PROTOCOL: SPD422-113

TITLE:	A Phase 1, Open-label, Single-sequence, Non-randomized, Crossover, Drug-Drug Interaction Study to Evaluate the Effect of Omeprazole on the Pharmacokinetics of SPD422 (anagrelide hydrochloride) in Healthy Adult Subjects
DRUG:	SPD422, Anagrelide Hydrochloride
IND:	Non-IND
EUDRACT NO.:	Non-EUDRACT
SPONSOR:	Shire 300 Shire Way, Lexington, MA 02421 USA
PRINCIPAL/ COORDINATING INVESTIGATOR:	[redacted]
PROTOCOL HISTORY:	Original Protocol: 04Oct2018

PROTOCOL SIGNATURE PAGE

Sponsor's (Shire) Approval

[redacted] **Date:** [redacted]

Investigator's Acknowledgement

I have read this protocol for Shire Study SPD422-113.

Title: A Phase 1, Open-label, Single-sequence, Non-randomized, Crossover, Drug-Drug Interaction Study to Evaluate the Effect of Omeprazole on the Pharmacokinetics of SPD422 (anagrelide hydrochloride) in Healthy Adult Subjects

I have fully discussed the objective(s) of this study and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a subject in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with International Conference on Harmonisation guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

I understand that the sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate my intention immediately in writing to the sponsor.

Investigator Name and Address: (please hand print or type)	***The investigator completes the bottom section of the protocol signature page***

Signature: _____ **Date:** _____

EMERGENCY CONTACT INFORMATION

In the event of a serious adverse event (SAE), the investigator must fax or e-mail the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form within 24 hours to the Shire Global Safety Department. Applicable fax numbers and e-mail address can be found on the form (sent under separate cover). A copy of this form must also be sent to the contract research organization (CRO)/Shire medical monitor by fax or e-mail using the details below.

For protocol- or safety-related issues, the investigator must contact the Shire medical monitor:

[redacted]

Telephone (24 hour coverage)
[redacted] (business hours)
[redacted] (after business hours)

Email: [redacted]

ADDITIONAL CONTACT INFORMATION

In case of any other issues, including non-safety-related issues or if the medical monitor is unable to be reached, the investigator must contact the Shire Study Manager:

[redacted]
Office Telephone: [redacted] (business hours)

If unavailable, please contact:

[redacted]
Telephone: [redacted] (business hours)
[redacted] (24-hour coverage)

PRODUCT QUALITY COMPLAINTS

Investigators are required to report investigational product quality complaints to Shire within 24 hours. This includes any instances wherein the quality or performance of a Shire product (marketed or investigational) does not meet expectations (eg, inadequate or faulty closure, product contamination) or that the product did not meet the specifications defined in the application for the product (eg, wrong product such that the label and contents are different products). For instructions on reporting AEs related to product complaints, see Section 8.

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Origin of Product Quality Complaint	E-mail Address
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European Union and Rest of World	[redacted]

Telephone numbers (provided for reference if needed):

Shire, Lexington, MA (USA)
[redacted]

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ABBREVIATIONS

AE	adverse event
AUC	area under the curve
β-hCG	beta-human chorionic gonadotropin
BID	twice daily
BMI	body mass index
CI	confidence interval
CRA	clinical research associate
CRF	case report form
CRC	clinical research center
CRO	contract research organization
EC	ethics committee
ECG	electrocardiogram
EM	extensive metabolizer
ET	essential thrombocythemia
EU	European Union
FDA	Food and Drug Administration
GCP	good clinical practice
HBsAG	hepatitis B surface antigen
HCV	hepatitis C virus antibody
HIV	human immunodeficiency virus
ICH	International Conference on Harmonisation
IM/PM	intermediate/poor metabolizer
IP	investigation product
IRB	institutional review board
IRT	interactive response technology
PK	pharmacokinetics
QD	once daily
SAE	serious adverse event
SAP	statistical analysis plan
TEAEs	treatment emergent adverse events
TSH	thyroid stimulating hormone
T4	thyroxine
T3	triiodothyronine
US	United States

STUDY SYNOPSIS

Protocol number: SPD422-113	**Drug:** Anagrelide Hydrochloride
Title of the study: A Phase 1, Open-label, Single-sequence, Non-randomized, Crossover, Drug-Drug Interaction Study to Evaluate the Effect of Omeprazole on the Pharmacokinetics of SPD422 (anagrelide hydrochloride) in Healthy Adult Subjects	
Number of subjects: Approximately 35 subjects will be screened to ensure the enrollment and completion of approximately 20 subjects.	
Investigator(s): [redacted]	
Site(s) and Region(s): [redacted]	
Study period (planned): Feb 2018 – May 2018	**Clinical phase:** Phase 1
Objectives: **Primary:** The primary objective of this study is to assess the effect of a CYP1A2 inducer (omeprazole) on the pharmacokinetics (PK) of anagrelide when administered concurrently. **Secondary:** The secondary objective of this study is to assess the safety and tolerability of anagrelide, omeprazole, and co-administered anagrelide plus omeprazole.	
Rationale: CYP1A2 inducers may modify the pharmacokinetics of anagrelide and its metabolites when given concurrently. In this study the effect of a commonly used CYP1A2 inducer (omeprazole) on the PK of anagrelide will be assessed.	
Investigational product, dose, and mode of administration: • SPD422 anagrelide hydrochloride 1 mg administered orally as two 0.5 mg capsules • Omeprazole 40 mg administered orally as a single 40 mg capsule once daily (QD) for 7 days	
Methodology: This is a Phase 1, open-label, single-sequence, non-randomized, multiple-dose, crossover pharmacokinetic study to assess the effect of a CYP1A2 inducer (omeprazole 40 mg) on the pharmacokinetics of anagrelide (1 mg) when administered concurrently in healthy subjects. Up to 35 male and female subjects, aged 18-45 years will be screened to ensure the enrollment and completion of approximately 20 subjects. Subjects who discontinue the study will not be replaced. The study includes a screening period of up to 28 days, a treatment period (9 days) and a follow-up phone call (7±2 days after the last dose of investigational drug is administered). The maximum duration of study participation for any subject is 46 days, if the maximum screening, treatment and follow-up durations are used. Following the screening visit, subjects who meet the protocol specific inclusion and exclusion criteria will return to the Clinical Research Center (CRC) on Day -1 to reconfirm eligibility criteria for participation in the study. Subjects who continue to meet the eligibility criteria will be admitted to the CRC on Day -1. Subjects will be confined at the CRC from Day -1 until after completion of the 24 hour post dose assessments on the morning of Day 9. Administration of study drug on Days 1-8 should be at the same time each morning for each subject during the study. Study procedures, safety and pharmacokinetic assessments will be collected and reported at scheduled time points during the study.	

Day 1: Anagrelide 1 mg, (administered orally as two 0.5 mg capsules) will be administered to all study subjects at approximately 8:00 in the morning on Day 1. Subjects are required to fast for 10 hours prior to and until 4 hours following administration of anagrelide on Day 1.

Days 2-7: Omeprazole 40 mg will be administered orally QD on Days 2-7 to all subjects. Omeprazole will be administered at approximately 8:00 in the morning, prior to breakfast.

Day 8: Anagrelide 1 mg and omeprazole 40 mg will be administered concurrently to all subjects at approximately 8:00 in the morning on Day 8. Subjects are required to fast for 10 hours prior to and until 4 hours following administration of anagrelide and omeprazole on Day 8.

Day 9: Subjects will be discharged from the CRC on Day 9 following the completion of all study procedures and assessments.

Subjects who discontinue early from the study will complete the Early Termination assessments.

- Safety Assessments include but are not limited to adverse events, physical examinations, 12-lead electrocardiograms (ECG), vital signs (blood pressure and pulse) and safety laboratory tests (hematology, chemistry, and urinalysis).
- Pharmacokinetic assessments:
- Omeprazole: Trough and serial blood samples for PK analysis will be collected for the determination of plasma omeprazole concentrations. Trough samples will be collected predose on Days 6 and 7. Serial samples will be collected on Day 8 starting at predose and up to 24 hours post dose. The samples will be collected according to the Schedule of Assessments.
- Anagrelide: Serial blood samples for PK analysis will be collected for the determination of plasma concentrations of anagrelide, 3-hydroxy-anagrelide and RL603 on Day 1 and Day 8 starting at predose and up to 12 hours post dose. The blood samples will be collected according to the Schedule of Assessments.

A follow up telephone call (or in-person follow-up contact at the investigator's discretion) will take place 7± 2 days following the subject's last dose of investigational product to collect information on any ongoing or new adverse events (AEs), serious adverse events (SAEs) or concomitant medications, as appropriate.

Inclusion Criteria:

The subject will not be considered eligible for the study without meeting all of the criteria below.

Subjects cannot be enrolled before all inclusion criteria (including test results) are confirmed.

1. Has given, personally signed, and dated informed consent to participate in the study, in accordance with the International Conference on Harmonization Good Clinical Practice Guideline E6 (1996) and applicable regulations, before completing any study-related procedures.
2. Age 18-45 years inclusive at the time of consent. The date of signing informed consent is defined as the beginning of the Screening Period. This inclusion criterion will be assessed only at the Screening Visit.
3. Male, or non-pregnant, non-lactating female who agrees to comply with any applicable contraceptive requirements of the protocol or females of non-childbearing potential. A female of non-childbearing potential (defined as a female who is post-menopausal [amenorrhea for at least 12 consecutive months], has had a hysterectomy, bilateral tubal ligation, bilateral oophorectomy or bilateral salpingectomy.
4. Satisfactory medical assessment with no clinically significant or relevant abnormal findings as determined by medical/surgical history, physical examination, vital signs, 12-lead electrocardiogram, and clinical laboratory evaluation (hematology, biochemistry, thyroid function, and urinalysis) that are likely to interfere with the subject's participation or ability to complete the study as assessed by the investigator.

5. An understanding, ability, and willingness to fully comply with study procedures and restrictions.
6. Body mass index (BMI) between 18.5 and 30.0 kg/m^2 inclusive; assessed only at the screening visit.
7. Able to swallow (multiple capsules or tablets at 1 time or consecutively at 1 time) all investigational product.
8. Healthy as determined by the investigator on the basis of screening evaluations.

Exclusion Criteria:

Subjects are excluded from the study if any of the following exclusion criteria are met at the Screening visit (Days -28 through Day -2) or on Day -1 through the morning of Day 1, just prior to dosing:

1. Current or recurrent disease or conditions (eg, cardiovascular, renal, liver, gastrointestinal, malignancy or other conditions) that could affect the absorption, action, or disposition of either omeprazole or anagrelide or its metabolites, or could affect clinical assessments or clinical laboratory evaluations.
2. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to fully comply with the requirements of the study or complete the study, or any condition that presents undue risk from the investigational product or study procedures.
3. Significant illness, as judged by the investigator, within the 2 weeks of administration of the first dose of investigational product.
4. Use of any medication (including prescription, over-the-counter, herbal, multivitamin, oral contraceptives and other hormonal contraceptive treatments, or homeopathic preparations) within the 30 days prior to the first dose of study drug or during the study through Day 9 (occasional use of acetaminophen is allowed).
5. Treatment with any known hepatic and/or P450 enzyme-altering agents, including CYP1A2 inducers or inhibitors within 30 days prior to the first dose of investigational product. This includes(*):

Inhibition/Inducer Index	Medication
Strong inhibitor	ciprofloxacin, enoxacin, fluvoxamine, and zafirlukast
Moderate inhibitor	methoxsalen, mexiletine, and oral contraceptives
Moderate inducer	phenytoin, rifampin, ritonavir, smoking, teriflunomide
Inducer	lansoprazole

* FDA Draft Guidance for Industry: Drug interaction studies - study design, data analysis, implications for dosing, and labeling recommendations. 2012.

6. A history of any of the following medical conditions:
    - History of previous bone marrow suppression.
    - History of hypersensitivity to the investigational product.
    - History of adverse hematologic reaction, (such as neutropenia, thrombocytopenia, anemia) to any drug.
    - History of symptomatic or clinically meaningful orthostatic hypotension or syncope, as assessed by the investigator.
    - History of controlled or uncontrolled hypertension or a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg at the Screening Visit or Day -1.
    - Subject has any history of seizure disorder.
    - History or presence of known structural cardiac abnormalities, syncope, cardiac conduction problems (PR interval >220 ms, second or third-degree heart block, bundle branch block [except congenital right bundle branch block], or prolonged QTc interval) or exercise-related cardiac events.

- History of alcohol or other substance abuse within the last year.

