute respiratory failure requiring endotracheal intubation and mechanical ventilation and extended hospitalization in one case; the need for hospitalization, supportive oxygen therapy, and reduced performance in another, and; a positive dechallenge and rechallenge case providing reasonable evidence of a causal association, consideration should be given to elevating the information regarding this adverse event and appropriate management to the Warnings and Precautions section of the labeling.

OSE recommended to delete the proposed sentence (b) (4) for allergic alveolitis adverse events "because it is unnecessary given the adverse events captured from spontaneous reports listed in post-marketing experience is separate from the listing of adverse events identified in clinical trials and the frequency of the event cannot be reliably estimated from spontaneous reports".

Intentional Overdose

The sponsor found the following 2 cases of intentional overdose from their post-marketing database search.

SGB1-2003-00318: On (b) (6), a 24-year-old Caucasian male (brother of a female patient taking agrylin) took an intentional overdose of "104 capsules (0.5 mg) of agrylin with 17 can of beer and had vomited twice. He was admitted to the hospital and was found to be intoxicated and sleepy. No other adverse events were reported and he was discharged following an observation period of 48 hours.

SUS2-1995-00002: A 56-year-old female patient had been taking anagrelide since November 24,1995 for CML. At 2 a.m. on (b) (6) she took a deliberate overdose of 12 x 0.5mg anagrelide and 18 x 20 mg Prozac. Following her overdose she had a sinus tachycardia >112 beats/min. Anagrelide was temporily stopped and the sinus tachycardia resolved. Anagrelide was restarted on December 14, 1995. Medical history included thrombocythemia with transformation to CML, hypothyroidism and depression. Concomitant medication included thyroxine, aspirin, allopurinol and hydroxyurea.

No literature report of intentional overdose was found.

AERS Search/OSE Consult

A search of AERS conducted by OSE identified one case of intentional overdose that the sponsor has included above as the first case (25-year-old male) among a total of 592 adverse event reports contained in AERS for anagrelide as of November 5, 2007.

For the proposed labeling, OSE recommended to delete wording "(b) (4)" in the proposed sentence under OVERDOSE section of labeling "because it is vague and any additional cases will render this phrase obsolete".

Conclusions and Recommendations

This reviewer recommends that this labeling supplement should be approved currently with the following labeling revisions.

1. See pharmacology/toxicology review for revision in Carcinogenesis, Mutagenesis, Impairment of Fertility subsection of the PRECAUTIONS section and additions in Pregnancy and Nonclinical toxicology sections.

2. For allergic alveolitis, the following should be included in the WARNINGS section after Hepatic subsection.

   (b) (4)

   (b) (4)

   mproved after discontinuation of anagrelide (See ADVERSE REACTIONS).

   The proposed additions should be modified as follows in the end of ADVERSE REACTIONS section:

   (b) (4)

   Postmarketing Reports

   (b) (4)

4. Under Acute Toxicity and Symptoms subsection of the OVERDOSAGE section, the proposed labeling changes should be revised as follows:

   "OVERDOSAGE

Acute Toxicity and Symptoms
Single oral doses of anagrelide hydrochloride at 2,500, 1,500 and 200 mg/kg in mice, rats and monkeys, respectively, were not lethal. Symptoms of acute toxicity were: decreased motor activity in mice and rats and softened stools and decreased appetite in monkeys.

There have been (b) (4) postmarketing case reports of intentional overdose with anagrelide hydrochloride. Reported symptoms include sinus tachycardia and vomiting. Symptoms resolved with conservative management. Platelet reduction from anagrelide therapy is dose-related; therefore, thrombocytopenia, which can potentially cause bleeding, is expected from overdosage. Should overdosage occur, cardiac and central nervous system toxicity can also be expected."

Additional clinical information has been requested from the sponsor regarding cancer data in anagrelide clinical trials as described under Reviewer's comments (page 7) and the response is pending currently. Further OSE consult will be needed to assess the cancer risk in human based on cancer data from anagrelide clinical trials when the requested information is received. Additional labeling changes may be needed when these data are reviewed by the Division and OSE.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Min Lu
6/6/2008 10:54:54 AM
MEDICAL OFFICER

Kathy Robie-Suh
6/6/2008 11:30:02 AM
MEDICAL OFFICER

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 20-333/S-013

PHARMACOLOGY AND TOXICOLOGY REVIEWS

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	20-333
SERIAL NUMBER:	SLR 013
DATE RECEIVED BY CENTER:	09/27/07
PRODUCT:	AGRYLIN (Anagrelide hydrochloride)
INTENDED CLINICAL POPULATION:	Patients needing platelet count reduction
SPONSOR:	SHIRE
DOCUMENTS REVIEWED:	10 Vols, 3000 pages, in EDR
REVIEW DIVISION:	Div of Med Imaging and Hem Drug Prod (HFD-160)
PHARM/TOX REVIEWER:	David E. Bailey, Ph.D.
PHARM/TOX SUPERVISOR:	Adebayo A. Laniyonu, Ph.D.
ACTING DIVISION DIRECTOR:	Rafel Dwaine Rieves, M.D.
PROJECT MANAGER:	Hyon-Zu Lee, Pharm. D.

Date of review submission to Division File System (DFS): 02/25/08

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability:

This is a labeling supplement for an approved drug.

B. Recommendation for nonclinical studies: None

C. Recommendations on labeling:

The proposed changes to the labeling by the sponsor are in yellow. The nonclinical pharmacology and toxicology edits are in blue plus strikeout. The unhighlighted paragraphs are as the approved labeling was written some years ago.

Carcinogenesis, Mutagenesis, Impairment of Fertility: In a two year rat carcinogenicity study a higher incidence of uterine adenocarcinoma, relative to controls, was observed in females receiving 30mg/kg/day (at least 174 times human AUC exposure after a 1mg twice daily dose). Adrenal phaeochromocytomas were increased relative to controls in males receiving 3mg/kg/day and above, and in females receiving 10mg/kg/day and above (at least 10 and 18 times respectively human AUC exposure after a 1mg twice daily dose) (b) (4) (b) (4) .

Anagrelide hydrochloride was not genotoxic in the Ames test, the mouse lymphoma cell (L5178Y, $TK^{+/-}$) forward mutation test, the human lymphocyte chromosome aberration test, or the mouse micronucleus test. Anagrelide hydrochloride at oral doses up to 240 mg/kg/day (1,440 mg/m^2/day, 195 times the recommended maximum human dose based on body surface area) was found to have no effect on fertility and reproductive performance of male rats. However, in female rats, at oral doses of 60 mg/kg/day (360 mg/m^2/day, 49 times the recommended maximum human dose based on
body surface area) or higher, it disrupted implantation when administered in early pregnancy and retarded or blocked parturition when administered in late pregnancy.

Pregnancy: Pregnancy Category C.
(i) Teratogenic Effects
Teratology studies have been performed in pregnant rats at oral doses up to 900 mg/kg/day (5,400 mg/m^2/day, 730 times the recommended maximum human dose based on body surface area) and in pregnant rabbits at oral doses up to 20 mg/kg/day (240 mg/m^2/day, 32 times the recommended maximum human dose based on body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to anagrelide hydrochloride.
(ii) Nonteratogenic Effects
A fertility and reproductive performance study performed in female rats revealed that anagrelide hydrochloride at oral doses of 60 mg/kg/day (360 mg/m^2/day, 49 times the recommended maximum human dose based on body surface area) or higher disrupted implantation and exerted adverse effect on embryo/fetal survival.

A perinatal and postnatal study performed in female rats revealed that anagrelide hydrochloride at oral doses of 60 mg/kg/day (360 mg/m^2/day, 49 times the recommended maximum human dose based on body surface area) or higher produced delay or blockage of parturition, deaths of nondelivering pregnant dams and their fully developed fetuses, and increased mortality in the pups born.

There are however, no adequate and well controlled studies with anagrelide hydrochloride in pregnant women. Because animal reproduction studies are not always predictive of human response, anagrelide hydrochloride should be used during pregnancy only if clearly needed.

Nonclinical toxicology:

A significant increase in non-neoplastic lesions were observed in anagrelide treated males and females in the adrenal (medullary hyperplasia), heart (myocardial hypertrophy and chamber distension), kidney (hydronephrosis, tubular dilation and urothelial hyperplasia) and bone (femur enostosis). Vascular effects were observed in tissues of the pancreas (arteritis/periarteritis, intimal proliferation and medial hypertrophy), kidney (arteritis/periarteritis, intimal proliferation and medial hypertrophy) and sciatic nerve (vascular mineralization), and testes (tubular atrophy and vascular infarct) in anagrelide treated males.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The sponsor conducted this study to determine the effects of Anagrelide on the incidence and morphology of tumors following oral (dietary) administration to the rat for 104 weeks. A carcinogenicity study was not requested by the FDA since the drug is only administered periodically and for only one week at a time. The study protocol was not submitted to the agency for concurrence prior to initiation of the study. There were 2 control groups and a total of 5 groups per gender designated Groups 1-5 with dose levels of Anagrelide of 0, 0, 3, 10, and 30 mg/kg/day. Due to low survival in the 2 top dose groups, the dose levels were lowered and the study was terminated early. Males in the high dose group were terminated week 80 and females week 89. All remaining groups were terminated during weeks 97-98.

Generally, body weight and food intake values were greater in Anagrelide treated groups. At necropsy, enlarged adrenal, heart and kidney were observed in males and females with renal pelvic dilatation seen in males only.

Non-neoplastic microscopic lesions included significant heart, adrenal, bone, kidney and testicular lesions. Vascular effects were observed in pancreas, kidney, testes and sciatic nerve.

There was an increase in the incidence of significant, drug-related neoplastic lesions in Anagrelide treated animals with benign phaeochromocytoma in adrenal in males and females and adenocarcinoma in uterus in females.

B. Pharmacologic activity

Anagrelide reduces the elevated platelet count in patients with thrombocythemia secondary to myeloproliferative disorders.

C. Nonclinical safety issues relevant to clinical use: None

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 20-333
Review number: SLR 092507
Sequence number/date/type of submission: SLR 013 SEPT-25-2007
Information to sponsor: Yes, Labeling
Sponsor and/or agent: Shire Development Inc., Wayne, PA
Manufacturer for drug substance: Not indicated

Reviewer name: David E. Bailey, Ph.D.
Division name: Medical Imaging and Hematology Drug Products (DMIHP)
HFD #: 160
Review completion date: February 5, 2008

Drug:

Trade name: Agrylin
Generic name: Anagrelide Hydrochloride Monohydrate
Code name:
Chemical name: 6,7-Dichoro-1,5-dihydroimidazo[2,1-b]-quinazolin-2(2H)-one hydrochloride monohydrate

CAS registry number:
Molecular weight: 310.55
Structure:

Relevant INDs/NDAs/DMFs: None indicated
Drug class: Platelet reducing agent
Intended clinical population: Patients with elevated blood platelet counts

Clinical formulation: 0.5 mg tablet
Route of administration: Oral
Clinical dose: 0.5 mg qid or 1.0 mg bid for 1 week. Not to exceed 10 mg/day or 2.5 mg/dose

Disclaimer: Tabular information is constructed by the reviewer. Graphical information is taken from the sponsor's submitted report.

Studies reviewed within this submission:

Anagrelide: Oral (Dietary) Oncogenicity Study in the Rat

Studies not reviewed within this submission: **None**

2.6.2 PHARMACOLOGY

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2.6.2.4 Safety pharmacology
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2.6.4.9 Discussion and Conclusions
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2.6.5 PHARMACOKINETICS TABULATED SUMMARY

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary
2.6.6.2 Single-dose toxicity
2.6.6.3 Repeat-dose toxicity
2.6.6.4 Genetic toxicology

2.6.6.5 Carcinogenicity

Study title: Anagrelide: Oral (Dietary) Oncogenicity Study in the Rat.

Key study findings: A carcinogenicity study was not requested by the FDA since the drug is only administered periodically and for only one week at a time. Clinical dose is 0.5 mg qid or 1.0 mg bid for 7 days, with maximum dose being 10 mg/day or 2.5 mg/dose. However, European regulators required a carcinogenicity study, so this study was conducted in rats at initial doses of Anagrelide of 0, 0, 3, 10 and 30 mg/kg/day. The highest dose was a multiple of approximately 170 times the MHD. Upon completion of the study the final report was submitted in the U.S. prior to revising the label.

Non-neoplastic lesions were observed in the adrenal, heart, kidney, testes and bone. Vascular effects were observed in tissues of the pancreas, kidney, testes and sciatic nerve.

Adequacy of the carcinogenicity study and appropriateness of the test model:
The study appears adequate to assess the carcinogenic potential in rats. However, the study has inherent problems, with the top 2 doses selected too high, resulting in poor survival rates and early termination during weeks 97-98. Had the protocol been submitted to the agency for concurrence, there are some protocol changes that would have been recommended for optimum conduct of the study, including individual housing instead of group housing of 5 animals/cage which was employed, and not bleeding the main study carcinogenicity animals for toxicokinetics.

Evaluation of tumor findings:
There was a significant, drug related increase in the incidence of neoplastic lesions in Anagrelide treated animals with benign phaeochromocytoma in adrenal in males and females, and adenocarcinoma in uterus in females.

Study no.:	ROO812-SPD422
Volume #, and page #:	9 Volumes, 3000 pages submitted to EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 2, 20042004
In-Life dates:	April 16, 2004 – February 28, 2006.
Report date:	August 14, 2007
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	Lot # CML 348/03-RS6, purity 92.9%
CAC concurrence:	No. Protocol was not submitted for CAC concurrence. Study required by European regulators but not in U.S.

Design: This study was designed to evaluate the carcinogenic potential of Anagrelide in a 104-week dietary study in rats. Overall study design is shown in the table below.

Group		Dose Level [a]	Number of Animals Assigned	Weeks Dosed	Week Survivors Killed
Number	**Description**	**mg/kg/day**			
	Males				
1	Control 1	0	60	1-96	97
2	Control 2	0	60	1-96	97
3	Low dose	3	60	1-96	97
4	Mid dose	10	60	1-66	
		7 [b]		67-88	
		0 [c]		89-96	97
5	High dose	30	60	1-66	
		15 [d]		67-79	80
	Females				
1	Control 1	0	60	1-97	98
2	Control 2	0	60	1-97	98
3	Low dose	3	60	1-97	98
4	Mid dose	10	60	1-97	98
5	High dose	30 [e]	60	1-88	89

[a] Dose expressed as free base.
[b] Dose reduced to 7 mg/kg due to mortality.
[c] Treatment discontinued due to mortality.
[d] Dose reduced to 15 mg/kg due to mortality week 67, all survivors killed week 80.
[e] All surviving females in group killed week 89 due to mortality.

Methods

Doses: 0, 0, 3, 10 and 30 mg/kg/day by diet.
Basis of dose selection: AUC
Species/strain: Charles River (b) (4), Sprague Dawley [Crl:CD®(SD)IGSBR]

Number/sex/group: 60
Route: Oral, dietary
Frequency of dosing: Ad lib in diet

Toxicokinetics: Main study animals, 5/sex/group at 4, 52 and 78 wks.
Age: 7-8 weeks at initiation.
Animal housing: Gang housed, 5/sex/cage.

Dietary restriction: None
Drug stability/homogeneity: Diets prepared weekly, with duplicate samples of lowest and highest dose groups checked weeks 1, 13, 26, 39, 52, 65, 78 and 91.

Dual controls employed: Yes
Interim sacrifices: Not by design.
Protocol deviations: Doses were lowered and animals were sacrificed early.

Rational for dose selection: Range finding study information was not provided in this study report. The rational for dose selection is unclear from this report although the sponsor indicates that doses were on the AUC basis. The sponsor selected doses that gave exaggerated AUC multiples based on the human AUC. Human AUC values were not included, however the report indicated that the AUC for the rat increased with time as did the clinical dose. The report also indicated that for the 3 mg/kg group the AUC would be expected to increase over time and provide an initial dose multiple of 1.8, increasing with time to 5.9 times the human AUC. For the 30 mg/kg group exposure was expected to increase over time and initially provide a dose multiple of 20 and increasing with time to 60 times the human exposure.

Based on the results of this study, high dose exposure increased to approximately 170 times the MHD and mid dose exposure increased to approximately 57 times the MGD. It is not surprising that there were deaths and the study had to be terminated early with such exaggerated high doses.

The sponsor's range finding study was apparently inadequate to predict effects of high exposure levels for extended periods of time.

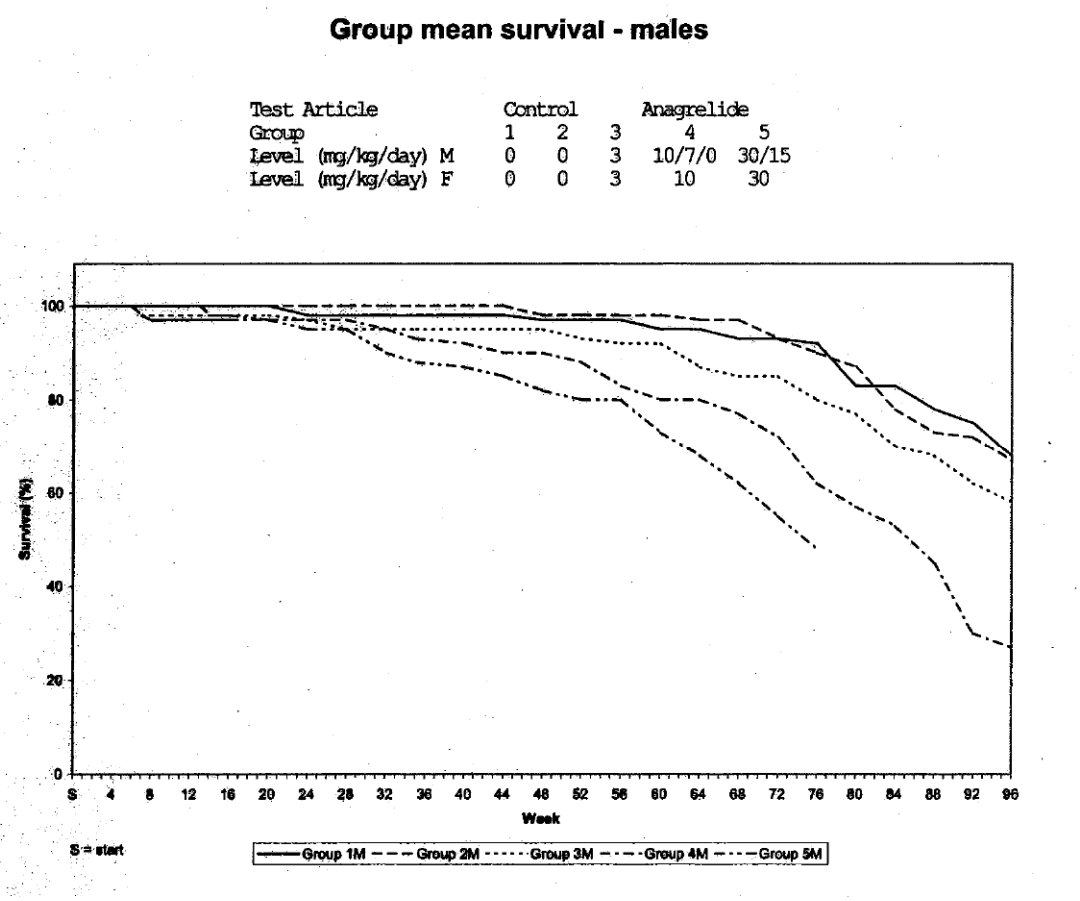
Results:

Mortality: As can be seen in the table below, mortality was generally the greatest in the two highest dose groups with mortality being dose related. Males of the highest dose group were all sacrificed during week 80, and females of that group were all sacrificed during week 89. All survivors in the remaining groups were terminated during week 97 for males and 98 for females.

	Group #				
	1	**2**	**3**	**4**	**5**
Dose (mg/kg)	0 (Control)	0 (Control)	3 mg/kg	10/7/0 mg/kg	30/15 mg/kg
	Males				
# Animals Assigned	60	60	60	60	60
Found Dead	6	4	9	23	24
Moribund Sacrifice	16	16	19	21	11
Total Deaths	22	20	28	44	35
Surviving at final sac	38	40	32	16	25
Week of Final sac	97	97	97	97	80
	Females				
# Animals Assigned	60	60	60	60	60
Found Dead	6	2	5	2	7
Moribund Sacrifice	30	23	24	30	28
Total Deaths	36	25	29	32	35
Surviving at final sac	24	35	31	28	25
Week of final sac	98	98	98	98	89

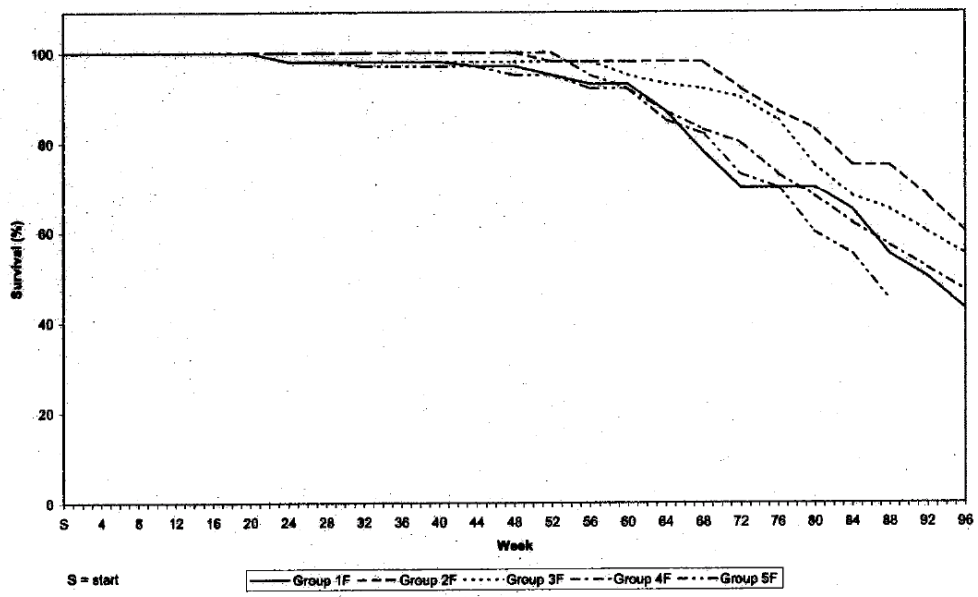
Survival graphs:

As can be seen in the following survival graphs, deaths began occurring in males at week 8 and in females at week 20.



Group mean survival - females

Test Article	Control			Anagrelide	
Group	1	2	3	4	5
Level (mg/kg/day) M	0	0	3	10/7/0	30/15
Level (mg/kg/day) F	0	0	3	10	30



Clinical signs:

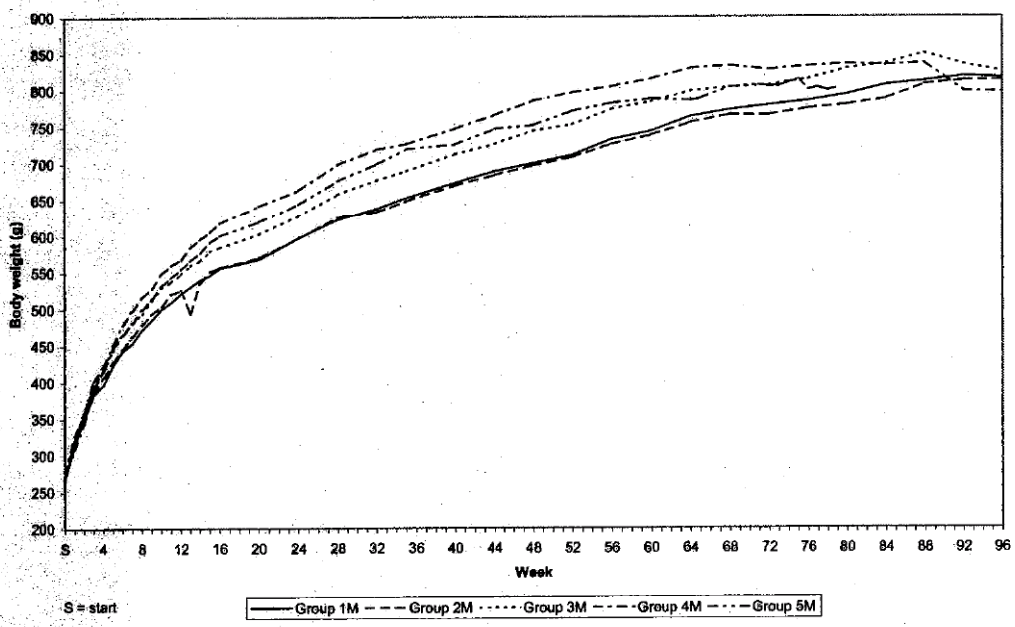
The primary clinical observation was that of a protruding sternum which occurred at a higher incidence in all Anagrelide treated groups, and was somewhat more prominent in males. Otherwise, as the animals aged there was an increase in the incidence of ruffled fur, hair loss, staining and skin sores that occurred sporadically in all groups including controls.

Body weights:

As can be seen in the growth curves below, body weights for males were generally greater in the Anagrelide treated groups as compared to controls. This is presumed to be the result of an increase in food intake. This was true for females also, for the first year of the study, but thereafter, female body weights were less for Anagrelide treated groups.

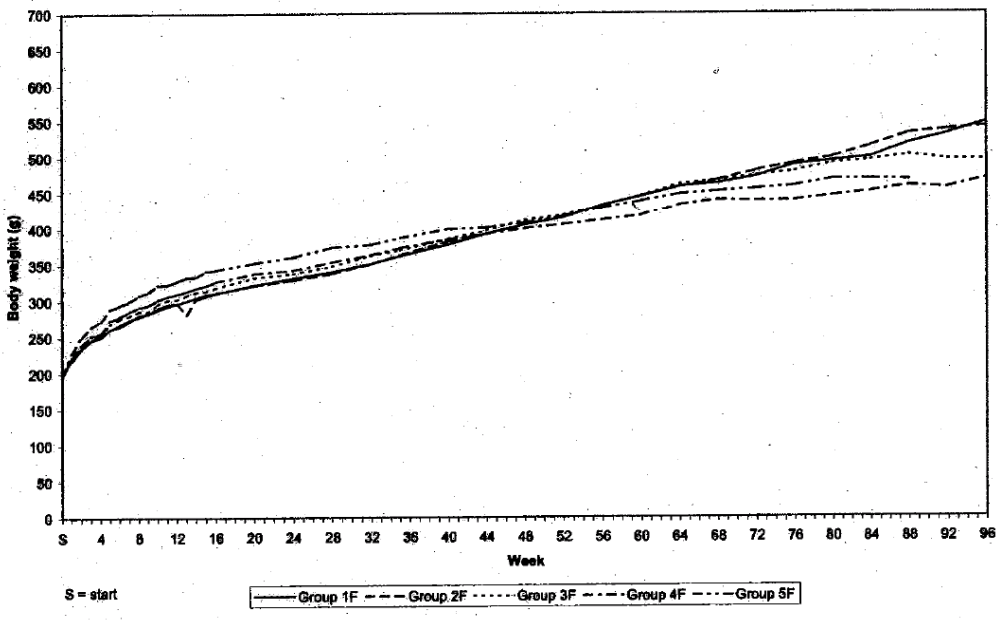
Group mean body weights - males

Test Article	Control			Anagrelide	
Group	1	2	3	4	5
Level (mg/kg/day) M	0	0	3	10/7/0	30/15
Level (mg/kg/day) F	0	0	3	10	30



Group mean body weights - females

Test Article	Control			Anagrelide	
Group	1	2	3	4	5
Level (mg/kg/day) M	0	0	3	10/7/0	30/15
Level (mg/kg/day) F	0	0	3	10	30

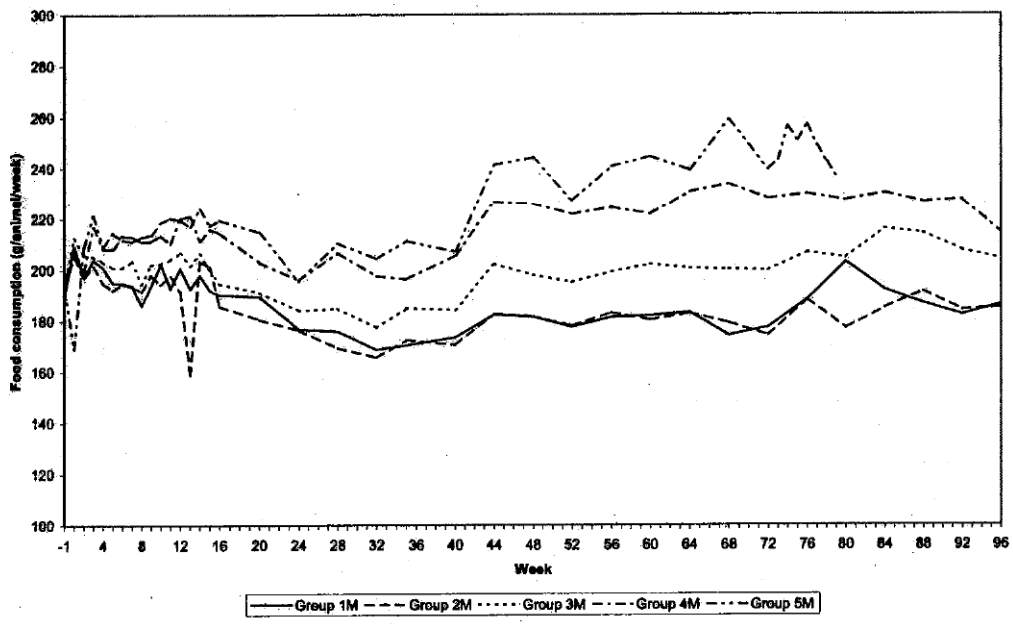


Food consumption:

Food consumption was generally increased in all Anagrelide treated groups, with a resultant increase in body weight. See the graphs below.