7. A subject's alcohol consumption that fulfils one of the following: (Note: One alcohol unit=1 beer [12 oz]=1 wine [5 oz]=1 liquor [1.5 oz])
    - Has consumed alcohol within 2 days prior to the first dose of investigational product.
    - Male subjects who consume more than 3 units* of alcohol per day.
    - Female subjects who consume more than 2* units of alcohol per day.
8. Positive screening test results for alcohol, drugs of abuse, or pregnancy (females of childbearing potential only) at the Screening Visit or Day –1.
9. A positive human immunodeficiency virus (HIV) antibody screen, hepatitis B surface antigen (HBsAG) or hepatitis C virus antibody (HCV) screen.
10. Use of tobacco in any form (eg, smoking or chewing) or other nicotine-containing products in any form (eg, gum, patch) within 30 days prior to the first dose of investigational product and during the in-house stay at the CRC.
11. A positive urine cotinine test that is ≥50 ng/mL at either the Screening Visit or on Day -1.
12. Routine consumption of more than 2 units of caffeine per day or subjects who experience caffeine-withdrawal headaches or have a history of caffeine-withdrawal headaches. (One caffeine unit is contained in the following items: one 6-oz cup of coffee, two 12-oz cans of cola, one 12-oz cup of tea, and three 1-oz chocolate bars. Decaffeinated coffee, tea, or cola are not considered to contain caffeine.)
13. Consumption of grapefruit, Seville oranges, and/or products containing these items within 7 days prior to the first dose of investigational product.
14. Donation of blood or blood products (egg, plasma, or platelets) within 60 days prior to the first dose of investigational product.
15. Known or suspected intolerance or hypersensitivity to the investigational products (ie, anagrelide or omeprazole) or closely related compounds, or any of the stated ingredients.
16. Within 30 days prior to the first dose of investigational product:
    - Have used an investigational product (if elimination half-life is <6 days, otherwise 5 half-lives).
    - Have been enrolled in a clinical study (including vaccine studies) that, in the Investigator's opinion, may impact this Shire-sponsored study.
17. Prior screen failure, participation, or enrollment in this study.
18. History of sensitivity to heparin or heparin-induced thrombocytopenia.

Maximum duration of subject involvement in the study:

- Planned duration of screening period: 28 days
- Planned duration of treatment period: 9 days
- Planned duration of follow-up: 7 ±2 days

Endpoints and statistical analysis:

Endpoints and statistical analysis:

Three analysis populations are defined for this study: the enrolled, safety, and pharmacokinetic sets:

The enrolled set consists of all subjects for whom an enrollment number has been assigned. Usually, these are the subjects who meet the study inclusion/exclusion criteria and sign the informed consent form.

The safety set consists of subjects who are administered at least 1 dose of investigational product (anagrelide or omeprazole) and have at least 1 post-dose safety assessment.

The pharmacokinetic set consists of subjects who receive at least 1 dose of study drug and have evaluable PK data (defined as complete concentration-time profile to obtain meaningful estimates of PK parameters) available for 1 dose regimen. The PK analyses will be based on this population.

Primary endpoint:

SPD422 will be administered under fasting conditions on Days 1 and 8. Omeprazole will be administered prior to breakfast on Days 2-7 and under fasting conditions on Day 8. PK evaluations of anagrelide concentrations will be performed on Day 1 and Day 8, and PK evaluations of omeprazole concentrations will be following coadministration with anagrelide on Day 8.

Pharmacokinetic parameters will be determined from the plasma concentration-time data for anagrelide and omeprazole by non-compartmental analysis. The pharmacokinetic parameters will include, but not be limited to:

AUC0-¥: Area under the curve extrapolated to infinity, calculated using the observed value of the last non-zero concentration (anagrelide and metabolites)

AUC0-t:: Area under the curve from time 0 to the last time point of sample collection (anagrelide and metabolites)

AUC0-tau: Area under the concentration-time curve from time zero to the end of the dosing interval (24 hours post dose) (omeprazole only)

CL/F: Total body clearance for extravascular administration.

Cmax: Maximum concentration occurring at tmax

Cmin: The minimum concentration observed over the dosing interval (omeprazole only)

t½: Terminal half-life

tmax: Time of maximum observed concentration sampled during a dosing interval

Vz/F:Volume of distribution associated with the terminal slope following extravascular administration.

The primary pharmacokinetic parameters will include the Cmax, AUC0-t and AUC0-∞ for anagrelide and its active metabolite, 3-OH-anagrelide (BCH24426).

The geometric mean relative bioavailability and 90% confidence limits for the effect of omeprazole will be evaluated (eg, AUCanagrelide+omeprazole/AUCanagrelide).

Because the CYP2C19-mediated metabolism of omeprazole is subject to genetic polymorphism (approximately 2/3 subjects are CYP2C19 extensive metabolizers (EMs) with lower omeprazole AUC and 1/3 subjects are CYP2C19 intermediate/poor metabolizers IMs/PMs with higher omeprazole AUC), the geometric mean relative bioavailability and 90% confidence interval (CI) will be calculate for (1) all subjects, (2) EMs, and (3) IMs/PMs. The observed omeprazole $AUC_{0\text{-}tau}$ will be used to define the CYP2C19 metabolizer status of subjects, with $AUC_{0\text{-}tau}$ <5,200 ng•h/mL (<15 µmol/L) classified as CYP2C19 EM and $AUC_{0\text{-}tau}$ ≥5,200 ng•h/mL (≥15 µmol/L) classified as CYP2C19 IM/PM.

Secondary endpoint:

Safety will be assessed for the following evaluations for anagrelide alone, omeprazole alone, and anagrelide and omeprazole combination treatments:

Number, severity, seriousness, and causality of treatment emergent adverse events (TEAEs)

Changes in physical examination, vital signs, electrocardiogram (ECGs), and clinical laboratory results (hematology, chemistry, and urinalysis) from baseline to post-baseline time points.

Sample Size Justification:

Approximately 20 subjects will be enrolled. Subjects who discontinue will not be replaced.

Sample size estimation is to achieve a desired precision of the effect of interest, targeting the width of the 90% confidence interval.

From a previous crossover study (SPD422-110), the within-subject coefficient variation in anagrelide log-transformed AUC was estimated to be 0.132. Allowing for a slightly larger within-subject CV of 0.15, a sample size of 11 subjects is required to estimate the mean difference between anagrelide + omeprazole and anagrelide alone in the log-transformed AUC with an error margin of at most ±0.2231 at 90% confidence with a probability of 90%. Thus, if the true geometric mean treatment ratio is 0.6, the lower and upper 90% confidence bounds will be estimated to be within 0.48 and 0.75 (exp[ln(0.6) – 0.2231] and exp[ln(0.6) + 0.2231]) with 90% probability. Therefore, with an overall sample size of 20 subjects, anticipated 13 to 14 CYP2C19 EM subjects and 6 to 7 CYP2C19 IM/PM subjects, this statistical power will be met for the overall population and the CYP2C19 EM population. The confidence bounds for the smaller population of CYP2C19 IM/PM subjects will be slightly wider.

Statistical Methodology for Pharmacokinetic Endpoint(s):

The following analyses will be conducted on the Pharmacokinetic Analysis Set. All statistical analyses will be presented for the CYP2C19 EM group, the CYP2C19 IM/PM group, and overall.

Summary statistics (number of observations, mean, standard deviation, coefficient of variation, median, maximum, minimum, and geometric mean) will be determined for all pharmacokinetic parameters and presented by treatment. Plasma concentrations of anagrelide, 3-hydroxy-anagrelide, RL603, and omeprazole at each nominal sampling time will also be summarized by treatment using descriptive statistics.

The log-transformed pharmacokinetic parameters will be compared between the 2 treatments (anagrelide plus omeprazole and anagrelide alone) using an analysis of variance model for a single-sequence crossover design with treatment and subject as fixed effects will be used. The magnitude of the effect of omeprazole on the pharmacokinetic profile of anagrelide and its metabolites will be evaluated by the point estimate and ninety percent (90%) confidence intervals for the treatment difference on the log-transformed parameters back-calculated to the original scale for the comparisons of Cmax, AUC0 t, and AUC0 ∞ (test treatment=anagrelide plus omeprazole, reference treatment=anagrelide alone).

Statistical Methodology for Safety Endpoint(s):

The safety endpoints will be summarized with descriptive statistics for the safety set.

Relevant safety endpoints as well as their changes from baseline will be summarized. Baseline is defined as the last assessment prior to the first dose of investigational product. Potentially clinically important findings will also be summarized or listed. The potentially clinically important values will be defined in the statistical analysis plan (SAP).

Adverse events will be coded using the Medical Dictionary for Regulatory Activities. The number of events, incidence, and percentage of treatment-emergent adverse events will be calculated overall, by system organ class, by preferred term, and by treatment group. Treatment emergent adverse events will be further summarized by severity and relationship to investigational product. Adverse events related to investigational product, AEs leading to withdrawal, SAEs, and deaths will be similarly summarized/listed.

Safety will be assessed by TEAEs, new findings from physical examination, evaluation of blood pressure, heart rate, new findings from 12-lead ECG, and clinical laboratory test results (biochemistry, hematology, and urinalysis).

Clinical laboratory tests, vital signs, and ECG findings will be summarized by treatment group and visit. Potentially clinically important findings will also be summarized or listed.

STUDY SCHEDULE(S)

Table 1: Schedule of Assessments

	Screening Period Day -28 to Day -2	**Treatment Period**										**Early Termination** [a]	**Follow Up Phone Contact 7± 2 days** [b]
		Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9		
Informed Consent	X												
In-Patient[c]		X	X	X	X	X	X	X	X	X	X		
Inclusion/Exclusion Criteria	X	X											
Demographics	X												
Medical & Medication History	X												
Physical Examination	X	X	X								X	X	
Height, weight and calculate BMI[d]	X												
Vital Signs (blood pressure and pulse)[e]	X	X	X							X	X	X	
12-lead ECG [e]	X	X	X							X	X	X	
Drugs of abuse and alcohol screen	X	X											
Urine cotinine	X	X											
HIV, HBsAg, and HCV screen	X												
Serum Pregnancy (all females)	X	X									X	X	
Biochemistry, hematology, and urinalysis	X	X	X							X	X	X	
TSH and T4	X												
Omeprazole PK Sample Collection[f]								X [f]	X [f]	X	X		
Anagrelide PK Sample Collection			X							X			
Administration of omeprazole[g,]				X	X	X	X	X	X	X[i]			
Administration of anagrelide [h, i]			X [i]							X [i]			
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X

Table 1: Schedule of Assessments

	Screening Period Day -28 to Day -2	**Treatment Period**										**Early Termination** [a]	**Follow Up Phone Contact 7± 2 days** [b]
		Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9		
Adverse events/serious adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X

BMI=body mass index; ECG=electrocardiogram; HIV=human immunodeficiency virus, HBsAG=hepatitis B surface antigen, HCV=hepatitis C virus; CBP=childbearing potential, TSH=thyroid stimulating hormone, T4=thyroxine, PK=pharmacokinetic

[a] An attempt to perform the early termination assessments and procedures will be made for any subject who withdraws or is removed from the study.
[b] A follow up telephone contact (or in-person follow-up contact at the investigator's discretion) will take place 7± 2 days following the subject's last dose of anagrelide.
[c] Subjects will remain in-patient at the Clinical Research Center (CRC) starting on Day -1 until discharged following the 24 hour post dose PK sample and safety assessments on the morning of Day 9.
[d] Height to be measured without shoes and weight to be taken in light clothes
[e] Subjects should be resting in a supine position for at least 5 minutes prior to collecting vital signs and ECGs. Vital sign assessments during the screening visit only include blood pressure, pulse, respiratory rate and temperature.
[f] Omeprazole PK sample collections on Days 6 and Day 7 are to be taken at pre-dose only.
[g] Omeprazole is to be administered prior to breakfast.
[h] Anagrelide should be taken by the subject at 8:00 am. If more than 1 subject is receiving anagrelide on the same day, the dosing interval between subjects may be a stagger of 3 minutes between dose administrations.
[i] Subjects are required to fast for approximately 10 hours prior to and until 4 hours post dose (after PK and safety assessments) on Days 1 and 8

Table 2: Schedule of Assessments – Detail of Treatment Period Day 1

Study Procedure/Relative to IP Administration	Administration of Anagrelide[a]	Anagrelide PK Sample Collection	Physical Examination	Vital Signs[b] (BP and pulse)	12-lead ECG[b]	Biochemistry, hematology, and urinalysis	Concomitant Medications	Adverse Events/Serious Adverse Events
Pre-dose[c]		X		X	X	X	X	X
0	X[d]						X	X
0.5 hours		X					X	X
1 hour		X		X			X	X
1.5 hours		X					X	X
2 hours		X		X			X	X
2.5 hours		X					X	X
3 hours		X					X	X
4 hours		X		X			X	X
8 hours		X		X			X	X
12 hour		X	X	X	X	X	X	X

ECG=electrocardiogram; PK=pharmacokinetic; IP=Investigation Product
[a] The subjects should be administered anagrelide starting at 8:00 am.
[b] Subjects should be resting in a supine position for at least 5 minutes prior to collecting vital signs and ECGs.
[c] Pre-dose assessments should be performed within 30 minutes prior to dose.
[d] Subjects are required to fast for approximately 10 hours prior to and until 4 hours post-dose (after PK and safety assessments) on Day 1 and 8

Table 3: Schedule of Assessments – Detail of Treatment Period Day 8

Study Procedure/Relative to IP Administration	Administration of Anagrelide & Omeprazole[a]	Anagrelide PK Sample Collection	Omeprazole PK Sample Collection	Physical Examination	Vital Signs[b] (BP and pulse)	12-lead ECG[b]	Biochemistry, hematology, and urinalysis	Concomitant Medications	Adverse Events/Serious Adverse Events
Pre-dose[c]		X	X		X	X	X	X	X
0	X[d]							X	X
0.5 hours		X	X					X	X
1 hour		X	X		X			X	X
1.5 hours		X	X					X	X
2 hours		X	X		X			X	X
2.5 hours		X	X					X	X
3 hours		X	X					X	X
4 hours		X	X		X			X	X
8 hours		X	X		X			X	X
12 hour		X	X		X	X		X	X
24 hour			X	X	X	X	X	X	X

ECG=electrocardiogram; PK=pharmacokinetic; IP=Investigation Product

[a] The subjects should be administered anagrelide and omeprazole concurrently starting at 8:00 am.