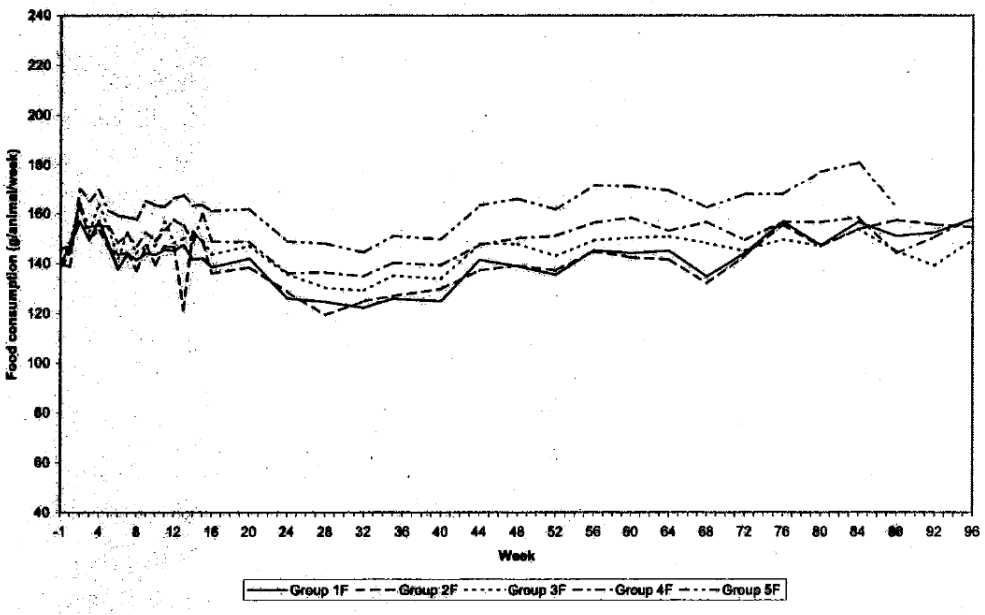
Group mean food consumption - males

Test Article	Control			Anagrelide	
Group	1	2	3	4	5
Level (mg/kg/day) M	0	0	3	10/7/0	30/15
Level (mg/kg/day) F	0	0	3	10	30



Group mean food consumption - females

Test Article	Control			Anagrelide	
Group	1	2	3	4	5
Level (mg/kg/day) M	0	0	3	10/7/0	30/15
Level (mg/kg/day) F	0	0	3	10	30



Ophthalmology:

Unaffected by treatment.

Hematology:

Only WBC and RBC counts were recorded and only at termination. WBC counts were unaffected by treatment. Mean RBC counts were insignificantly lower (4-7%) in the Anegrelide treated groups for both sexes. A drug or dose response was not observed.

Gross pathology:

The primary observations at necropsy that are considered drug or dose related include protruding sternum, enlarged adrenals, heart and kidney in females. Enlarged adrenals, heart, and kidney with pelvic dilatation were observed in males.

Histopathology:

Non-neoplastic microscopic lesions are shown in the table below. Significant heart, adrenal, bone, kidney and testicular lesions were noted. Vascular effects were observed in pancreas, kidney, testes and sciatic nerve.

Group	**1 & 2**	**3**	**4**	**5**	**1 & 2**	**3**	**4**	**5**
# Examined	**120**	**60**	**60**	**59**	**120**	**60**	**60**	**59**
	Males				**Females**			
Heart								
Myocardial hypertrophy	0	2	11	18	0	2	2	2
Chamber distension	4	8	15	18	0	2	2	4
Adrenal hyperplasia								
Focal medullary	27	27	29	35	21	19	26	26
Diffuse medullary	0	0	3	1	0	0	0	0
	Blood Vessels							
# Examined	120	59	59	57	120	59	59	58
Pancreas (Vascular)								
Arteritis/periarteritis	1	15	21	14	1	4	16	23
Intimal proliferation	0	5	6	3	0	1	6	11
Medial hypertrophy	0	18	39	30	0	2	19	24
Kidney (Vascular)								
Arteritis/periarteritis	0	4	11	7	0	0	1	2
Intimal proliferation	0	0	2	2	0	0	0	0
Medial hypertrophy	0	3	22	19	0	0	2	6
Testis (Vascular)								
Infarct	0	0	5	3	-	-	-	-
Sciatic nerve (Vascular)								
Vascular mineralization	3	13	20	21	1	1	4	8
	Bone effects							
Femur enostosis	0	1	2	6	0	0	1	2
	Kidney effects							
Urothelial hyperplasia	7	11	12	9	17	14	25	36
Tubular dilation	2	6	15	16	0	2	1	5
Hydronephrosis	15	18	33	40	14	6	14	23
Chronic nephrophathy	101	56	58	56	67	40	49	57
	Testicular effects							
Tubular								
Atrophy	25	21	32	36	-	-	-	-
Mineralisation	11	10	14	16				

Neoplastic lesions: The table below lists the observed tumors that are listed as "potentially" significant in the statistical review. There was an increase in the incidence of neoplastic lesions in Anagrelide treated animals with benign and metastatic phaeochromocytoma in adrenal, interstitial cell adenoma in testes, and follicular cell adenoma in thyroid in males, and benign phaeochromocytoma in adrenal and adenocarcinoma in uterus in females.

Since the malignant phaeochromocytomas in adrenal, interstitial cell adenoma in testes and follicular cell adenoma in thyroid are considered common tumors the p values were not adequate ($P \leq 0.005$) to meet the criteria for significant, drug-related tumors.

Therefore, the only significant, drug-related increase in neoplastic tumors includes benign phaeochromocytoma in adrenal in males and females and adenoma in uterus of females.

Group	1 & 2	3	4	5	p-values[b]		
	Incidence (%)[a]				Trend	Control vs Mid	Control vs Hi
Males							
# Examined	**120**	**60**	**59**	**58**			
Adrenal							
B -phaeochromocytoma	13(11)	19(32)	31(53)	20(34)	0.0000	0.0000	0.0000
M-phaeochromocytoma	3(3)	6(10)	9(15)	4(7)	0.0150	0.0000	0.0814
Testis							
Interstitial cell adenoma	7(6)	3(5)	3(5)	7(12)	0.0134	0.5910	0.0686
Thyroid							
Follicular cell adenoma	2(2)	0(0)	6(10)	3(5)	0.0193	0.0105	0.0483
Females							
# Examined	**120**	**60**	**60**	**58**			
Adrenal							
B -phaeochromocytoma	7(6)	1(2)	11(18)	16(28)	0.0000	0.0014	0.0000
Uterus							
Adenocarcinoma	3(3)	0(0)	3(5)	11(19)	0.0000	0.2139	0.0000

[a] Percentages rounded to nearest whole percent.
[b] p-values taken from CDER statistical review.

Toxicokinetics:

It can be seen from the table below that exposure is greater than proportional to dose, and that at the same dose, exposure increases over time. AUC values for males are generally greater than for females. This is true except for week 52 and 78 for high dose group, where female values are greater than for males.

		C_{max}(ng/mL)			$AUC_{(0-t)}$(ng.h/mL)		
Group	Sex	Week 4	Week 52	Week 78	Week 4	Week 52	Week 78
3	Male	26.5	55.9	93.4	416	915	1430
	Female	13.6	18.4	22.9	188	283	393
4	Male	141	679	475	1920	8240	5930
	Female	68.3	80.8	134	730	1100	1900
5	Male	980	3440	1370	13500	58700	21700
	Female	555	1190	1450	6820	117100	22300

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Embryofetal development

Prenatal and postnatal development

2.6.6.7 Local tolerance

2.6.6.8 Special toxicology studies

2.6.6.9 Discussion and Conclusions

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

This study was designed to asses the effects of Anagrelide on the incidence and morphology of tumors following oral (dietary) administration to Sprague Dawley rats with doses of 0, 0, 3, 10, and 30 mg/kg/day for 104 weeks. Significant increases in the incidence of adrenal neoplastic tumors were observed in males and females, and in uterus in females.

Non-neoplastic lesions were observed in the adrenal, heart, kidney, testes and bone. Vascular effects were observed in tissues of the pancreas, kidney, testes and sciatic nerve.

Unresolved toxicology issues: None

Suggested labeling: Please see page 3 of this review for suggested labeling.

Signatures:

Reviewer Signature: David E. Bailey, Ph.D.

Supervisory Signature: Adebayo A. Laniyonu, Ph.D.

APPENDIX/ATTACHMENTS : EXECUTIVE CAC MEETING MINUTES.

Executive CAC
Date of meeting: February 19, 2008

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Paul Brown, Ph.D., OND IO, Member
John Leighton, Ph.D., DDOP, Member
Adebayo A. Laniyonu, Ph.D., DMIHP, Supervisory Pharmacologist
David E. Bailey, Ph.D., DMIHP, Presenting Reviewer

Author of Draft: David E. Bailey, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA #: 20-333 **DRUG Name:** AGRYLIN® (Anagrelide hydrochloride monohydrate)

Sponsor: Shire Development Incorporated, Wayne, PA

Anagrelide is orally active and reduces the elevated platelet count in patients with thrombocythemia secondary to myeloproliferative disorders. In support of this indication, the sponsor submitted a report of a 104-week oral (dietary) study in rats that was required by European regulators. The study was not required in the US since the maximum duration of clinical treatment is 10 mg/day for 1 week. However, the sponsor submitted the study since there were drug related tumors and labeling was being revised.

Rat Carcinogenicity Study

In a 104-week oral (dietary) carcinogenicity study, Anagrelide was administered to groups of Sprague Dawley rats at initial doses of 0, 0, 3, 10 and 30 mg/kg/day. There were 2 identical control groups. Groups included 60 rats/sex/group and gang housed with 5 rats/cage. Doses were selected based on comparative AUC data for rats and humans. The initial doses were too high and due to deaths in males, the mid-dose group males were reduced to 7 mg/kg/day from week 67-88, and reduced further to 0 mg/kg/day from week 89 to terminal sacrifice during weeks 97-98. High dose males were reduced to 15 mg/kg/day during weeks 67-79 and the group sacrificed during week 80. All surviving groups were terminated during weeks 97-98. Drug related non-neoplastic lesions were observed in adrenal, bone, heart, kidney and testes. Vascular effects were observed in pancreas, kidney, testes and sciatic nerve. There was a drug related increase in the incidence of significant neoplastic tumors in Anagrelide treated animals with benign phaeochromocytoma in adrenal of males, and benign phaeochromocytoma in adrenal, and adenocarcinoma in uterus of females.

Executive CAC Recommendations and Conclusions:

The Committee noted a number of concerns with the study, including using initial dose levels that were too high in males (mid- and high-dose) and females (high-dose) which led to early deaths and study termination; using group housing (5 animals/cage) with a dietary study; and bleeding of main study animals during the study.

The Committee encourages sponsors to obtain a protocol review prior to starting carcinogenicity studies to optimize their conduct and interpretation of results.

The Committee found that the study was positive for benign phaeochromocytomas in adrenals for both males and females and adencocarcinoma in the uterus of females (although this last finding was significant only at a dose that exceeded the MTD).

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

Cc:\ /Division File, DMIHP
/ALaniyonu, DMIHP
/DBailey, DMIHP
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/ASeifried, OND IO

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/s/

David Jacobson-
Kram
2/21/2008
11:47:18 AM

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/s/

David Bailey
2/27/2008 11:03:02 AM
PHARMACOLOGIST

Adebayo Laniyonu
2/28/2008 12:18:42 PM
PHARMACOLOGIST

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 20-333/S-013

STATISTICAL REVIEWS

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Pharmacoepidemiology and Statistical Science
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CARCINOGENICITY STUDY

NDA Number:	20,333 / Serial 003
Drug Name:	Agrylin® (Anagrelide Hydrochloride Monohydrate) Capsules, 0.5 mg
Indication:	Blood platelet count reduction
Applicant:	Shire Development, Inc.
Date:	Submitted 21 September 2007
Review Priority:	Standard
Biometrics Division:	Division 6
Statistical Reviewer:	Steve Thomson
Concurring Reviewer:	Team Leader: Karl Lin, Ph. D.
Medical Division:	Medical Imaging and Hematology Products
Toxicologist:	Reviewer: David E. Bailey, Ph.D. Supervisor: Laniyonu, Adebayo, Ph.D.
Project Manager:	Hyon-Zu Lee
Keywords:	Bayesian analysis, Carcinogenicity, Cox regression, Kaplan-Meier product limit, Survival analysis, Trend test

Table of Contents

1. EXECUTIVE SUMMARY

According to the report provided by the Sponsor the objective of this study was "to determine the effects of Anagrelide, on the incidence and morphology of tumours following oral (dietary) administration to the rat for 104 weeks." (page 27 of Sponsor's Report) Due to lowered survival actual study duration was shorter than two years. The sponsor is the Shire Pharmaceuticals Development Ltd., in Chineham, Basingstroke, England. The studies were conducted by the (b) (4).

1.1. Conclusions and Recommendations

This submission summarizes the results of a rat study of the carcinogenic potential of Anagrelide when administered with diet for two years. There were five treatment groups per gender, including two supposedly identical vehicle controls, and three treatment groups with nominal dose levels of Anagrelide at 3, 10, and 30 mg/kg/day respectively. The latter three dose groups, labeled in the Sponsor's report as Groups 3-5, were labeled as Low, Medium, and High, respectively. Each dose group had 60 animals per gender. Thus the pooled controls included 120 animals. Further, due to high mortality, the medium dose group in males was reduced to a nominal 7 mg/kg/day at Week 67, with dosing completely discontinued at Week 89. The high dose group in males was reduced to a nominal 15 mg/kg/day at Week 67. High dose males were sacrificed in Week 80, while High dose females were sacrifice in Week 89. Note that animals were housed together in groups of five, and since treatment was administered with diet, doses may be only approximate.

The statistical significances of the tests of differences in survival across treatment groups are given below. Since differences between the two vehicle controls should be due solely to randomization, for the tests below these two control groups are pooled to a single control group. That is, the tests of homogeneity and trend in survival over pooled Groups 1 and 2, and, 3 through 5 are tests of homogeneity and trend over the four dose groups, starting from the two pooled controls.

The Cox test in Table 1 below is usually called the logrank test, while the K-W test, i.e., Kruskal-Wallis test, is more commonly called the Wilcoxon test or the generalized Wilcoxon test. Note that the Wilcoxon test places more weight on earlier events than does the logrank test.

In males the test of the null hypothesis of homogeneity in survival over the four dose groups is statistically significant (Cox $p<0.00005$ and K-W $p<0.00005$) and in females the tests are close to statistical significance (Cox $p=0.0800$ and K-W $p=0.0742$). From the Kaplan-Meier estimated survival curves, given in Appendix 1, in males the statistically significant results are associated with clear dose response. Further, approximate parallelism in the log scale implies that the Cox proportional model would fit quite well. In females, the treatment differences were much smaller than in males. The survival curves of the low Anagrelide dose (3 mg/kg/day) group and the pooled controls are closely intertwined, with slightly increasing mortality

associated with the medium and high dose groups (10 and 30 mg/kg/day, respectively). For both genders, the test of trend was statistically significant (In males, both Cox and K-W $p<0.00005$, in females both $p\leq0.0116$). For males the departure from trend was barely statistically significant (both $p=0.0342$). For females the departure from trend was not statistically significant (both $p\geq0.8263$). Appendix 2 includes an experimental Bayesian analysis of these issues.

Table 1. Statistical Significances of Tests of Homogeneity and Trend in Survival

	Males		Females	
	Cox	K-W	Cox	K-W
Homogeneity over Groups 1+2, 3-5	0.0000	0.0000	0.0800	0.0742
Trend over Groups 1+2, 3-5	0.0000	0.0000	0.0116	0.0103
Departure from trend in 1+2, 3-5	0.0342	0.0342	0.8263	0.8445

Appendix 2 includes an experimental Bayesian analysis of dose related survival. In male rats this also confirms the strong trend in increasing mortality over dose. In female rats, results are somewhat more equivocal. There is evidence that the pooled controls and the low dose groups have significantly lower mortality than the high dose group, and there is a suggestion of increasing mortality over dose.

Since this is a one species study, for rare tumors both the Peto test of trend and the Peto test of pairwise comparisons between the high dose group and the controls should be tested at a 0.05 (5%) level. The corresponding tests for common tumors should be tested at a 0.01 level. This approach is intended to balance both Type I error and Type II error (i.e., the error of concluding there is no evidence of a relation to tumorgenicity when there actually is such a relation). Note this differs from the usual Haseman-Lin-Rahman rules for two species/two genders cited by the Sponsor (please see Section 1.3.1.4. for details).

From the incidence in the control group all potentially statistically significant tumors cited in Table 2 below would be considered as common tumors. For a roughly 10% error rate in each comparison one can consider the test to be statistically significant if the test has a p-value of 1% or less. In this table, both male and female benign phaeochromocytomas displayed highly statistically significant trends and differences between the high dose group and the pooled controls (all four $p < 0.00005$). The differences between the pooled controls and the medium dose groups were also statistically significant (males: $p<0.00005$, females: $p=0.0014$). In male rats the test of trend in malignant phaeochromocytomas was not quite statistically significant ($p= 0.0150$), but the test comparing the medium dose group to controls was statistically significant ($p<0.00005$). In male rats the tests of trends in interstitial cell adenoma of the testis and the test of trends in follicular cell adenoma of the thyroid were fairly close, but did not achieve statistical significance ($p=0.0134$ and $p=0.0193$, respectively). Note that the difference between the medium dose group and the pooled controls was quite close to statistical significance for the latter tumor ($p=0.0105$). In female rats both the test of trend and the test of differences between the high dose group and the pooled controls were highly statistically significant in adenocarcinoma of the uterus (both $p < 0.00005$).

Table 2. Potentially Statistically Significant Neoplasms in Rats

	Incidence:				p-values:	Hi vs	Med vs
	Ctrls	Low	Med	High	Trend	Ctrls	Ctrls
MALES:							
ADRENAL							
BENIGN PHAEOCHROMOCYTOMA	13	19	31	20	0.0000	0.0000	0.0000
MALIGNANT PHAEOCHROMOCYTOMA	3	6	9	4	0.0150	0.0814	0.0000
TESTIS							
INTERSTITIAL CELL ADENOMA	7	3	3	7	0.0134	0.0686	0.5910
THYROID							
FOLLICULAR CELL ADENOMA	2	0	6	3	0.0193	0.0483	0.0105
FEMALES:							
ADRENAL							
BENIGN PHAEOCHROMOCYTOMA	7	1	11	16	0.0000	0.0000	0.0014
UTERUS							
ADENOCARCINOMA	3	0	3	11	0.0000	0.0000	0.2139

Note that more complete incidence tables are provided in Appendix 4. Appendix 3 reproduces the Sponsor's detailed summary of neoplasms and the results of Peto tests. Appendix 5 presents the results of a so-called poly-k adjustment of the Cochran-Armitage test for trend as well as pairwise comparisons. This test does not require accurate assessment of whether or not a tumor is fatal, as do the Peto tests. It generally confirms the analysis presented above. Finally, Appendix 6 presents a Bayesian assessment of the incidence of neoplasms. It also is generally consistent with the analysis above.

1.2. Brief Overview of the Studies

This submission had one rat study:

Study Report 2082/018 Anagrelide: Oral (Dietary) Oncogenicity Study in the Rat,

According to the report provided by the Sponsor, the objective of this study was "to determine the effects of Anagrelide, on the incidence and morphology of tumours following oral (dietary) administration to the rat for 104 weeks." However, as noted earlier the study was terminated early due to high mortality in some dose groups (97 weeks for males, 98 for females). The sponsor is the Shire Pharmaceuticals Development Ltd., in Chineham, Basingstroke, England. The studies were conducted by the (b) (4), (b) (4) Two groups of 60 rats/sex/group were designated as Controls and received the vehicle. Three further treatment groups of 60 male and 60 female Crl: CD® (SD)IGSBR rats/group were administered the test article in the diet. Note that animals in each treatment group were initially housed in multiples of five. Nominal dose levels were 3, 10, and 30 mg/kg/day, based on average within cage food consumption.

1.3. Statistical Issues and Findings

1.3.1. Statistical Issues

In this section, several issues, typical of statistical analyses of these studies, are considered. These issues include details of the survival analyses, tests on tumorigenicity, multiplicity of tests on neoplasms, and the validity of the designs.

1.3.1.1. Control Groups:

The Sponsor separately tabulates measures of mortality, tumorigenicity, food consumption, etc. among the two controls. In the Sponsor's report these are labeled as treatment groups 1 and 2 among the five treatment groups. But supposedly these control groups are identical and should be pooled for analysis. The data sets provided to the FDA do not distinguish between the two control groups, and list only four treatment groups. For consistency with the Sponsors analysis, for the FDA analysis the pooled controls are labeled as Groups 1 & 2 or as "Controls".

1.3.1.2. Survival Analysis:

Note that the Cox test is usually called the logrank test, while the K-W, i.e., Kruskal-Wallis test, is more commonly called the Wilcoxon test or the generalized Wilcoxon test. Note that the Wilcoxon test places more weight on earlier events than does the logrank test.
Both the Cox logrank and Kruskal-Wallis-Wilcoxon tests were used to test homogeneity of survival among the treatment groups. Tests of dose related trend using a Cox proportional odds model were also performed. The number of such tests raises issues of multiple testing, but from the point of view of finding differences among treatment groups (i.e., reducing the probability of Type II error), this should be acceptable. Appendix 1 reviews the animal survival analyses in some detail. The Sponsor's analyses are summarized in Section 3.2.1.1.

The Sponsor notes that "Although administration of Anagrelide over the first 6-9 months was generally well tolerated, higher mortality in males given 10 or 30mg/kg/day became apparent as the study progressed. . . . Lowering of the dose level for males, from 10 and 30mg/kg/day to 7 and 15mg/kg/day respectively, in Week 66 of treatment, did not result in a diminution of the deterioration in clinical condition. Consequently, it became necessary to sacrifice surviving males given 30/15mg/kg/day early, in Week 80 of treatment, when the number of surviving animals declined to 25. Although treatment of surviving males given 10/7mg/kg/day ceased in Week 89, the rate and incidence of mortality in this group did not subside and surviving animals in this group were sacrificed in Week 97. Surviving females given 30mg/kg/day were sacrificed in Week 89 of treatment, when the number of survivors declined to 25. All surviving animals were sacrificed in Week 97 (males) or Week 98 (females), when the number of surviving females in Group 1 (Control) declined to 24." (page 29 of the Sponsor's Report) It is this reviewer's opinion that the rule of sacrificing animals when only 25 remain seems premature, especially when this bound is only exceeded in one of two putatively identical control groups and not in other dose groups.

1.3.1.3. Tests on Neoplasms:

Three different assessments of tumorigenicity are provided in the appendices. Appendix 3 presents the Sponsor's Peto analysis, while Appendix 4 presents the corresponding FDA Peto analysis. This is has been the usual primary carcinogenicity analysis utilized in submissions to CDER. However, the Society of Toxicological Pathology had a town hall meeting in June 2001 where this approach was criticized. The primary alternative discussed in the commentary on this meeting (STP Peto Working Group, 2002) is the poly-k modification of the Cochran-Armitage test of trend for tumor incidence, presented in Appendix 5. Appendix 6 includes an experimental Bayesian analysis of tumorigenicity.

The FDA Peto style of tumorigenicity analyses of fatal tumors are based on the time of death, and for observable tumors based on time of detection. Both are analyzed at the time of detection with an analysis equivalent to the death rate method. Non-fatal tumors found at the time of the animals' death are labeled as incidental, and were analyzed by the so-called prevalence method. For the FDA analyses all three results were pooled. The tests on these neoplasms used in the FDA analysis were basically tests of trend. In the FDA Peto analysis, significance levels of two tests are provided: 1) a test of trend over from the pooled controls and the three Anagrelide treatment groups (i.e., Sponsor Groups 1&2, 3-5) and 2) a test comparing the high dose of Anagrelide to the pooled controls. Note that the so-called poly-k tests, reported in Appendix 5, also provide the results of pairwise tests comparing the medium and low dose groups to the pooled controls.

1.3.1.4. Multiplicity of Tests on Neoplasms:

Testing the various neoplasms involved a large number of statistical tests, which in turn necessitated an adjustment in experiment-wise Type I error. One, perhaps the usual, approach for two species, two gender, two year studies with testing for trend over four doses and comparing the high dose group to controls follows the Haseman-Lin-Rahman rules for the Peto analysis. Based on his extensive experience with such analyses, for pairwise tests between the high dose group and controls in two species, Haseman (1983) claimed that for a roughly 0.10 (10%) overall false positive error rate, rare tumors should be tested at a 0.05 (5%) level, and common tumors (with a historical control incidence greater than 1%) at a 0.01 level. For a standard chronic study in two species (i.e., rats and rats) study, based on simulations and their experience, Lin & Rahman (1998) proposed a further p-value adjustment for tests of trend. That is, for a roughly 0.10 (10%) overall false positive error rate in tests of trend, rare tumors should be tested at a 0.025 (2.5%) level and common tumors at a 0.005 (0.5%) level. This is the adjustment used by the Sponsor. However, since this is a one species study, for rare tumors both the test of trend and the test of pairwise comparison between the high dose group and the appropriate control should be tested at a 0.05 (5%) level. The corresponding tests for common tumors should be tested at a 0.01 level. In this analysis we will use the observed incidence in the pooled control group to decide if a tumor is rare or common. This approach is intended to balance both Type I error and Type II error (i.e., the error of concluding there is no evidence of a relation to tumorgenicity when there actually is such a relation).

Further, note that strictly speaking, these rules only control the overall errors of the test of trend in Anagrelide and the corresponding comparison between the high dose Anagrelide group and the pooled controls (i.e., tests 1 and 2 in 1.3.1.3 above). It is not clear how the error rate would apply to other possible tests. This problem is exacerbated in the Sponsor's analysis since the Sponsor also tests differences between each treatment group and the pooled controls.

Preliminary unpublished results from (b) (4) (personal communication) suggest that the (b) (4) analysis. As discussed in Berger and Savage (1966) one can consider that the Bayesian methods (as in Appendices 2 and 6), incorporate an automatic correction for multiplicity.

1.3.1.5. Housing of Animals:

According to the Sponsor's report: "The animals were housed in groups of five in cages that conform with the 'Code of practice for the housing and care of animals used in scientific procedures' (Home Office, London, 1989)." (page 37) Note that multiple housing of animals may cause statistical problems in the analysis. With dietary administration of treatment it would be difficult to control actual dosing within each dosing group of five animals. At each point in time the Sponsor ascribes the mean food consumed to each animal in the cage. So differences in food consumption and hence dose within a cage are ignored. Competition for diet might induce negative correlations in treatment response, while proximity might induce positive correlations in treatment response. Further, animals housed together might fight each other. The skins of some animals could be damaged, and this damage might be associated with skin tumors. Thus, with this multiple housing, from a statistical design point of view, the appropriate treatment unit would generally be the group of five animals housed together. Apparently these possible correlations are generally ignored, and the treatment unit is assumed to be the individual animal.
.

It should be noted that because of this housing the within treatment estimated variances may be too large or too small, resulting in conservative or liberal tests (in terms of Type I error). Unless it has been clearly shown that tumor incidence is independent of cage, from a purely statistical point of view, this reviewer would generally recommend single housing of animals. However, the net result of the analysis in Appendix 7 is that there is evidence of a weak cage effect in male rats, but little evidence in female rats.

1.3.1.6. Validity of the Designs:

When determining the validity of designs there are two key points:
1) adequate drug exposure,
2) tumor challenge to the tested animals.

1) is related to whether or not sufficient animals survived long enough to be at risk of forming late-developing tumors and 2) is related to the Maximum Tolerated Dose (MTD), designed to achieve the greatest likelihood of tumorigenicity.

Lin and Ali (1994), quoting work by Haseman, have suggested that a survival rate of about 25 animals, out of 50 or more animals, between weeks 80-90 of a two-year study may be

considered a sufficient number of survivors as well as one measure of adequate exposure. Note this criterion does seem to be satisfied.

Chu, Ceuto, and Ward (1981), citing earlier work by Sontag *et al* (1976) recommend that the MTD "is taken as 'the highest dose that causes no more than a 10% weight decrement as compared to the appropriate control groups, and does not produce mortality, clinical signs of toxicity, or pathologic lesions (other than those that may be related to a neoplastic response) that would be predicted to shorten the animal's natural life span' " The values in the following tables were computed from the WEIGHTS and FOOD data sets. Table 3 gives the final body weight and the final percent weight change relative to the pooled controls in each study. Note that this criterion seems to fail in both male and female rats. The relative weight decrement in female rats may indicate that the MTD was exceeded, while the weight relative increments in all the treated groups in male rats may indicate that the high dose is under the MTD.

Table 3: Relative Weight Change (compared to control)

Study 2082/018: Group – Nominal Dose (mg/kg/day)	Males			Females		
	Baseline	Week 79 change	% from Control	Baseline	Week 88 Change	% from control
Controls – 0	269.7	510.4		198.5	328.2	
Low - 3	272.3	543.4	+6.4%	197.9	305.2	-7.0%
Medium -10	276.0	557.7	+9.3%	195.5	266.6	-18.8%
High -30	269.1	536.7	+5.1%	198.2	268.1	-18.3%

From Table 4, below, in males treatment groups seem to be generally associated with higher mean food consumption in treated groups compared to controls, while in females only the high dose group had higher mean food consumption.

Table 4: Mean Food Consumption (g/animal/week)

Study 2082/018: Dose Label	Males			Females		
	Dose Initial/Final (mg/kg/day)	Week 79	% from Control	Dose Initial/Final (mg/kg/day)	Week 88	% from Control
Controls	0	187.8		0	149.8	
Low	3	205.0	+9.1%	3	144.4	-3.6%
Medium	10/7/0	227.5	+21.1%	10	144.0	-3.8%
High	30/15	250.0	+33.1%	30	162.5	+8.5%

Note that excess mortality in the higher dose groups not associated with or concomitant with a tumor, may be associated with toxicity. This may suggest that the MTD was exceeded. One way to assess this is to measure mortality not associated with any identified tumor. Table 5 below indicates the number of animals in each dose group that died of a natural death or moribund sacrifice, but did not show any tumors:

Table 5: Observed Animal Mortality with no Identified Tumor

Study 2082/018 Dose Label	Males			Females		
	Dose Initial/Final (mg/kg/day)	Died without tumor	Other	Dose Initial/Final (mg/kg/day)	Died without tumor	Other
Controls	0	4	116	0	4	116
Low	3	9	51	3	2	58
Medium	10/7/0	14	46	10	1	59
High	30/15	16	44	30	5	55

To compare the incidence of deaths without tumors we can specify the usual survival tests where animals that die with a tumor or are sacrificed are considered as censored. For males the tests of homogeneity in survival over dose are strongly rejected (both logrank and Wilcoxon $p<0.00005$). For females the corresponding tests are not statistically significant, though perhaps close to statistical significance (logrank $p=0.0914$, Wilcoxon $p=0.0714$). Although this is a decision for the toxicologist, this is evidence that in male rats the MTD was exceeded in both the high dose and the medium dose groups. If these were premature deaths due to toxicity it would explain the lower incidence of some tumors in the high dose group than in lower dose groups.