[b] Subjects should be resting in a supine position for at least 5 minutes prior to collecting vital signs and ECGs.

[c] Pre-dose assessments should be performed within 30 minutes prior to dose.

[d] Subjects are required to fast for approximately 10 hours prior to and until 4 hours post-dose (after PK and safety assessments) on Days 1 and Day 8.

1. BACKGROUND INFORMATION

1.1 Indication and Current Treatment Options

Anagrelide is a selective inhibitor of megakaryocyte growth and development, which is used to lower the high platelet count in patients with essential thrombocythemia (ET). The effect of anagrelide on the platelet count of patients with ET is highly variable, requiring careful dose titration to lower platelet counts to ≤600 x 10^9/L and optimally to ≤450 x 10^9/L. Treatment with Anagrelide is typically initiated at 1 mg/day (administered as 0.5 mg twice daily [BID]) for one week, followed by careful titration to individually subject-optimized dose regimen balancing lowered platelet counts and tolerability.

1.2 Product Background

Anagrelide (AGRYLIN®; XAGRID®; Shire Pharmaceuticals Group PLC), licensed in 45 territories including the European Union (EU), is an orally active quinazoline derivative and the only platelet-lowering agent showing selectivity for megakaryocytes. Anagrelide is believed to inhibit TPO/c-mpl receptor-mediated events in megakaryocytes, leading to reduced maturation of the megakaryocytes and ultimately to decreased platelet numbers. Clinical studies in subjects support a hypothesis of dose-related reduction in platelet production resulting from a decrease in megakaryocyte hypermaturation.

Anagrelide selectively lowers platelets due to inhibitory effects due to megakaryocytopoiesis. The action of anagrelide has recently been determined to suppress expression of the key transcription factors. GATA-1 and FOG-1, downstream of TPO/c-mpl receptor-mediated events. These factors are required at all stages of megakaryocytopoiesis from differentiation to maturation. Anagrelide downregulates expression of GATA-1 and FOC-1 without altering TPO/c-mpl-mediated signaling, an action independent of cAMP PDE III inhibition. Anagrelide does not interfere with erythropoietin-stimulated erythropoiesis, or leukocytopoiesis (Ahluwalia et al., 2010).

Refer to the investigator's brochure provided under separate cover for more information.

1.2.1 Clinical Information

Anagrelide administered to healthy subjects during Phase 1 clinical studies demonstrated a consistent safety profile at 1mg/day dosing. In studies SPD422-107 and SPD422-110, there were no serious adverse events. Treatment-emergent events were primarily mild, and the most common events (>10%) were dizziness, postural dizziness, headache, feeling hot, procedural site pain, and palpitations. There were no clinically significant electrocardiogram (ECG) changes reported in either study. In healthy subjects receiving a single dose of anagrelide 2.5mg (study SPD422-111, Thorough QT study), 73% of subjects reported headache, and >20% of subjects reported dizziness and nausea. One subject was withdrawn from the study due to treatment-emergent T-wave inversion on ECG at the 2.5 mg dose. In that study, the high dose of anagrelide 2.5 mg rapidly increased mean heart rate during the first 1.5 hours after administration, with the largest mean time-matched increase of +29.1 bpm occurring at 2 hours after administration.

Additionally, anagrelide 2.5 mg increased the QTcNi (patient-specific corrected QT) and QTcF (Fridericia corrected QT) with an upper 2-sided 90% confidence interval (CI) exceeded 10msec at 1 and 1.5 hours (values of 15.7 msec and 13.6 msec, respectively) for QtcNi and at 1 hour for QTcF (value of 12.7 msec). These changes corresponded to the time of t_{max} for anagrelide and its active metabolite, 3-hydroxy-anagrelide, and the magnitude of the change at these time points met the definition for a positive thorough QT study. However, the QTcNi and QTcF values were increased only when the heart rate was increasing rapidly at 0.5-1.5 hours after administration of anagrelide 2.5mg, and the interpretation of the increased QTcNi and QTcF may be confounded by the rapid increase in heart rate and the corresponding QT-RR hysteresis that occurs when the heart rate changes rapidly.

Anagrelide is metabolized via cytochrome P450 type 1A2 (CYP1A2) to form its active metabolite, 3-hydroxy-anagrelide (also known as BCH24426) which undergoes both renal excretion and further metabolism via CYP1A2 to form the inactive metabolite, RL603. Administration of anagrelide with a CYP1A2 inducer could potentially increase the clearance of anagrelide as well as increase both formation rate and clearance of the active metabolite. Shire has been requested by the European Medicines Agency to assess the effect of the CYP1A2 inducer omeprazole on the pharmacokinetics (PK) of anagrelide when given concurrently.

Omeprazole is metabolized by CYP2C19, an important polymorphically expressed enzyme. Based on the incidence of this genetic polymorphism in the general population, approximately 2/3 of the subjects in this study are expected to be CYP2C19 extensive metabolizers (EMs), and the remaining 1/3 of the subjects in this study will be CYP2C19 intermediate/poor metabolizers (IMs/PMs). The CYP2C19 IMs/PMs will have higher omeprazole plasma concentrations leading to a greater CYP1A2 induction than the CYP2C19 EMs. It is important to evaluate the effect of omeprazole on the pharmacokinetic profile of anagrelide and its active metabolite in EMs and IMs/PMs as well as overall (Rost et al., 1992; Rost and Roots, 1994;Nousbaum et al., 1994). The area under the curve (AUC) of omeprazole will be used to classify subjects into the CYP2C19 EM or IM/PM categories in this study (Rost et al., 1992). Increased knowledge and understanding of the effect of the CYP1A2 inducer omeprazole on the pharmacokinetic profile of anagrelide and the varying impact of CYP2C19 polymorphisms on the magnitude of omeprazole's CYP1A2 induction effect on the pharmacokinetic profile of anagrelide are helpful in optimizing personalized anagrelide drug therapy.

Always refer to the latest version of the SPD422 anagrelide hydrochloride investigator's brochure for the overall risk/benefit assessment and the most accurate and current information regarding the drug metabolism, pharmacokinetics, efficacy, and safety of SPD422 anagrelide hydrochloride.

2. STUDY OBJECTIVES AND PURPOSE

2.1 Rationale for the Study

The European Medicines Agency requested that Shire assess the effect of the CYP1A2 inducer omeprazole on the pharmacokinetics (PK) of anagrelide when given concurrently to provide additional guidance to the prescribing physician on this potential drug-drug interaction. CYP1A2 inducers may modify the pharmacokinetics of anagrelide and its metabolites when given concurrently. In this study the effect of a commonly used CYP1A2 inducer (omeprazole) on the pharmacokinetics of anagrelide will be assessed**.**

2.2 Study Objectives

2.2.1 Primary Objectives

The primary objective of this study is to assess the effect of a CYP1A2 inducer (omeprazole) on the pharmacokinetics of anagrelide when administered concurrently.

2.2.2 Secondary Objectives

The secondary objective of this study is to assess the safety and tolerability of anagrelide, omeprazole, and co-administered anagrelide plus omeprazole.

3. STUDY DESIGN

3.1 Study Design and Flow Chart

This is a Phase 1, open-label, single-sequence, non-randomized, multiple-dose, crossover pharmacokinetic study to assess the effect of a CYP1A2 inducer (omeprazole 40 mg once daily [QD]) on the pharmacokinetics of anagrelide (1 mg) when administered concurrently in healthy subjects. Up to 35 healthy male and female subjects, aged 18-45 years will be screened to ensure enrollment and completion of 20 subjects. Subjects who discontinue from the study will not be replaced. The study design is shown in the study schema in Figure 1.

3.1.1 Dose selection, rationale and administration

Therapeutic doses of omeprazole typically range from 20 to 40 mg once-daily for most indications, but may be as high as 60 mg once-daily for pathological hypersecretory conditions. Omeprazole is safe and well tolerated in this dose range. Omeprazole 40 mg QD produces substantial CYP1A2 induction with 1 week of dosing (Rost et al., 1992; Rost and Roots, 1994; Nousbaum et al., 1994).

Anagrelide 1 mg is a typical starting dose for treating ET patients, and it is sufficient to provide a robust PK profile for both parent anagrelide and the 3-hydroxy metabolite in healthy subjects at a safe and tolerable single-dose (SPD422-103, SPD422-104, SPD422-109, SPD422-110). Higher single-doses of anagrelide 2.5 mg have led to substantial increases in heart rate (mean increase +29 bpm) observed in a thorough QTc study (Troy et al., 2014), therefore this drug-drug interaction study will use the 1mg dose which is below 2.5 mg.

For each study subject, dosing should occur at approximately the same time point in the morning on Days 1 through Day 8. A previous study (SPD422-109) demonstrated that food delays the onset of absorption and slows the rate of absorption of anagrelide, with a 20% increase in mean anagrelide AUC and 29% decrease in mean 3-hydroxy-anagrelide AUC. This effect is not considered clinically important. In general, clinical pharmacology studies are conducted with fasted administration to minimize the potential confounding effect of administration with a meal on the interpretation of the study results. Therefore, the subjects are required to fast for approximately 10 hours prior to and until 4 hours following administration of anagrelide on the primary pharmacokinetic sampling days (Days 1 and 8). Omeprazole will be administered at 8:00 in the morning, prior to breakfast on Days 2-7.

3.1.2 Screening Period and Day -1

The maximum duration of the screening period is 28 days before the administration of the first dose of anagrelide on Day 1. At the initial visit to the clinical research center (CRC) the subject will first give informed consent. This will be the date that he/she enters the screening period. After giving consent, the subject's eligibility will be evaluated against the inclusion and exclusion criteria and they will undergo the procedures outlined in Table 1.

Following the screening visit, subjects who meet the protocol specific inclusion and exclusion criteria will return to the CRC on Day -1 to reconfirm eligibility criteria for participation in the study. Subjects who continue to meet the eligibility criteria will be admitted to the CRC on Day -1.

3.1.3 Treatment Period (Day 1-9)

Subjects will be admitted to the CRC after eligibility is confirmed on Day -1. Subjects will be confined at the CRC from Day -1 until after completion of the 24 hour post dose assessments on the morning of Day 9. Administration of study drug on Days 1-8 should be at the same time each morning for each subject during the study.

Study procedures, safety and pharmacokinetic assessments will be collected and reported at scheduled time points during the study. The timing of these assessments are described in Table 1, Table 2, and Table 3.

Day 1: Anagrelide 1 mg, (administered orally as two 0.5 mg capsules) will be administered to all study subjects at approximately 8:00 in the morning on Day 1. Subjects are required to fast for 10 hours prior to and until 4 hours following administration of anagrelide on Day 1.

Days 2-7: Omeprazole 40 mg will be administered orally QD on Days 2-7 to all subjects. Omeprazole will be administered at approximately 8:00 in the morning, prior to breakfast.

Day 8: Anagrelide 1 mg and omeprazole 40 mg will be administered concurrently to all subjects at approximately 8:00 in the morning on Day 8. Subjects are required to fast for 10 hours prior to and until 4 hours following administration of anagrelide and omeprazole on Day 8.

Day 9: Subjects will be discharged from the CRC on Day 9 following the completion of all study procedures and assessments.

Subjects who discontinue early from the study will complete the Early Termination assessments.

- Safety Assessments include but are not limited to assessment of adverse events, physical examinations, 12-lead ECG, vital signs (blood pressure and pulse) and safety laboratory tests (hematology, chemistry, and urinalysis).
- Pharmacokinetic assessments:
    - Omeprazole: plasma concentrations of omeprazole
    - Anagrelide: plasma concentrations of anagrelide, 3-hydroxy-anagrelide and RL603

3.1.4 Follow-up

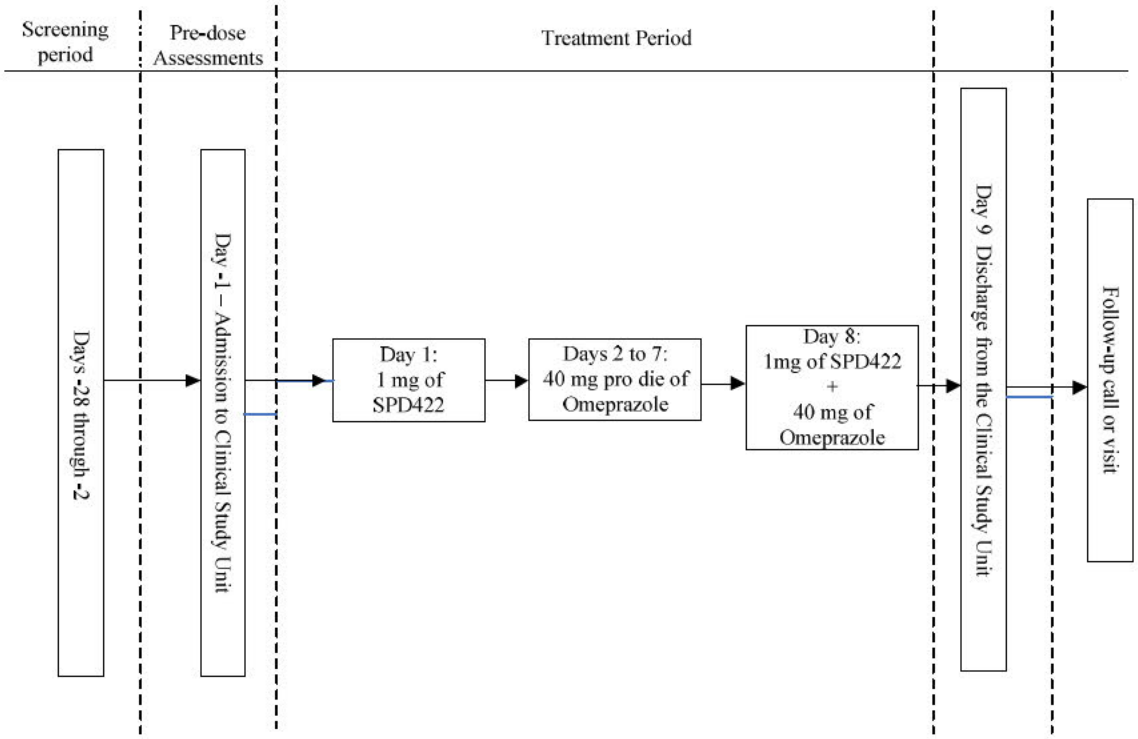
A follow up telephone call (or in-person follow-up contact at the investigator's discretion) will take place 7± 2 days following the subject's last dose of investigational product to collect information on any ongoing or new adverse events (AEs), serious adverse events (SAEs) or concomitant medications, as appropriate.

3.1.5 Primary and Secondary Endpoints

This study will evaluate the effect of omeprazole coadministration on the pharmacokinetic profile of anagrelide and its metabolites. The primary endpoints will be C_{max} and AUC of anagrelide and 3-hydroxyanagrelide compared between anagrelide administered alone and anagrelide administered after omperazole 40 mg QD for 7 days.

Safety and tolerability will be assessed throughout the study by routine recording of ECGs, vital signs, safety laboratory assessments, and AEs.

Figure 1: Study Design Flow Chart



- SPD422 will be administered under fasting conditions on Days 1 and 8
- Omeprazole will be administered prior to breakfast on Days 2-7 and under fasting conditions on Day 8
- PK assessments for anagrelide on Day 1 and Day 8, and for omeprazole on Day 8

3.2 Duration and Study Completion Definition

The subject's maximum duration of participation is expected to be approximately 46 days if the maximum screening, treatment and follow-up durations are used.

- Maximum planned duration of the screening period: 28 days
- Planned duration for the treatment period: 9 days
- Planned duration for the follow up: 7±2 days

The study will be completed in approximately 3 months.

The Study Completion Date is defined as the date the final subject, across all sites, completes their final protocol-defined assessment. Please note that this includes the follow-up visit or contact, whichever is later. The Study Completion Date is used to ascertain timing for study results posting and reporting.

3.3 Sites and Regions

This study will be conducted at a single center Clinical Research Center in the United States.

4. STUDY POPULATION

Each subject must participate in the informed consent process and provide written informed consent/assent before any procedures specified in the protocol are performed.

4.1 Inclusion Criteria

The subject will not be considered eligible for the study without meeting all of the criteria below.

Subjects cannot be enrolled before all inclusion criteria (including test results) are confirmed.

1. Has given, personally signed, and dated informed consent to participate in the study, in accordance with the International Conference on Harmonization Good Clinical Practice Guideline E6 (1996) and applicable regulations, before completing any study-related procedures.

2. Age 18-45 years inclusive at the time of consent. The date of signing informed consent is defined as the beginning of the Screening Period. This inclusion criterion will be assessed only at the Screening Visit.

3. Male, or non-pregnant, non-lactating female who agrees to comply with any applicable contraceptive requirements of the protocol or females of non-childbearing potential. A female of non-childbearing potential (defined as a female who is post-menopausal [amenorrhea for at least 12 consecutive months], has had a hysterectomy, bilateral tubal ligation, bilateral oophorectomy or bilateral salpingectomy.

4. Satisfactory medical assessment with no clinically significant or relevant abnormal findings as determined by medical/surgical history, physical examination, vital signs, 12-lead electrocardiogram, and clinical laboratory evaluation (hematology, biochemistry, thyroid function, and urinalysis) that are likely to interfere with the subject's participation or ability to complete the study as assessed by the investigator.

5. An understanding, ability, and willingness to fully comply with study procedures and restrictions.

6. Body mass index (BMI) between 18.5 and 30.0 kg/m² inclusive; assessed only at the screening visit.

7. Able to swallow (multiple capsules or tablets at 1 time or consecutively at 1 time) all investigational product.

8. Healthy as determined by the investigator on the basis of screening evaluations.

4.2 Exclusion Criteria

Subjects are excluded from the study if any of the following exclusion criteria are met at the Screening visit (Days -28 through Day -2) or on Day -1 through the morning of Day 1, just prior to dosing:

1. Current or recurrent disease or conditions (eg,, cardiovascular, renal, liver, gastrointestinal, malignancy or other conditions) that could affect the absorption, action, or disposition of either omeprazole or anagrelide or its metabolites, or could affect clinical assessments or clinical laboratory evaluations.
2. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to fully comply with the requirements of the study or complete the study, or any condition that presents undue risk from the investigational product or study procedures.
3. Significant illness, as judged by the investigator, within the 2 weeks of administration of the first dose of investigational product.
4. Use of any medication (including prescription, over-the-counter, herbal, multivitamin, oral contraceptives and other hormonal contraceptive treatments, or homeopathic preparations) within the 30 days prior to the first dose of study drug or during the study through Day 9 (occasional use of acetaminophen is allowed).
5. Treatment with any known hepatic and/or P450 enzyme-altering agents, including CYP1A2 inducers or inhibitors within 30 days prior to the first dose of investigational product. This includes(*):

Inhibition/Inducer Index	Medication
Strong inhibitor	ciprofloxacin, enoxacin, fluvoxamine, and zafirlukast
Moderate inhibitor	methoxsalen, mexiletine, and oral contraceptives
Moderate inducer	phenytoin, rifampin, ritonavir, smoking, teriflunomide
Inducer	lansoprazole

* FDA Draft Guidance for Industry: Drug interaction studies - study design, data analysis, implications for dosing, and labeling recommendations. 2012.

6. A history of any of the following medical conditions:
    - History of previous bone marrow suppression.
    - History of hypersensitivity to the investigational product.
    - History of adverse hematologic reaction, (such as neutropenia, thrombocytopenia, anemia) to any drug.

- History of symptomatic or clinically meaningful orthostatic hypotension or syncope, as assessed by the investigator.
- History of controlled or uncontrolled hypertension or a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg at the Screening Visit or Day -1.
- Subject has any history of seizure disorder.
- History or presence of known structural cardiac abnormalities, syncope, cardiac conduction problems (PR interval >220 ms, second or third-degree heart block, bundle branch block [except congenital right bundle branch block], or prolonged QTc interval) or exercise-related cardiac events.
- History of alcohol or other substance abuse within the last year.

7. A subject's alcohol consumption that fulfils one of the following: (Note: One alcohol unit=1 beer [12 oz]=1 wine [5 oz]=1 liquor [1.5 oz])
    - Has consumed alcohol within 2 days prior to the first dose of investigational product.
    - Male subjects who consume more than 3 units* of alcohol per day.
    - Female subjects who consume more than 2* units of alcohol per day.
8. Positive screening test results for alcohol, drugs of abuse, or pregnancy (females of childbearing potential only) at the Screening Visit or Day –1.
9. A positive human immunodeficiency virus (HIV) antibody screen, hepatitis B surface antigen (HBsAG) or hepatitis C virus antibody (HCV) screen.
10. Use of tobacco in any form (eg, smoking or chewing) or other nicotine-containing products in any form (eg, gum, patch) within 30 days prior to the first dose of investigational product and during the in-house stay at the CRC.
11. A positive urine cotinine test that is ≥50 ng/mL at either the Screening Visit or on Day -1.
12. Routine consumption of more than 2 units of caffeine per day or subjects who experience caffeine-withdrawal headaches or have a history of caffeine-withdrawal headaches. (One caffeine unit is contained in the following items: one 6-oz cup of coffee, two 12-oz cans of cola, one 12-oz cup of tea, and three 1-oz chocolate bars. Decaffeinated coffee, tea, or cola are not considered to contain caffeine.)
13. Consumption of grapefruit, Seville oranges, and/or products containing these items within 7 days prior to the first dose of investigational product.
14. Donation of blood or blood products (egg, plasma, or platelets) within 60 days prior to the first dose of investigational product.
15. Known or suspected intolerance or hypersensitivity to the investigational products (ie, anagrelide or omeprazole) or closely related compounds, or any of the stated ingredients.

16. Within 30 days prior to the first dose of investigational product:
    - Have used an investigational product (if elimination half-life is <6 days, otherwise 5 half-lives).
    - Have been enrolled in a clinical study (including vaccine studies) that, in the Investigator's opinion, may impact this Shire-sponsored study.
17. Prior screen failure, participation, or enrollment in this study.
18. History of sensitivity to heparin or heparin-induced thrombocytopenia.

4.3 Restrictions

1. Subjects should refrain from strenuous physical exercise 48 hours prior to admission to the CRC and during the in-house stay at the CRC.
2. Subjects should refrain from alcohol 48 hours prior to admission to the CRC and during the in-house stay at the CRC.
3. Subjects should refrain from foods or beverages containing caffeine/xanthine 48 hours prior to admission to the CRC and during the in-house stay at the CRC.
4. On Day 1 and Day 8 subjects will be required to fast for approximately 10 hours prior to administration of investigational product and continuing through 4 hours after administration. In addition, water intake will be restricted (except for water to administer the dose) starting 4 hours prior and continuing up to 2 hours after administration of the investigational product.
5. Subjects will be required to follow standardized meal schedules and eat the meals provided by the site while housed in the CRC. No outside food or beverages (including gum, mints, etc.) will be permitted. Menus will be identical for all subjects at the CRC. Copies of the menus will be provided to the sponsor for approval prior to the start of the study. While confined, the total daily nutritional composition should be approximately 50% carbohydrate, 35% fat, and 15% protein. The daily caloric intake per subject should not exceed approximately 3200 kcal.

4.4 Reproductive Potential

4.4.1 Female Contraception

Sexually active females of childbearing potential should be using an acceptable form of contraception. Females of childbearing potential must be advised to use acceptable contraceptives throughout the study period and for 30 days following the last dose of investigational product. Females of childbearing potential who are not currently sexually active must agree to use acceptable contraception, as defined below, if they become sexually active during the period of the study and 30 days following the last dose of investigational product.

Female subjects should be either:

- Postmenopausal (12 consecutive months of spontaneous amenorrhea and ≥age 51 years)

- Surgically sterile (having undergone one of the following surgical acts: hysterectomy, bilateral tubal ligation, bilateral oophorectomy or bilateral salpingectomy) and at least 6 weeks post-sterilization, or
- Females of childbearing potential with a negative urine and/or serum β-hCG pregnancy test at screening and at Day -1. Females of childbearing potential must agree to abstain from sexual activity that could result in pregnancy or agree to use acceptable methods of contraception.

Acceptable methods of contraception are:

- Intrauterine devices plus condoms
- Double-barrier methods (eg, condoms and diaphragms with spermicidal gel or foam)

4.4.2 Male Contraception

Not Applicable.

4.5 Discontinuation of Subjects

A subject may withdraw from the study at any time for any reason without prejudice to their future medical care by the physician or at the institution. The investigator or sponsor may withdraw the subject at any time (eg, in the interest of subject safety). The investigator is encouraged to discuss withdrawal of a subject from investigational product with the medical monitor when possible.

If investigational product is discontinued, regardless of the reason, the early withdrawal evaluations listed in Table 1 are to be performed as completely as possible. Whenever possible, all discontinued subjects should also undergo the protocol-specified follow-up. Comments (spontaneous or elicited) or complaints made by the subject must be recorded in the source documents. The reason for termination and date of stopping investigational product must be recorded in the case report form (CRF) and source documents.

Subjects who discontinue will not be replaced.

4.5.1 Reasons for Discontinuation

The reason for withdrawal must be determined by the investigator and recorded in the subject's medical record and on the CRF. If a subject is withdrawn for more than 1 reason, each reason should be documented in the source document and the most clinically relevant reason should be entered on the CRF.

Reasons for discontinuation include but are not limited to:

- Adverse event
- Protocol deviation
- Withdrawal by subject

- Lost to follow-up
- Other

4.5.2 Subjects "Lost to Follow-up" Prior to Last Scheduled Visit

A minimum of 3 documented attempts must be made to contact any subject lost to follow-up at any time point prior to the last scheduled contact (office visit or telephone contact). At least 1 of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that they return to the site for final safety evaluations and return any unused investigational product.

5. PRIOR AND CONCOMITANT TREATMENT

5.1 Prior Treatment

Prior treatment includes all treatment (including but not limited to herbal treatments, vitamins, non-pharmacological treatment such as psychotherapy, as appropriate) received within 30 days (or pharmacokinetic equivalent of 5 half-lives, whichever is longer) of the date of first dose of investigational product. Prior treatment information must be recorded on the appropriate CRF page.