1.3.2. Statistical Findings

Please see Section 1.1 above.

2. INTRODUCTION

2.1. Overview

Results from a study in Sprague Dawley Crl:CD® (SD)IGSBR strain rats were submitted to assess the carcinogenic potential of Anagrelide when administered with the animals' diets.

2.2. Data Sources

Four SAS transport files were provided by the Sponsor and placed in the CDER electronic data room (edr):

Food.xpt Tumor.xpt
Mortal.xpt Weights.xpt

Although there were two supposedly identical control groups, these groups were not distinguishable in the data sets above, and were pooled for all FDA generated tables and analyses. The Sponsor supplied analyses that distinguished between these control groups.

3. STATISTICAL EVALUATION

3.1. Evaluation of Efficacy

NA

3.2. Evaluation of Safety

More detailed results on the study are presented below.

3.2.1. Study 2082/018 Anagrelide: Oral (Dietary) Oncogenicity Study in the Rat

STUDY DURATION: Up to 97 Weeks (Males), 98 Weeks (Females).
DOSING STARTING DATE: April 16, 2004.
NECROPSY DATE: February 24, 2006.
EARLY DOSING TERMINATION: Males: Medium Dose Group 4 Week 89.
High Dose Group 5 Week 79.
Females: High Dose Group 5 Week 89.
TERMINAL SACRIFICE: Males: High Dose Group 5 Week 80.
Dose Groups 1-4 Week 97.
Females: High Dose Group 5 Week 89.
Dose Groups 1-4 Week 98.
STUDY ENDING DATE (Final Report dated): October 19, 2006.
RAT STRAIN: Sprague Dawley Crl:CD® (SD)IGSBR strain rats.
ROUTE: Oral Dietary, (b) (4) ground diet (b) (4)

Five treatment groups, Groups 1-5, were formed for each of male and female CD rats (60/gender in each group). According to the report: "All animals were observed daily for signs of ill health or overt toxicity. In addition, each animal was given a detailed physical examination at weekly intervals, which included palpation for tissue masses. An individual record was maintained of the clinical condition of each animal." (page 39 of the Sponsor's Report)

An alternative layout of the study design is summarized in the following Table 6:

Table 6: Study Design

Group		Dose (mg/kg/day)	Initial number of animals	Weeks dosed	Week surviving animals killed
Males	Control 1	0	60	1-96	97
	Control 2	0	60	1-96	97
	Low	3	60	1-96	97
	Medium	10	60	1-66	
		7		67-88	
		0		89-96	97
	High	30	60	1-66	
		15		67-79	80
Females	Control 1	0	60	1-97	98
	Control 2	0	60	1-97	98
	Low	3	60	1-97	98
	Medium	10	60	1-97	98
	High	30	60	1-89	89

3.2.1.1. Allocation to Treatment

"The animals were assigned to treatment groups during the acclimatisation period using a randomisation procedure based on stratified body weight. Control animals were housed on separate racks from animals given the test article." (page 38) Animals were approximately seven to eight weeks old at first dosing.

3.2.1.2. Animal Enviroment

During the study, animals were housed in groups of five. "Throughout the study the animals had access ad libitum to (b) (4) Rat Maintenance Diet (b) (4) Ground (b) (4)). Each batch of diet was analysed for specific constituents and contaminants. Mains water was provided ad libitum via an automatic watering system. From during Week 24 (27 September 2004) onwards, animal water was provided ad libitum via water bottles. The water was periodically analysed for specific contaminants. The supply of water to Group 2 animals was temporarily interrupted in Week 13 due to a malfunction in the automatic watering system, and was considered not to have affected the study integrity or outcome." (page 38)

"The amount of food consumed by each cage of animals was determined commencing one week pre-treatment, weekly for the first 16 weeks and then approximately one week in every four thereafter. Due to a general loss in clinical condition, food consumption for males given 30/15mg/kg/day was recorded weekly in Weeks 72 to 79 in order to monitor the condition of the animals more closely. Consumption was calculated as g/animal/week. Lower than expected food consumption was recorded for animals in one of the Control groups (Control 2) in Week 13, due to a technical problem with the automated watering system, which resulted in flooded hoppers. The animals did not show any clinical indication of stress, and consumed a sufficient amount of food thereafter. As this was a single occurrence, this was considered not to have affected the study integrity or outcome." (page 39)

3.2.1.3. Sponsor's Results and Conclusions

This section will present a summary of the Sponsor's analysis on survivability and tumorigenicity in rats.

Survival analysis:

Simple mortality results are summarized in the following table:

Table 7: Sponsor's Summary Survival Counts

	Group/Sex/Dose Level (mg/kg/day)									
Group (Dose)	1M (0)	2M (0)	3M (3)	4M (10/7/0)	5M (30/15)	1F (0)	2F (0)	3F (3)	4F (10)	5F (30)
Number animals/group	60	60	60	60	60	60	60	60	60	60
Found dead	6	4	9	23	24	6	2	5	2	7
Killed early	16	16	19	21	11	30	23	24	30	28
Total decedents	22	20	28	44	35	36	25	29	32	35

According to the Sponsor: "Males demonstrated an overall increasing dose response in mortality across the groups, for all groups to the start of cull for animals given 30/15mg/kg/day ($P<0.001$) and for Control 1, Control 2, the 3mg/kg/day and 10/7/0mg/kg/day dose groups for the duration of the study ($P<0.001$). The mortality in the males given 10/7/0mg/kg/day or 30/15mg/kg/day was significantly higher than that in the combined control groups ($P<0.001$ for all tests). There was no significant difference in mortality between the two control groups ($P\geq0.05$ for both analyses).

"Females demonstrated an overall increasing dose response in mortality across all five groups, to the start of the cull for animals given 30mg/kg/day ($P=0.002$). Animals given 30mg/kg/day demonstrated significantly higher mortality than the combined control groups ($P=0.004$). There was a statistically significant difference in the mortality between the two control groups ($P=0.019$). When Control 1 alone was used, there was a significant overall increasing dose response ($P=0.026$). When Control 2 alone was used, there was a significant overall increasing dose response ($P<0.001$), and animals given 10 or 30mg/kg/day demonstrated significantly higher mortality than the control animals ($P=0.020$ and $P<0.001$ respectively).

"Over the full duration of the study there was a significant difference in female mortality between the two control groups ($P=0.027$), but the overall dose response and pairwise tests for Control 1, Control 2, the 3mg/kg/day and 10mg/kg/day dose groups did not achieve statistical significance ($P\geq0.05$), regardless of which controls were used.

"None of the tests for decreasing dose response achieved statistical significance for either sex ($P\geq0.05$)." (page 52 of Sponsor's Report)

Tumorigenicity analysis:

The Sponsor indicates that the following tumour types gave rise to results statistically significant at the 5% level (please see Sponsor's results on neoplasms in Appendix 4):

(a) adrenal medullary tumour, in males and females
(b) thyroid follicular cell adenoma, in males
(c) skin/appendage squamous cell tumour, in males
(d) uterus adenoma/carcinoma
(e) uterus stromal tumour
(f) skin+subcutis lipoma, in females

Table 8. Summary of Selected Tumorigenicity Findings

		Males					Females				
		Ctr1	Ctr2	Low	Med	High	Ctr1	Ctr2	Low	Med	High
Adrenals	# examined	60	60	60	59	58	60	60	60	60	58
B-phaeochromocytoma	Incidence	7	6	19	31	20	3	4	1	11	16
M-phaeochromocytoma	Incidence	2	1	6	9	4	0	1	1	0	1
Uterus	# examined	-	-	-	-	-	60	60	60	59	58
Adenocarcinoma	Incidence	-	-	-	-	-	3	0	0	3	11

The Sponsor's analysis follows the Haseman-Lin-Rahman rules described in section 1.3.1.4 above. However, since this is a one species study, for rare tumors both the test of trend and the test of pairwise comparison between the high dose group and the appropriate control should be tested at a 0.05 (5%) level. The corresponding tests for common tumors should be tested at a 0.01 level. According to the Sponsor: "With the classification for type (d) being rare and the rest common, the results of note were:

• Male adrenal medullary tumours:
To the start of the 30/15mg/kg/day cull, fatals and non-fatals combined, overall dose response (P=0.004), controls v 10/7/0mg/kg/day (P=0.001), controls v 30/15mg/kg/day (P=0.002). Non-fatals only, overall dose response (P=0.004), controls v 10/7/0mg/kg/day (P=0.007), controls v 30/15mg/kg/day (P=0.002). For the duration of the study, fatals and non-fatals combined and non-fatals only, overall dose response (P<0.001), controls v Group 3 (P<0.001), controls v 10/7/0mg/kg/day (P<0.001).

• Female adrenal medullary tumours:
To the start of the 30mg/kg/day cull, all non-fatal, overall dose response (P=0.003).
For the duration of the study, all non-fatal, overall dose response (P=0.001), controls v 10mg/kg/day (P=0.006).
• Female uterus adenoma/carcinoma:
To the start of the 30mg/kg/day cull, fatals and non-fatals combined, overall dose response (P<0.001), controls v 30mg/kg/day (P=0.006). Fatals only, overall dose response (P<0.001), controls v 30mg/kg/day (P=0.002).

In the tests for decreasing dose response there were no significant findings for the males (P≥0.05 for all tests). The following tumour type gave rise to results statistically significant at

the 5% level, in females: adrenal cortical tumour (for the duration of the study; overall dose response (P=0.002), combined controls v Group 3 (P=0.020) and combined controls v 10/7/0mg/kg/day (P=0.030)).

For males, there was no significant difference in tumour incidence between the two control groups for any tumour type analysed (P≥0.05 for all tests). For females, to the start of the 30mg/kg/day cull, there was evidence of a difference between the two control groups for mammary gland epithelial tumours (P=0.021). When Control 2 alone was used, the females demonstrated a significant overall increasing dose response (P=0.027); the 3, 10 and 30mg/kg/day dose groups demonstrated a significantly higher incidence than the control group (P=0.048, P=0.014, P=0.007 respectively).

Over the duration of the study, there was a significant difference between the two female control groups for uterus adenoma/carcinoma (P=0.047) and uterus stromal tumours (P=0.013). When control Group 1 alone was used, there was a significant increasing dose response in tumour incidence for stromal tumours (P=0.035); the 3 and 10mg/kg/day dose groups also demonstrated significantly higher tumour incidence than the control group (P=0.017, P=0.014 respectively). When Control 2 alone was used, there was a significant overall dose response for uterus adenoma/carcinoma (P=0.007), and the 10mg/kg/day dose group showed a significantly higher tumour incidence than the control (P=0.038).” (page 65)

In a one species study, both the test of trend and the test of pairwise comparison between the high dose Group and the appropriate control should be tested at a 0.05 (5%) level for rare tumors, while common tumors should be tested at a 0.01 (1%) level. Following these criteria, in both male and female rats, trends over the Anagrelide treatment groups (pooled Groups 1 & 2, and Groups 3-5) and the comparisons between the pooled controls and the Anagrelide high dose group (Group 5) in both hepatocellular adenoma and hepatocellular adenoma/carcinoma of the liver were statistically significant (all $p \leq 0.0023$).

3.2.1.4. FDA Reviewer's Results

This section will present the current Agency findings on survival and tumorigenicity in male and female rats.

Survival analysis:

The following tables (Table 9 for male rats, Table 10 for female rats) summarize the mortality results for the dose groups. The data were grouped for the specified time period, and present the number of deaths during the time interval over the number at risk at the beginning of the interval. The percentage cited is the percent survived at the end of the interval.

Table 9. Summary of Male Rats Survival (Anagrelide: dose/kg/day)

Period (Weeks)	Vehicle Control	Low 3 mg/kg/day	Medium 10 mg/kg/day	High 30 mg/kg/day
0-50	3/120[1] 97.5%[2]	3/60 95%	7/60 88.3%	12/60 80%
51-78	15/117 85%	10/57 78.3%	18/53 58.3%	21/48 45%
79	0/102	1/47 76.7%	0/35	2/27 41.7%
Terminal 80-97	102	46	35	25
81-96	24/102 76.5%	14/46 53.3%	19/35 20%	
Terminal 97	78	32	16	

[1] number deaths / number at risk
[2] per cent survival to end of period.

In these tables all animals that died during the terminal sacrifice period are counted as having been sacrificed, even those that died of other causes.

Table 10. Summary of Female Rats Survival (Anagrelide: dose/kg/day)

Period (Weeks)	Vehicle Control	Low 3 mg/kg/day	Medium 10 mg/kg/day	High 30 mg/kg/day
0-50	2/120[1] 98.3%[2]	1/60 98.3%	0/60 100%	3/60 95%
51-78	26/118 76.7%	11/59 80%	19/60 68.3%	18/57 65.0%
79-88	14/92 65.0%	9/48 65.0%	7/41 56.7%	12/39 45%
Terminal 89-98	78	39	34	27
89-97	19/78 49.1%	8/39 51.7%	6/34 46.7%	
Terminal 98	59	31	28	

[1] number deaths / number at risk
[2] per cent survival to end of period.

Note again, that in the tables above that the number of females in the first labeled terminal period includes all animals that died of any cause in the first defined terminal period (weeks 89-98). This period is defined when the number of survivors in the second control group reached 15, and all animals in that group were sacrificed. This grouping is used in the FDA Peto analysis program. The second terminal period only includes those animals classified by the Sponsor's CRO as a "Terminal Sacrifice."

In males the hypotheses of homogeneity in survival is rejected both over all groups (Cox $p<0.00005$ and K-W $p<0.00005$) and in females the hypotheses are close to statistical significance (Cox $p=0.0800$ and K-W $p=0.0742$). From the Kaplan-Meier estimated survival curves, given in Appendix 1, in males the statistically significant results are associated with clear dose response. Further, approximate parallelism in the log scale implies that the Cox proportional model would fit quite well. In females, the treatment differences were much smaller than in males. The survival curves of the low Anagrelide dose (3 mg/kg/day) group and the pooled controls are closely intertwined, with slightly increasing mortality associated with the medium and high dose groups (10 and 30 mg/kg/day, respectively). For both genders, the test of trend was statistically significant (In males, both Cox and K-W $p<0.00005$, in females both $p\leq 0.0116$). For males the departure from trend was barely statistically significant (both $p=0.0342$). For females the departure from trend was not statistically significant (both $p\geq 0.8263$).

As discussed in Section 1.3.1.6 it is possible that the excess mortality in the high dose group among male rats may be due to exceeding the maximum tolerated dose (MTD).

Tumorigenicity analysis:

The four treatment groups provided in the Sponsor's data set were the pooled controls and three treatment groups with nominal dose levels of Anagrelide at 3, 10, and 30 mg/kg/day respectively. The latter three dose groups, Sponsor Groups 3-5, were labeled as Low, Medium, and High, respectively. Table 11 below lists tumors that are potentially statistically significant. From the incidence in the control group all of these tumors would be considered as common tumors, and hence for a roughly 10% error rate in each comparison one can consider these to be statistically significant if the test of corresponding test has a p-value 1% or less. In this table, both male and female benign phaeochromocytomas showed highly statistically significant trends and differences between the high dose group and the pooled controls (all $p < 0.00005$). The differences between the pooled controls and the medium dose groups were also statistically significant (males: $p<0.00005$, females: $p=0.0014$). In male rats the test of trend in malignant phaeochromocytomas was not quite statistically significant ($p= 0.0150$), but the test comparing the medium dose group to controls was statistically significant ($p<0.00005$). In male rats the tests of trends in interstitial cell adenoma of the testis and the test of trends in follicular cell adenoma of the thyroid were fairly close, but did not achieve statistical significance ($p=0.0134$ and $p=0.0193$, respectively). Note that the difference between the medium dose group and the pooled controls was quite close to statistical significance for the latter tumor ($p=0.0105$). In female rats both the test of trend and the test of differences between the high dose group and the pooled controls was highly statistically significant in adenocarcinoma of the uterus (both $p < 0.00005$). More complete tables are presented in Appendix 4.

Table 11. Potentially Statistically Significant Neoplasms in Rats

	Incidence:				p-values:	Hi vs	Med vs
	Ctrls	Low	Med	High	Trend	Ctrls	Ctrls
MALES:							
ADRENAL							
BENIGN PHAEOCHROMOCYTOMA	13	19	31	20	0.0000	0.0000	0.0000
MALIGNANT PHAEOCHROMOCYTOMA	3	6	9	4	0.0150	0.0814	0.0000
TESTIS							
INTERSTITIAL CELL ADENOMA	7	3	3	7	0.0134	0.0686	0.5910
THYROID							
FOLLICULAR CELL ADENOMA	2	0	6	3	0.0193	0.0483	0.0105
FEMALES:							
ADRENAL							
BENIGN PHAEOCHROMOCYTOMA	7	1	11	16	0.0000	0.0000	0.0014
UTERUS							
ADENOCARCINOMA	3	0	3	11	0.0000	0.0000	0.2139

Appendix 3 reproduces the Sponsor's detailed summary of neoplasms and the results of Peto tests. Appendix 5 presents the resulta of a so-called poly-k adjustment of the Cochran-Armitage test for trend as well as pairwise comparisons. This test does not require accurate assessment of whether or not a tumor is fatal, as do the Peto tests above. It generally confirms the analysis presented above. Finally, Appendix 6 presents a Bayesian assessment of the incidence of neoplasms. It also is very consistent with the analysis above.

4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

NA

5. SUMMARY AND CONCLUSIONS

5.1. Statistical Issues and Collective Evidence

Please see Section 1.3 above.

5.2. Conclusions and Recommendations

Please see section 1.1 above.

APPENDICES:

Appendix 1. Survival Analysis

The statistical significance of the tests of differences in survival across treatment groups are given below. Note that differences between the two controls should be solely due to randomization, so even had the Sponsor identified the separate control groups, for the tests below these two control groups are pooled to a single control group. A test for homogeneity is a test that survival is equal across treatment groups, while the test of trend is a test of dose related trend. Note that the Cox test is usually called the logrank test, while the K-W, i.e., Kruskal-Wallis test, is more commonly called the Wilcoxon test.

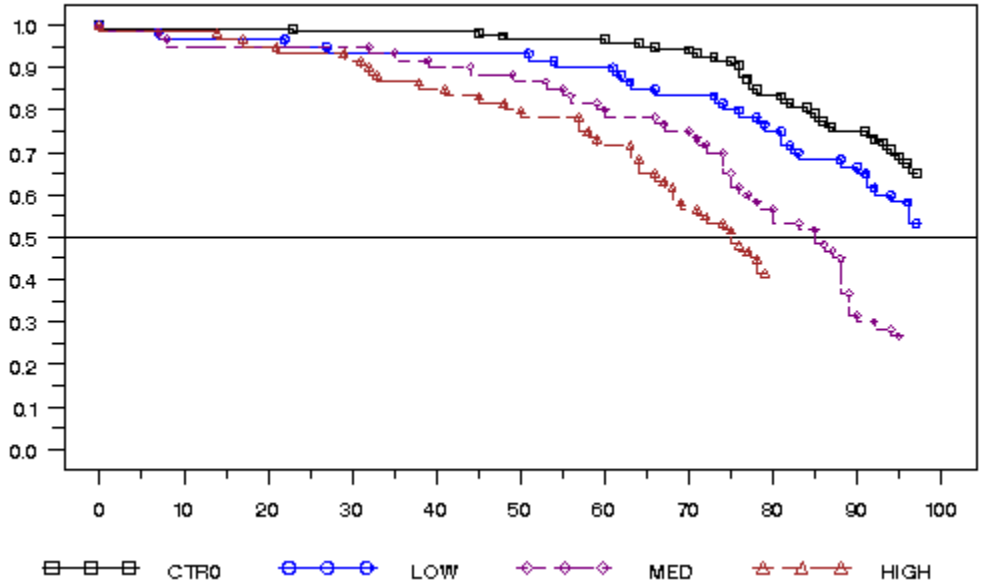
Table A.1.1 Statistical Significances of Tests of Homogeneity and Trend in Survival

	Males		Females	
	Cox	K-W	Cox	K-W
Homogeneity over Groups 1+2, 3-5	0.0000	0.0000	0.0800	0.0742
Trend over Groups 1+2, 3-5	0.0000	0.0000	0.0116	0.0103
Departure from trend in 1+2, 3-5	0.0342	0.0342	0.8263	0.8445

In males the hypotheses of homogeneity in survival is rejected both over all groups (Cox $p<0.00005$ and K-W $p<0.00005$) and in females the hypotheses are close to statistical significance (Cox $p=0.0800$ and K-W $p=0.0742$). From the Kaplan-Meier estimated survival curves, given in Appendix 1, in males the statistically significant results are associated with clear dose response. Further, approximate parallelism in the log scale implies that the Cox proportional model would fit quite well. In females, the treatment differences were much smaller than in males. The survival curves of the low Anagrelide dose (3 mg/kg/day) group and the pooled controls are closely intertwined, with slightly increasing mortality associated with the medium and high dose groups (10 and 30 mg/kg/day, respectively). For both genders, the test of trend was statistically significant (In males, both Cox and K-W $p<0.00005$, in females both $p\leq0.0116$). For males the departure from trend was barely statistically significant (both $p=0.0342$). For females the departure from trend was not statistically significant (both $p\geq0.8263$). Appendix 2 includes an experimental Bayesian analysis of these issues.

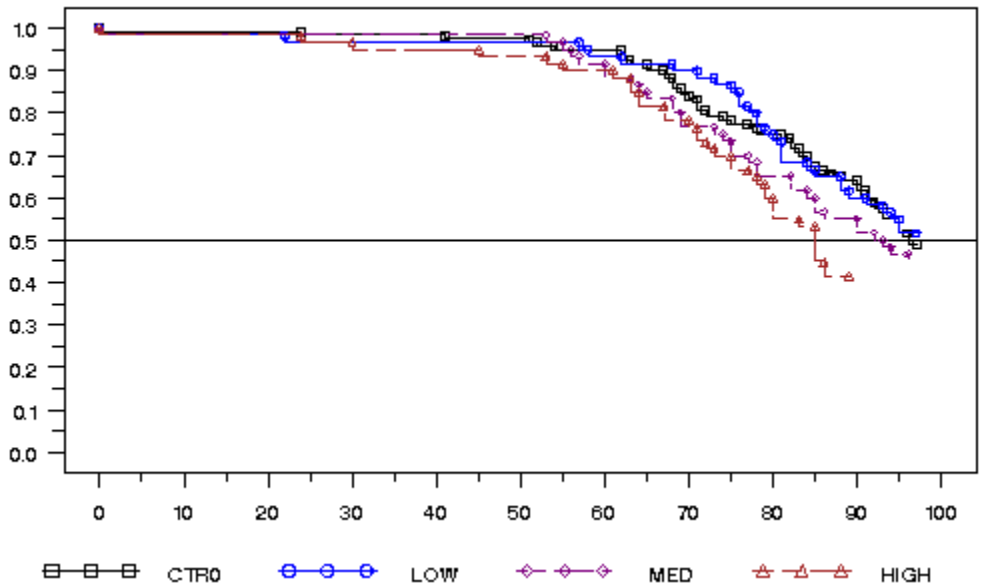
The figures A.1.1 and A.1.2, below, display these Kaplan-Meier estimated survival curves for the two genders. In males there is a clear decrement in survival over increasing dose.

Figure A.1.1 Kaplan-Meier Survival Curves for Male Rats



In females, the treatment differences were much smaller than in males. The survival curves of the low Anagrelide dose (3 mg/kg/day) group and the pooled controls are closely intertwined, with slightly increasing mortality associated with the medium and high dose groups (10 and 30 mg/kg/day, respectively).

Figure A.1.2 Kaplan-Meier Survival Curves for Female Rats



Appendix 2. Bayesian Analysis of Survival

Let $S(t)$ be the survival function, i.e., with T denoting the survival time,

$S(t) = Pr(T > t)$,

and $f(t)$ the density of T. The instantaneous hazard function is $h(t) = f(t)/S(t)$ with cumulative hazard:

$$H(t_i) = \int_0^{t_i} h(u)du$$

So $f(t) = h(t)\, S(t)$. Also $log(S(t)) = -H(t)$, so $S(t) = e^{-H(t)}$. Then $f(t) = h(t)\, e^{-H(t)}$.

The standard Cox regression form of the proportional hazards model for survival specifies the hazard function:

$h(t \mid x) = h_0(t)\, exp(x^t\beta)$.

Frequentist analysis of this model uses asymptotics to analyze the linear predictor, ignoring the baseline hazard $h_0(t)$. A Bayesian analysis requires priors on all parameters, including the baseline hazard. Perhaps the simplest Bayesian model would postulate a within interval constant baseline hazard. For this analysis, the intervals, in days, were chosen as (0,175], (175,350], (350,480], (480,525], (525, terminal]. This analysis assumes a within interval constant baseline hazard.

Thus we need to specify an appropriate prior for the baseline hazard. Note that the baseline hazard is essentially the hazard of the control group. An unbounded uniform prior on the baseline hazards is improper but, at least in this case, results in a proper posterior distribution, and, partly for experimental reasons, was chosen as the prior for this analysis. The priors on regression parameters were a well dispersed normal distribution (i.e., N(0.0,100,000)).

Note there were four treatment groups, including the pooled controls, with three treatment groups with nominal dose levels of Anagrelide at 3, 10, and 30 mg/kg/day respectively. In the formulation above, the baseline hazard is partially confounded with the specification of treatment effects (i.e., a multiplicative constant can be moved to either the baseline hazard or the term with covariates). So there are only three degrees of freedom for testing differences among the four treatment groups.

When parameterizing each treatment group separately, using so called dummy coding, we can define, for each treatment group i, except the control dose:

δ_i = 1 for the ith treatment group,
0 otherwise.

With this parameterization each labeled effect actually represents the differential effect of the specified treatment over the effect of the control group.

At least three possible models are suggested:

Over all the four treatment groups:
(1) Parameterization of no differences in survival across treatment groups with vehicle, (i.e., constant dose effect) $x_i^t\beta = \beta_0$.
(2) Parameterization of a differential effect over the controls, over the treatment groups, i.e.: $x_i^t\beta = \beta_0 + \beta_1*\delta_1 + \beta_2*\delta_2 + \beta_3*\delta_3$.
(3) Parameterization of a linear effect of dose, $x_i^t\beta = \beta_0 + \beta_1*$ dose.

Note again, that for each of these models $\exp(\beta_0)$ is confounded with the baseline hazard $h_0(t)$ and is not estimated. In model (2) above, β_k measures the differences between the k^{th} dose in the model and the high dose group. The program used for this analysis was the experimental SAS® procedure, PROC BPHREG. Because this is a new procedure and is still considered to be experimental, this analysis, at best, can only be considered to be supporting.

One possible approach to model selection is to use of the so-called information criteria measures. These attempt to assess the information about the parameters in the model. One such measure is the so-called Bayesian Information Criterion (BIC), defined as -2 x the maximized log likelihood – (# of free parameters to be estimated) x log (# of observations). In general the model with smallest BIC is considered to be the best among the listed models:

BIC	Males	Females
Constant	3055.1	3226.1
Trend	3031.6	3224.9
Different groups	3032.3	3234.6

In general, for model selection in Bayesian models this reviewer would prefer to use the so-called Deviance Information Criterion (DIC). However, the test version SAS BPHREG procedure contains a programming error when computing the DICs (personal communication from the SAS technical help). Using the BIC, for both genders the model with simple trend seems to be best, although in males it is essentially equivalent to the model with where groups differ.

Table A.2.1, below, summarizes the estimated posterior distributions of the treatment group parameters. The two right most columns provide the lower and upper endpoints of the estimated so-called highest posterior density interval. One way to translate this to a hypothesis testing framework is to suggest that if 0 is in the posterior interval we would conclude that the parameter could be zero. Note that in males whether we consider all treatment groups separately or the simple trend parameter, all intervals exclude zero. This can be interpreted as strong evidence in males that all of the pooled controls, low, and medium dose groups have significantly lower mortality than the high dose group. Moreover, there is a clear trend in increasing mortality over dose. In female rats, results are somewhat more equivocal. There is evidence that the pooled controls and the low dose groups have significantly lower mortality than the high dose group, and there is a suggestion of increasing mortality over dose. However,

in female rats the evidence of a difference in mortality between the high dose and the medium dose groups is quite weak (since the credible interval contains 0 at some distance from the boundaries).

Table A.2.1 Posterior Summaries of Treatment Parameters in the Rats Study

		Standard	Quantiles			HPD Credible	
Parameter	Mean	Deviation	25%	50%	75%	Interval	
Males							
Over all doses different							
Controls vs High	-1.6031	0.2445	-1.7677	-1.6018	-1.4394	-2.1000	-1.1387
Low vs High	-1.2174	0.2692	-1.3977	-1.2148	-1.0356	-1.7514	-0.6988
Medium vs High	-0.4690	0.2386	-0.6296	-0.4721	-0.3088	-0.9357	-0.00135
Trend							
dose	0.0508	0.00721	0.0460	0.0508	0.0556	0.0367	0.0650
Females							
Over all doses different							
Controls vs High	-0.4990	0.2146	-0.6436	-0.5014	-0.3550	-0.9118	-0.0626
Low vs High	-0.5812	0.2554	-0.7517	-0.5805	-0.4078	-1.0831	-0.0883
Medium vs High	-0.3773	0.2468	-0.5434	-0.3779	-0.2129	-0.8690	0.0948
Females Trend							
dose	0.0176	0.00692	0.0130	0.0177	0.0223	0.00428	0.0313

Appendix 3. Sponsor's Peto Tumorigenicity Analysis

Table A.3.1 belows, reproduces Table 21 of the Sponsor's report. The first part of the table displays the tumors that were pooled for analysis, apparently at the request of the Pathologist (see page 47 of report). The remainder of the table shows the number of neoplasms reported by the Sponsor in each organ by tumor combination in both male and female rats, respectively for Groups 1-5 as well as for Groups 1-4 (i.e., not including the high dose group). The significance level of the test for trend is labeled as "C,L,I,H". The remaining tests are pairwise comparisons. The Sponsor's summary of these results is given section 3.2.1.3 above.

Table A.3.1 Tumour incidence and statistical analysis (Table 21)

Tumour types analysed

Tissue type	Tumour type	Tissue	Finding
BR	M-MALIGNANT ASTROCYTOMA	BRAIN	M-MALIGNANT ASTROCYTOMA
B	B-BENIGN GRANULAR CELL MENINGIOMA	BRAIN	B-BENIGN GRANULAR CELL MENINGIOMA
HE	M-GRANULOCYTIC LEUKAEMIA	HAEMOLYMPHORETICULAR	M-GRANULOCYTIC LEUKAEMIA
SK	B-LIPOMA	SKIN + SUBCUTIS	B-LIPOMA
TE	B-INTERSTITIAL CELL ADENOMA	TESTIS	B-INTERSTITIAL CELL ADENOMA
TY	B-FOLLICULAR CELL ADENOMA	THYROID	B-FOLLICULAR CELL ADENOMA
VA	B-BENIGN GRANULAR CELL TUMOUR	VAGINA	B-BENIGN GRANULAR CELL TUMOUR
AD	CORTICAL TUMOUR	ADRENAL	B-CORTICAL ADENOMA
		ADRENAL	M-CORTICAL CARCINOMA
AD	MEDULLARY TUMOUR	ADRENAL	B-BENIGN PHAEOCHROMOCYTOMA
		ADRENAL	M-MALIGNANT HAEOCHROMOCYTOMA
HE	LYMPHOID TUMOUR	HAEMOLYMPHORETICULAR	M-MALIGNANT LYMPHOMA-LYMPHOCYTIC
		HAEMOLYMPHORETICULAR	M-MALIGNANT LYMPHOMA-PLEOMORPHIC
LI	HEPATOCELLULAR TUMOUR	LIVER	B-HEPATOCELLULAR ADENOMA
		LIVER	M-HEPATOCELLULAR CARCINOMA
LU	ALVEOLAR EPITHELIAL TUMOUR	LUNG	B-BRONCHIOLO-ALVEOLAR ADENOMA
		LUNG	M-BRONCHIOLO-ALVEOLAR CARCINOMA
MA	EPITHELIAL TUMOUR	MAMMARY GLAND	B-ADENOMA
		MAMMARY GLAND	B-FIBROADENOMA
		MAMMARY GLAND	M-ADENOCARCINOMA
PA	ISLET CELL TUMOUR	PANCREAS	B-ISLET CELL ADENOMA
		PANCREAS	M-ISLET CELL CARCINOMA
PI	ADENOMA/CARCINOMA	PITUITARY	B-ADENOMA
		PITUITARY	M-CARCINOMA
PT	ADENOMA/CARCINOMA	PARATHYROID	B-ADENOMA
		PARATHYROID	M-CARCINOMA
SK	S/A BASAL CELL TUMOUR	SKIN + SUBCUTIS	B-BENIGN BASAL CELL TUMOUR
		SKIN + SUBCUTIS	M-MALIGNANT BASAL CELL TUMOUR

Table A.3.1 (cont.) Tumour incidence and statistical analysis (Table 21)

Tumour types analysed

Tissue type	Tumour type	Tissue	Finding
SK	S/A FIBROBLASTIC TUMOUR	SKIN + SUBCUTIS	B-DERMAL FIBROMA
		SKIN + SUBCUTIS	B-FIBROLIPOMA
		SKIN + SUBCUTIS	B-FIBROMA
		SKIN + SUBCUTIS	M-FIBROSARCOMA
ST	SQUAMOUS CELL TUMOUR	STOMACH	B-SQUAMOUS CELL PAPILLOMA
		STOMACH	M-SQUAMOUS CELL CARCINOMA
TY	C-CELL TUMOUR	THYROID	B-C-CELL ADENOMA
		THYROID	M-C-CELL CARCINOMA
UT	ADENOMA/CARCINOMA	UTERUS	B-ADENOMA
		UTERUS	M-ADENOCARCINOMA
UT	STROMAL TUMOUR	UTERUS	B-STROMAL POLYP
		UTERUS	M-SARCOMA-NOS
		UTERUS	M-STROMAL SARCOMA
#	BLOOD VESSEL TUMOUR	FOOT/LEG	B-HAEMANGIOMA
		LIVER	M-HAEMANGIOSARCOMA
		MESENTERIC LYMPH NODE	B-HAEMANGIOMA
		SKIN + SUBCUTIS	M-HAEMANGIOSARCOMA
		SPLEEN	B-HAEMANGIOMA
		SPLEEN	M-HAEMANGIOSARCOMA
#	S/A SQUAMOUS CELL TUMOUR	FOOT/LEG	B-BENIGN HAIR FOLLICLE TUMOUR
		FOOT/LEG	M-SQUAMOUS CELL CARCINOMA
		SKIN + SUBCUTIS	B-BENIGN HAIR FOLLICLE TUMOUR
		SKIN + SUBCUTIS	B-SQUAMOUS CELL PAPILLOMA
		SKIN + SUBCUTIS	M-SQUAMOUS CELL CARCINOMA
		TAIL	B-KERATOACANTHOMA
		TAIL	B-SQUAMOUS CELL PAPILLOMA

= Merged tissues S/A = Skin/appendage

Tumour incidence in males
Numbers of tumour bearing animals & results of tests for increasing dose response
All groups, to the start of the Group 5 cull

Tissue code	Tumour type		Group 1 (C)	2 (C)	3 (L)	4 (I)	5 (H)	P-values C,L,I,H	CvL	CvI	CvH
SK	B-LIPOMA	Exam	10	8	14	25	35				
		F	0	1	0	0	1				
		NF	0	0	0	1	0				
		All	0	1	0	1	1	.327	1.00	.691	.486
TE	B-INTERSTITIAL CELL ADENOMA	Exam	10	8	14	24	33				
		NF	0	1	0	1	4	.064	1.00	.801	.312
TY	B-FOLLICULAR CELL ADENOMA	Exam	7	7	13	19	19				
		NF	0	0	0	3	0	.730	1.00	.190	1.00
AD	MEDULLARY TUMOUR	Exam	10	8	14	24	33				
		F	0	0	0	1	0				
		NF	0	0	2	7	10	.004 **	.169	.007 **	.002 **
		All	0	0	2	8	10	.004 **	.169	.001 **	.002 **

Table A.3.1 (cont.) Tumour incidence and statistical analysis (Table 21)

PI	ADENOMA/CARCINOMA	Exam	10	8	14	24	33			
		F	2	2	3	1	1			
		NF	1	2	0	6	2			
		All	3	4	3	7	3	.863	.734	.592	.928
SK	S/A FIBROBLASTIC TUMOUR	Exam	10	8	14	25	35			
		F	0	1	0	2	0			
		NF	1	1	0	0	0			
		All	1	2	0	2	0	.871	1.00	.640	1.00
TY	C-CELL TUMOUR	Exam	7	7	13	19	19			
		NF	1	0	0	1	2	.165	1.00	.825	.500
#	BLOOD VESSEL TUMOUR	Exam	10	8	14	25	35			
		F	0	0	0	1	0			
		NF	0	0	1	0	1			
		All	0	0	1	1	1	.398	.423	.316	.800
#	S/A SQUAMOUS CELL TUMOUR	Exam	10	8	14	25	35			
		F	0	1	0	0	1			
		NF	0	1	1	2	0			
		All	0	2	1	2	1	.719	.782	.678	.806

C = Control, L = Low dose, I = Intermediate dose, H = High dose
F = Fatal, NF = Non-fatal # = Merged tissues S/A = Skin/Appendage
* P<0.05, ** P<0.01, *** P<0.001

Tumour incidence in males
Numbers of tumour bearing animals & results of tests for increasing dose response
Groups 1, 2, 3 and 4 for the entire study

			Group				P-values		
Tissue code	Tumour type		1 (C)	2 (C)	3 (L)	4 (I)	C,L,I	CvL	CvI
BR	M-MALIGNANT ASTROCYTOMA	Exam	60	60	60	60			
		F	0	0	1	0			
		NF	1	1	1	0			
		All	1	1	2	0	.645	.355	1.00
SK	B-LIPOMA	Exam	60	60	60	60			
		F	0	1	0	0			
		NF	0	2	1	1			
		All	0	3	1	1	.621	.768	.791
TE	B-INTERSTITIAL CELL ADENOMA	Exam	60	60	60	59			
		NF	2	5	3	3	.319	.662	.459
TY	B-FOLLICULAR CELL ADENOMA	Exam	55	58	56	49			
		NF	1	1	0	6	.011 *	1.00	.047 *
AD	CORTICAL TUMOUR	Exam	60	60	60	59			
		NF	3	2	0	1	.758	1.00	.786

Table A.3.1 (cont.) Tumour incidence and statistical analysis (Table 21)

Tumour incidence in males
Numbers of tumour bearing animals & results of tests for increasing dose response
Groups 1, 2, 3 and 4 for the entire study

Tissue code	Tumour type		Group 1 (C)	2 (C)	3 (L)	4 (I)	P-values C,L,I	CvL	CvI
AD	MEDULLARY TUMOUR	Exam	60	60	60	59			
		F	0	0	1	1			
		NF	9	7	21	34	<.001 ***	<.001 ***	<.001 ***
		All	9	7	22	35	<.001 ***	<.001 ***	<.001 ***
HE	LYMPHOID TUMOUR	Exam	60	60	59	56			
		F	1	1	0	0			
		NF	0	1	1	0			
		All	1	2	1	0	.867	.757	1.00
LI	HEPATOCELLULAR TUMOUR	Exam	60	60	60	59			
		NF	3	4	2	0	.961	.824	1.00
LU	ALVEOLAR EPITHELIAL TUMOUR	Exam	60	60	60	60			
		NF	2	1	1	0	.924	.806	1.00
MA	EPITHELIAL TUMOUR	Exam	1	1	5	2			
		F	0	0	2	0			
		NF	1	0	3	1			
		All	1	0	5	1	.648	.238	1.00
PA	ISLET CELL TUMOUR	Exam	60	60	59	59			
		NF	5	1	2	2	.668	.766	.817
PI	ADENOMA/CARCINOMA	Exam	60	60	60	58			
		F	6	7	5	3			
		NF	15	22	16	15			
		All	21	29	21	18	.688	.705	.779
PT	ADENOMA/CARCINOMA	Exam	57	58	60	56			
		NF	2	2	2	0	.805	.588	1.00
SK	S/A BASAL CELL TUMOUR	Exam	60	60	60	60			
		F	0	0	0	1			
		NF	1	0	2	1			
		All	1	0	2	2	.080	.235	.197
SK	S/A FIBROBLASTIC TUMOUR	Exam	60	60	60	60			
		F	3	2	0	2			
		NF	6	4	4	5			
		All	9	6	4	7	.295	.914	.391
TY	C-CELL TUMOUR	Exam	55	58	56	49			
		NF	10	6	8	5	.609	.487	.722
#	BLOOD VESSEL TUMOUR	Exam	60	60	60	60			
		F	0	0	0	1			
		NF	2	4	4	2			
		All	2	4	4	3	.235	.386	.296

Table A.3.1 (cont.) Tumour incidence and statistical analysis (Table 21)

Tumour incidence in males
Numbers of tumour bearing animals & results of tests for increasing dose response
Groups 1, 2, 3 and 4 for the entire study

Tissue code	Tumour type		Group 1 (C)	2 (C)	3 (L)	4 (I)	P-values C,L,I	CvL	CvI
#	S/A SQUAMOUS CELL TUMOUR	Exam	60	60	60	60			
		F	0	1	2	0	.580	.232	1.00
		NF	6	9	13	10	.037 *	.036 *	.082
		All	6	10	15	10	.052	.013 *	.115

C = Control, L = Low dose, I = Intermediate dose
F = Fatal, NF = Non-fatal
# = Merged tissues
S/A = Skin/Appendage
* P<0.05
** P<0.01
*** P<0.001

Tumour incidence in females
Numbers of tumour bearing animals & results of tests for increasing dose response
All groups, to the start of the Group 5 cull

Tissue Code	Tumour type		1 (C)	2 (C)	3 (L)	4 (I)	5 (H)	C,L,I,H	CvL	CvI	CvH
			Group					P-values			
HE	M-GRANULOCYTIC LEUKAEMIA	Exam	27	15	23	25	35				
		F	1	0	0	1	0				
		NF	0	1	0	0	0				
		All	1	1	0	1	0	.807	1.00	.724	1.00
AD	MEDULLARY TUMOUR	Exam	27	15	23	26	33				
		NF	1	1	0	2	7	.003 **	1.00	.450	.031 *
MA	EPITHELIAL TUMOUR	Exam	27	15	23	26	35				
		F	12	4	9	13	13				
		NF	3	2	5	3	5				
		All	15	6	14	16	18	.058	.277	.101	.060
PI	ADENOMA/CARCINOMA	Exam	27	15	23	25	33				
		F	13	9	10	7	8				
		NF	8	1	6	8	13				
		All	21	10	16	15	21	.535	.665	.846	.641
TY	C-CELL TUMOUR	Exam	25	14	21	25	30				
		NF	2	0	2	2	4	.150	.449	.458	.226
UT	ADENOMA/CARCINOMA	Exam	27	15	23	25	33				
		F	0	0	0	0	5	<.001 ***	1.00	1.00	.002 **
		NF	2	0	0	1	2	.299	1.00	.726	.601
		All	2	0	0	1	7	<.001 ***	1.00	.726	.006 **

Table A.3.1 (cont.) Tumour incidence and statistical analysis (Table 21)

Tumour incidence in females
Numbers of tumour bearing animals & results of tests for increasing dose response
All groups, to the start of the Group 5 cull

Tissue Code	Tumour type		1 (C)	2 (C)	3 (L)	4 (I)	5 (H)	C,L,I,H	CvL	CvI	CvH
UT	STROMAL TUMOUR	Exam	27	15	23	25	33				
		F	0	0	0	3	2	.045 *	1.00	.031 *	.094
		NF	0	1	1	0	2	.245	.583	1.00	.413
		All	0	1	1	3	4	.030 *	.583	.109	.070

C = Control, L = Low dose, I = Intermediate dose, H = High dose
F = Fatal, NF = Non-fatal
* P<0.05
** P<0.01
*** P<0.001

Tumour incidence in females
Numbers of tumour bearing animals & results of tests for increasing dose response
Groups 1, 2, 3 and 4 for the entire study

Tissue code	Tumour type		Group 1 (C)	2 (C)	3 (L)	4 (I)	P-values C,L,I	CvL	CvI
HE	M-GRANULOCYTIC LEUKAEMIA	Exam	58	60	59	57			
		F	1	0	0	1			
		NF	0	1	0	0			
		All	1	1	0	1	.590	1.00	.721
SK	B-LIPOMA	Exam	60	59	60	59			
		NF	0	1	0	3	.037*	1.00	.085
TY	B-FOLLICULAR CELL ADENOMA	Exam	57	59	57	59			
		NF	0	2	1	0	.846	.642	1.00
VA	B-BENIGN GRANULAR CELL TUMOUR	Exam	60	60	60	59			
		NF	1	0	1	1	.408	.576	.528
AD	CORTICAL TUMOUR	Exam	60	60	60	60			
		NF	5	4	0	0	1.00	1.00	1.00
AD	MEDULLARY TUMOUR	Exam	60	60	60	60			
		NF	3	4	2	11	.001**	.866	.006**
MA	EPITHELIAL TUMOUR	Exam	58	60	60	60			
		F	15	9	10	15			
		NF	17	25	19	22			
		All	32	34	29	37	.158	.839	.166
PA	ISLET CELL TUMOUR	Exam	58	60	59	59			
		NF	1	2	0	0	1.00	1.00	1.00
PI	ADENOMA/CARCINOMA	Exam	59	60	60	59			
		F	17	12	13	8			
		NF	20	21	27	26			
		All	37	33	40	34	.691	.262	.689

Table A.3.1 (cont.) Tumour incidence and statistical analysis (Table 21)

Tumour incidence in females
Numbers of tumour bearing animals & results of tests for increasing dose response
Groups 1, 2, 3 and 4 for the entire study

Tissue code	Tumour type		Group 1 (C)	2 (C)	3 (L)	4 (I)	P-values C,L,I	CvL	CvI
PT	ADENOMA/CARCINOMA	Exam	59	56	53	59			
		NF	2	2	2	0	.920	.549	1.00
SK	S/A FIBROBLASTIC TUMOUR	Exam	60	59	60	59			
		F	2	0	0	1			
		NF	2	1	3	0			
		All	4	1	3	1	.783	.536	.891
ST	SQUAMOUS CELL TUMOUR	Exam	60	60	60	60			
ST	SQUAMOUS CELL TUMOUR	Exam	60	60	60	60			
		NF	0	2	0	1	.652	1.00	.771
TY	C-CELL TUMOUR	Exam	57	59	57	59			
		NF	7	6	5	5	.688	.762	.739
UT	ADENOMA/CARCINOMA	Exam	60	60	60	59			
		NF	4	0	0	4	.092	1.00	.203
UT	STROMAL TUMOUR	Exam	60	60	60	59			
		F	0	2	1	4			
		NF	0	4	5	2			
		All	0	6	6	6	.103	.160	.129
#	S/A SQUAMOUS CELL TUMOUR	Exam	60	59	60	59			
		F	0	0	0	1			
		NF	0	2	1	4			
		All	0	2	0	2	.257	1.00	.409

C = Control, L = Low dose, I = Intermediate dose
F = Fatal, NF = Non-fatal
# = Merged tissues
S/A = Skin/Appendage
* P<0.05
** P<0.01
*** P<0.001

Appendix 4. Peto Tumorigenicity Analysis

Tables A.4.1 and A.4.2, below, display the number of neoplasms in each organ by tumor combination in both male and female rats, respectively. These values are taken from the SAS datasets provided by the Sponsor. There originally were five treatment groups per gender, including two supposedly identical vehicle controls, and three treatment groups with nominal dose levels of Anagrelide at 3, 10, and 30 mg/kg/day respectively. However, the data sets provided by the Sponsor do distinguish between controls, and are pooled in all FDA tables and analyses. The other three dose groups, labeled in the Sponsor's report as Groups 3-5, were labeled as Low, Medium, and High, respectively. For each dose group, the tumor incidence is the number of animals where histopathological analysis detected a tumor. The Sponsor's table of neoplasms indicates that almost all animals in each treatment group were microscopically examined. Three p-values of tests of hypotheses for each tumor by gender combination are presented. The column labeled "Trend" provides the observed p-value of the tests of trend over the pooled vehicle controls, and the low, medium, high Anagrelide alone dose groups. The column labeled "Hi vs Ctrls" provides the significance levels of the tests comparing the high Anagrelide dose group (Group 5) and the pooled controls (Groups 1&2). Note that the high dose groups were sacrificed much earlier than the other dose groups. So a comparison of the medium dose group to controls may be useful and is provided. The FDA toxicologist only requested systemic pooling of hemangiomas and hemagiosarcomas. These results are summarized under the organ labled "Systemic".

For fewer than 10 tumor bearing animals in the comparison, the reported significance levels come from exact tests (i.e., assuming that the marginal totals for the number of animals with and without the neoplasm are fixed). For 10 or more tumor bearing animals, large sample, asymptotic tests are used.

As noted in Section 1.3.1.4., this is a one species two gender study, so to preserve overall Type I error to a rough 10%, for rare tumors both the test of trend and the test of pairwise comparison between the high dose group and the appropriate control should be tested at a 0.05 (5%) level. The corresponding tests for common tumors should be tested at a 0.01 level. When comparing the medium dose group to the pooled control group, it is not known to what degree specifying the same restriction actually controls familywise error. Clearly familywise error is increased but to some unknown degree.

Table A.4.1 below lists tumors that are potentially statistically significant. From the incidence in the control group all of these tumors would be considered as common tumors, and hence for a roughly 10% error rate in each comparison one can consider these to be statistically significant if the test of corresponding test has a p-value 1% or less. In this table, both male and female benign phaeochromocytomas showed highly statistically significant trends and differences between the high dose group and the pooled controls (all $p < 0.00005$). The differences between the pooled controls and the medium dose groups were also statistically significant (males: $p<0.00005$, females: $p=0.0014$). In male rats the test of trend in malignant

phaeochromocytomas was not quite statistically significant (p= 0.0150), but the test comparing the medium dose group to controls was statistically significant (p<0.00005). In male rats the tests of trends in interstitial cell adenoma of the testis and the test of trends in follicular cell adenoma of the thyroid were fairly close, but did not achieve statistical significance (p=0.0134 and p=0.0193, respectively). Note that the difference between the medium dose group and the pooled controls was quite close to statistical significance for the latter tumor (p=0.0105). In female rats both the test of trend and the test of differences between the high dose group and the pooled controls was highly statistically significant in adenocarcinoma of the uterus (both p < 0.00005).

Table A.4.1. Potentially Statistically Significant Neoplasms in Rats

	Incidence:				p-values:	Hi vs	Med vs
	Ctrls	Low	Med	High	Trend	Ctrls	Ctrls
MALES:							
ADRENAL							
BENIGN PHAEOCHROMOCYTOMA	13	19	31	20	0.0000	0.0000	0.0000
MALIGNANT PHAEOCHROMOCYTOMA	3	6	9	4	0.0150	0.0814	0.0000
TESTIS							
INTERSTITIAL CELL ADENOMA	7	3	3	7	0.0134	0.0686	0.5910
THYROID							
FOLLICULAR CELL ADENOMA	2	0	6	3	0.0193	0.0483	0.0105
FEMALES:							
ADRENAL							
BENIGN PHAEOCHROMOCYTOMA	7	1	11	16	0.0000	0.0000	0.0014
UTERUS							
ADENOCARCINOMA	3	0	3	11	0.0000	0.0000	0.2139

Tables A.4.2 and A.4.3 below display more complete results for male rats and female rats, respectively:

Table A.4.2. Tumorgenicity in Male Rats

	Incidence:				p-values:	Hi vs	Med vs
	Ctrls	Low	Med	High	Trend	Ctrls	Ctrls
ABDOMINAL CAVITY							
CARCINOMA - NOS	0	1	0	0	0.5698		
OSTEOSARCOMA	0	0	1	0	0.2933		0.2426
ADRENAL							
BENIGN PHAEOCHROMOCYTOMA	13	19	31	20	0.0000	0.0000	0.0000
CORTICAL ADENOMA	4	0	1	0	0.8006	1.0000	0.7342
CORTICAL CARCINOMA	1	0	0	1	0.2440	0.3763	1.0000
MALIGNANT PHAEOCHROMOCYTOMA	3	6	9	4	0.0150	0.0814	0.0000
BRAIN							
BENIGN GRANULAR CELL MENINGI	1	1	0	2	0.0584	0.1167	1.0000
MALIGNANT ASTROCYTOMA	2	2	0	1	0.3334	0.5216	1.0000
CONNECTIVE TISSUE							
MALIGNANT FIBROUS HISTIOCYTO	1	0	0	0	1.0000	1.0000	1.0000
MALIGNANT SCHWANNOMA	0	1	0	0	0.4916		

Table A.4.2. (cont.) Tumorgenicity in Male Rats

	Incidence:				p-values:	Hi vs	Med vs
	Ctrls	Low	Med	High	Trend	Ctrls	Ctrls
EPIDIDYMIS							
MALIGNANT MESOTHELIOMA	0	0	1	0	0.2682		0.2288
FOOT/LEG							
HAEMANGIOMA	0	0	0	1	0.2500	0.6667	
HAEMOLYMPHORETICULAR							
MALIGNANT LYMPHOMA - LYMPHOC	2	0	0	1	0.2773	0.4219	1.0000
MALIGNANT LYMPHOMA - PLEOMOR	1	1	0	0	0.7247	1.0000	1.0000
KIDNEY							
LIPOMA	0	0	0	1	0.1173	0.1875	
TRANSITIONAL CELL CARCINOMA	0	0	0	1	0.1529	0.2500	
TUBULAR CELL CARCINOMA	1	0	0	0	1.0000	1.0000	1.0000
LIVER							
HAEMANGIOSARCOMA	1	0	0	0	1.0000	1.0000	1.0000
HEPATOCELLULAR ADENOMA	7	0	0	1	0.6959	0.8213	1.0000
HEPATOCELLULAR CARCINOMA	0	2	0	0	0.5145		
LUNG							
BRONCHIOLO-ALVEOLAR ADENOMA	3	0	0	1	0.4054	0.5697	1.0000
BRONCHIOLO-ALVEOLAR CARCINOM	0	1	0	0	0.7191		
MESENTERIC LYMPH NODE							
HAEMANGIOMA	4	4	2	0	0.8456	1.0000	0.4167
PANCREAS							
ACINAR CELL ADENOMA	1	0	1	0	0.4677	1.0000	0.4068
ISLET CELL ADENOMA	6	1	2	0	0.8826	1.0000	0.6723
ISLET CELL CARCINOMA	0	1	0	0	0.4888		
PARATHYROID							
ADENOMA	4	2	0	1	0.6462	0.7837	1.0000
PITUITARY							
ADENOMA	49	21	18	9	0.8553	0.8848	0.4554
BENIGN CRANIOPHARYNGIOMA	0	1	0	0	0.4888		
CARCINOMA	1	0	0	0	1.0000	1.0000	1.0000
SKIN + SUBCUTIS							
BENIGN BASAL CELL TUMOUR	1	2	1	0	0.4889	1.0000	0.4420
BENIGN HAIR FOLLICLE TUMOUR	11	11	7	3	0.2834	0.4391	0.1015
DERMAL FIBROMA	3	2	3	0	0.5391	1.0000	0.1363
FIBROLIPOMA	0	1	0	0	0.4916		
FIBROMA	10	0	5	0	0.8295	0.9778	0.2595
FIBROSARCOMA	2	1	0	0	0.8363	1.0000	1.0000
HAEMANGIOSARCOMA	0	0	1	0	0.3741		0.3198
HISTIOCYTIC SARCOMA	2	0	0	0	1.0000		1.0000
LIPOMA	3	1	1	1	0.3479	0.5175	0.6916
MALIGNANT BASAL CELL TUMOUR	0	0	1	0	0.3056		0.2553
SEBACEOUS CELL ADENOMA	0	0	1	0	0.2718		0.2187
SQUAMOUS CELL CARCINOMA	1	2	0	0	0.7153	1.0000	1.0000
SQUAMOUS CELL PAPILLOMA	3	0	2	0	0.5308	1.0000	0.3087
SPLEEN							
HAEMANGIOMA	1	0	0	0	1.0000	1.0000	1.0000
HAEMANGIOSARCOMA	1	0	0	0	1.0000	1.0000	1.0000

Table A.4.2. (cont.) Tumorgenicity in Male Rats

	Incidence:				p-values:	Hi vs	Med vs
	Ctrls	Low	Med	High	Trend	Ctrls	Ctrls
STOMACH							
SARCOMA - NOS	1	0	0	0	1.0000	1.0000	1.0000
SQUAMOUS CELL PAPILLOMA	0	0	1	1	0.2152	0.4792	0.4898
Systemic							
Hemangioma	5	4	2	1	0.7531	0.9184	0.5051
Hemangiosarcoma	1	0	1	0	0.5419	1.0000	0.4754
Hemangioma/-sarcoma	6	4	3	1	0.7685	0.9344	0.3675
TAIL							
KERATOACANTHOMA	0	1	0	0	0.6024		
SQUAMOUS CELL PAPILLOMA	1	1	2	0	0.3643	1.0000	0.2732
TESTIS							
INTERSTITIAL CELL ADENOMA	7	3	3	7	0.0134	0.0686	0.5910
THYROID							
C-CELL ADENOMA	14	8	4	2	0.8398	0.9033	0.5985
C-CELL CARCINOMA	2	0	1	0	0.7238	1.0000	0.6807
FOLLICULAR CELL ADENOMA	2	0	6	3	0.0193	0.0483	0.0105
URINARY BLADDER							
TRANSITIONAL CELL CARCINOMA	0	1	0	0	0.4888		
ZYMBAL GLAND							
SQUAMOUS CELL CARCINOMA	1	0	0	0	1.0000	1.0000	1.0000

Table A.4.3. Tumorgenicity in Female Rats

	Incidence:				p-values:	Hi vs	Med vs
	Ctrls	Low	Med	High	Trend	Ctrls	Ctrls
ADRENAL							
BENIGN PHAEOCHROMOCYTOMA	7	1	11	16	0.0000	0.0000	0.0014
CORTICAL ADENOMA	9	0	0	1	0.8966	0.9423	1.0000
MALIGNANT PHAEOCHROMOCYTOMA	1	1	0	1	0.2863	0.4294	1.0000
BRAIN							
BENIGN GRANULAR CELL MENINGI	0	1	0	0	0.6273		
COLON							
ADENOCARCINOMA	0	0	0	1	0.1429	0.2464	
CONNECTIVE TISSUE							
LIPOMA	0	0	0	1	0.1627	0.2784	
FEMUR + MARROW							
OSTEOMA	1	0	0	0	1.0000	1.0000	1.0000
OSTEOSARCOMA	0	1	0	0	0.6273		
HAEMOLYMPHORETICULAR							
GRANULOCYTIC LEUKAEMIA	2	0	1	0	0.7943	1.0000	0.7102
MALIGNANT LYMPHOMA - LYMPHOC	1	0	0	0	1.0000	1.0000	1.0000
MALIGNANT LYMPHOMA - PLEOMOR	0	0	0	1	0.1466	0.2500	
HEART							
MALIGNANT ENDOCARDIAL SCHWAN	0	1	1	0	0.2877		0.2877
KIDNEY							
LIPOMA	0	1	0	0	0.5620		
LIVER							
HEPATOCELLULAR ADENOMA	1	0	1	0	0.5313	1.0000	0.4898

Table A.4.3. (cont.) Tumorgenicity in Female Rats

	Incidence:				p-values:	Hi vs	Med vs
	Ctrls	Low	Med	High	Trend	Ctrls	Ctrls
MAMMARY GLAND							
ADENOCARCINOMA	15	7	10	11	0.0308	0.0946	0.2252
ADENOMA	6	6	6	4	0.1713	0.2020	0.1317
FIBROADENOMA	59	24	31	19	0.4653	0.6842	0.2559
MESENTERIC LYMPH NODE							
HAEMANGIOMA	0	1	0	0	0.5583		
OVARY							
MALIGNANT THECOMA	0	0	1	0	0.3140		0.2838
PANCREAS							
ACINAR CELL ADENOCARCINOMA	0	1	0	0	0.5630		
ISLET CELL ADENOMA	3	0	0	1	0.4792	0.6868	1.0000
ISLET CELL CARCINOMA	0	0	0	1	0.2264	0.3636	
PARATHYROID							
ADENOMA	3	2	0	1	0.6113	0.7279	1.0000
CARCINOMA	1	0	0	0	1.0000	1.0000	1.0000
PITUITARY							
ADENOMA	60	36	27	26	0.3557	0.3213	0.6573
CARCINOMA	10	4	7	3	0.5745	0.7765	0.2262
SKIN + SUBCUTIS							
BENIGN BASAL CELL TUMOUR	0	1	0	0	0.5630		
BENIGN HAIR FOLLICLE TUMOUR	0	0	1	0	0.3109		0.2778
FIBROLIPOMA	1	0	0	0	1.0000	1.0000	1.0000
FIBROMA	1	3	1	1	0.3218	0.5388	0.4753
FIBROSARCOMA	3	0	0	0	1.0000	1.0000	1.0000
LIPOMA	1	0	3	0	0.4772	1.0000	0.0623
SQUAMOUS CELL CARCINOMA	1	0	0	0	1.0000	1.0000	1.0000
SQUAMOUS CELL PAPILLOMA	1	0	0	0	1.0000	1.0000	1.0000
STOMACH							
SQUAMOUS CELL CARCINOMA	0	0	1	0	0.4727		0.3971
SQUAMOUS CELL PAPILLOMA	2	0	0	0	1.0000	1.0000	1.0000
Systemic							
Hemangioma	0	1	0	0	0.5620		
Hemangioma/-sarcoma	0	1	0	0	0.5620		
THYMUS							
BENIGN THYMOMA	0	0	0	1	0.1391	0.2462	
MALIGNANT THYMOMA	1	0	0	0	1.0000	1.0000	1.0000
THYROID							
C-CELL ADENOMA	12	4	4	5	0.3926	0.5160	0.7611
C-CELL CARCINOMA	2	1	1	1	0.4235	0.7003	0.6346
FOLLICULAR CELL ADENOMA	2	1	0	0	0.9237	1.0000	1.0000

Table A.4.3. (cont.) Tumorgenicity in Female Rats

	Incidence:				p-values:	Hi vs	Med vs
	Ctrls	Low	Med	High	Trend	Ctrls	Ctrls
UTERUS							
ADENOCARCINOMA	3	0	3	11	0.0000	0.0000	0.2139
ADENOMA	1	0	1	1	0.1668	0.4294	0.4898
GRANULAR CELL TUMOUR	1	0	0	0	1.0000	1.0000	1.0000
HISTIOCYTIC SARCOMA	1	0	0	0	1.0000	1.0000	1.0000
KERATOACANTHOMA	1	0	0	0	1.0000	1.0000	1.0000
LEIOMYOMA	0	0	1	1	0.1427	0.3636	0.3881
SARCOMA - NOS	0	0	1	0	0.3700		0.3306
SQUAMOUS CELL CARCINOMA	1	0	0	0	1.0000	1.0000	1.0000
STROMAL POLYP	6	5	5	5	0.0539	0.0847	0.1927
STROMAL SARCOMA	0	1	0	0	0.4884		
VAGINA							
BENIGN GRANULAR CELL TUMOUR	1	1	1	1	0.2683	0.4294	0.4898
FIBROMA	0	1	0	0	0.5620		
STROMAL POLYP	1	0	0	0	1.0000	1.0000	1.0000
ZYMBAL GLAND							
SEBACEOUS CELL ADENOMA	0	1	0	0	0.6306		
SEBACEOUS CELL CARCINOMA	0	0	0	1	0.1453	0.2464	

Appendix 5. Poly-k Tumorigenicity Analysis

The tables below display the tumor incidence and the p-values using the poly-k adjustment to the Cochran-Armitage test of trend in dose. This is an experimental procedure designed and written by one of the FDA statisticians. The first p-value provides the results of the poly-k test of trend, here with k=3. The poly-k test modifies the original Cochran-Armitage test to adjust for differences in mortality (please see Bailer & Portier, 1988, Bieler & Williams, 1993). The next p-value corresponds to the original Cochran-Armitage test of trend. It is not adjusted for differences in mortality among treatment groups. Finally, the last three columns present the results of tests between the pooled controls and each of the low dose group, the medium dose group, the high dose group respectively. These tests are also adjusted for differences in mortality.