5.2 Concomitant Treatment

Concomitant treatment refers to all treatment taken between the dates of the first dose of investigational product and the end of the follow-up period, inclusive. Concomitant treatment information must be recorded on the appropriate CRF page.

5.2.1 Permitted Treatment

Subjects should refrain from taking any medications during the course of the study. Any medication which is considered necessary for the subject's safety and well-being may be given at the discretion of the investigator. The administration of all medications (including investigational products) must be listed on the appropriate CRF page.

6. INVESTIGATIONAL PRODUCT

6.1 Identity of Investigational Product

The test product is commercial anagrelide, which will be provided as 0.5mg capsules. Additional information is provided in the SPD422 anagrelide hydrochloride investigator's brochure.

6.1.1 Blinding the Treatment Assignment

Not applicable.

6.1.2 Commercially Obtained Investigational Product

Commercially available omeprazole 40 mg will be sourced by the site or Clinical Research Center.

6.2 Administration of Investigational Product(s)

6.2.1 Allocation of Subjects to Treatment

This is an open-label, non-randomized study.

Screening numbers are assigned to all subjects as they consent to take part in the study. The screening number is assigned to subjects according to the sequence of presentation for study participation. This will be a 4-digit number starting at 0001.

A 4-digit subject number, starting at 1001, will be allocated immediately prior to dosing after eligibility has been determined. If a subject number is allocated incorrectly, the study monitor must be notified as soon as the error is discovered. Once a subject number has been assigned, that number must not be used again if, for example, a subject is withdrawn from the study. For enrolled subjects, the subject number will be the identifying number used throughout the CRF.

6.2.2 Dosing

After satisfying all screening and entry criteria, anagrelide and/or omeprazole will be administered to all subjects according to the following schedule:

Day 1: Anagrelide 1 mg, (administered orally as two 0.5 mg capsules) will be administered to all subjects at approximately 8:00 in the morning on Day 1. Subjects are required to fast for 10 hours prior to and until 4 hours following administration of anagrelide on Day 1.

Days 2-7: Omeprazole 40 mg (administered orally, QD) will be administered to all subjects on Days 2-7. Omeprazole will be administered at approximately 8:00 in the morning, prior to breakfast.

Day 8: Anagrelide 1 mg and omeprazole 40 mg will be administered concurrently to all subjects at approximately 8:00 in the morning on Day 8. Subjects are required to fast for 10 hours prior to and until 4 hours following administration of anagrelide and omeprazole on Day 8.

6.2.3 Unblinding the Treatment Assignment

Not Applicable.

6.3 Labeling, Packaging, Storage, and Handling

6.3.1 Labeling

Labels containing study information and pack identification are applied to the investigational product(s) container.

In addition to the commercial label, all investigational product is labeled with a minimum of the protocol number, batch number and/or packaging reference, directions for use, the statements "For clinical trial use only" and/or "CAUTION: New Drug – Limited by Federal (or US) Law to Investigational Use, and the sponsor's name and address.

Additional labels may, on a case-by-case basis, be applied to the investigational product in order to satisfy local or institutional requirements, but must not:

- Contradict the clinical study label
- Obscure the clinical study label
- Identify the study subject by name

Additional labels may not be added without the sponsor's prior full agreement.

6.3.2 Packaging

Investigational product, anagrelide 0.5 mg capsules will be provided in the manufacturer's original package. In addition to the commercial label, each bottle will have supplemental label that will contain but will not be limited to: protocol number, batch number and/or packaging reference, directions for use, the statements "For clinical trial use only" and/or "CAUTION: New Drug – Limited by Federal (or US) Law to Investigational Use, and the sponsor's name and address.

Changes to sponsor-supplied packaging prior to dosing may not occur without full agreement in advance by the sponsor.

6.3.3 Storage

The investigator has overall responsibility for ensuring that investigational product is stored in a secure, limited-access location. Limited responsibility may be delegated to the pharmacy or member of the study team, but this delegation must be documented. Investigational products are distributed by the pharmacy or nominated member of the study team.

Investigational product must be stored in accordance with labeled storage conditions. Temperature monitoring is required at the storage location to ensure that the investigational product is maintained within an established temperature range.

The investigator is responsible for ensuring that the temperature is monitored throughout the duration of the study and that records are maintained; the temperature should be monitored continuously by using either an in house system, a mechanical recording device such as a calibrated chart recorder, or by manual means, such that both minimum and maximum thermometric values over a specific time period can be recorded and retrieved as required. Such a device (ie, certified min/max thermometer) would require manual resetting upon each recording. The sponsor must be notified immediately upon discovery of any excursion from the established range. Temperature excursions will require site investigation as to cause and remediation. The sponsor will determine the ultimate impact of excursions on the investigational product and will provide supportive documentation as necessary. Under no circumstances should the product be dispensed to subjects until the impact has been determined and the product is deemed appropriate for use by the sponsor.

The sponsor should be notified immediately if there are any changes to the storage area of the investigational product that could affect the integrity of the product(s), eg, fumigation of a storage room.

6.4 Drug Accountability

Investigators will be provided with sufficient amounts of the investigational product to carry out this protocol for the agreed-upon number of subjects. The investigator or designee will acknowledge receipt of the investigational product, documenting shipment content and condition. Accurate records of all investigational product dispensed, used, returned, and/or destroyed must be maintained as detailed further in this section.

The investigator has overall responsibility for administering/dispensing investigational product. Where permissible, tasks may be delegated to a qualified designee (eg, a pharmacist) who is adequately trained in the protocol and who works under the direct supervision of the investigator. This delegation must be documented in the applicable study delegation of authority form.

The investigator or his/her designee (as documented by the investigator in the applicable study delegation of authority form) will administer the investigational product only to subjects included in this study following the procedures set out in the study protocol. Each subject will be given only the investigational product carrying his/her treatment assignment. All administered medication will be documented on the CRFs and/or other investigational product record.

No investigational product stock or returned inventory from a Shire-sponsored study may be removed from the site where originally shipped without prior knowledge and consent by the sponsor. If such transfer is authorized by the sponsor, all applicable local, state, and national laws must be adhered to for the transfer.

At the end of the study, or as instructed by the sponsor, all unused stock, and empty/used investigational product packaging are to be sent to a nominated contractor on behalf of the sponsor. Investigational products being returned to the sponsor's designated contractors must be counted and verified by clinical site personnel and the sponsor (or designated contract research organization [CRO]). For unused supplies where the original supplied tamper-evident feature is verified as intact, the tamper-evident feature must not be broken and the labeled amount is to be documented in lieu of counting.

Shipment return forms, when used, must be signed prior to shipment from the site. Validated electronic return systems (ie, interactive response technology [IRT]) do not require a shipment form. Returned investigational products must be packed in a tamper-evident manner to ensure product integrity. Contact the sponsor for authorization to return any investigational product prior to shipment. Shipment of all returned investigational product must comply with local, state, and national laws.

Based on entries in the site drug accountability forms, it must be possible to reconcile investigational products delivered with those used and returned. All investigational products must be accounted for and all discrepancies investigated and documented to the sponsor's satisfaction.

6.5 Subject Compliance

Compliance must be assessed by observation of dosing by the investigator or designee. In addition, the CRC personnel should perform a hand-and-mouth check (mouth check is only required for oral dosing) of the subject to assure the investigational product has been ingested. The investigator/nominated person will record details on the drug accountability log(s) and/or source documents. In addition, details of the dosing time (time, date, dose level) will be captured in the appropriate CRF.

6.6 Retention of Bioavailability and Bioequivalence Testing Samples

Not Applicable.

7. STUDY PROCEDURES

7.1 Study Schedule

See Table 1, Table 2, and Table 3 for study procedures.

The following "priority order" will be in effect when more than 1 procedure or assessment is required at a particular time point.

- Spontaneous or solicited AE reporting
- Electrocardiogram
- Vital signs
- Pharmacokinetic blood sampling
- Clinical laboratory tests
- Physical examination

NOTE: Blood sampling for pharmacokinetic evaluation must be performed at the precise protocol-scheduled time. Actual sampling time(s) must be accurately recorded in the source document and appropriate CRF.

7.1.1 Screening Period

Screening procedures must be completed within 28 days prior to receiving the first dose of investigational product. All screening assessments and procedures are to be performed by the principal investigator or a qualified designee. See Table 1 for a complete list of screening procedures to be performed.

Written, signed, and dated informed consent from each subject prior to the performance of any study-related procedures must be obtained by the principal investigator or a designee. A copy of the signed informed consent form must be given to the subject for their records.

7.1.1.1 Screening Failure

A screen failure is a subject who has given informed consent and failed to meet the inclusion and/or met at least 1 of the exclusion criteria and has not been enrolled or administered investigational product(s).

For purposes of data collection, all subjects who give consent to the study but are not enrolled will be reported as screen failures even if they were otherwise fully eligible for the study (for example, alternates/reserve subjects).

7.1.1.2 Rescreening of Subjects

Subjects who fail to meet all inclusion/exclusion criteria will not be permitted to be rescreened for the study at any point.

Eligible subjects who meet all inclusion/exclusion criteria but are unable to participate in the study due to scheduling conflicts/timing may be rescreened based on investigator discretion and sponsor approval should their availability to participate fall outside the screening window. In these cases, a new screening number must be assigned for each subject who is rescreened and a new informed consent form must be signed.

7.1.2 Treatment Period

7.1.2.1 Admission to the Clinical Research Center (Day -1)

Following the screening visit, eligible subjects will return to the CRC on Day -1 of the study. See Table 1 for a list of procedures and assessments to be completed upon admission to the CRC. Subjects who successfully complete the pre-admission procedures and assessments will be admitted to the CRC on Day -1 and assigned a subject number on Day 1 as described in Section 6.2.1. Eligible subjects will be confined to the CRC from the morning of Day -1 until completion of all procedures and assessments on Day 9.

7.1.2.2 Treatment Period (Day 1 through Day 9)

Subjects will be admitted to the CRC after eligibility is confirmed on Day -1. Subjects will be confined at the CRC from Day -1 until after completion of the 24 hour post dose assessments on the morning of Day 9. Administration of study drug on Days 1-8 should be at the same time each morning for each subject during the study.

Study procedures, safety and pharmacokinetic assessments will be collected and reported at scheduled time points during the study. Subjects will undergo assessments and procedures as specified by visit in Table 1, Table 2, and Table 3.

- Day 1: Anagrelide 1 mg, (administered orally as two 0.5 mg capsules) will be administered to study subjects at approximately 8:00 in the morning on Day 1. Subjects are required to fast for 10 hours prior to and until 4 hours following administration of anagrelide on Day 1. The procedures, assessments and time points for Day 1 are presented in Table 2.

- Days 2-7: Omeprazole 40 mg will be administered orally QD on Days 2-7. Omeprazole will be administered at approximately 8:00 in the morning, prior to breakfast.

- Day 8: Anagrelide 1 mg and omeprazole 40 mg will be administered concurrently in a fasted state (10 hours prior to and until 4 hours following administration of anagrelide and omeprazole) at approximately 8:00 in the morning on Day 8. The procedures, assessments and time points for Day 8 are presented in Table 3.

- Day 9: Subjects will be discharged from the CRC on Day 9 following the completion of all study procedures and assessments.

Subjects who discontinue early from the study will complete the Early Termination assessments in Table 1.

7.1.3 Follow-up

A follow up telephone call (or in-person follow-up contact at the investigator's discretion) will take place 7 ±2 days following the subject's last dose of investigational product to collect information on any ongoing or new AEs, SAEs or concomitant medications, as appropriate. All AEs and SAEs that are not resolved at the time of this contact will be followed to closure (see Section 8.1).

7.1.4 Additional Care of Subjects after the Study

No aftercare is planned for this study.

7.2 Study Evaluations and Procedures

7.2.1 Demographic and Other Baseline Characteristics

Demographic characteristics such as age, sex, weight, height and body mass index will be collected according to Table 1.

7.2.2 Safety

The name and address of each third-party vendor (eg, clinical laboratory) used in this study will be maintained in the investigator's and sponsor's files.

Actual safety assessment times will be monitored and recorded. The sponsor's expectation is that the investigator will ensure that every effort is made to perform all assessments at the precise protocol-scheduled time. Any safety assessment that deviates from the scheduled assessment time set forth in the protocol by more than ±15 minutes will be considered a protocol deviation.

7.2.2.1 Medical and Medication History

A complete medical and medication history, as well as demographic information, will be performed at the screening visit/time points described in Table 1 by a qualified licensed physician, physician's assistant, or a nurse practitioner. The medical history will be reviewed and recorded, including:

- Date of birth
- Sex
- Race and ethnicity
- Recent ingestion of medication (30 days prior to entering the screening period)
- History of respiratory, cardiovascular, renal, gastrointestinal, hepatic, endocrine, hematological, neurological, psychiatric, and other diseases
- Smoking habits

7.2.2.2 Physical Examination (Including Height and Weight)

A complete physical examination will be performed at the time points described in Table 1, Table 2, and Table 3 by a qualified licensed physician, physician's assistant, or nurse practitioner.