As noted in the report, at the Society of Toxicological Pathology "town hall" meeting in June 2001 the poly-k modification of the Cochran-Armitage test of trend seemed to have been recommended over the Peto tests. However, as presented here these tests use the asymptotic p-values and assume large samples. For cases with 10 or more tumors these asymptotic approximations are probably quite accurate, but may be problematic with only a few tumors. Also the effect of the multiplicity of tests needs to be investigated further. Tentatively the Haseman-Lin-Rahman rules discussed in Section 1.3.1.4. of the report seem to apply, here with the modification for a single species study.

Table A.5.1 below lists tumors that seem to have a sufficiently large number of events to use the asymptotic tests and are flagged as potentially statistically significant. Since these would all be considered as common tumors, for a roughly 10% error rate in each comparison one can consider these to be statistically significant if the test of corresponding test has a p-value 1% or less. In this table, both male and female benign phaeochromocytomas showed highly statistically significant trends and differences between the high dose group and the pooled controls (all $p < 0.00005$). The test comparing the medium dose group to controls was also statistically significant (p=0.0001

Table A.5.1. Potentially Statistically Significant Results of Poly-k tests for Neoplasms

	Incidence:				P-values:		Low vs	Med vs	High vs
	Ctrls	Low	Med	High	Poly-k Trend	Cochran Trend	Ctrls	Ctrls	Ctrls
Males									
ADRENAL									
BENIGN PHAEOCHROMOCYTOMA	13	19	31	20	.0000	.0001	.0001	.0000	.0000
MALIGNANT PHAEOCHROMOCYTOMA	3	6	9	4	.0608	.0870	.0093	.0001	.0412
Females									
ADRENAL									
BENIGN PHAEOCHROMOCYTOMA	7	1	11	16	.0000	.0000	.9034	.0021	.0000

Tables A.5.2 and A.5.3 below provide more complete incidence tables:

Table A.5.2. Results of Poly-k tests for Neoplasms in Male Rats

	Incidence:				P-values:		Low vs	Med vs	High vs
	Ctrls	Low	Med	High	Poly-k Trend	Cochran Trend	Ctrls	Ctrls	Ctrls
ABDOMINAL CAVITY									
CARCINOMA - NOS	0	1	0	0	.6324	.0786	.0727	.	.
OSTEOSARCOMA	0	0	1	0	.3603	.0786	.	.0607	.
ADRENAL BENIGN PHAEOCHROMOCYTOMA	13	19	31	20	.0000	.0001	.0001	.0000	.0000
CORTICAL ADENOMA	4	0	1	0	.8647	.9231	.9100	.6204	.8712
CORTICAL CARCINOMA	1	0	0	1	.1893	.3080	.7456	.7095	.2447
MALIGNANT PHAEOCHROMOCYTOMA	3	6	9	4	.0608	.0870	.0093	.0001	.0412
BRAIN									
BENIGN GRANULAR CELL MENINGIOMA	1	1	0	2	.0757	.1090	.2861	.7095	.0715
MALIGNANT ASTROCYTOMA	2	2	0	1	.5251	.5000	.2127	.7836	.4072
CONNECTIVE TISSUE									
MALIGNANT FIBROUS HISTIOCYTOMA	1	0	0	0	.7572	.7602	.7455	.7094	.7118
MALIGNANT SCHWANNOMA	0	1	0	0	.6315	.0786	.0716	.	.
EPIDIDYMIS									
MALIGNANT MESOTHELIOMA	0	0	1	0	.3606	.0786	.	.0601	.
FOOT/LEG									
BENIGN HAIR FOLLICLE TUMOUR	0	0	1	1	.0513	.0786	.	.0601	.0608
HAEMANGIOMA	0	0	0	1	.0256	.0786	.	.	.0616
OSTEOMA	1	0	0	0	.7572	.7602	.7450	.7089	.7118
HAEMOLYMPHORETICULAR									
MALIGNANT LYMPHOMA - LYMPHOCYTIC	2	0	0	1	.3775	.5000	.8255	.7830	.4089
MALIGNANT LYMPHOMA - PLEOMORPHIC	1	1	0	0	.7675	.7602	.2850	.7088	.7116
KIDNEY									
LIPOMA	0	0	0	1	.0256	.0786	.	.	.0608
TRANSITIONAL CELL CARCINOMA	0	0	0	1	.0256	.0786	.	.	.0612
TUBULAR CELL CARCINOMA	1	0	0	0	.7570	.7602	.7445	.7084	.7110
LIVER									
HAEMANGIOSARCOMA	1	0	0	0	.7572	.7602	.7456	.7095	.7118
HEPATOCELLULAR ADENOMA	7	0	0	1	.8568	.8989	.9638	.9333	.8052
HEPATOCELLULAR CARCINOMA	0	2	0	0	.6840	.0224	.0183	.	.
LUNG									
BRONCHIOLO-ALVEOLAR ADENOMA	3	0	0	1	.5306	.6393	.8761	.8328	.5276
BRONCHIOLO-ALVEOLAR CARCINOMA	0	1	0	0	.6316	.0786	.0722	.	.
MAMMARY GLAND									
ADENOCARCINOMA	0	1	0	0	.6315	.0786	.0716	.	.
FIBROADENOMA	1	4	1	1	.4605	.3080	.0094	.2404	.2433
MESENTERIC LYMPH NODE									
HAEMANGIOMA	4	4	2	0	.8653	.9231	.1276	.3579	.8712
ORAL CAVITY									
SQUAMOUS CELL CARCINOM	0	1	0	0	.6315	.0786	.0721	.	.
PANCREAS									
ACINAR CELL ADENOMA	1	0	1	0	.5959	.7602	.7456	.2421	.7118
ISLET CELL ADENOMA	6	1	2	0	.9023	.9605	.8333	.5384	.9191
ISLET CELL CARCINOMA	0	1	0	0	.6315	.0786	.0716	.	.

Table A.5.2. (cont.) Results of Poly-k tests for Neoplasms in Male Rats

	Incidence:				P-values:		Low vs Ctrls	Med vs Ctrls	High vs Ctrls
	Ctrls	Low	Med	High	Poly-k Trend	Cochran Trend			
PARATHYROID									
ADENOMA	4	2	0	1	.7197	.7388	.4552	.8687	.6240
PITUITARY									
ADENOMA	49	21	18	9	.9896	.9998	.5944	.6165	.9894
BENIGN CRANIOPHARYNGIOMA	0	1	0	0	.6315	.0786	.0716	.	.
CARCINOMA	1	0	0	0	.7572	.7602	.7448	.7088	.7116
SKIN + SUBCUTIS									
BENIGN BASAL CELL TUMOUR	1	2	1	0	.6949	.7602	.0942	.2402	.7118
BENIGN HAIR FOLLICLE TUMOUR	11	11	7	3	.7330	.8368	.0211	.1203	.6759
DERMAL FIBROMA	3	2	3	0	.7290	.8910	.3363	.1083	.8355
FIBROLIPOMA	0	1	0	0	.6315	.0786	.0716	.	.
FIBROMA	10	0	5	0	.9186	.9891	.9841	.2804	.9664
FIBROSARCOMA	2	1	0	0	.8420	.8420	.4673	.7828	.7862
HAEMANGIOSARCOMA	0	0	1	0	.3593	.0786	.	.0610	.
HISTIOCYTIC SARCOMA	2	0	0	0	.8390	.8420	.8257	.7832	.7862
LIPOMA	3	1	1	1	.5257	.6393	.6021	.5246	.5321
MALIGNANT BASAL CELL TUMOUR	0	0	1	0	.3606	.0786	.	.0606	.
SEBACEOUS CELL ADENOMA	0	0	1	0	.3605	.0786	.	.0606	.
SQUAMOUS CELL CARCINOMA	1	2	0	0	.7851	.7602	.0944	.7082	.7107
SQUAMOUS CELL PAPILLOMA	3	0	2	0	.7341	.8910	.8761	.2596	.8355
SPLEEN									
HAEMANGIOMA	1	0	0	0	.7572	.7602	.7456	.7095	.7118
HAEMANGIOSARCOMA	1	0	0	0	.7572	.7602	.7456	.7095	.7118
STOMACH									
SARCOMA - NOS	1	0	0	0	.7572	.7602	.7449	.7088	.7117
SQUAMOUS CELL PAPILLOMA	0	0	1	1	.0522	.0786	.	.0606	.0614
Systemic									
Hemangioma	5	4	2	1	.7473	.8101	.1989	.4505	.7063
Hemangioma/-sarcoma	6	4	3	1	.8117	.8989	.3625	.413	.8121
Hemangiosarcoma	1	0	1	0	.7269	.8420	.8261	.4051	.7862
TAIL									
KERATOACANTHOMA	0	1	0	0	.6321	.0786	.0726	.	.
SQUAMOUS CELL PAPILLOMA	1	1	2	0	.5643	.7602	.2861	.0707	.7118
TESTIS									
INTERSTITIAL CELL ADENOMA	7	3	3	7	.0210	.0848	.5301	.4052	.0277
THYROID									
C-CELL ADENOMA	14	8	4	2	.9274	.9676	.2964	.6710	.8987
C-CELL CARCINOMA	2	0	1	0	.7266	.8420	.8252	.3997	.7860
FOLLICULAR CELL ADENOMA	2	0	6	3	.0196	.1004	.8256	.0015	.0552
URINARY BLADDER									
TRANSITIONAL CELL CARCINOMA	0	1	0	0	.6315	.0786	.0719	.	.
ZYMBAL GLAND									
SQUAMOUS CELL CARCINOMA	1	0	0	0	.7572	.7602	.7449	.7088	.7117

Table A.5.3. Results of Poly-k tests for Neoplasms in Female Rats

	Incidence:				P-values:		Low vs Ctrls	Med vs Ctrls	High vs Ctrls	
	Ctrls	Low	Med	High	Poly-k Trend	Cochran Trend				
ADRENAL										
BENIGN PHAEOCHROMOCYTOMA	7	1	11	16	.0000	.0000	.9034	.0021	.0000	
CORTICAL ADENOMA	9	0	0	1	.9359	.8933	.9874	.9828	.9261	
MALIGNANT PHAEOCHROMOCYTOMA	1	1	0	1	.3344	.3080	.3120	.7506	.2823	
BONE										
OSTEOSARCOMA	0	1	0	0	.6746	.0786	.0797	.	.	
BRAIN										
BENIGN GRANULAR CELL MENINGIOMA	0	1	0	0	.6753	.0786	.0802	.	.	
COLON										
ADENOCARCINOMA	0	0	0	1	.0272	.0786	.	.	.0706	
CONNECTIVE TISSUE										
LIPOMA	0	0	0	1	.0272	.0786	.	.	.0706	
FEMUR + MARROW										
OSTEOMA	1	0	0	0	.7668	.7602	.7627	.7506	.7430	
OSTEOSARCOMA	0	1	0	0	.6746	.0786	.0797	.	.	
FOOT/LEG										
SQUAMOUS CELL CARCINOMA	0	0	1	0	.4124	.0786	.	.0745	.	
HAEMOLYMPHORETICULAR										
GRANULOCYTIC LEUKAEMIA	2	0	1	0	.7627	.8420	.8432	.4800	.8215	
MALIGNANT LYMPHOMA - LYMPHOCYTIC	1	0	0	0	.7667	.7602	.7611	.7490	.7416	
MALIGNANT LYMPHOMA - PLEOMORPHIC	0	0	0	1	.0272	.0786	.	.	.0706	
HEART										
MALIGNANT ENDOCARDIAL SCHWANNOMA	0	1	1	0	.5652	.0786	.0796	.0738	.	
KIDNEY										
LIPOMA	0	1	0	0	.6741	.0786	.0790	.	.	
LIVER										
HEPATOCELLULAR ADENOMA	1	0	1	0	.6411	.7602	.7627	.2930	.7430	
MAMMARY GLAND										
ADENOCARCINOMA	15	7	10	11	.0806	.2088	.5913	.2012	.1093	
ADENOMA	6	6	6	4	.3604	.3232	.1114	.0919	.2789	
FIBROADENOMA	59	24	31	19	.9274	.9870	.9210	.3366	.9676	
MESENTERIC LYMPH NODE										
HAEMANGIOMA	0	1	0	0	.6741	.0786	.0792	.	.	
ORAL CAVITY										
SQUAMOUS CELL CARCINOMA	1	0	0	0	.7667	.7602	.7619	.7497	.7425	
OVARY										
MALIGNANT THECOMA	0	0	1	0	.4131	.0786	.	.0735	.	
PANCREAS										
ACINAR CELL ADENOCARCINOMA	0	1	0	0	.6741	.0786	.0792	.	.	
ISLET CELL ADENOMA	3	0	0	1	.5542	.6393	.8944	.8815	.5988	ISLET
CELL CARCINOMA	0	0	0	1	.0272	.0786	.	.	.0709	
PARATHYROID										
ADENOMA	3	2	0	1	.6849	.6393	.3806	.8809	.5979	
CARCINOMA	1	0	0	0	.7668	.7602	.7626	.7505	.7430	

Table A.5.3. (cont.) Results of Poly-k tests for Neoplasms in Female Rats

	Incidence:				P-values:		Low vs	Med vs	High vs
	Ctrls	Low	Med	High	Poly-k Trend	Cochran Trend	Ctrls	Ctrls	Ctrls
PITUITARY									
ADENOMA	60	36	27	26	.7282	.8000	.0873	.6179	.5910
CARCINOMA	10	4	7	3	.6651	.7917	.6660	.1932	.7424
SKIN + SUBCUTIS									
BENIGN BASAL CELL TUMOUR	0	1	0	0	.6741	.0786	.0790	.	.
BENIGN HAIR FOLLICLE TUMOUR	0	0	1	0	.4131	.0786	.	.0735	.
FIBROLIPOMA	1	0	0	0	.7668	.7602	.7627	.7506	.7430
FIBROMA	1	3	1	1	.4938	.3080	.0371	.2935	.2841
FIBROSARCOMA	3	0	0	0	.8985	.8910	.8933	.8803	.8719
LIPOMA	1	0	3	0	.5147	.7602	.7627	.0311	.7430
SQUAMOUS CELL CARCINOMA	1	0	0	0	.7668	.7602	.7627	.7506	.7430
SQUAMOUS CELL PAPILLOMA	1	0	0	0	.7667	.7602	.7618	.7497	.7424
STOMACH									
SQUAMOUS CELL CARCINOMA	0	0	1	0	.4124	.0786	.	.0745	.
SQUAMOUS CELL PAPILLOMA	2	0	0	0	.8496	.8420	.8443	.8308	.8226
Systemic									
Hemangioma	0	1	0	0	.6741	.0786	.0792	.	.
Hemangioma/-sarcoma	0	1	0	0	.6741	.0786	.0792	.	.
THYMUS									
BENIGN THYMOMA	0	0	0	1	.0272	.0786	.	.	.0706
MALIGNANT THYMOMA	1	0	0	0	.7668	.7602	.7627	.7506	.7430
THYROID									
C-CELL ADENOMA	12	4	4	5	.5179	.6404	.7934	.7414	.5685
C-CELL CARCINOMA	2	1	1	1	.4602	.5000	.5037	.4784	.4642
FOLLICULAR CELL ADENOMA	2	1	0	0	.8654	.8420	.5058	.8311	.8228
UTERUS									
ADENOCARCINOMA	3	0	3	11	.0000	.0001	.8937	.1687	.0000
ADENOMA	1	0	1	1	.2075	.3080	.7627	.2930	.2823
GRANULAR CELL TUMOUR	1	0	0	0	.7668	.7602	.7627	.7506	.7430
HISTIOCYTIC SARCOMA	1	0	0	0	.7668	.7602	.7627	.7506	.7430
KERATOACANTHOMA	1	0	0	0	.7668	.7602	.7622	.7501	.7429
LEIOMYOMA	0	0	1	1	.0652	.0786	.	.0743	.0710
SARCOMA - NOS	0	0	1	0	.4126	.0786	.	.0743	.
SQUAMOUS CELL CARCINOMA	1	0	0	0	.7668	.7602	.7621	.7500	.7428
STROMAL POLYP	6	5	5	5	.1862	.1901	.1953	.1659	.1490
STROMAL SARCOMA	0	1	0	0	.6741	.0786	.0791	.	.
VAGINA									
BENIGN GRANULAR CELL TUMOUR	1	1	1	1	.3150	.3080	.3150	.2930	.2823
FIBROMA	0	1	0	0	.6741	.0786	.0790	.	.
STROMAL POLYP	1	0	0	0	.7668	.7602	.7626	.7505	.7430
ZYMBAL GLAND									
SEBACEOUS CELL ADENOMA	0	1	0	0	.6749	.0786	.0799	.	.
SEBACEOUS CELL CARCINOMA	0	0	0	1	.0272	.0786	.	.	.0706

Appendix 6. Bayesian Tumorigenicity Analysis

The frequentist approach to testing in the presence of multiplicities is to adjust the type I error rate (i.e., the probability of rejecting a true hypothesis of no differences). For example, the Haseman-Lin-Rahman rules for the Peto tests described in Section 1.3.1.4. and also noted in Appendices 3 & 4, above, are designed to control the type I error for tests of trend and for pairwise tests each at about a 10% error rate. The Bayesian approach is less tied to Type I error, and assesses the probability of each of the multiple events on the basis of all information in the trial, including other events. The fact that these are conditional on observed data allows one to specify analyses conditional on data based criteria. The criterion used here was that there should be at least one tumor in the high dose group and and one or more tumor in the remaining dose groups.

For this analysis we define a mixed two-stage/three-stage hierarchical models for tests of trend and and pairwise comparisons. For testing trend, we define p_{ijk} as the probability of tumor i in subject j in treatment group k. That is, with i = 1 to n_t tumors and j = 1 to n_s tumors, and dose d_k, leaving the experiment at time t_j and subject effect δ_j:

$\text{logit}(p_{ijk}) = \alpha_i + \beta_i d_k + \gamma_i t_j + \delta_j$, k=1,..,4, i=1,…, n_t, j=1,…,n_s.

with random subject effect $\delta_j \sim N(\mu_\delta, \sigma_\delta^2)$. We assign model priors:

$\alpha_i \sim N(\mu_\alpha, \sigma_\alpha^2)$

$\beta_i \sim \pi_i I_{[0]} + (1- \pi_i)N(\mu_\beta, \sigma_\beta^2)$ for i=1, . . . , n_t.

and,

$\pi_i \sim$ Beta(1,3)

$\gamma_i \sim N(\mu_g, \sigma_g^2)$ for j=1, . . . , n_s.

with $\mu_\delta = \mu_\alpha = \mu_\beta = \mu_g = \mu_s = 0$ and $\sigma_\delta^2 = 100$,

$\sigma_\alpha^2, \sigma_\beta^2, \sigma_g^2 \sim$ Inverse Gamma(1,3).

The model for pairwise comparisons is similar:

$\text{logit}(p_{ijk}) = \alpha_i + \beta_{ik} + \gamma_i t_j + \delta_j$, k=2,3,4, i=1,…, n_t, j=1,…,n_s, with $\beta_{i0} = 0$.

and,

$\pi_{ik} \sim$ Beta(1,3) for k=2,3,4.

$\beta_{ik} \sim \pi_{ik} I_{[0]} + (1- \pi_{ik})N(\mu_{\beta k}, \sigma_{\beta k}^2)$ for i=1, . . . , n_t, k = 2,3,4.

Note that with this parameterization, for k = 2,3,4, the β_{ik} represent the deviation of treatment effect from the controls.

These should represent reasonably noninformative priors on parameters.

These models were implemented in WinBUGS 1.4. As noted before, the choice of tumors chosen for analysis is conditioned on there being at least one tumor in the high dose group and and one or more tumor in the remaining dose groups. Tables A.4.1 and A.4.2 below indicate the observed frequency of tumors and the estimated probability that the linear dose effect (i.e., slope) is zero, followed by the probability that the differential effect of the the high

dose over the control effect is zero. The rightmost column is the estimated probability that the differential effect of the medium dose over the control effect is zero.

Thus, in both male and female rats there is strong evidence of a trend over dose in benign phaechromocytoma in the adrenals (probability of 0 difference is 0.014 in males and less than 0.00005 in females). Similarly, in males and females there is strong evidence that tumor incidence is much higher in the high dose group than in the controls (probabilities in both genders are less than 0.00005). The evidence of a difference in benign phaechromocytoma between the medium dose groups and controls is somewhat debatable (probability of 0 is less than 0.00005 in males and is 0.0572 in females). In male rats there is evidence of a difference between the medium dose group and controls also seems to be nonzero (probability of 0 difference is 0.0305). There is also some evidence of a difference between the medium dose group and controls in terms of follicular cell adenoma in the thyroid (probability of 0 is 0.0415). Note that the lesser tumor incidence in the high dose group would be consistent with the observation that the MTD may have been exceeded (please see Section 1.3.1.6). In female rats there is strong evidence of a trend in adenocarcinoma of the uterus (probability of 0 is 0.0019), as well as evidence of a difference between the high dose group than and controls (probability of 0 is 0.0081).

Table A.6.1 Incidence of Tumors in Males Used in Bayesian Analysis

						Probabilities		
						slope	High vs	Med vs
Organ	Tumor	cntrl	low	med	high	= 0	Cntrl	Cntrl
ADRENAL	BENIGN PHAEOCHROMOCYTOMA	13	19	31	20	0.014	0.0000	0.0000
	MALIGNANT PHAEOCHROMOCYTOMA	3	6	9	4	0.9294	0.2572	0.0305
	CORTICAL CARCINOMA	1	0	0	1	0.9369	0.7656	0.8919
BRAIN	MALIGNANT ASTROCYTOMA	2	2	0	1	0.9321	0.8308	0.9126
	BENIGN GRANULAR CELL MENINGIOMA	1	1	0	2	0.9375	0.5847	0.8892
FOOT/LEG	BENIGN HAIR FOLLICLE TUMOUR	0	0	1	1	0.9283	0.7195	0.7424
HAEMOLYMPHORETICULAR								
	MALIGNANT LYMPHOMA-LYMPHOCYTIC	2	0	0	1	0.9374	0.8066	0.9029
LIVER	HEPATOCELLULAR ADENOMA	7	0	0	1	0.8023	0.906	0.9238
LUNG	BRONCHIOLO-ALVEOLAR ADENOMA	3	0	0	1	0.9361	0.862	0.9204
MAMMARY GLAND	FIBROADENOMA	1	4	1	1	0.9425	0.7716	0.8058
PITUITARY	ADENOMA	49	21	18	9	0.2965	0.947	0.9549
PARATHYROID	ADENOMA	4	2	0	1	0.9187	0.8928	0.9325
SKIN + SUBCUTIS	LIPOMA	3	1	1	1	0.9143	0.8716	0.8917
	BENIGN HAIR FOLLICLE TUMOUR	11	11	7	3	0.9583	0.8814	0.7139
STOMACH	SQUAMOUS CELL PAPILLOMA	0	0	1	1	0.9174	0.6817	0.7026
Systemic	Hemangioma	5	4	2	1	0.8759	0.9215	0.9081
TESTIS	INTERSTITIAL CELL ADENOMA	7	3	3	7	0.8388	0.1304	0.8906
THYROID	FOLLICULAR CELL ADENOMA	2	0	6	3	0.9591	0.2853	0.0415
	C-CELL ADENOMA	14	8	4	2	0.7682	0.9496	0.9523

Table A.6.2 Incidence of Tumors in Females Used in Bayesian Analysis

						Probabilities		
Organ	Tumor	cntrl	low	med	high	slope = 0	High vs Cntrl	Med vs Cntrl
ADRENAL	BENIGN PHAEOCHROMOCYTOMA	7	1	11	16	0.0000	0.0000	0.0572
	MALIGNANT PHAEOCHROMOCYTOMA	1	1	0	1	0.9502	0.8033	0.8413
	CORTICAL ADENOMA	9	0	0	1	0.7769	0.7513	0.6824
MAMMARY GLAND	FIBROADENOMA	59	24	31	19	0.9371	0.7689	0.7761
	ADENOMA	6	6	6	4	0.9591	0.8192	0.7098
	ADENOCARCINOMA	15	7	10	11	0.9423	0.7467	0.826
PANCREAS	ISLET CELL ADENOMA	3	0	0	1	0.9681	0.8272	0.8248
PITUITARY	ADENOMA	60	36	27	26	0.9681	0.8935	0.8979
	CARCINOMA	10	4	7	3	0.9451	0.8813	0.7917
PARATHYROID	ADENOMA	3	2	0	1	0.9786	0.8436	0.8439
SKIN + SUBCUTIS	FIBROMA	1	3	1	1	0.9552	0.805	0.8136
THYROID	C-CELL ADENOMA	12	4	4	5	0.9786	0.8919	0.9045
	C-CELL CARCINOMA	2	1	1	1	0.9552	0.8407	0.8429
UTERUS	ADENOCARCINOMA	3	0	3	11	0.0019	0.0081	0.7644
	STROMAL POLYP	6	5	5	5	0.9603	0.8023	0.8224
	LEIOMYOMA	0	0	1	1	0.8876	0.7508	0.7587
	ADENOMA	1	0	1	1	0.9406	0.7985	0.8073
VAGINA	BENIGN GRANULAR CELL TUMOUR	1	1	1	1	0.9501	0.8134	0.8167

Table A.6.3 Incidence of Tumors in Males Not Used in Bayesian Analysis

Organ	Tumor	cntrl	low	med	high
ABDOMINAL CAVITY	CARCINOMA - NOS	0	1	0	0
	OSTEOSARCOMA	0	0	1	0
ADRENAL	CORTICAL ADENOMA	4	0	1	0
CONNECTIVE TISSUE					
	MALIGNANT FIBROUS HISTIOCYTOMA	1	0	0	0
	MALIGNANT SCHWANNOMA	0	1	0	0
EPIDIDYMIS	MALIGNANT MESOTHELIOMA	0	0	1	0
FOOT/LEG	OSTEOMA	1	0	0	0
HAEMOLYMPHORETICULAR					
	MALIGNANT LYMPHOMA-PLEOMORPHIC	1	1	0	0
KIDNEY	TUBULAR CELL CARCINOMA	1	0	0	0
	TRANSITIONAL CELL CARCINOMA	0	0	0	1
	LIPOMA	0	0	0	1
LIVER	HEPATOCELLULAR CARCINOMA	0	2	0	0
LUNG	BRONCHIOLO-ALVEOLAR CARCINOMA	0	1	0	0
MAMMARY GLAND	ADENOCARCINOMA	0	1	0	0
ORAL CAVITY	SQUAMOUS CELL CARCINOMA	0	1	0	0
PANCREAS	ISLET CELL ADENOMA	6	1	2	0
	ACINAR CELL ADENOMA	1	0	1	0
	ISLET CELL CARCINOMA	0	1	0	0
PITUITARY	CARCINOMA	1	0	0	0
	BENIGN CRANIOPHARYNGIOMA	0	1	0	0

Table A.6.3 (cont.) Incidence of Tumors in Males Not Used in Bayesian Analysis

Organ	Tumor	cntrl	low	med	high
SKIN + SUBCUTIS	SQUAMOUS CELL CARCINOMA	1	2	0	0
	FIBROSARCOMA	2	1	0	0
	FIBROMA	10	0	5	0
	SQUAMOUS CELL PAPILLOMA	3	0	2	0
	MALIGNANT BASAL CELL TUMOUR	0	0	1	0
	SEBACEOUS CELL ADENOMA	0	0	1	0
	DERMAL FIBROMA	3	2	3	0
	BENIGN BASAL CELL TUMOUR	1	2	1	0
	HISTIOCYTIC SARCOMA	2	0	0	0
	FIBROLIPOMA	0	1	0	0
STOMACH	SARCOMA - NOS	1	0	0	0
Systemic	Hemangiosarcoma	1	0	1	0
TAIL	KERATOACANTHOMA	0	1	0	0
	SQUAMOUS CELL PAPILLOMA	1	1	2	0
THYROID	C-CELL CARCINOMA	2	0	1	0
URINARY BLADDER	TRANSITIONAL CELL CARCINOMA	0	1	0	0
ZYMBAL GLAND	SQUAMOUS CELL CARCINOMA	1	0	0	0

Table A.6.4 Incidence of Tumors in Females Not Used in Bayesian Analysis

Organ	Tumor	cntrl	low	med	high
BONE	OSTEOSARCOMA	0	1	0	0
BRAIN	BENIGN GRANULAR CELL MENINGIOMA	0	1	0	0
COLON	ADENOCARCINOMA	0	0	0	1
CONNECTIVE TISSUE	LIPOMA	0	0	0	1
FEMUR + MARROW	OSTEOMA	1	0	0	0
FEMUR + MARROW	OSTEOSARCOMA	0	1	0	0
FOOT/LEG	SQUAMOUS CELL CARCINOMA	0	0	1	0
HAEMOLYMPHORETICULAR	GRANULOCYTIC LEUKAEMIA	2	0	1	0
	MALIGNANT LYMPHOMA-PLEOMORPHIC	0	0	0	1
	MALIGNANT LYMPHOMA-LYMPHOCYTIC	1	0	0	0
HEART	MALIG. ENDOCARDIAL SCHWANNOMA	0	1	1	0
KIDNEY	LIPOMA	0	1	0	0
LIVER	HEPATOCELLULAR ADENOMA	1	0	1	0
ORAL CAVITY	SQUAMOUS CELL CARCINOMA	1	0	0	0
OVARY	MALIGNANT THECOMA	0	0	1	0
PANCREAS	ISLET CELL CARCINOMA	0	0	0	1
	ACINAR CELL ADENOCARCINOMA	0	1	0	0
PARATHYROID	CARCINOMA	1	0	0	0
SKIN + SUBCUTIS	SQUAMOUS CELL CARCINOMA	1	0	0	0
	LIPOMA	1	0	3	0
	FIBROSARCOMA	3	0	0	0
	BENIGN HAIR FOLLICLE TUMOUR	0	0	1	0
	SQUAMOUS CELL PAPILLOMA	1	0	0	0
	BENIGN BASAL CELL TUMOUR	0	1	0	0
	FIBROLIPOMA	1	0	0	0

Table A.6.4 Incidence of Tumors in Females Not Used in Bayesian Analysis

Organ	Tumor	cntrl	low	med	high
STOMACH	SQUAMOUS CELL PAPILLOMA	2	0	0	0
	SQUAMOUS CELL CARCINOMA	0	0	1	0
Systemic	Hemangioma	0	1	0	0
THYMUS	MALIGNANT THYMOMA	1	0	0	0
	BENIGN THYMOMA	0	0	0	1
THYROID	FOLLICULAR CELL ADENOMA	2	1	0	0
UTERUS	SQUAMOUS CELL CARCINOMA	1	0	0	0
	KERATOACANTHOMA	1	0	0	0
	STROMAL SARCOMA	0	1	0	0
	HISTIOCYTIC SARCOMA	1	0	0	0
	GRANULAR CELL TUMOUR	1	0	0	0
	SARCOMA - NOS	0	0	1	0
VAGINA	STROMAL POLYP	1	0	0	0
	FIBROMA	0	1	0	0
ZYMBAL GLAND	SEBACEOUS CELL ADENOMA	0	1	0	0
	SEBACEOUS CELL CARCINOMA	0	0	0	1

Appendix 7. Effect of Multiple Housing

According to the Sponsor's report: "The animals were housed in groups of five in cages that conform with the 'Code of practice for the housing and care of animals used in scientific procedures' (Home Office, London, 1989)." (page 37) As discussed in Section 1.3.1.5 above, multiple housing of animals may cause statistical problems in the analysis. Competition for diet might induce negative correlations in treatment response, while proximity might induce positive correlations in treatment response. With this multiple housing, from a statistical design point of view, the appropriate treatment unit would generally be the group of five animals housed together. Because of this housing the within treatment estimated variances may be too large or too small, resulting in conservative or liberal tests (in terms of Type I error). Unless it has been clearly shown that tumor incidence is independent of cage, from a purely statistical point of view, this reviewer would generally recommend single housing of animals.

There a number of ways of investigating the effect of cages. One of the most important would be to assess the effect of cage on actual dose. However, the food consumption reported by the Sponsor for each animal is simply the mean consumption within the cage. That is, individual variation in dose within a cage is not recoverable. However, animals were weighed individually. One possible, though indirect, way to assess effect of dose is to assess cage effects on weight at each point in time, i.e. day of measurement. When one does an analysis of variance with cage nested within dose group, if there were no effect of dose at all we would expect an F-ratio for cage to be about of about 1, resulting in a statistically nonsignificant test. The table below summarizes the information on these F ratios for cage. Note that after 8 months there is little evidence of a cage effect on weight, but before that time point, there is some evidence a cage effect. For change from baseline in weight before before 14 months for males and 8 months for females there is for evidence of a cage effect, but no strong evidence later.

Table A.7.1 Weight and Change in Weight over Cages

Variable	Gender	Days	F-ratio	
Weight				
	Males	8-85	Not Significant	
		92-281	Significant	1.4-2.5
		281-561	Not Significant	(with two exceptions)
	Females	8-246	Significant	1.6-4.7
		>246	Not Significant	
Change from baseline in weight				
	Males	8-421	Significant	1.4-6.5
		>421	Not Significant	(with two exceptions)
	Females	8-246	Significant	1.6-7.9
		>246	Not Significant	

Although this could be used as evidence that there may be a difference in received dose due to the group housing, it would probably be more relevant to study the effect on

tumorigenicity. One way to assess this would be to count the total number of tumors on an animal as summarized in Table A.7.2, below. Typically one models such count data as some variation of a Poisson distribution. However, here the overall mean of the tumor counts is 1.69 with a variance of 1.71, quite consistent with the proposition that the counts follow a simple Poisson distribution.

Table A.7.2 Incidence of Tumors in Males Used for Bayesian Analysis

Male Rats		Count							
Dose group		0	1	2	3	4	5	6	8	All
Controls	n	20	49	36	9	5	1	.	.	120
	%	16.7	40.8	30.0	7.5	4.2	0.8	.	.	
Low	n	11	22	14	3	8	1	.	1	60
	%	18.3	36.7	23.3	5.0	13.3	1.7	.	1.7	
Medium	n	15	10	17	12	4	1	1	.	60
	%	25.0	16.7	28.3	20.0	6.7	1.7	1.7	.	
High	n	21	24	10	2	2	1	.	.	60
	%	35.0	40.0	16.7	3.3	3.3	1.7	.	.	

Female Rats		Count								
Dose group		0	1	2	3	4	5	6	7	All
Controls	n	15	36	41	14	9	4	.	1	120
	%	12.5	30.0	34.2	11.7	7.5	3.3	.	0.8	
Low	n	6	21	17	12	4	.	.	.	60
	%	10.0	35.0	28.3	20.0	6.7	.	.	.	
Medium	n	4	21	18	11	1	4	1	.	60
	%	6.7	35.0	30.0	18.3	1.7	6.7	1.7	.	
High	n	10	16	18	7	6	1	1	1	60
	%	16.7	26.7	30.0	11.7	10.0	1.7	1.7	1.7	

To assess the effect of cage, we analyze the square root of the tumor count. This is a typical variance stabilizing transform for Poisson data. A mixed model for this square root transformed count would have a random cage effects nested within the fixed effect dose groups. Note that early termination of the higher dose groups will mask later tumor development and reduce the observed tumor count. With a mixed model for cage nested in dose group, we find the following F-ratios for cage effects:

	F-ratio	p-value
Male rats	1.31	0.0853
Female rats	1.03	0.4224

Note that in females there is no evidence of differences in total tumors due to cage effects. However, the evidence of cage effects seems to be equivocal in male rats. Thus, overall, there may be evidence of a weak cage effect in male rats, but little evidence in female rats.

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/s/

Steven Thomson
12/4/2007 11:06:22 AM
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Karl Lin
12/5/2007 11:43:52 AM
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Concur with review

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 20-333/S-013

OTHER REVIEWS

Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Surveillance and Epidemiology

Date:	January 2, 2007
To:	Rafel D. Rieves, MD Acting Director, Division of Medical Imaging and Hematology Products
Thru:	Ann McMahon, MD MS Deputy Director, Division of Drug Risk Evaluation For Mark Avigan, MD CM Director, Division of Drug Risk Evaluation
From:	Betsy Scroggs, Pharm. D. Safety Evaluator, Division of Drug Risk Evaluation Corrinne Kulick, Pharm. D. Safety Evaluator, Division of Drug Risk Evaluation
Consult by:	Laura Governale, Pharm.D., MBA Drug Use Data Analysis, Team Leader Office of Surveillance, Research, and Communication Support
Subject:	Cancer, allergic alveolitis, interstitial pneumonitis, and overdose
Drug Name(s):	Agrylin (anagrelide hydrochloride)
Submission Number:	S-013
Application Type/Number:	NDA 20-333
Applicant/sponsor:	Shire
OSE RCM #:	2007-1378

CONTENTS

EXECUTIVE SUMMARY

This consult is in response to a request made June 19, 2007 by the Division of Medical Imaging and Hematology Products (DMIHP) to review the Adverse Event Reporting System (AERS) database and literature for reports of cancer, allergic alveolitis, interstitial pneumonitis, and overdose associated with Agrylin (anagrelide hydrochloride) capsules. This request follows a "Changes Being Effected" (CBE) supplement submitted by the sponsor, Shire, which describes the findings of the carcinogenicity study (R00812-SPD422) and includes a summary and detailed description of the pre and postmarketing reports of cancer in patients receiving anagrelide. Shire has proposed to revise the Carcinogenesis, Mutagenesis, Impairment of Fertility section of the product labeling to include a statement regarding the two-year rat carcinogenicity study. In addition, the supplement provides for the inclusion of two events *i.e.*, allergic alveolitis and intentional overdoses. For consistency with the European Summary of Product Characteristics, Shire has proposed to create a postmarketing subsection in the Adverse Reactions section to include allergic alveolitis (without frequency estimates), and revise the Overdosage section of the product labeling to include information regarding intentional overdose.

A search of AERS through November 5, 2007 identified 592 cases for anagrelide (raw count, all adverse events, foreign and domestic).

CANCER

An AERS search performed November 5, 2007 identified 10 unduplicated reports of cancer associated with anagrelide. There were six reports of solid tumors and four reports of leukemia. The reported solid tumor related events were lymphoma (2), laryngeal cancer (1), prostate cancer (1), brain cancer (1) and skin cancer (1). Notably, there were no reports of uterine adenocarcinoma. The reported leukemia-related events were CML (2), AML (1) and acute leukemia (1). All ten cases either contained insufficient information to assess the drug-event relationship or were complicated by underlying disease state/risk factors or use of concomitant medications. Although a possible relationship cannot be excluded, the current case series does not support a clear drug event relationship between anagrelide and the development of cancer.

The proposed labeling on cancer is acceptable from our standpoint since the sponsor did not propose any additions to the product label with regard to their analyses of reported cases of cancer in humans associated with anagrelide

INTERSTITIAL LUNG DISEASE

A search of AERS through November 5, 2007, using the search strategy provided in the Office of Surveillance and Epidemiology's (OSE) case definition for interstitial lung diseases (ILD), identified 12 unduplicated cases. No additional cases were identified from the literature. Of the 12 total cases, one case was excluded because the patient was diagnosed with pneumonia. Eleven cases were reviewed to analyze the potential association between anagrelide and ILD. Of the 11 reports, 8 met the OSE case definition of ILD because of a clinical diagnosis of allergic alveolitis (3), eosinophilic pneumonia (1), pneumonitis (3), or interstitial lung disease (1) and are included in the case series. In all 8 cases the onset of the pulmonary event was consistent with the clinical profile for

ILD (*i.e.*, 1 week to 3.5 years) and manifest while patients were taking anagrelide. Although patients may have received previous or concomitant therapy with a pharmacologic agent that is labeled for this event (i.e., hydroxyurea, lisinopril, hydrochlorothiazide, or hydrochlorothiazide/triamterene), there is a temporal relationship of the event with the initiation of anagrelide for all reported cases. This evidence, in conjunction with a definitive report of a positive dechallenge and rechallenge in one case, supports a plausible association between anagrelide and ILD. Given these findings, we find the sponsors proposed addition of this information in the Adverse Reactions – Postmarketing Experience section of the product label inadequate to describe the potential for this event following anagrelide exposure. Because of the rapid decline in respiratory function culminating in acute respiratory failure requiring endotracheal intubation and mechanical ventilation and extended hospitalization in one case; the need for hospitalization, supportive oxygen therapy, and reduced performance in another, and; a positive dechallenge and rechallenge case providing reasonable evidence of a causal association, consideration should be given to elevating the information regarding this adverse event and appropriate management to the Warnings and Precautions section of the labeling (see Conclusions and Recommendations).

OVERDOSE

In addition, a search of AERS through November 5, 2007 identified three cases of overdose associated with anagrelide. No cases were identified from the literature. Two cases were excluded from the case series of adverse events regarded as intentional overdose associated with anagrelide because the overdose involved an agent other than anagrelide, *i.e.*, carbon monoxide (1), and because although the patient took the total daily dose (10 mg) in a single dose rather than two separate doses, the reported outcome (death) was due to drug discontinuation and resulting thrombocythemia rather than drug misuse (1). The remaining AERS case was reviewed to analyze the consequences of intentional overdose of anagrelide. Information from this case of a patient who reportedly ingested "104 capsules (57 mg)" without clinical sequela, does not suggest a safety signal at this time or provide additional information to Shire's proposed labeling change regarding intentional overdose. However, consideration should be given to the inclusion of additional details from the European experience (if known) with respect to the dose ingested and describing any supportive measures.

1 BACKGROUND

This consult is in response to a request made by DMIHP to review the AERS database and literature for reports of cancer, allergic alveolitis, interstitial pneumonitis, and overdose associated with anagrelide. This request follows a CBE supplement submitted June 13, 2007 by Shire. This CBE supplement was requested by DMIHP April 13, 2007, in response to Shire's March 6, 2007 IND Safety Report (Serial No. 196) that described the occurrence of adenocarcinoma among female animals exposed to anagrelide in the 2 year rat carcinogenicity study R00812-SPD422. Therefore, this submission includes a summary and detailed description of the pre and postmarketing reports of cancer in patients receiving anagrelide. In addition, the supplement provides for the inclusion of two events, *i.e.*, allergic alveolitis and intentional overdoses. These two events were added to the European Summary of Product Characteristics for anagrelide as requested by

the European Medicines Agency (EMEA), in response to the 3rd anagrelide Periodic Safety Update Report (PSU 031) submitted to the EMEA in May 2006.

With regard to the findings of the carcinogenicity study (R00812-SPD422) the sponsor proposes the following revisions to the PRECAUTIONS -Carcinogenesis, Mutagenesis, Impairment of Fertility section of the product label (in pertinent part):

> ~~No long-term studies in animals have been performed to evaluate carcinogenic potential of anagrelide hydrochloride.~~
>
> In a two year rat carcinogenicity study a higher incidence of uterine adenocarcinoma, relative to controls, was observed in females receiving 30mg/kg/day (at least 174 times human AUC exposure after a 1mg twice daily dose). Adrenal phaeochromocytomas were increased relative to controls in males receiving 3mg/kg/day and above, and in females receiving 10mg/kg/day and above (at least 10 and 18 times respectively human AUC exposure after a 1mg twice daily dose). [redacted] (b) (4)
>
> .

The sponsor did not propose any additions to the product label with regard to their analyses of reported cases of cancer in humans associated with anagrelide.

With regard to allergic alveolitis, the sponsor proposes the addition of the following information as a POSTMARKETING REPORTS subsection of the ADVERSE REACTIONS section of the product labeling:

> **POSTMARKETING REPORTS**
>
> [redacted] (b) (4)

With regard to overdosage, the sponsor proposes the following revisions to the OVERDOSAGE- Acute Toxicity and Symptoms section of the product labeling (in pertinent part):

> ~~There are no reports of overdosage with anagrelide hydrochloride.~~
>
> There have been [redacted] (b) (4) postmarketing case reports of intentional overdose with anagrelide hydrochloride. Reported symptoms include sinus tachycardia and vomiting. Symptoms resolved with conservative management.

1.1 REGULATORY HISTORY/PRODUCT LABELING

Anagrelide was approved on March 14, 1997 for the treatment of essential thrombocythemia and subsequently approved on December 16, 1998 to include patients

[1] SLR-013 minor amendment submitted December 4, 2007 containing labeling correction for editorial error.

with thrombocythemia secondary to myeloproliferative disorders. The INDICATIONS AND USAGE section of the product labeling for anagrelide states[2]:

> AGRYLIN® Capsules are indicated for the treatment of patients with thrombocythemia, secondary to myeloproliferative disorders, to reduce the elevated platelet count and the risk of thrombosis and to ameliorate associated symptoms including thrombo-hemorrhagic events (see CLINICAL STUDIES, DOSAGE AND ADMINISTRATION).

The PRECAUTIONS section of the anagrelide label currently contains the following with regard to animal experience:

> **Carcinogenesis, Mutagenesis, Impairment of Fertility:** No long-term studies in animals have been performed to evaluate carcinogenic potential of anagrelide hydrochloride. Anagrelide hydrochloride was not genotoxic in the Ames test, the mouse lymphoma cell (L5178Y, TK+/-) forward mutation test, the human lymphocyte chromosome aberration test, or the mouse micronucleus test. Anagrelide hydrochloride at oral doses up to 240 mg/kg/day (1,440 mg/m^2/day, 195 times the recommended maximum human dose based on body surface area) was found to have no effect on fertility and reproductive performance of male rats. However, in female rats, at oral doses of 60 mg/kg/day (360 mg/m^2/day, 49 times the recommended maximum human dose based on body surface area) or higher, it disrupted implantation when administered in early pregnancy and retarded or blocked parturition when administered in late pregnancy.

The anagrelide label currently does not include information regarding cancer risk in humans. In addition, the anagrelide label currently does not include information regarding "allergic alveolitis" or "interstitial pneumonitis." However, the following information regarding pulmonary events appears in the ADVERSE REACTIONS section of the label:

> While most reported adverse events during anagrelide therapy have been mild in intensity and have decreased in frequency with continued therapy, serious adverse events were reported in these patients. These include the following: … pericardial effusion, pleural effusion, pulmonary infiltrates, pulmonary fibrosis, pulmonary hypertension….

The anagrelide label contains the following with regard to overdosage:

> **OVERDOSAGE**
> Acute Toxicity and Symptoms
> Single oral doses of anagrelide hydrochloride at 2,500, 1,500, and 200 mg/kg in mice, rats and monkeys, respectively, were not lethal. Symptoms

[2] AGRYLIN® (anagrelide hydrochloride) Capsules, Shire US Inc., 02/2006

of acute toxicity were: decreased motor activity in mice and rats and softened stools and decreased appetite in monkeys.

There are no reports of overdosage with anagrelide hydrochloride. Platelet reduction from anagrelide therapy is dose-related; therefore, thrombocytopenia, which can potentially cause bleeding, is expected from overdosage. Should overdosage occur, cardiac and central nervous system toxicity can also be expected.

Management and Treatment
In case of overdosage, close clinical supervision of the patient is required; this especially includes monitoring of the platelet count for thrombocytopenia. Dosage should be decreased or stopped, as appropriate, until the platelet count returns to within the normal range.

2 METHODS AND MATERIALS

2.1 AERS SELECTION OF CASES

To provide an overview of the number of reports for anagrelide, a search of the AERS database was performed November 5, 2007 for all adverse events associated with the active ingredient anagrelide or the brand name Agrylin. The selection of cases for each safety issue of concern, *i.e.*, cancer, allergic alveolitis, interstitial pneumonitis, and overdose, is described below.

CANCER

Events of interest (MedDRA preferred terms) were identified in the November 5, 2007 AERS search using results found under the system organ class (SOC) "Neoplasms Benign, Malignant And Unspecified (Incl Cysts And Polyps)." The AERS database was searched using the active ingredient anagrelide or the brand name Agrylin and the following MedDRA preferred terms: acute leukaemia, acute myeloid leukaemia, benign hydatidiform mole, central nervous system neoplasm, chronic myeloid leukaemia, Hodgkin's disease, laryngeal cancer, leukaemia, lymphoproliferative disorder, marrow hyperplasia, myelodysplastic syndrome, myelofibrosis, myeloid metaplasia, myeloproliferative disorder, neoplasm, Non-Hodgkin's lymphoma, prostate cancer, refractory anaemia with ringed sideroblasts, and seborrhoeic keratosis. (Further discussion with the medical officer narrowed our case series to cancer[3] cases only).

INTERSTITIAL LUNG DISEASE

The events "allergic alveolitis" and "interstitial pneumonitis" are members of the ILDs, a large heterogeneous group of diffuse parenchymal lung disorders generally characterized

[3] Cancer: a term for diseases in which abnormal cells divide without control. Cancer cells can invade nearby tissues and can spread to other parts of the body through the blood and lymph systems. There are several main types of cancer. Carcinoma is cancer that begins in the skin or in tissues that line or cover internal organs. Sarcoma is cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Leukemia is cancer that starts in blood-forming tissue such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and enter the blood. Lymphoma and multiple myeloma are cancers that begin in the cells of the immune system. Central nervous system cancers are cancers that begin in the tissues of the brain and spinal cord. [National Cancer Institute accesssed December 17, 2007 (http://www.cancer.gov/Templates/db_alpha.aspx?CdrID=45333]

by a process of inflammation and fibrosis. In order to capture all cases in AERS that are potentially relevant to the safety issues of concern, *i.e.*, "allergic alveolitis" or "interstitial pneumonitis," the AERS database was searched using the active ingredient anagrelide or the brand name Agrylin and the following MedDRA search terms for ILD[4]:

Lower respiratory tract inflammatory and immunologic conditions (HLT) including the preferred terms alveolitis, alveolitis fibrosing, pneumonitis

Parenchymal lung disorders NEC (HLT) including the preferred terms cryptogenic organizing pneumonia, interstitial lung disease, lung infiltration, pulmonary fibrosis, pulmonary toxicity

OVERDOSE

In order to capture all cases in AERS that are potentially relevant to the safety issue of concern, *i.e.*, intentional overdose, the AERS database was searched using the active ingredient anagrelide or the brand name Agrylin and the following MedDRA search terms for Overdose and Suicide:

Overdoses (HLT) including the preferred terms accidental overdose, intentional overdose, multiple drug overdose, multiple drug overdose accidental, and multiple drug overdose intentional

Intentional drug misuse (PT)

Suicidal and self-injurious behaviour (HLT) including the preferred terms completed suicide, intentional self-injury, suicide attempt.

2.2 LITERATURE SEARCH

CANCER

A PubMed search was performed using the active ingredient anagrelide and the terms cancer, lymphoma, leukemia or leukaemia, adenocarcinoma, carcinoma, and nervous system cancer or neoplasms, and prostate cancer. No additional literature reports were found during our search of PubMed to add to the 4 literature reports yielded November 5, 2007 from the AERS database. Three of our four AERS literature reports were submitted by the firm in this submission (SLR-013).

INTERSTITIAL LUNG DISEASE

A PubMed search was performed using the active ingredient anagrelide and the terms "alveolitis," "interstitial pneumonitis," "interstitial lung disease," or "pulmonary toxicity." This search yielded two published case reports both reported to AERS.

[4] Pratt. Robert, Interstitial Lund Disease. OSE Working Case Definitions for Postmarketing ADR Review. Accessed online Nov. 5, 2007: http//cdernet.cder.gov/ods. (see Appendix)

OVERDOSE

In addition, a PubMed search was performed using the active ingredient anagrelide and the term "overdose" or "overdosage." This search did not retrieve any literature reports.

2.3 DRUG USE

DETERMINING SETTINGS OF CARE AND DATA SOURCES USED

The IMS Health, IMS National Sales Perspectives™ (see Appendix 2) was used to determine the various retail and non-retail channels of distribution for anagrelide (data not provided). The examination of wholesale sales data by number of capsules sold from January 2007 through September 2007 indicate that approximately (b)(4)% of anagrelide was distributed to outpatient pharmacy settings.[5] Outpatient pharmacy settings include chain, independent, food stores with pharmacies, and mail order pharmacies ((b)(4)%). Outpatient utilization patterns were examined.

DATA SOURCES

Proprietary drug use databases licensed by the Agency were used to conduct this analysis.

Total dispensed prescriptions for anagrelide were examined using Verispan, LLC: Vector One®: National (VONA) for calendar years 1997 through September 2007. We also examined number of patients who received a prescription for anagrelide in the outpatient setting using Verispan, LLC: Vector One®: Total Patient Tracker (TPT) by year from 2002 to 2006 and year-to-date September 2007, and also cumulatively from year 2002 to September 2007. Diagnoses associated with the use of anagrelide, as reported by office-based physicians, were measured by Verispan, LLC: Physician Drug and Diagnosis Audit (PDDA) for calendar years 1997 through September 2007.

3 RESULTS

3.1 AERS AND LITERATURE ANALYSIS

A total of 592 adverse event reports are contained in AERS for anagrelide (raw count, all adverse events, foreign and domestic).

CANCER

An AERS search performed November 5, 2007 for the brand name Agrylin or anagrelide and the preferred terms identified under the system organ class "Neoplasms Benign, Malignant And Unspecified (Incl Cysts And Polyps)" yielded twenty-eight raw reports. Follow up conversation with the medical officer confirmed that the focused events of interest were cancer cases associated with anagrelide use.. Therefore, the final case series for review consisted of 10 unduplicated cancer cases. Please see table below for further inclusion/exclusion numbers of cases.

[5] IMS Health, IMS Nationals Sales Perspectives™, Data extracted 11-8-07. File: 0711anag.dvr

Cases identified in SOC, Neoplasms Benign, Malignant and Unspecified (n = 28)	Number of cases
One literature report submitted to AERS contained 3 cases	+ 2 (i.e., 1 report = 3 cases)
Duplicate report	1
Non-cancer events	19
Total cases for review	10

The characteristics of the cases are provided in Table 1 and a line listing of the cases are presented in Appendix 4.

Table 1. Characteristics of Cancer Cases as of November 5, 2007 (n = 10)	
Age (years) [n= 4]	Mean 61.5 Median 65.5 Range 43 to 72
Gender (n= 7)	Male 4 Female 3
Report Source (n= 10)	US 3 Foreign 7
Report Type (n= 10)	Direct 2 Expedited 8
Indication (n= 8)	Thrombocythemia 7 Myelofibrosis 1
Diagnosis or Inclusion PT (n=10)	Acute leukaemia (1), acute myeloid leukaemia (1), central nervous system neoplasm (1), chronic myeloid leukaemia (2), Hodgkin's disease (1), squamous cell carcinoma (larynx) (1) non-Hodgkin's lymphoma (1), prostate cancer (1), squamous cell carcinoma (skin) (1)
1^0 Outcome (n= 9)	Death 2 Life threatening 2 Hospitalization 1 Required intervention 1 Medically serious 6
Time to onset (n=10)	Mean 67.5 weeks Median 16.4 weeks Range 2 weeks to 4 years
Other medications (n= 6)	phenytoin 1 epoetin alpha 1 busulfan 1 hydroxyurea 4 aspirin 1 interferon alpha 1

All ten cases either contained insufficient information to assess the drug-event relationship or were complicated by underlying disease state/risk factors or use of concomitant medications. Accordingly, no index cases are included. Please refer to Appendix #4 for line listing of the 10 cancer cases.

INTERSTITIAL LUNG DISEASE

The AERS search using the criteria discussed in Section 2.1- Interstitial Lung Disease yielded 12 unique cases. Two published cases retrieved from the literature were also

reported to AERS. Of the 12 total cases, one case was excluded because the patient was diagnosed with pneumonia.

Based on OSE's working case definition for postmarketing adverse drug reaction review of ILD, the following satisfies inclusion criteria into the case series[4]:

> A clinical diagnosis of interstitial lung disease or interstitial pneumonia or diffuse parenchymal lung disease or alveolitis or similar terminology. Respiratory symptoms and radiologic evidence of bilateral diffuse parenchymal opacities are considered supporting information. There is usually improvement in symptoms and imaging after the drug is discontinued.

Of the 11 reports, 8 met the OSE case definition inclusion criteria for ILD because of a clinical diagnosis of allergic alveolitis (3), eosinophilic pneumonia (1), pneumonitis (3), or interstitial lung disease (1). The remaining 3 reports did not meet OSE's case definition of ILD, however, lack of information in the reports could not preclude a drug-event association. As such, these 3 reports were not included in the case series, but are discussed separately (see Discussion).

The characteristics of the 8 reports included in this series are provided in Table 2 and a line listing of all 11 reports are presented in Appendix 1.