The physical examination will include a review of the following body systems:

- General appearance
- Skin
- Head, eyes, ears, nose, and throat
- Spine/neck/thyroid
- Musculoskeletal
- Respiratory
- Cardiovascular
- Neurological
- Abdomen (including liver and kidneys)

Abnormalities identified at the screening visit will be documented in the subject's source documents and on the medical history CRF. Changes after the screening visit that are considered clinically significant will be captured as AEs on the AE CRF page, as deemed by the investigator.

7.2.2.3 Adverse Event Collection

At each study visit, subjects will be questioned in a general way to ascertain if AEs have occurred since the previous visit (eg, "Have you had any health problems since your last visit?"). Adverse events are collected from the time informed consent is signed. (Please refer to Section 8, Adverse and Serious Adverse Events Assessment.)

7.2.2.4 Vital Signs

Blood Pressure and Pulse Rate

Blood pressure and pulse rate will be measured at times specified in Table 1, Table 2, and Table 3 of this protocol. Additional blood pressure and pulse rate measurements may be performed, as determined by the investigator, in order to ensure appropriate monitoring of subject safety and accurate recording of vital sign measurements. Any changes from baseline which are deemed clinically significant by the investigator are to be recorded as an AE.

The same method for obtaining blood pressure measurement (auscultatory or oscillometric) should be used throughout the study for all subjects (and documented). In addition, the conditions of vital sign measurements should be controlled and as consistent as possible during the study, in order to minimize external variability of the readings.

It is advised that measurements be collected at a comfortable room temperature with little to no background noise, using the same (appropriately sized) cuff placed at the same location of the same arm during the study. The bladder deflation rate should be deflated (calibrated for oscillometric method or manually by auscultatory method) at a rate of 2-3 mmHg/s (and the first and last audible sounds recorded as systolic and diastolic pressure) after at least 5 minutes of rest in the assumed position.

The cuff should have a bladder length that is 80% and a width that is at least 40% of arm circumference (a length-to-width ratio of 2:1).

The subject should be asked to remove all clothing that covers the location of cuff placement. The subject should be instructed to relax as much as possible for at least 5 minutes prior to collection. The subject should remain quiet during this time and through the measurement.

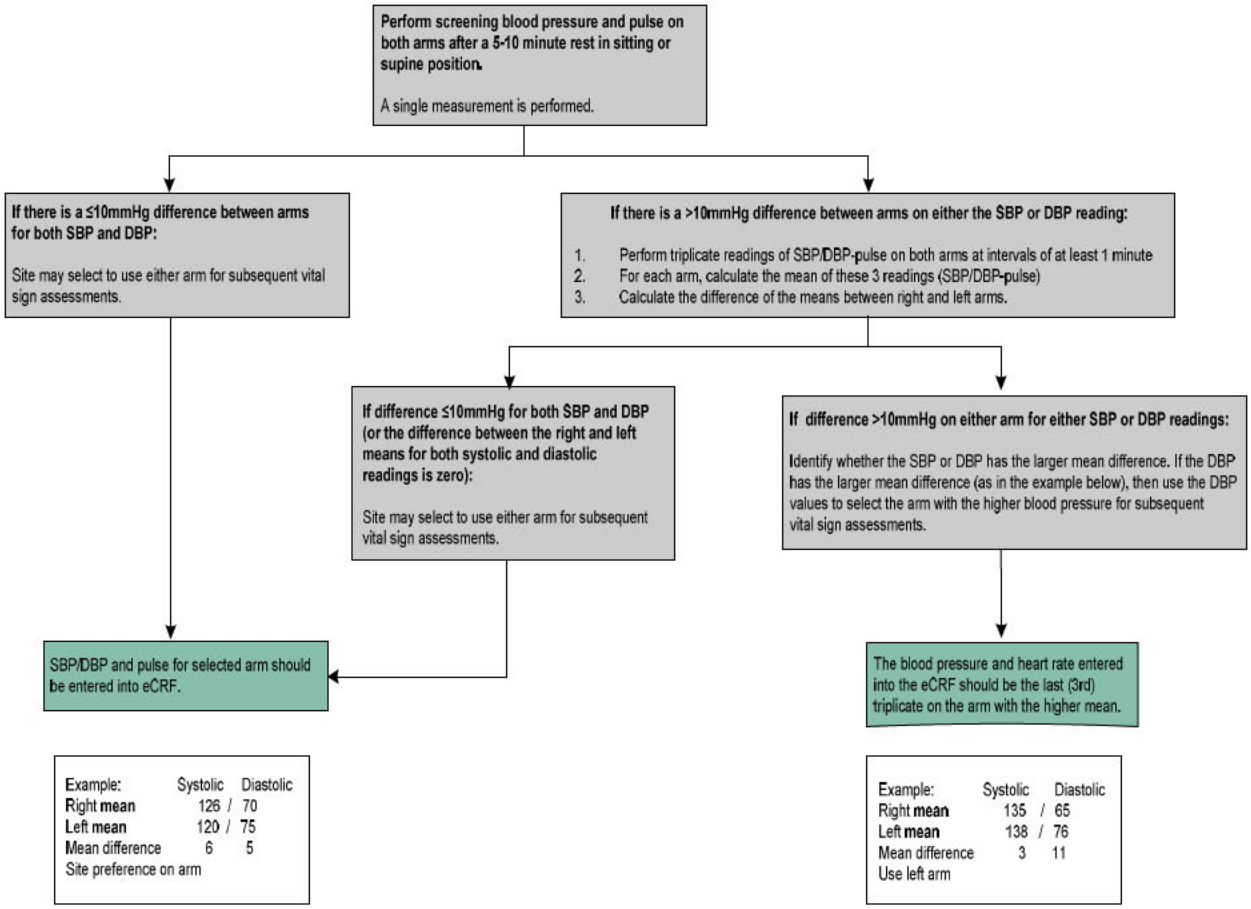
The subject should be lying comfortably, with the legs uncrossed. The arm should be supported with a pillow, such that the middle of the cuff on the upper arm is at the level of the right atrium (approximately halfway between the bed and the level of the sternum).One reading (supine systolic blood pressure/diastolic blood pressure-heart rate) should be taken at each timepoint.

The use of automated devices for measuring pulse rate is acceptable, although, when done manually, pulse rate will be measured in the brachial/radial artery for at least 30 seconds. When the timing of these measurements coincides with a blood collection, blood pressure and pulse rate should be obtained prior to the nominal time of the blood collection.

At the screening visit, blood pressure should be compared between both arms. When there is a consistent inter-arm difference confirmed over 3 consecutive measurements (>10 mmHg), the arm with the higher blood pressure should be used for inclusion at screening and the last measurement recorded in the CRF. The same (right or left) arm with the higher blood pressure will be used throughout the study.

For details on blood pressure and pulse procedures for healthy subjects, see Figure 2.

Figure 2: Procedures for Screening Vital Signs (Blood Pressure – Pulse) – Healthy Subjects Only



DBP=diastolic blood pressure; eCRF=electronic case report form; SBP=systolic blood pressure

7.2.2.5 Clinical Laboratory Evaluations

All clinical laboratory assays will be performed according to the laboratory's normal procedures. Reference ranges are to be supplied by the laboratory and will be used to assess the clinical laboratory data for clinical significance and out-of-range pathological changes. The investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant. Abnormal clinical laboratory values, which are unexpected or not explained by the subject's clinical condition, may, at the discretion of the investigator or sponsor, be repeated as soon as possible until confirmed, explained, or resolved.

The following clinical laboratory assessments will be performed:

Biochemistry

Blood samples (8.5 mL) for serum biochemistry will be collected into a gel separator tube at the time points described in Table 1, Table 2, and Table 3. The following parameters will be assessed:

Sodium	Phosphorus	β-hCG [a]
Potassium	Total protein	
Glucose	Total CO_2 (Bicarbonate)	
Blood urea nitrogen	Albumin	
Creatinine	Aspartate transaminase	
Calcium	Alanine transaminase	
Chloride	Gamma glutamyl transferase	
Thyroid stimulating hormone (TSH)	Alkaline phosphatase	
Thyroxine (T4 total)	Total bilirubin	
Triiodothyronine (T3)	Uric acid	

[a]β-hCG=beta-human chorionic gonadotropin. Females only.

Hematology

Blood samples (4 mL) for hematology will be collected into a ethylenediaminetetraacetic acid tube at the time points described in Table 1, Table 2, and Table 3. The following parameters will be assessed:

Hemoglobin	Total neutrophils (absolute)
Hematocrit	Eosinophils (absolute)
Red blood cells	Monocytes (absolute)
Platelet count	Basophils (absolute)
White blood cell count; total and differential	Lymphocytes (absolute)

Urinalysis

A urine sample for urinalysis will be collected at the time points described in Table 1, Table 2, and Table 3. The following parameters will be assessed:

pH	Blood	Nitrites
Glucose	Ketones	Leukocyte esterase
Protein	Bilirubin	Specific gravity

Microscopic examination will be conducted if protein and/or blood is/are detected during urinalysis. At a minimum the microscopic examination will consist of red blood cells, white blood cells, casts, and bacteria.

7.2.2.6 Pregnancy Test

For all female subjects (regardless of reproductive potential status), a serum pregnancy test will be performed at the visits specified in Table 1; or if pregnancy is suspected; or on withdrawal of the subject from the study.

7.2.2.7 Drug and Alcohol Screen

A urine screen for drugs of abuse and alcohol will be performed at the time points described in Table 1. Additional drug and alcohol screens may be performed at the investigator's discretion.

Urine samples are to be tested for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiate metabolite, and phencyclidine.

Results of urine drug and alcohol screens will be reviewed and verified by the study monitor, but will not be collected in the CRF database.

Any positive result for drugs of abuse or alcohol at screening or on Day -1 will exclude the subject from further participation in the study.

7.2.2.8 Serology Screen

At the screening visit, a blood sample of approximately 8.5 mL will be drawn into a serum separator tube to test for the presence of HIV, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) antibody.

The test results must be confirmed negative prior to enrollment in the study. If a test result is positive, the subject will be excluded from entering the study. Results of the virology screen will be reviewed and verified by the study monitor, but will not be collected in the CRF database.

7.2.2.9 Electrocardiogram

Twelve-lead ECGs will be performed at the times specified in Table 1, Table 2, and Table 3 using the standard methods/equipment at the CRC.

The following parameters will be recorded on the appropriate CRF page: heart rate, PR, RR, QRS, and QT intervals. The QTcB and QTcF will be derived from the data in the database. The investigator's assessment of the ECG tracing as normal or abnormal must be documented, and if abnormal, his/her determination of whether the abnormality is clinically significant or not will be documented on the tracing and recorded in the CRF.

The subject should be asked to remove all clothing that covers the location of lead placement. The subject must be resting in the supine position for at least 5 minutes prior to collecting the ECG.

In some cases, it may be appropriate to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality. It is important that leads are placed in the same positions each time in order to achieve precise ECG recordings.

One complete recording, including a 10-second rhythm strip, should be taken at each time point. It should be immediately assessed as a valid recording and if not valid, it should be repeated. Invalid recordings will not be entered in the CRF. The ECG collected predose on Day 1 will serve as the subject's baseline ECG.

7.2.3 Pharmacokinetic Procedures

The name and address of the bioanalytical laboratory(ies) for this study will be maintained in the investigator's files at the/each site and in the Trial Master File at the sponsor.

Actual pharmacokinetic blood sample collection times versus time of dosing will be monitored. The sponsor's expectation is that the investigator will ensure that every effort is made to collect all pharmacokinetic blood samples at the precise protocol scheduled time. Pharmacokinetic blood collection must not deviate from the nominal collection time set forth in the protocol by more than ±5 minutes from samples drawn within 4 hours post dose or by more than ±15 minutes for samples drawn beyond 4 hours post dose. Samples drawn outside these parameters will be considered a protocol deviation.

7.2.3.1 Blood Sample Collection and Handling Procedures

Blood samples will be collected at the time specified in Table 1, Table 2, and Table 3 to measure plasma concentrations of anagrelide, its active metabolite, BCH24426 (3-hydroxy-anagrelide), RL603, and omeprazole. Other potential metabolites may also be determined as appropriate.

A full description of the PK blood collection, handling, storage, and shipping can be found in the laboratory manual.

Plasma sample tubes for bioanalysis must be freezer-safe and identified with freezer-safe labels provided by the CRC. The labels will contain the following information:

- Study number (SPD422-113)
- Subject identifier
- Analyte (anagrelide or omeprazole)
- Nominal day (Day 1, Day 6…)
- Nominal time
- Matrix identifier (plasma)
- Split (primary or backup or aliquot 1 or aliquot 2)

7.2.3.2 Shipment of Pharmacokinetic Samples

All pharmacokinetic samples should be placed in a cryo-box, double-bagged to contain leaks and packed with a sufficient quantity of dry ice to ensure that they remain frozen for at least 72 hours to allow for delays in shipment. All applicable shipping regulations must be followed. Shipments should be scheduled so that no samples arrive on the weekend and should be shipped Monday-Wednesday only. Samples should be transported to ensure that they arrive at the bioanalytical laboratory between the hours of 9:00 AM and 4:00 PM. The recipient and primary Shire contact must be notified by telephone or e-mail when the samples are shipped and they must be provided with the shipment tracking number.

Full directions for shipment of all PK samples, (along with the corresponding documentation) can be found in the laboratory manual provided under separate cover.

Pharmacokinetic samples will be stored nominally at -80°C for anagrelide and -20°C for omeprazole at YBS prior to and after analysis until their disposal is authorized by Shire.