Table 2. Characteristics of Interstitial Lung Disease Cases as of 10/5/07 (n=8)	
Report Source (n=8)	US 4 Foreign 4
Report Type (n=8)	Direct 1 (HCP) Expedited 5 Periodic 2
Age (years) [n=7]	Mean 63 Median 65 Range 44 to 81
Gender (n=8)	Male 4 Female 4
Indication (n=8)	Thrombocythemia 8
Inclusion Diagnosis	Diagnosis (n=8): Allergic alveolitis (3), eosinophilic pneumonia (1), pneumonitis (3), interstitial lung disease (1)
1^0 Outcome (n=8)	LT 1; HO 5; DS 1; OT 1
Time to onset (n=10)	Mean 37 weeks Median 21 weeks Range 1 week to 3.5 years
Dechallenge (n=8) Rechallenge (n=1)	Yes 7; No 1 Yes 1

Life-threatening (LT); Hospitalization (HO); Disability or Permanent Damage (DS); Other (OT)

Index narratives are described below:

1) ISR #4199165, Expedited (15-Day), US[6]

This case involves a 60-year-old white woman with chronic myeloid leukemia (CML) who had been treated with hydroxyurea for 7 years (2 gm/d). Anagrelide (1 mg BID) was added to hydroxyurea to help control thrombocytosis. Five weeks later the patient presented to the hospital complaining of chest pain, palpitations and shortness of breath that began one week after anagrelide was initiated and became progressively worse. The patient's medical history included type 2 non–insulin- dependent diabetes mellitus, essential hypertension, chronic renal insufficiency, and gout. The patient did not smoke or consume alcohol, denied substance abuse, and had no known drug allergies. Medications prior to hospitalization included hydroxyurea 2 g/d, anagrelide 1 mg twice

[6] Raghavan M, Mazer MA, Brink DJ. Severe hypersensitivity pneumonitis associated with anagrelide. Ann Pharmacother. 2003; 37(9):1228-31.

daily, allopurinol 300 mg/d, glipizide 10 mg twice daily, lisinopril 20 mg/d, furosemide 40 mg/d, and erythropoietin 40,000 units subcutaneous twice weekly.

On admission chest X-ray showed chronic mediastinal lymphadenopathy and new diffuse, bilateral interstitial infiltrates. A high-resolution computed tomography scan of the chest demonstrated extensive multifocal ground glass attenuation and patchy alveolar consolidation involving both lungs. Cardiac catheterization revealed normal left and right ventricular function, severe pulmonary hypertension (pulmonary artery pressure 83/40 mm Hg), and excluded significant coronary artery disease. The symptoms and signs of respiratory failure continued to worsen. Chest X-rays revealed progressive interstitial infiltrates and, 8 days following admission to the hospital, the patient required endotracheal intubation and mechanical ventilation in the intensive care unit. The initial PaO2/FiO2 ratio was 72, suggesting severe respiratory failure. Cultures of blood, urine, sputum, and autoimmune serologies were unremarkable. The erythrocyte sedimentation rate was 95 mm/h. Bronchoalveolar lavage revealed a preponderance of lymphocytes, suggesting hypersensitivity phenomenon, but was otherwise negative for malignancy and did not reveal an infectious etiology for the infiltrates. An objective causality assessment revealed that an adverse drug event was probable.

Anagrelide and hydroxyurea were discontinued and intravenous methylprednisone therapy was initiated. The patient improved within 48 hours with clearing of the infiltrates, rise in PaO2/FiO2 ratio to174, and a decrease in the erythrocyte sedimentation rate to 29 mm/h. Patient was weaned from the ventilator and discharged 10 days later on a tapering course of oral steroids. Three weeks post discharge patient had no dyspnea and complete resolution of infiltrates on chest x-ray. CML treated with imatinib.

2) ISR #4952088, Expedited (15-Day), Foreign[7]

This case involves a 45-year-old man with a 6.5 year history of essential thrombocythemia for which he had been taking anagrelide (2 mg BID) for 3.5 years. The patient was admitted to the hospital after a 3 day history of lethargy, productive cough, dyspnea, and pyrexia and a 1 day history of left sided pleuritic chest pain. The patient was an ex-smoker of 3 years.

On admission the patient was clammy, febrile, tachycardic (120 beats/min) and tachypnoeic (22/min) with oxygen saturations of 92% on air and bronchial breathing in the left lung. Chest X-ray showed extensive consolidation at the left base and a probable combination of consolidation and effusion. Blood tests revealed elevated WBC count (11.3×10^9/L) and C-reactive protein (CRP) (429 mg/L), hypoxia (pO_2 8.7) and new renal impairment (urea 18.2 mmol/L; creatinine 251 mmol/L). Diagnosis was left lower lobe pneumonia and the patient was started on intravenous co-amoxicillin/ clavulanic acid and clarithromycin and blood and sputum cultures were taken. The patient became hypotensive and had increasing oxygen demands and was placed in the high dependency unit (HDU) and received continuous positive airway pressure (CPAP).

[7] Spencer EM, Lawrence DS. 'Double hit' from streptococcal pneumonia and hypersensitivity pneumonitis associated with anagrelide. Clin Lab Haematol. 2006; 28(1):63-5.

Day 2- A repeat chest X-ray showed deterioration with bilateral pneumonia and blood cultures were positive for penicillin-sensitive Streptococcus pneumoniae. Clarithromycin was discontinued.

Day 6- The patient had made little improvement still required high fraction of inspired oxygen (FiO_2); therefore, antibiotics were switched to benzylpenicillin with gentamicin for synergy.

Day 7- The patient's renal function had returned to normal, but, his fever, CRP (262 mg/L), WBC (33.9 x 10^9/L) were not resolving. A computed tomography chest scan showed an extensive left lower lobe pneumonic consolidation with an anterolateral pleural fluid collection and a smaller fluid collection at the posterior right base. Aspirated fluid had a high pH [7.92 (left) and 8.22 (right)]. There was no growth of organisms from the fluid.

Day 11- The patient's condition or Chest X-ray were not improved so gentamicin was changed to levofloxacin. The patient remained severely hypoxic requiring high levels of FiO_2 and bi-level positive airway pressure (BiPAP) ventilatory support.

Day 13- There was still no improvement. Anagrelide was suspected as causing hypersensitivity pneumonitis and renal failure and subsequently discontinued. Prednisolone 60 mg/d was started. Over the next 3 days, he improved dramatically and was weaned off BiPAP. His oxygen requirements reduced and he was moved from HDU to the respiratory ward where penicillin was stopped.

Day 20- The patient's CRP was normal and his WBC count was 11.8 x 10^9/L with normal renal function.

Day 22- The patient was discharged on a tapering course of prednisolone. He was mobile with oxygen saturations of 97% on air.

2 weeks post discharge- The patient complained of reduced stamina and Chest X-ray showed left basal consolidation.

10 weeks post discharge- The patient was symptom free and the Chest X-ray was normal. On stopping the anagrelide, the platelet count was 170 x 10^9/L and it steadily rose to 526 x 10^9/L. The patient was started on hydroxyurea to manage thrombocythemia.

3) ISR #3919225, Periodic, US

This case involves a 74 year-old female with essential thrombocythemia who had previously taken hydroxyurea. The patient's PMH includes asthma, allergic rhinitis, and coronary artery disease. Medications prior to hospitalization include: Premarin, salmeterol inhaler (ii puffs BID), loratadine (10 mg QD PRN), budesonide nasal spray (QD), fluticasone 100 mcg inhaler (ii puffs BID), diltiazem 120 mg (QD), ASA 81 mg (QD), furosemide (PRN- discontinued one month prior to event), hydrochlorothiazide 50 mg/triamterene 75 mg (discontinued one week prior to event).

12-Oct-1999- 24-Oct-2000- The patient was taking anagrelide 0.5 mg BID

(b) (6) (seven weeks post discontinuation of anagrelide)- The patient was hospitalized for acute onset of SOB, cough, pulmonary infiltrates, prominent malaise and fatigue, and dyspnea. Chest X-ray revealed extensive infiltrates and CT scan revealed diffuse alveolar filling right posterior upper and lower lobes. Laboratory values were as

follows: WBC count 12,000-14,000/mm^3 at baseline increased to 18,000/mm^3 and then to 25,000/mm^3 and then to 25,000/mm^3 on admission to hospital.

12-Feb-2001- The patient was rechallenged with anagrelide (0.5 mg BID 5 days per week; 0.5 mg TID two days per week).

16-Jul-2001 (5 months post rechallenge)- The patient was experiencing the same symptoms as her initial presentation of eosinophilic pneumonia.

18-Jul-2001- Anagrelide was discontinued, and hydroxyurea was started.

1-Aug-2001- Event resolved. The relationship between anagrelide and the event was assessed as probable.

OVERDOSE

The AERS search using the criteria discussed in Section 2.1- Overdose yielded three unique cases. One was included in the case series of adverse events regarded as intentional overdose associated with anagrelide. Two cases were excluded because the overdose involved an agent other than anagrelide, *i.e.*, carbon monoxide (1) or because although the patient took the total daily dose (10 mg) in a single dose rather than two separate doses, the reported outcome (death) was due to drug discontinuation (thrombocythemia) rather than drug misuse (1).

The index narrative is described below:

1) ISR #4198363, Expedited (15-Day), Foreign

This case involves a 25 year-old male sibling of a female patient prescribed anagrelide 0.5 mg for primary thrombocythemia. The male reportedly intentionally ingested "104 capsules (57 mg)" of anagrelide along with 17 "tins" of beer. He was reported to have vomited on two occasions prior to his admittance to the hospital 5.5 hours post ingestion, held for observation, and discharged 48 hours later. On admission he was reported to have been intoxicated and somnolent. No "ill effects" were noted during observation. Platelet count on admission was 323 x 10^9/L and on discharge was 259 x 10^9/L.

3.2 DRUG USE

DISPENSED ANAGRELIDE OUTPATIENT PRESCRIPTIONS

From March 1997 through September 2007, over (b) (4) prescriptions for anagrelide were dispensed through outpatient retail pharmacies (Appendix 3: Table 1). Approximately (b) (4)% of all anagrelide prescriptions during this time period were for the 0.5 mg strength. Generic versions of anagrelide entered the market in August 2004. As of year-to-date September 2007, (b) (4) is the market leader with approximately (b) (4)% ((b) (4) dispensed prescriptions from January – September 2007) of the anagrelide market, followed by (b) (4) ((b) (4)% or (b) (4) prescriptions), (b) (4) ((b) (4)% or (b) (4) prescriptions), and (b) (4) ((b) (4)% or (b) (4) prescriptions). Agrylin prescriptions

[8] Verispan, Vector One®: National. Years 1997 – September 2007. Extracted 11-07. File: VONA 2007-1378 anagrelide strength 97-SEP07 xls

accounted for only (b) (4) ((b) (4) prescriptions) of the anagrelide market during this time period.

PATIENTS RECEIVING OUTPATIENT PRESCRIPTIONS FOR ANAGRELIDE

On average, approximately (b) (4) unique patients received a prescription for anagrelide in the outpatient retail pharmacy setting annually from year 2002 through 2006 (Appendix 3: Table 2). Similar to dispensed prescription data, as of year-to-date September 2007, the majority of patients were receiving the generic product and only (b) (4) patients (b) (4)%) were receiving the branded product.

For the entire period of January 2002 through September 2007, approximately (b) (4) unique patients received a prescription for anagrelide in the outpatient retail pharmacy setting.[9]

DIAGNOSES ASSOCIATED WITH THE USE OF ANAGRELIDE

The top three diagnoses or indications associated with the use of anagrelide as reported by office-based physician practices were "lymphoproliferative disease not otherwise specified" (ICD-9 238.7), "unspecified diseases of blood and blood-forming organs" (ICD-9 289.9), and "polycythemia vera" (ICD-9 238.4).[10] These accounted for (b) (4)%, (b) (4)% and (b) (4)% of diagnosis encounters, respectively, during the entire time period between year 1997 through September 2007

4 DISCUSSION

4.1 ADVERSE EVENT INDEX CASES AND LITERATURE

CANCER

Ten reports were included in this case series of adverse events (i.e., occurrence of cancer of any kind) associated with anagrelide. After hands on review of the 10 cases, none were identified as a safety signal "index case." The following discussion describes the general case findings.

Four of the reports (including the two reports of death) supplied scant information and could not be further evaluated.

In grouping the adverse events by cancer type, two main cancer types were identified, solid tumors (6) and leukemias (4). Concerning solid tumor types, adenocarcinoma of the uterus, the main reason for the request, was not identified in our case series, therefore its absence is notable (no signal for the primary event of concern).

Under the solid tumor category (lymphoma 2, laryngeal 1, prostate 1, brain cancer 1, and skin cancer 1), the laryngeal squamous cell carcinoma occurred in a male of unknown age with a history of essential thrombocythemia and smoking. In a patient submitted

[9] Verispan, Vector One®: Total Patient Tracker. Years 2002 – September 2007. Extracted 11-07. File: TPT 2007-1378 anagrelide Jan02-Sep07 Aggregate brand generic xls

[10] Verispan, Physician Drug and Diagnosis Audit. Years 1997 – September 2007. Extracted 11-07. File: PDDA 2007-1378 anagrelide Dx4 xls

report, PSAs rose and prostate cancer was diagnosed in a 72 year-old male with myelofibrosis 14 days after initiating anagrelide, an unlikely timeframe for cancer development. In the four remaining solid tumor cases, lymphomas, brain and skin, there was not enough information to evaluate these cases.

All four leukemia patients (CML 2, AML 1, acute leukemia 1) took anagrelide for essential thrombocythemia. One of the two CML patients could not be further evaluated due to lack of information, whereas the remaining three all had history of hydroxyurea use. In terms of confounding medications, four patients had history of hydroxyurea use. Regarding hydroxyurea use in other patients, since hydroxyurea is a mainstay of therapy in this patient population, it is likely that the remaining patients had also been exposed to hydroxyurea at some time in their medical history.

INTERSTITIAL LUNG DISEASE

Eight reports were included in this case series of adverse events regarded as ILD associated with anagrelide. Three additional cases that did not meet OSE's case definition for ILD are mentioned below.

Three representative cases from the case series are highlighted because of specific attributes of the case. The first case (ISR #4199165)[66] provides evidence of a serious pulmonary reaction requiring intubation and mechanical ventilation and prolonged hospitalization. Although this patient received hydroxyurea[11] (labeled for acute pulmonary reactions and pulmonary fibrosis) for 7 years prior to the initiation of anagrelide, no pulmonary events were reported relating to this drug. However, one week following the addition of anagrelide onto the hydroxyurea regimen for synergistic therapy, the patient began to experience escalating symptoms of chest pain, palpitations, and SOB culminating in respiratory failure 5 weeks later. In addition, environmental, infectious, and malignant etiologies were excluded and chest imaging studies and bronchoalveolar lavage were suggestive of hypersensitivity pneumonitis. This close temporal relationship between the initiation of anagrelide and the onset of symptoms strongly implicates anagrelide, not hydroxyurea, as the causative agent. Furthermore, the event abated upon discontinuation of anagrelide and hydroxyurea and initiation of steroid therapy.

The second case (ISR #4952088)[7] provides evidence of a serious case of acute hypersensitivity pneumonitis secondary to bacterial pneumonia (*S. pneumoniae*) requiring prolonged hospitalization and ventilatory support in a patient who had been taking anagrelide for 3.5 years without incident. This patient was diagnosed with pneumonia on admission to the hospital and received empiric as well an organism specific antibiotic therapies and ventilatory support (*i.e.*, CPAP and BiPAP) without improvement. However, on day 13 of the hospitalization anagrelide was discontinued as it was the suspected causative agent, prednisone was initiated, and the patient progressed. The fact that this patient did not respond to appropriate antibiotic therapy and improved after anagrelide was discontinued and steroids initiated suggests hypersensitivity pneumonitis induced by anagrelide. In addition, this case led the authors to conclude that taking anagrelide for 3.5 years may have initiated a sub-clinical inflammatory response in

[11] Hydroxyurea (hydroxyurea) Capsule, Barr Laboratories, Inc., 02/2006

the lung that when stressed by the pneumonia triggered a fulminant hypersensitivity pneumonitis. This speculation appears consistent with the established labeled toxicological effects of anagrelide.

The third case (ISR #3919225) provides evidence of a positive dechallenge and rechallenge following anagrelide therapy. Although the patient was started on hydrochlorothiazide/triamterene[12] one month prior to the first hospitalization, a drug labeled as associated with allergic pneumonitis relative to its thiazide component, no pulmonary events were reported with this drug. Hydrochlorothiazide/triamterene was administered for approximately three weeks and discontinued one week prior to hospitalization because of diarrhea and was not administered concomitantly with anagrelide. Moreover, the average time to onset of symptoms following hydrochlorothiazide is reported as 44 minutes and the onset acute and dramatic.[13] Therefore, it is unlikely hydrochlorothiazide/triamterene had a role in triggering the first event. The patient was rechallenged with anagrelide in the absence of hydrochlorothiazide /triamterene and 5 months later experienced the same symptoms as "her initial presentation diagnoses of eosinophilic pneumonia."

The remaining 5 cases met the inclusion criteria for ILD because of a clinical diagnosis of allergic alveolitis (1) or pneumonitis (4). In all cases, the pulmonary event was temporally associated with anagrelide administration (median=14 weeks), radiographic imaging of the lung confirmed diagnosis (4), and there was improvement in symptoms or imaging after anagrelide was discontinued or steroids were administered (4). In the one case where improvement was not seen following discontinuation of the drug, the patient was receiving concomitant therapy with hydroxyurea[11] therapy, a drug labeled for acute pulmonary reactions and pulmonary fibrosis.

Three additional cases that did not meet OSE's case definition for ILD merit consideration based on the reporting PT's, clinical narrative, and radiographic imaging suggestive of ILD. Moreover, the reports lacked sufficient medical supportive information indicating that the pulmonary event either existed or was ongoing prior to the administration of anagrelide or provide a differential diagnosis to preclude a potential drug-event association.

OVERDOSE

One report was included in the case series of adverse events regarded as intentional overdose associated with anagrelide. This case (ISR #4198363) provides evidence of a male who reportedly intentionally ingested 104 anagrelide 0.5 mg capsules. However, because there was no clinically significant adverse consequence, including pronounced pharmacological effect on platelet count, this case is unremarkable.

4.2 DRUG USE

From March 1997 through September 2007, nearly (b) (4) prescriptions for anagrelide have been dispensed through outpatient retail pharmacies. The majority of them were for

[12] Hydrochlorothiazide (Hydrochlorothiazide) capsule, Mylan Pharmaceuticals, Inc., 05/05

[13] Biron P, Dessureault J, and Napke E. Acute allergic interstitial pneumonitis induced by hydrochlorothiazide. CMAJ. 1991; 145(1):28–34.

the 0.5 mg strength. As of year-to-date September 2007, Agrylin® prescriptions accounted for only (b) (4) ((b) (4) prescriptions) of the anagrelide market.

For the entire period of January 2002 through September 2007, approximately (b) (4) unique patients have received a prescription for anagrelide in the outpatient retail pharmacy setting.

The top three diagnoses or indications associated with the use of anagrelide as reported by office-based physician practices were "lymphoproliferative disease not otherwise specified" ((b) (4)%), "unspecified diseases of blood and blood-forming organs" ((b) (4)%), and "polycythemia vera" ((b) (4)%).

5 CONCLUSIONS AND RECOMMENDATIONS

In summary, an AERS and literature search was performed to retrieve and analyze all postmarketing reports of cancer, allergic alveolitis, interstitial pneumonitis, and overdose associated with anagrelide.

CANCER

An AERS search performed November 5, 2007 identified 10 unduplicated reports of cancer associated with anagrelide. There were six reports of solid tumors and 4 reports of leukemia. The reported solid tumor related events were lymphoma (2), laryngeal cancer (1), prostate cancer (1), brain cancer (1), and skin cancer (1). Notably, there were no reports of uterine adenocarcinoma. The reported leukemia-related events were CML (2), AML (1) and acute leukemia (1). All ten cases either contained insufficient information to assess the drug-event relationship or were complicated by underlying disease state/risk factors or use of concomitant medications. Although a possible relationship cannot be excluded, the current case series does not support a clear drug event relationship between anagrelide and the development of cancer.

With regard to the occurrence of cancer associated with anagrelide, the proposed labeling addition is acceptable from our standpoint. The sponsor did not propose any additions to the product label with regard to their analyses of reported cases of cancer in humans associated with anagrelide

INTERSTITIAL LUNG DISEASE

With regard to interstitial lung diseases associated with anagrelide, specifically allergic alveolitis and interstitial pneumonia, and the proposed labeling addition, we find the sponsors proposed addition of this information in the ADVERSE REACTIONS – Postmarketing Experience section of the product label inadequate to describe the potential for this event following anagrelide exposure. Because of the rapid decline in respiratory function beginning one week following the initiation of anagrelide therapy and culminating in acute respiratory failure requiring endotracheal intubation and mechanical ventilation and extended hospitalization in one case; the need for hospitalization, supportive oxygen therapy, and reduced performance in another, and; a positive dechallenge and rechallenge case providing reasonable evidence of a causal association, consideration should be given to elevating the information regarding this adverse event and appropriate management to the Warnings and Precautions section of the labeling.

In the event the proposed language and placement of the information is deemed sufficient then consideration should be given to deletion of the second sentence in the proposed text (*i.e.*, "(b) (4) ") because it is unnecessary given the adverse events captured from spontaneous reports listed in postmarketing experience is separate from the listing of adverse events identified in clinical trials and the frequency of the event cannot be reliably estimated from spontaneous reports.

OVERDOSE

With regard to overdose associated with anagrelide and the proposed labeling revision, the information from the one case of a patient who reportedly ingested "104 capsules (57 mg)" without clinical sequela, does not suggest a safety signal at this time or provide additional information to Shire's proposed labeling change regarding intentional overdose. However, consideration should be given to the inclusion of additional details from the European experience (if known) with respect to the dose ingested and describing any supportive measures. In addition, consideration should be given to deleting "(b) (4) (b) (4)" from the text because it is vague and any additional cases will render this phrase obsolete.

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APPENDICES

APPENDIX 1: LINE DESCRIPTION OF ILD CASES

ISR	Source	Type	Age/Gender	Outcome	Indication Inclusion Diagnosis or PT	Time to Onset (weeks)	Treatment	Dechallenge	Rechallenge	Comment
4199165[6]	US	Expedited	60/F	LT, HO	Thrombocythemia Allergic Alveolitis	1	methylprednisone	Y	N	Received hydroxyurea x 7 yr w/o incident; started anagrelide w/ hydroxyurea and symptoms emerged w/in on week. Pt hosp and intubated w/ ventilation. Anagrelide/hydroxyurea d/c'd and steroids started with abatement. Meds: allopurinol 300 mg/d, glipizide 10 mg twice daily, lisinopril 20 mg/d, furosemide 40 mg/d, and erythropoietin 40,000 units subcutaneous twice weekly.
4952088[7]	Foreign	Expedited	45/M	HO	Thrombocythemia Allergic Alveolitis	168	Amoxicillin/clavulanic, clarithromycin; benzylpenicillin w/ gentamicin; levaquin; prednisolone 60 mg QD	Y	N	Pt had extended hospital stay requiring ventilatory support, extensive antibiotic therapy was unsuccessful until anagrelide (and hydroxyurea) d/c'd on day 13 of hospitalization.
3919225	US	Periodic	74/F	HO, RI	Essential Thrombocythemia Eosinophilic Pneumonia	54/18	U	Y	Y	Pt had taken hydroxyurea without incident previously. Meds: Premarin, salmeterol inhaler (ii puffs BID), loratadine (10 mg QD PRN), budesonide nasal spray (QD), fluticasone 100 mcg inhaler (ii puffs BID), diltiazem 120 mg (QD), ASA 81 mg (QD), furosemide (PRN- discontinued one month prior to event), hydrochlorothiazide 50 mg/triamterene 75 mg (discontinued one week prior to event). Initial event began 7 weeks following d/c of anagrelide (post 48 weeks on tx); dx eosinophilic pneumonia; intervention and outcome not provided 8 weeks post first incident patient was rechallenged; after 18 weeks of anagrelide pt had second event. Anagrelide d/c'd hydroxyurea started.

3880412	Foreign	Expedited	44/F	DS, OT	Thrombocythemia Allergic Alveolitis	24	U	Y	N	PMH: recurrent pulmonary emboli, recurrent thromboembolic disease, iron def. anemia, splenectomy Meds: Prevacid 30 mg QD, PVK 250 mg AD, Amitriptyline 50 mg QD, hydroxyurea 1.5 g taken for several years and d/c'd 1.5 mo post initiation of anagrelide. ADVERSE EVENT: Allergic Alveolitis, Pulmonary Infiltration: A high resolution CT scan showed subtle changes suggestive of early lung parenchymal infiltration, possibly consistent with a drug reaction. PFT's showed a restrictive lung defect. Anagrelide d/c'd (post 7 mo tx); hydroxyurea restarted. Breathlessness gradually improved and repeat PFT's approximately six weeks after stopping anagrelide, showed an improvement of 25%. This was felt to be in keeping with an allergic response to anagrelide.
4198165	Foreign	Expedited	U/M	HO	Essential Thrombocythemia Interstitial pneumonitis	U	steroids	Y	N	Dx: acute interstitial pneumonia; anagrelide only drug provided; patient was admitted to the intensive care unit with, what the physician believed to be, acute interstitial pneumonitis, treated with antibiotics but only started to make a recovery on treatment with steroids and on stopping anagrelide hydrochloride; reporter considered event to be caused by anagrelide
4284069	US	Direct	81/F	HO	Essential Thrombocythemia Pneumonitis	1.2	U	Y	N	Agrylin 1mg TID. Four days later she began to feel tired. One week later was tachycardic at 104. plts=234. Decreased Agrylin to twice a day. Ten days post initiation, Chest x-ray showed interstitial infiltrates and she was treated with steroids for a suspected pneumonitis. Agrylin stopped on day of admission. Her platelets reached a low of 44,000 on (b) (6) but then escalated to over 1 million by (b) (6). At that point hydrea was started at 500 mg, with improvement of infiltrates on chestr x-ray. Meds: Prevacid 10 mg QD, Coreg 6.25 mg BID; HCTZ 25 mg QD, Cozaar 100 mg QD, APAP w/ codeine.
4809258	Foreign	Expedited	72/M	HO	Thrombocythemia Interstitial Lung Disease	4	Ceftriaxone, clarithromycin and IV steroids	Y	N	30 days post initiation of anagrelide, pt had diffuse infiltrative bilateral pneumonia with fever and regressive "inflammatory syndrome;" oxygen required w/ ~30 day hospitalization. No concomitant meds listed.

3262040	US	Periodic	65/M	OT	Thrombocythemia Interstitial Pneumonitis	32	U	N	N	Patient taking hydroxyurea 25 mo; anagrelide (0.5 mg po TID) added on, 8 mo later pt reported increasing dyspnea; chest X:ray basilar interstitial pulmonary infiltrates; CT thorax: diffuse interstitial inflammatory changes pronounced in the left lung base. Anagrelide d/c'd; 2 weeks later hydroxyurea d/c'd 8 weeks post d/c anagrelide (6 weeks post d/c hydroxyurea) open lung biopsy showed chronic interstitial pneumonitis with fibrosis.
3204280	US	Direct	59/F	HO	U Lung Infiltrate, Dyspnea	U	U	U	N	Pt had became acutely ill, Temp 103^0, marked dyspnea, low BP, and bilateral pulmonary infiltrates (new since last CXR); anagrelide only drug provided; temp decreased day after anagrelide d/c'd; no info provided on resolution of pulmonary symptoms
3212835	US	Expedited	48/F	LT, HO	Polycythemia rubra vera Lung Infiltrate, Dyspnea	28	U	U	N	Patient had several TIAs; admitted to hospital and placed on Heparin. 48 hours later developed fever with progressive dyspnea, pulmonary infiltrates, effusions, and left ventricular dysfunction. E.F. -35%; No significant coronary artery disease; exudate on effusion. Patient had been slowly tapered off Interferon and placed on Agrylin. Dosage was adjusted very slowly to control platelet count <600K. Dose was 2.0mg TID. After last 0.5m added, patient complained of palpitations and headache with visual changes. Drug d/c'd Meds: Indocin
3919236	US	Periodic	70/M	HO	Primary Thrombocythemia Lung Disorder, Lung Infiltrate	U	U	U	U	Anagrelide 3 mg QD x 6-12 months. SOB, CT scan –multiple subpleural noduar opacities in the lateral aspect of the rt hemithorax; several reticulonodular densities in rt lower lobe. Bronchoscopy identified no lesions or malignant cells. Anagrelide only drug provided which was continued post event. Relationship noted "maybe" PMH sign NSCLC s/p left pneumonectomy 20y prior, HTN, COPD;

Underline indicated drugs labeled for ILD

Life-threatening (LT), Hospitalization (HO), Disability (DS), Required Intervention (RI), Other (OT), Pulmonary Function Test (PFT)

APPENDIX 2: DATABASE DESCRIPTIONS

Verispan, LLC: Vector One®: National (VONA)

Verispan's VONA measures retail dispensing of prescriptions or the frequency with which drugs move out of retail pharmacies into the hands of consumers via formal prescriptions. Information on the physician specialty, the patient's age and gender, and estimates for the numbers of patients that are continuing or new to therapy are available.

The Vector One® database integrates prescription activity from a variety of sources including national retail chains, mass merchandisers, mail order pharmacies, pharmacy benefits managers and their data systems, and provider groups. Vector One® receives over 1.5 billion prescription claims per year, representing over 100 million unique patients. Since 2002 Vector One® has captured information on over 8 billion prescriptions representing 200 million unique patients.

Prescriptions are captured from a sample of approximately 59,000 pharmacies throughout the US. The pharmacies in the data base account for nearly all retail pharmacies and represent nearly half of retail prescriptions dispensed nationwide. Verispan receives all prescriptions from approximately one-third of the stores and a significant sample of prescriptions from the remaining stores.

Verispan, LLC: Vector One®: Total Patient Tracker (TPT)

Verispan's Total Patient Tracker is a national-level projected audit designed to estimate the total number of unique patients across all drugs and therapeutic classes in the retail outpatient setting.

TPT derives its data from the Vector One® database which integrates prescription activity from a variety of sources including national retail chains, mail order pharmacies, mass merchandisers, pharmacy benefits managers and their data systems. Vector One® receives over 2 billion prescription claims per year, which represents over 160 million patients tracked across time.