7.2.3.3 Plasma Drug Assay Methodology

Plasma sample analysis will be performed according to the relevant Standard Operating Procedures at the contract bioanalytical lab.

Plasma concentrations will be measured using the most current validated bioanalytical methods. In addition, selected plasma samples may be used to investigate incurred sample reproducibility (full details will be described in the bioanalytical study plan). The presence of other metabolites or artifacts may be monitored or quantified as appropriate. Raw data will be stored in the archive of the designated bioanalytical contract laboratory.

Table 4: Volume of Blood to Be Drawn from Each Subject

Assessment		Sample Volume (mL)	Number of Samples	Total Volume (mL)
Anagrelide Pharmacokinetic samples[a]		6[a]	20	120
Omeprazole Pharmacokinetic samples		2	13	26
HBsAg, HIV, HCV		8.5	1	8.5
Safety	Biochemistry and β-hCG[b]	8.5	5	42.5
	Hematology	4	5	20
Thyroid function		5	1	5
Total mL				222

β-hCG=beta-human chorionic gonadotropin; HBsAg=hepatitis B surface antigen; HCV=hepatitis C virus; HIV=human immunodeficiency virus

[a] If a catheter is used, the first mL is to be discarded; then take 4 mL into appropriate tube for pharmacokinetic sample. A total of 5 mL of blood drawn has been used in determination of sample volume.

[b] β-hCG testing for females only.

During this study, it is expected that approximately 222 mLs of blood will be drawn from all subjects, regardless of sex.

Note: The amount of blood to be drawn for each assessment is an estimate. The amount of blood to be drawn may vary according to the instructions provided by the manufacturer or laboratory for an individual assessment; however, the total volume drawn over the course of the study should be approximately 222 mLs. When more than 1 blood assessment is to be done at the time point/period, if they require the same type of tube, the assessments may be combined.

8. ADVERSE AND SERIOUS ADVERSE EVENTS ASSESSMENT

8.1 Definition of Adverse Events, Period of Observation, Recording of Adverse Events

An **AE** is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (International Conference on Harmonisation [ICH] Guidance E2A 1995).

All AEs are collected from the time the informed consent is signed until the defined follow-up period stated in Section 7.1.3. This includes events occurring during the screening phase of the study, regardless of whether or not investigational product is administered. Where possible, a diagnosis rather than a list of symptoms should be recorded. If a diagnosis has not been made, then each symptom should be listed individually. All AEs should be captured on the appropriate AE pages in the CRF and in source documents. In addition to untoward AEs, unexpected benefits outside the investigational product indication should also be captured on the AE CRF.

All AEs must be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to normal), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.

8.1.1 Severity Categorization

The severity of AEs must be recorded during the course of the event including the start and stop dates for each change in severity. An event that changes in severity should be captured as a new event. Worsening of pre-treatment events, after initiation of investigational product, must be recorded as new AEs (for example, if a subject experiences mild intermittent dyspepsia prior to dosing of investigational product, but the dyspepsia becomes severe and more frequent after first dose of investigational product has been administered, a new AE of severe dyspepsia [with the appropriate date of onset] is recorded on the appropriate CRF).

The medical assessment of severity is determined by using the following definitions:

Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

8.1.2 Relationship Categorization

A physician/investigator must make the assessment of relationship to investigational product for each AE. The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the investigational product. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related". Otherwise, if there is any valid reason, even if undetermined or untested, for suspecting a possible cause-and-effect relationship between the investigational product and the occurrence of the AE, then the AE should be considered "related". The causality assessment must be documented in the source document.

The following additional guidance may be helpful:

Term	Relationship Definition
Related	The temporal relationship between the event and the administration of the investigational product is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.
Not Related	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the investigational product and the event.

8.1.3 Outcome Categorization

The outcome of AEs must be recorded during the course of the study on the CRF. Outcomes are as follows:

- Fatal
- Not Recovered/Not Resolved
- Recovered/Resolved
- Recovered/Resolved With Sequelae
- Recovering/Resolving
- Unknown

8.1.4 Clinical Laboratory and Other Safety Evaluations

A change in the value of a clinical laboratory, vital sign, or ECG assessment can represent an AE if the change is clinically relevant or if, during treatment with the investigational product, a shift of a parameter is observed from a normal value to an abnormal value, or a further worsening of an already abnormal value. When evaluating such changes, the extent of deviation from the reference range, the duration until return to the reference range, either while continuing treatment or after the end of treatment with the investigational product, and the range of variation of the respective parameter within its reference range, must be taken into consideration.

If, at the end of the treatment phase, there are abnormal clinical laboratory, vital sign, or ECG values which were not present at the pretreatment value observed closest to the start of study treatment, further investigations should be performed until the values return to within the reference range or until a plausible explanation (eg, concomitant disease) is found for the abnormal values.

The investigator should decide, based on the above criteria and the clinical condition of a subject, whether a change in a clinical laboratory, vital sign, or ECG parameter is clinically significant and therefore represents an AE.

8.1.5 Pregnancy

All pregnancies are to be reported from the time informed consent is signed until the defined follow-up period stated in Section 7.1.3.

Any report of pregnancy for any female study participant must be reported within 24 hours to the Shire Global Safety Department using the Shire Investigational and Marketed Products Pregnancy Report Form. A copy of the Shire Investigational and Marketed Products Pregnancy Report Form (and any applicable follow-up reports) must also be sent to the CRO/Shire medical monitor using the details specified in the emergency contact information section of the protocol. The pregnant female study participant must be withdrawn from the study.

Every effort should be made to gather information regarding the pregnancy outcome and condition of the infant. It is the responsibility of the investigator to obtain this information within 30 calendar days after the initial notification and approximately 30 calendar days post partum.

Pregnancy complications such as spontaneous abortion/miscarriage or congenital abnormality are considered SAEs and must be reported using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form. Note: An elective abortion is not considered an SAE.

In addition to the above, if the investigator determines that the pregnancy meets serious criteria, it must be reported as an SAE using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form as well as the Shire Investigational and Marketed Products Pregnancy Report Form. The test date of the first positive serum/urine β-hCG test or ultrasound result will determine the pregnancy onset date.

8.1.6 Abuse, Misuse, Overdose, and Medication Error

Abuse, misuse, overdose, or medication error (as defined below) must be reported to the sponsor according to the SAE reporting procedure whether or not they result in an AE/SAE as described in Section 8.2. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors unless these result in an SAE.

The categories below are not mutually exclusive; the event can meet more than 1 category.

- **Abuse** – Persistent or sporadic intentional intake of investigational product when used for a non-medical purpose (eg, to alter one's state of consciousness or get high) in a manner that may be detrimental to the individual and/or society

- **Misuse** – Intentional use of investigational product other than as directed or indicated at any dose (Note: this includes a situation where the investigational product is not used as directed at the dose prescribed by the protocol)

- **Overdose** – Intentional or unintentional intake of a dose of investigational product higher than the protocol-prescribed dose

- **Medication Error** – An error made in prescribing, dispensing, administration, and/or use of an investigational product. For studies, medication errors are reportable to the sponsor only as defined below.

Medication errors should be collected/reported for all products under investigation.

The administration and/or use of an expired investigational product should be considered as a reportable medication error.

8.2 Serious Adverse Event Procedures

8.2.1 Reference Safety Information

The reference for safety information for this study is the investigator's brochure which the sponsor has provided under separate cover to all investigators.

The reference for safety information for omeprazole (PRILOSEC®) is the FDA-approved package insert which the sponsor has provided under separate cover to all investigators

8.2.2 Reporting Procedures

All initial and follow-up SAE reports must be reported by the investigator to the Shire Global Safety Department and the CRO/Shire medical monitor within 24 hours of the first awareness of the event. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors (see Section 8.1.6) unless they result in an SAE.

The investigator must complete, sign, and date the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form and verify the accuracy of the information recorded on the form with the corresponding source documents (Note: Source documents are not to be sent unless requested) and fax or e-mail the form to the Shire Global Safety Department. A copy of the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form (and any applicable follow-up reports) must also be sent to the CRO/Shire medical monitor using the details specified in the emergency contact information section of the protocol.

8.2.3 Serious Adverse Event Definition

A ***serious adverse event*** **(SAE)** is any untoward medical occurrence (whether considered to be related to investigational product or not) that at any dose:

- Results in death
- Is life-threatening. Note: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization. Note: Hospitalizations that are the result of elective or previously scheduled surgery for pre-existing conditions and have not worsened after initiation of treatment should not be classified as SAEs. For example, an admission for a previously scheduled ventral hernia repair would not be classified as an SAE; however, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meet(s) serious criteria must be reported as SAE(s).
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Is an important medical event. Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization; or the development of drug dependency or drug abuse.

8.2.4 Serious Adverse Event Collection Time Frame

All SAEs (regardless of relationship to study) are collected from the time the subject signs the informed consent until the defined follow-up period stated in Section 7.1.3 and must be reported to the Shire Global Safety Department and the CRO/Shire medical monitor within 24 hours of the first awareness of the event.

In addition, any SAE(s) considered "related" to the investigational product and discovered by the investigator at any interval after the study has completed must be reported to the Shire Global Safety Department within 24 hours of the first awareness of the event.

8.2.5 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalizations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms experienced by the subject after signing the informed consent form, or leading up to the onset date of the SAE, or following the resolution date of the SAE, must be recorded as an AE, if appropriate.

8.2.6 Fatal Outcome

Any SAE that results in the subject's death (ie, the SAE was noted as the primary cause of death) must have fatal checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at the time of death that did not contribute to the subject's death, the outcome should be considered not resolved, without a resolution date recorded.

For any SAE that results in the subject's death or any ongoing events at the time of death, unless another investigational product action was previously taken (eg, drug interrupted, reduced, withdrawn), the action taken with the investigational product should be recorded as "dose not changed" or "not applicable" (if the subject never received investigational product). The investigational product action of withdrawn should not be selected solely as a result of the subject's death.

8.2.7 Regulatory Agency, Institutional Review Board, Ethics Committee, and Site Reporting

The sponsor is responsible for notifying the relevant regulatory authorities of related, unexpected SAEs.

In addition the sponsor is responsible for notifying active sites of all related, unexpected SAEs occurring during all interventional studies across the anagrelide program.

The investigator is responsible for notifying the local IRB, local EC, or the relevant local regulatory authority of all SAEs that occur at his or her site as required.

9. DATA MANAGEMENT AND STATISTICAL METHODS

9.1 Data Collection

The investigators' authorized site personnel must enter the information required by the protocol on the CRF. A study monitor will visit each site in accordance with the monitoring plan and review the CRF data against the source data for completeness and accuracy. Discrepancies between source data and data entered on the CRF will be addressed by qualified site personnel. When a data discrepancy warrants correction, the correction will be made by authorized site personnel. Data collection procedures will be discussed with the site at the site initiation visit and/or at the investigator's meeting. Once a subject is randomized, it is expected that site personnel will complete the CRF entry within approximately 3 business days of the subject's visit.

9.2 Clinical Data Management

Data are to be entered into a clinical database as specified in the CRO's data management plan. Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections are documented in an auditable manner.

9.3 Data Handling Considerations

Not applicable.

9.4 Statistical Analysis Process

The study will be analyzed by the sponsor or its agent.

The statistical analysis plan (SAP) will provide the statistical methods and definitions for the analysis of the pharmacokinetic, pharmacodynamic, and safety data, as well as describe the approaches to be taken for summarizing other study information such as subject disposition, demographics and baseline characteristics, investigational product exposure, and prior and concomitant medications. The SAP will also include a description of how missing, unused and spurious data will be addressed.

To preserve the integrity of the statistical analysis and study conclusions, the SAP will be finalized prior to database lock.

All statistical analyses will be performed using SAS® (SAS Institute, Cary, NC 27513) version 9.4 or higher.

9.5 Planned Interim Analysis, Adaptive Design, and Data Monitoring Committee

There is no planned interim analysis, adaptive design, or Data Monitoring Committee in this study.

9.6 Sample Size Calculation and Power Considerations

Approximately 20 subjects will be enrolled. Subjects who discontinue will not be replaced.

Sample size estimation is to achieve a desired precision of the effect of interest, targeting the width of the 90% confidence interval.

From a previous crossover study (SPD422-110), the within-subject coefficient variation in anagrelide log-transformed AUC was estimated to be 0.132. Allowing for a slightly larger within-subject CV of 0.15, a sample size of 11 subjects is required to estimate the mean difference between anagrelide + omeprazole and anagrelide alone in the log-transformed AUC with an error margin of at most ±0.2231 at 90% confidence with a probability of 90%. Thus, if the true geometric mean treatment ratio is 0.6, the lower and upper 90% confidence bounds will be estimated to be within 0.48 and 0.75 (exp[ln(0.6) – 0.2231] and exp[ln(0.6) + 0.2231]) with 90% probability. Therefore, with an overall sample size of 20 subjects, anticipated 13 to 14 CYP2C19 EM subjects and 6 to 7 CYP2C19 IM/PM subjects, this statistical power will be met for the overall population and the CYP2C19 EM population. The confidence bounds for the smaller population of CYP2C19 IM/PM subjects will be slightly wider.

9.7 Study Population

The enrolled set consists of all subjects for whom an enrollment number has been assigned. Usually, these are the subjects who meet the study inclusion/exclusion criteria and sign the informed consent form.