Verispan, LLC: Physician Drug & Diagnosis Audit (PDDA)

Verispan's Physician Drug & Diagnosis Audit (PDDA) is a monthly survey designed to provide descriptive information on the patterns and treatment of diseases encountered in office-based physician practices in the U.S. The survey consists of data collected from approximately 3,100 office-based physicians representing 29 specialties across the United States that report on all patient activity during one typical workday per month. These data may include profiles and trends of diagnoses, patients, drug products mentioned during the office visit and treatment patterns. The data are then projected nationally by physician specialty and region to reflect national prescribing patterns.

Verispan uses the term "drug uses" to refer to mentions of a drug in association with a diagnosis during an office-based patient visit. This term may be duplicated by the number of diagnosis for which the drug is mentioned. It is important to note that a "drug use" does not necessarily result in prescription being generated. Rather, the term indicates that a given drug was mentioned during an office visit.

APPENDIX 3: TABLES AND FIGURES

Table 1: Total dispensed prescriptions for anagrlide from outpatient retail pharmacies by firm, Years 1997 through September 2007.

	Retail TRxs											
	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	JAN-SEP'07	JAN'02 - SEP'07
TOTAL MARKET	(b) (4)											(b) (4)

Source: Vector One: National. Years 1997 - September 2007. Extracted November 2007. File: VONA 2007-1378 anagrelide TRx 97-SEP07

Table 2: Total number of patients receiving a prescription for anagrelide in the outpatient retail pharmacy setting, Years 2002 - September 2007.

	2002		2003		2004		2005		2006		JAN'07 - SEP'07	
Product Brand	Projected Patient Count	*Total Patient Share*	Projected Patient Count	*Total Patient Share*	Projected Patient Count	*Total Patient Share*	Projected Patient Count	*Total Patient Share*	Projected Patient Count	*Total Patient Share*	Projected Patient Count	*Total Patient Share*
Grand Total												(b) (4)
AGRYLIN												
ANAGRELIDE												

* Patients may switch from one product to another brand over a time period resulting in double counting among individual product brands For this reason, subtotals may not sum to Grand Total

Source: Verispan, Vector One: Total Patient Tracker, Years 2002 - September 2007 Extracted November 2007 Files: TPT 2007-1378 anagrelide Jan07-Sep07 Aggregate xls, TPT 2007-1378 anagrelide 02-06 Display xls

APPENDIX 4: LINE DESCRIPTION OF CANCER CASES

ISR No. Country	Rpt Type	Age (4)	SEX (6)	Indication for anagrelide use (8)	Adverse Event (10)	Time to Onset (days) (5)	Outcomes (9)	Time to progression (years) (2)	Comments
3104385-8 US	Direct	U	M	essential thrombocythaemia	Laryngeal cancer, squamous cell carcinoma	1483	LT, HO, RI	NR	smoker, h/o of other ET meds NR, Agrylin cont'd
3336421-8 US	15-day	72	M	myelofibrosis	Prostate cancer	14	OT, MS	NR	histology unk, timing is implausible, h/o epogen, Agrylin dc'd
3816337-0 US	Direct	U	F	NR	Central nervous system neoplasm	NR	NR	NR	no other information
4615243-1 DK	15-day	43	M	essential thrombocythaemia	AML	115	LT	14.8	ET & Meds Confound case Lit. Report: leukemia, h/o busulfan & HU, blast crisis 1998, Agrylin dc'd
5015792-0 ES	15-day	71	F	essential thrombocythaemia	CML	21	OT, MS	NR	ET & Meds Confound case Lit. Report leukemia, 6 yr h/o hydroxyurea Agrylin dc'd
5086068-0 SG	15-day	60	F	essential thrombocythaemia	Acute leukaemia	730	OT, MS	4.4	ET & Meds Confound case Lit. Report, h/o interferon, hydroxyurea, ASA
5110892-9 FR	15-day	U	U	essential thrombocythaemia	CML	NR	DE, OT, MS	NR	ET confounds case Lit. Report , no other information
5110892-9 FR	15-day	U	U	essential thrombocythaemia	Non Hodgkins Lymphoma	NR	DE, OT, MS	NR	Lit. Report , no other information
5110892-9 FR	15-day	U	U	essential thrombocythaemia	Hodgkins Lymphoma	NR	OT, MS	NR	Lit. Report, no other information
5449658-9 GB	15-day	U	M	NR	Skin cancer squamous cell carcinoma	NR	OT	NR	Med confounds case Lit Report, h/o hydroxyurea, unk if agrylin cont'd

APPENDIX 5: OSE Case Definition

Interstitial lung disease

1. Disease/Adverse Event

Definition/Epidemiology:

The interstitial lung diseases (ILDs) are a large heterogeneous group of diffuse parenchymal lung disorders generally characterized by a process of inflammation and fibrosis within the interstitium.[14] However, the term interstitial is misleading because the process often spills over into the alveoli and lumen of small airways as well as the vasculature and pleura. ILD is also referred to as alveolitis or interstitial pneumonia, which is synonymous with interstitial pneumonitis. ILDs are classified together because of similar clinical, radiographic, physiologic, and pathologic manifestations. Diffuse lung diseases such as bronchial asthma and chronic obstructive pulmonary disease are excluded from this classification.

A two-year epidemiologic study of Bernalillo County, New Mexico from 1988 to 1990 estimated the prevalence rates per 100,000 of ILD in males and females to be 80.9 and 67.2, respectively. The incidence rates per 100,000/year were also estimated by gender and found to be 31.5 for males and 26.1 for females.

Etiology and Pathophysiology:

The most common identifiable causes of ILD are related to occupational exposures, such as inhalation of asbestos or dust during mining operations; environmental exposures to animal antigens, which can result in hypersensitivity pneumonitis; radiation; infections; connective tissue diseases; and drug-induced events. Some well-known drugs responsible for ILD include methotrexate, bleomycin, amiodarone, and nitrofurantoin. There are also idiopathic interstitial pneumonias that have similar presentations to the ILDs of known causes and associations. Classification schemes for the ILDs may be based on etiology and clinical syndrome or by their underlying histologic pattern of lung injury and repair. An abbreviated list of ILDs classified by causes and clinical syndrome is presented in Table 1.

Although there is no single pathogenic mechanism responsible for ILD, one general concept is that an initial and likely recurrent injury to the alveolar epithelial lining is followed by a chronic inflammatory response by the lung in an attempt at repair. This process may eventually result in fibroblast proliferation, disordered collagen

[14] The parenchymal interstitium represents an anatomic space that is lined by alveolar epithelial cells and capillary endothelial cells.

deposition, and the eventual structural changes that are responsible for clinical symptoms and physiologic abnormalities.[15]

6.1.1 Table 1. Causes and categories of interstitial lung disease

1.1.1.1 Inhaled agents

- Inorganic: asbestos, beryllium, silica
- Organic: animal and bird antigens, farm antigens

1.1.1.1.1 Drug- and treatment-induced

- Antiarrhythmics
- Antibiotics
- Antidepressants
- Anti-inflammatory agents
- Chemotherapeutic agents
- Oxygen
- Radiation

1.1.1.1.2 Connective tissue disease

- Ankylosing spondylitis
- Polymyositis/dermatomyositis
- Rhematoid arthritis
- Scleroderma
- Systemic lupus erythematosus

1.1.1.1.3 Infections

- Atypical pneumonias
- Tuberculosis

1.1.1.1.4 Malignant

[15] The general concept that injury evokes inflammation, which in turn signals fibroproliferation, does not hold up in some of the idiopathic ILDs. In the case of idiopathic pulmonary fibrosis (IPF), minimal interstitial inflammation is seen and the pathologic process is believed to result from disordered and persistent epithelial-fibroblast remodeling of the lung interstitium.

Clinical manifestations and diagnosis:

The clinical assessment of a patient with ILD often requires a combination of history and physical examination, laboratory investigation, lung function testing, chest imaging studies, bronchoalveolar lavage, and histologic examination.

- Progressive dyspnea is by far the most common symptom, but cough and fatigue may also be prominent. The presence of other symptoms and associated signs, e.g., fever, rash, hemoptysis, or chest pain, can vary depending on the underlying source of disease. The time over which symptoms develop is also variable. Generally, drug-induced ILD develops after a few weeks to a few months. However, a time to onset of several years can be seen in patients with amiodarone or chemotherapy lung. A few reports have also described the onset of lung disease years *after* cessation of exposure to a drug.
- The chest X-ray remains the most practical step for the detection and classification of ILD, though high-resolution computed tomography (HRCT) scanning offers significantly greater accuracy in making a diagnosis. Notably, the extent and perceived severity of the radiographic changes often do not correlate with symptoms or physiologic abnormalities.
- Laboratory investigation alone rarely permits the clinician to either rule in or rule out a specific ILD, but the results may support a suspected diagnosis. In addition, patients may present with lung function abnormalities on office spirometry, particularly a restrictive ventilatory pattern, but abnormalities in pulmonary function testing do not point to a specific diagnosis.
- Bronchoalveolar lavage (BAL) allows determination of the cellular contents and products of the distal air spaces of the lung. It may be useful to exclude infections and tumors and may aid in distinguishing certain forms of ILD when the clinical picture is compatible. Although transbronchial biopsy may yield a histologic diagnosis, surgical lung biopsy is the gold standard and allows for a specific diagnosis to be established in over 90% of cases.

Treatment:

Some, but not all, ILDs are treatment-responsive. Corticosteroids with and without other immunosuppressive agents, such as azathioprine or cyclophosphamide, remain the mainstay of therapy. For the responsive ILD, corticosteroids are a highly appropriate form of treatment. Many patients with ILD, however, first receive these drugs after their disease has evolved to the poorly responsive fibrotic stage.

2. OSE Working Definition

The diagnosis of drug-induced lung disease is largely dependent on finding a temporal association between exposure to a drug and the development of signs or symptoms. Exclusion of other potential etiologies is also important, because drugs induce lung diseases that can closely resemble ILDs from other causes; the differential diagnosis might include lung disease related to infection; hemodynamics; radiation; collagen vascular disease; cancer; or occupational and environmental exposures.

The following satisfies the inclusion criteria for **interstitial lung disease**:

- A clinical diagnosis[16] of interstitial lung disease or interstitial pneumonia or diffuse parenchymal lung disease or alveolitis or similar terminology. Respiratory symptoms and radiologic evidence of bilateral diffuse parenchymal opacities are considered supporting information. There is usually improvement in symptoms and imaging after the drug is discontinued.

Some patients with ILD may present with symptoms but have a normal chest radiograph, whereas others have an abnormal chest radiograph but have not yet developed symptoms. Also, removal of a drug is followed by improvement in the majority of patients, though cases that have progressed to pulmonary fibrosis may be poorly reversible.

3. AERS Search Strategy (MedDRA version 10.1)

- HLT - Lower respiratory tract inflammatory and immunologic conditions
  - Includes the following PTs: alveolitis, alveolitis fibrosing, pneumonitis
- HLT - Parenchymal lung disorders NEC
  - Includes the following PTs: cryptogenic organizing pneumonia, interstitial lung disease, lung infiltration, pulmonary fibrosis, pulmonary toxicity

Additional MedDRA terms for consideration:

PTs - Acute respiratory distress syndrome, obliterative bronchiolitis, chest X-ray abnormal, lung disorder

[16] It is important to emphasize the challenging nature of making a *specific ILD clinical diagnosis.* This was illustrated in a prospective, single-center study of 59 patients consecutively referred for further diagnostic evaluation of new-onset ILD. The study compared the accuracy of a clinical diagnosis by an ILD expert with the histopathologic diagnosis. The diagnosis made on clinical grounds alone was correct in only 60% of confirmed cases of ILD other than IPF. (Raghu G, et al. The accuracy of the clinical diagnosis of new-onset idiopathic pulmonary fibrosis and other interstitial lung disease. *CHEST* 1999; 116:1168-1174.) The findings of that study complement a comparison of the specific clinical diagnoses made by three pulmonologists with the histopathologic diagnosis for 91 patients with suspected IPF. Overall, IPF was present in 54 cases and not present in 37 cases. The probability of interobserver agreement regarding a specific ILD diagnosis was only 0.49. (Hunninghake GW, et al. Utility of a lung biopsy for the diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2001; 164:193-196.)

4. References

1. Schwarz MI, King TE, and Raghu G. Approach to the evaluation and diagnosis of interstitial lung disease. In: Schwarz MI and King TE, editors. *Interstitial Lung Disease* 2003; 1-30. Hamilton, Ontario: B.C. Decker, Inc.
2. Camus P. Drug-induced infiltrative lung disease. In: Schwarz MI and King TE, editors. *Interstitial Lung Disease* 2003; 485-534. Hamilton, Ontario: B.C. Decker, Inc.
3. Limper AH. Diagnostic criteria for the clinic and for investigations of drug induced lung disease. 99th International Conference, American Thoracic Society. Seattle. May 18, 2003.
4. Rottoli P and Bargagli E. Is bronchoalveolar lavage obsolete in the diagnosis of interstitial lung disease? *Curr Opin Pulm Med* 2003; 9:418-425.
5. Collard HR and King TE. Demystifying idiopathic interstitial pneumonia. *Arch Intern Med* 2003; 163:17-29.
6. Ryu JF, Olson EJ, Midthun DE, et al. Diagnostic approach to the patient with diffuse lung disease. *Mayo Clin Proc* 2002; 77:1221-1227.
7. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. *Am J Respir Crit Care Med* 2002; 165:277-304.
8. Flaherty KR and Martinez FJ. Diagnosing interstitial lung disease: A practical approach to a difficult problem. *Cleve Clin J Med* 2001; 68:33-49.
9. Ozkan M, Dweik RA, Ahmad M. Drug-induced lung disease. *Cleve Clin J Med* 2001; 68:782-795.
10. Coultas DB, Zumwalt RE, Black WC, et al. The epidemiology of interstitial lung diseases. *Am J Respir Crit Care Med* 1994; 150:967-972.

Author: Robert Pratt

Date of development: January 2005

MedDRA revision: December 14, 2007

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/s/

Corrinne Kulick
1/2/2008 05:12:53 PM
DRUG SAFETY OFFICE REVIEWER

Ann W McMahon
1/2/2008 05:22:56 PM
DRUG SAFETY OFFICE REVIEWER

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 20-333/S-013

ADMINISTRATIVE and CORRESPONDENCE DOCUMENTS

Lee, Hyon-Zu

From: Lee, Hyon-Zu
Sent: Thursday, June 12, 2008 3:34 PM
To: 'Lomri, Zohra'
Subject: RE: NDA 20-333 Agrylin: labeling
Attachments: NDA 20333 6.11.08 edit pi-marked.doc

Zohra,

I talked to the Pharm/Tox reviewer and as part of the new labeling, we are adding non-neoplastic findings in toxicology studies.

Also, we made the following changes:

"**Nonclinical toxicology:**
In the 2-year rat study, a ~~A~~ significant increase…"

Please see attached and let me know by tomorrow AM your response.

Thanks,
Hyon-Zu

From: Lomri, Zohra [mailto:ZLomri@shire.com]
Sent: Thursday, June 12, 2008 9:47 AM
To: Lee, Hyon-Zu
Subject: RE: NDA 20-333 Agrylin: labeling

Hello Hyon-Zu, the review is ongoing. I still need final feedback from key individuals at Shire, and should be able to get back to you before noon. As a heads up, we may want clarifications for the addition of the following text:

Nonclinical toxicology:

A significant increase in non-neoplastic lesions were observed in anagrelide treated males and females in the adrenal (medullary hyperplasia), heart (myocardial hypertrophy and chamber distension), kidney (hydronephrosis, tubular dilation and urothelial hyperplasia) and bone (femur enostosis). Vascular effects were observed in tissues of the pancreas (arteritis/periarteritis, intimal proliferation and medial hypertrophy), kidney (arteritis/periarteritis, intimal proliferation and medial hypertrophy), sciatic nerve (vascular mineralization), and testes (tubular atrophy and vascular infarct) in anagrelide treated males.

I am currently in a meeting and the best way to reach me is via email.

Thanks for your patience

Kind regards

Zohra Lomri
Global Regulatory Affairs
Tel (484) 595 - 8364
Cell (484) 802 - 7859
email zlomri@shire.com

Following this page, 12 pages withheld in full - draft labeling (b)(4)

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/s/

Hyon Z Lee
6/12/2008 03:58:48 PM
CSO

Lee, Hyon-Zu

From: Lee, Hyon-Zu
Sent: Wednesday, June 11, 2008 4:25 PM
To: 'Lomri, Zohra'
Subject: RE: NDA 20-333 Agrylin: labeling
Follow Up Flag: Follow up
Flag Status: Yellow
Attachments: NDA 20333 6.11.08 edit pi-marked.doc

Zohra,

We feel that the severity of these events and rapid improvement of condition after discontinuation of anagrelide qualify this AE to be in Warnings section of labeling. All reported events were serious and some of them were life-threatening and resulted in acute respiratory failure requiring mechanical ventilation.

We have made additional minor revisions in the WARNINGS and ADVERSE REACTIONS sections. Please see attached and provide a clean copy in Word without numbering if you agree.

Thanks,
Hyon-Zu

From: Lomri, Zohra [mailto:ZLomri@shire.com]
Sent: Wednesday, June 11, 2008 10:00 AM
To: Lee, Hyon-Zu
Subject: RE: NDA 20-333 Agrylin: labeling

Dear Hyon-Zu,

At this point, and after thorough review of your comments by our clinicians, we have no major issues with your recommendations. Nonetheless, we'd appreciate some clarifications around your decision to add the language of "Interstitial Lung Diseases" in the WARNINGS section in addition to the ADVERSE REACTIONS section as the current guidances are open to interpretation. Additionally we propose the following revision:

> **Interstitial Lung Diseases:**
> Interstitial Lung Diseases (including allergic alveolitis, eosinophilic pneumonia, and interstitial pneumonitis, (b) (4) have been reported to be associated with the use of anagrelide in post-marketing reports. The time of onset (b) (4) from 1 week to several years after initiating anagrelide. In most cases, the symptoms improved after discontinuation of anagrelide (See ADVERSE REACTIONS)

Shire proposes removal of " (b) (4)" on the basis that it is too broad a term when discussing Interstitial Lung Diseases and does not contribute to the overall understanding for the prescribing physician.

Shire also proposes removal of " (b) (4)" on the basis of redundancy (the terms are (b) (4)).

I look forward to hearing from you.

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/s/

Hyon Z Lee
6/12/2008 09:30:25 AM
CSO

Lee, Hyon-Zu

From: Lee, Hyon-Zu
Sent: Tuesday, June 10, 2008 11:48 AM
To: 'Lomri, Zohra'
Subject: RE: NDA 20-333 Agrylin: labeling
Follow Up Flag: Follow up
Flag Status: Yellow
Attachments: NDA 20333 PT edit.pi-marked.doc

Hi,

We have made additional revisions in the WARNINGS section and Postmarketing Reports subsection. The subheading '(b) (4)' has been revised to "Interstitial Lung Diseases" and subsections have been revised.

Again, please let me know by 11 am tomorrow if you agree.

Thanks,
Hyon-Zu

From: Lee, Hyon-Zu
Sent: Tuesday, June 10, 2008 10:06 AM
To: 'Lomri, Zohra'
Subject: NDA 20-333 Agrylin: labeling

Ms. Lomri,

We have the following recommendations to your proposed labeling. Our revisions are highlighted in yellow and blue.

Please see attached and let me know by 11 AM tomorrow (June 11, 2008) if you agree with our labeling. If you agree, please email a clean copy without numbering.

Thank you,

Hyon-Zu Lee, Pharm.D.
Regulatory Project Manager
Division of Medical Imaging and Hematology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research

Office; 301-796-2050
Fax; 301-796-9849
Hyon.Lee@fda.hhs.gov

Following this page, 12 pages withheld in full - draft labeling (b)(4)

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/s/

Hyon Z Lee
6/11/2008 10:30:33 AM
CSO

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

REQUEST FOR CONSULTATION

TO *(Office/Division)*: OSE/DDRE
Attention: Samuel Chan

FROM (*Name, Office/Division, and Phone Number of Requestor)*: Division of Medical Imaging and Hematology Products (HFD-160)
Hyon-Zu Lee, (301) 796-1292

DATE	IND NO.	NDA NO.	TYPE OF DOCUMENT	DATE OF DOCUMENT
June 15, 2007		20-333	SLR	June 13, 2007

NAME OF DRUG	PRIORITY CONSIDERATION	CLASSIFICATION OF DRUG	DESIRED COMPLETION DATE
Agrylin	standard	Miscellaneous blood products	September 13, 2007

NAME OF FIRM: Shire Development Inc.

REASON FOR REQUEST

I. GENERAL

- ☐ NEW PROTOCOL
- ☐ PROGRESS REPORT
- ☐ NEW CORRESPONDENCE
- ☐ DRUG ADVERTISING
- ☐ ADVERSE REACTION REPORT
- ☐ MANUFACTURING CHANGE / ADDITION
- ☐ MEETING PLANNED BY
- ☐ PRE-NDA MEETING
- ☐ END-OF-PHASE 2a MEETING
- ☐ END-OF-PHASE 2 MEETING
- ☐ RESUBMISSION
- ☐ SAFETY / EFFICACY
- ☐ PAPER NDA
- ☐ CONTROL SUPPLEMENT
- ☐ RESPONSE TO DEFICIENCY LETTER
- ☐ FINAL PRINTED LABELING
- ☐ LABELING REVISION
- ☐ ORIGINAL NEW CORRESPONDENCE
- ☐ FORMULATIVE REVIEW
- ☒ OTHER *(SPECIFY BELOW)*:

II. BIOMETRICS

- ☐ PRIORITY P NDA REVIEW
- ☐ END-OF-PHASE 2 MEETING
- ☐ CONTROLLED STUDIES
- ☐ PROTOCOL REVIEW
- ☐ OTHER (SPECIFY BELOW):
- ☐ CHEMISTRY REVIEW
- ☐ PHARMACOLOGY
- ☐ BIOPHARMACEUTICS
- ☐ OTHER (SPECIFY BELOW):

III. BIOPHARMACEUTICS

- ☐ DISSOLUTION
- ☐ BIOAVAILABILTY STUDIES
- ☐ PHASE 4 STUDIES
- ☐ DEFICIENCY LETTER RESPONSE
- ☐ PROTOCOL - BIOPHARMACEUTICS
- ☐ IN-VIVO WAIVER REQUEST

IV. DRUG SAFETY

- ☐ PHASE 4 SURVEILLANCE/EPIDEMIOLOGY PROTOCOL
- ☐ DRUG USE, e.g., POPULATION EXPOSURE, ASSOCIATED DIAGNOSES
- ☐ CASE REPORTS OF SPECIFIC REACTIONS (List below)
- ☐ COMPARATIVE RISK ASSESSMENT ON GENERIC DRUG GROUP
- ☐ REVIEW OF MARKETING EXPERIENCE, DRUG USE AND SAFETY
- ☐ SUMMARY OF ADVERSE EXPERIENCE
- ☐ POISON RISK ANALYSIS

V. SCIENTIFIC INVESTIGATIONS

- ☐ CLINICAL
- ☐ NONCLINICAL

COMMENTS / SPECIAL INSTRUCTIONS:

The sponsor submitted a labeling supplement to update safety information of anagrelide labeling. The labeling changes include the additions of uterine adenocarcinoma observed in animal carcinogenicity study, allergic alveolitis reported in post-marketing database, and overdose cases in post-marketing reports. The Division is requesting consultation of post-marketing database searching and literature reports for anagrelide for adverse events including cancer, allergic alveolitis, interstitial pneumonitis, and overdose.

Please note that the submission is in the EDR dated June 13, 2007.

The HFD-160 Medical Officer is Min Lu (301-796-1406), and the Regulatory Project Manager is Hyon-Zu Lee (301-796-2192).

We would like to have your consult review by September 13, 2007.
Thank you for your assistance, and feel free to call me if you have any questions

SIGNATURE OF REQUESTOR Hyon-Zu Lee	METHOD OF DELIVERY (Check one) ☒ DFS ☐ EMAIL ☐ MAIL ☐ HAND
PRINTED NAME AND SIGNATURE OF RECEIVER	PRINTED NAME AND SIGNATURE OF DELIVERER

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/s/

Hyon Z Lee

6/15/2007 05:52:54 PM

Lee, Hyon-Zu

From: Lee, Hyon-Zu
Sent: Thursday, April 10, 2008 2:57 PM
To: 'Lomri, Zohra'
Subject: NDA 20-333 Agrylin/S-013
Importance: High

Ms. Lomri,

We are reviewing the labeling supplement (S-013) submitted on June 13, 2007 and have the following information requests:

1. Please compare the total cancer incidence rate in US general population based on SEER data with the rates in the data in anagrelide clinical trial patients.

2. Please compare the incidence rates for all involved types of cancers (seen in anagrelide trials and shown in Table 1 in Clinical Assessment) in US general population with rates from the data in anagrelide clinical trial patients.

3. Provide the above comparisons in males and females separately.

4. Provide copies of SEER printouts for the above statistics analyses, if they are available.

5. Provide the age distribution of anagrelide treated patients in the clinical trials.

When you receive this email, please let me know when you are able to submit the information as we need the information as soon as possible.

Thank you,

Hyon-Zu Lee, Pharm.D.
Regulatory Project Manager
Division of Medical Imaging and Hematology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research

Office; 301-796-2050
Fax; 301-796-9849
Hyon.Lee@fda.hhs.gov

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/s/

Hyon Z Lee
4/10/2008 03:20:18 PM
CSO

Lee, Hyon-Zu

From: Lee, Hyon-Zu
Sent: Tuesday, March 11, 2008 10:56 AM
To: 'Lomri, Zohra'
Subject: RE: NDA 20-333 anagrelide hydrochloride CBE of 06-13-07

Zohra,

We have the following additional information request:

- You have included cancer cases (54 leukemia/myeloproliferative disorders and 71 other cancers) from clinical trial data. Is any of clinical trials randomized, controlled study? Please provide comparative summary of cancer cases by treatment group (Agrylin and control) from clinical trials if the data are available.

Please respond as soon as possible.

Thanks,
Hyon-Zu

From: Lomri, Zohra [mailto:ZLomri@shire.com]
Sent: Thursday, February 21, 2008 4:54 PM
To: Lee, Hyon-Zu
Subject: RE: NDA 20-333 anagrelide hydrochloride CBE of 06-13-07

Hello Hyon-Zu,

To your request for the submission of "any clinical data regarding allergic alveolitis and intentional overdose to support the proposed labeling changes. Please provide those data including post-marketing reports and literature summary or point out the location of the submission if they were submitted as soon as possible".

Please note that this exercise was conducted by Shire to support the same labeling change in Europe and that a clinical overview is attached (attachment labeled clinical overview). The section of the report you are interested start at page 100 as the previous pages deal with the administrative European regulatory requirements. The period covered in the overview ends in 2006, so additional attachments covering 2006 till present are also attached

1. For allergic alveolitis:
   a. Literature search:
   b. For post-marketing and clinical databases
2. For intentional overdose
   a. Literature search: to provide tomorrow (I need to confirm the time period covered, but so far nothing has showed up)
   b. For post-marketing: Shire has received two cases of overdose in association with anagrelide which are summarised below and included in the "overdose database search" attachment: (this attachment covers the anagrelide HCl from NDA approval until the present so no additional attachment are provided)

SGB1-2003-00318: This is a report concerning a 24-year-old Caucasian male who took an intentional overdose of 104 capsules of anagrelide (0.5mg) with alcohol (17 cans of beer)
and was reported to have vomited. He was admitted to hospital and was found to be intoxicated and

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/s/

Hyon Z Lee
3/11/2008 11:02:41 AM
CSO

Lee, Hyon-Zu

From: Lee, Hyon-Zu
Sent: Thursday, February 14, 2008 4:55 PM
To: 'Lomri, Zohra'
Subject: RE: NDA 20-333 Agrylin (anagrelide hydrochloride) CBE dated 6/13/07

Zohra,

Please submit any clinical data regarding allergic alveolitis and intentional overdose to support the proposed labeling changes. Please provide those data including post-marketing reports and literature summary or point out the location of the submission if they were submitted as soon as possible.

Thanks,

Hyon-Zu Lee, Pharm.D.
Regulatory Project Manager
Division of Medical Imaging and Hematology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research

Office; 301-796-2050
Fax; 301-796-9849
Hyon.Lee@fda.hhs.gov

From: Lomri, Zohra [mailto:ZLomri@shire.com]
Sent: Thursday, January 24, 2008 10:56 AM
To: Lee, Hyon-Zu
Subject: RE: NDA 20-333 Agrylin (anagrelide hydrochloride) CBE dated 6/13/07

Good morning,

I am following up on the CAC meeting, has it been held? if not has a date been set for this meeting? Any feedback would be greatly appreciated.

Kind regards

Zohra Lomri
Global Regulatory Affairs
Tel (484) 595 - 8364
email zlomri@shire.com

From: Lee, Hyon-Zu [mailto:Hyon.Lee@fda.hhs.gov]

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/s/

Hyon Z Lee
2/14/2008 05:01:54 PM
CSO

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

NDA 20-333/S-013 **CBE-0 SUPPLEMENT**

Shire Development, Inc.
Attention : Valerie Waltman
Manager, Regulatory Affairs
725 Chesterbrook Blvd.
Wayne, PA 19087

Dear Ms. Waltman:

We have received your supplemental drug application submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for the following:

Name of Drug Product: Agrylin® (anagrelide hydrochloride) Capsules

NDA Number: 20-333

Supplement number: S-013

Date of supplement: June 13, 2007

Date of receipt: June 13, 2007

This supplemental application, submitted as "Supplement - Changes Being Effected" proposes the following changes: Revisions to the **Carcinogenesis, Mutagenesis, Impairment of Fertility** subsection of the **PRECAUTIONS** section.

Unless we notify you within 60 days of the receipt date that the application is not sufficiently complete to permit a substantive review, we will file the application on August 12, 2007 in accordance with 21 CFR 314.101(a). If the application is filed, the user fee goal date will be December 13, 2007.

Please cite the application number listed above at the top of the first page of all submissions to this application. Send all submissions, electronic or paper, including those sent by overnight mail or courier, to the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Medical Imaging and Hematology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, call me at 301-796-2050.

Sincerely,

{See appended electronic signature page}

Hyon-Zu Lee, Pharm. D.
Regulatory Project Manager
Division of Medical Imaging and Hematology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research

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/s/

Hyon Z Lee
6/15/2007 11:14:05 AM