The safety set consists of all subjects who have taken at least 1 dose of investigational product (anagrelide or omeprazole) and have at least 1 post-dose safety assessment.

The pharmacokinetic set consists of subjects who receive at least 1 dose of study drug and has at least 1 measureable post-dose plasma concentration. The PK analyses will be based on this population.

9.8 Pharmacokinetic and Pharmacodynamic Analyses

9.8.1 Pharmacokinetic Analysis

All the pharmacokinetic analyses will be performed using the pharmacokinetic set.

Pharmacokinetic parameters will be determined from the plasma concentration-time data for anagrelide and its metabolites and omeprazole by non-compartmental analysis and all calculations will be based on the actual time since dose. The pharmacokinetic parameters will include, but not be limited to:

$AUC_{0-\infty}$	Area under the curve extrapolated to infinity, calculated using the observed value of the last non-zero concentration (anagrelide and metabolites)
AUC_{0-t}	Area under the curve from time 0 to the last time point of sample collection (anagrelide and metabolites)

$AUC_{0\text{-}tau}$	Area under the concentration-time curve from time zero to the end of the dosing interval (24 hours post dose) (omeprazole only)
CL/F	Total body clearance for extravascular administration.
C_{max}	Maximum concentration occurring at t_{max}
C_{min}	The minimum concentration observed over the dosing interval (omeprazole only)
t½	Terminal half-life
t_{max}	Time of maximum observed concentration sampled during a dosing interval
Vz/F	Volume of distribution associated with the terminal slope following extravascular administration.

The primary pharmacokinetic parameters will include the C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ for anagrelide and its active metabolite, 3-OH-anagrelide (BCH24426).

The geometric mean relative bioavailability and 90% confidence limits for the effect of omeprazole will be evaluated (eg, $AUC_{anagrelide+omeprazole}/AUC_{anagrelide}$).

Because the CYP2C19-mediated metabolism of omeprazole is subject to genetic polymorphism (approximately 2/3 subjects are CYP2C19 extensive metabolizers with lower omeprazole AUC and 1/3 subjects are CYP2C19 intermediate/poor metabolizers with higher omeprazole AUC), the geometric mean relative bioavailability and 90% CI will be calculate for (1) all subjects, (2) EMs, and (3) IMs/PMs

The observed omeprazole $AUC_{0\text{-}tau}$ will be used to define the CYP2C19 metabolizer status of subjects, with $AUC_{0\text{-}tau}$ <5,200 ng•h/mL (<15 µmol/L) classified as CYP2C19 EM and $AUC_{0\text{-}tau}$ ≥5,200 ng•h/mL (≥15 µmol/L) classified as CYP2C19 IM/PM.

9.8.1.1 Statistical Analysis of Pharmacokinetic Parameters

The following analyses will be conducted on the Pharmacokinetic Analysis Set. All statistical analyses will be presented for the CYP2C19 EM group, the CYP2C19 IM/PM group, and overall.

Summary statistics (number of observations, mean, standard deviation, coefficient of variation, median, maximum, minimum, and geometric mean) will be determined for all pharmacokinetic parameters and presented by treatment. Plasma concentrations of anagrelide, 3-hydroxy-anagrelide, RL603, and omeprazole at each nominal sampling time will also be summarized by treatment using descriptive statistics.

The log-transformed pharmacokinetic parameters will be compared between the 2 treatments (anagrelide plus omeprazole and anagrelide alone) using a 2-factor analysis of variance model for a single-sequence crossover design with fixed factors for treatment and subject. The magnitude of the effect of omeprazole on the pharmacokinetic profile of anagrelide and its metabolites will be evaluated by the point estimate and ninety percent (90%) confidence intervals for the treatment difference on the log-transformed parameters back-calculated to the original scale for the comparisons of C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$ (test treatment=anagrelide plus omeprazole, reference treatment=anagrelide alone).

9.9 Safety Analyses

The safety endpoints will be summarized with descriptive statistics for the safety set.

Relevant safety endpoints as well as their changes from baseline will be summarized. Baseline is defined as the last assessment prior to the first dose of investigational product. Potentially clinically important findings will also be summarized or listed. The potentially clinically important values will be defined in the SAP.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities. The number of events, incidence, and percentage of treatment-emergent adverse events will be calculated overall, by system organ class, by preferred term, and by treatment group. Treatment-emergent adverse events will be further summarized by severity and relationship to investigational product. Adverse events related to investigational product, AEs leading to withdrawal, SAEs, and deaths will be similarly summarized/listed.

Safety will be assessed by treatment emergent adverse events (TEAEs), new findings from physical examination, evaluation of blood pressure, heart rate , new findings from 12-lead ECG, and clinical laboratory test results (biochemistry, hematology, and urinalysis).

Clinical laboratory tests, vital signs, and ECG findings will be summarized by treatment group and visit. Potentially clinically important findings will also be summarized or listed.

9.10 Other Analyses

No other analyses are planned in this study.

10. SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

This study is conducted in accordance with current applicable regulations, ICH, EU Directive 2001/20/EC and its updates, and local ethical and legal requirements.

The name and address of each third-party vendor (eg, CRO) used in this study will be maintained in the investigator's and sponsor's files, as appropriate.

10.1 Sponsor's Responsibilities

10.1.1 Good Clinical Practice Compliance

The study sponsor and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with all applicable industry regulations, ICH Good Clinical Practice (GCP) Guideline E6 (1996), EU Directive 2001/20/EC, as well as all applicable national and local laws and regulations.

Visits to sites are conducted by representatives of the study sponsor and/or the company organizing/managing the research on behalf of the sponsor to inspect study data, subjects' medical records, and CRFs in accordance with current GCP and the respective local and inter/national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The sponsor ensures that local regulatory authority requirements are met before the start of the study. The sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of investigational product for shipment to the site.

10.1.2 Public Posting of Study Information

The sponsor is responsible for posting appropriate study information on applicable websites. Information included in clinical study registries may include participating investigators' names and contact information.

10.1.3 Submission of Summary of Clinical Study Report to Competent Authorities of Member States Concerned and Ethics Committees

The sponsor will provide a summary of the clinical study report to the competent authority of the member state(s) concerned as required by regulatory requirement(s) and to comply with the Community guideline on GCP. This requirement will be fulfilled within 6 months of the end of the study completion date for pediatric studies and within 1 year for non-pediatric studies as per guidance.

10.1.4 Study Suspension, Termination, and Completion

The sponsor may suspend or terminate the study, or part of the study, at any time for any reason. If the study is suspended or terminated, the sponsor will ensure that applicable sites, regulatory agencies and IRBs/ECs are notified as appropriate.

Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

10.2 Investigator's Responsibilities

10.2.1 Good Clinical Practice Compliance

The investigator must undertake to perform the study in accordance with ICH GCP Guideline E6 (1996), EU Directive 2001/20/EC, and applicable regulatory requirements and guidelines.

It is the investigator's responsibility to ensure that adequate time and appropriately trained resources are available at the site prior to commitment to participate in this study. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant study-related tasks, and shall, upon request of the sponsor, provide documented evidence of any licenses and certifications necessary to demonstrate such qualification. Curriculum vitae for investigators and sub-investigators are provided to the study sponsor (or designee) before starting the study.

If a potential research subject has a primary care physician, the investigator should, with the subject's consent, inform them of the subject's participation in the study.

Agreement with the final clinical study report is documented by the signed and dated signature of the principal investigator, in compliance with Directive 2001/83/EC as amended by Directive 2003/63/EC and ICH Guidance E3 (1995).

10.2.2 Protocol Adherence and Investigator Agreement

The investigator and any co-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study at their site, the investigator will promptly inform the sponsor and the IRB/EC and provide them with a detailed written explanation. The investigator will also return all investigational product, containers, and other study materials to the sponsor. Upon study completion, the investigator will provide the sponsor, IRB/EC, and regulatory agency with final reports and summaries as required by (inter)national regulations.

Communication with local IRBs/ECs, to ensure accurate and timely information is provided at all phases during the study, may be done by the sponsor, applicable CRO, investigator, or, for multicenter studies, the coordinating principal investigator, according to national provisions, and will be documented in the investigator agreement.

10.2.3 Documentation and Retention of Records

10.2.3.1 Case Report Forms

Case report forms are supplied by the CRO and should be handled in accordance with instructions from the sponsor.

The investigator is responsible for maintaining adequate and accurate medical records from which accurate information is recorded onto CRFs, which have been designed to record all observations and other data pertinent to the clinical investigation. Case report forms must be completed by the investigator or designee as stated in the site delegation log.

All data will have separate source documentation; no data will be recorded directly onto the CRF.

All data sent to the sponsor must be endorsed by the investigator.

The clinical research associate (CRA)/study monitor will verify the contents against the source data per the monitoring plan. If the data are unclear or contradictory, queries are sent for corrections or verification of data.

10.2.3.2 Recording, Access, and Retention of Source Data and Study Documents

Original source data to be reviewed during this study will include, but are not limited to: subject's medical file, and original clinical laboratory reports.

All key data must be recorded in the subject's medical records.

The investigator must permit authorized representatives of the sponsor; the respective national, local, or foreign regulatory authorities; the IRB/EC; and auditors to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The CRA/study monitor (and auditors, IRB/EC, or regulatory inspectors) may check the CRF entries against the source documents. The consent form includes a statement by which the subject agrees to the monitor/auditor from the sponsor or its representatives, national or local regulatory authorities, or IRB/EC, having access to source data (eg, subject's medical file, appointment books, original laboratory reports, X-rays, etc.).

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory agency (eg, the US FDA, EMA, UK Medicines and Healthcare products Regulatory Agency) or an auditor.

Essential documents must be maintained according to ICH GCP requirements and may not be destroyed without written permission from the sponsor.

10.2.3.3 Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US FDA (as well as other US national and local regulatory authorities), the EMA, the Medicines and Healthcare products Regulatory Agency, other regulatory authorities, the sponsor or its representatives, and the IRB/EC for each site.

10.2.3.4 Financial Disclosure

The investigator is required to disclose any financial arrangement during the study and for 1 year after, whereby the outcome of the study could be influenced by the value of the compensation for conducting the study, or other payments the investigator received from the sponsor. The following information is collected: any significant payments from the sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in investigational product; any significant equity interest in the sponsor or subsidiaries as defined in 21 CFR 54 2(b) (1998).

10.3 Ethical Considerations

10.3.1 Informed Consent

It is the responsibility of the investigator to obtain written informed consent from all study subjects prior to any study-related procedures including screening assessments. All consent documentation must be in accordance with applicable regulations and GCP. Each subject or the subject's legally authorized representative, as applicable, is requested to sign and date the subject informed consent form or a certified translation if applicable, after the subject has received and read (or been read) the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. A copy of the informed consent documentation (ie, a complete set of subject information sheets and fully executed signature pages) must be given to the subject or the subject's legally authorized representative, as applicable. This document may require translation into the local language. Signed consent forms must remain in each subject's study file and must be available for verification at any time.

The principal investigator provides the sponsor with a copy of the consent form that was reviewed by the IRB/EC and received their favorable opinion/approval. A copy of the IRB/EC's written favorable opinion/approval of these documents must be provided to the sponsor prior to the start of the study unless it is agreed to and documented (abiding by regulatory guidelines and national provisions) prior to study start that another party (ie, sponsor or coordinating principal investigator) is responsible for this action. Additionally, if the IRB/EC requires modification of the sample subject information and consent document provided by the sponsor, the documentation supporting this requirement must be provided to the sponsor.

10.3.2 Institutional Review Board or Ethics Committee

For sites outside the EU, it is the responsibility of the investigator to submit this protocol, the informed consent document (approved by the sponsor or their designee), relevant supporting information and all types of subject recruitment information to the IRB/EC for review, and all must be approved prior to site initiation.

The applicant for an EC opinion can be the sponsor or investigator for sites within the EU; for multicenter studies, the applicant can be the coordinating principal investigator or sponsor, according to national provisions.

Responsibility for coordinating with IRBs/ECs is defined in the clinical trial agreement.

Prior to implementing changes in the study, the sponsor and the IRB/EC must approve any revisions of all informed consent documents and amendments to the protocol unless there is a subject safety issue.

Investigational product supplies will not be released until the sponsor has received written IRB/EC approval of and copies of revised documents.

For sites outside the EU, the investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol, but in any case at least once a year; for sites within the EU, this can be done by the sponsor or investigator, or, for multicenter studies, it can be done by the coordinating principal investigator, according to national provisions. The investigator must also keep the local IRB/EC informed of any serious and significant AEs.

10.4 Privacy and Confidentiality

All US-based sites and laboratories or entities providing support for this study, must, where applicable, comply with HIPAA of 1996. A site that is not a covered entity as defined by HIPAA must provide documentation of this fact to the sponsor.

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the study, the sponsor and/or its representatives' reviews their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the sponsor; third parties with whom the sponsor may develop, register, or market SPD422 (anagrelide); national or local regulatory authorities; and the IRB(s)/EC(s) which gave approval for the study to proceed. The sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number; however, their initials and date of birth may also be collected and used to assist the sponsor to verify the accuracy of the data (eg, to confirm that laboratory results have been assigned to the correct subject).

The results of studies – containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth – will be recorded. They may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

10.5 Study Results/Publication Policy

Shire will endeavor to publish the results of all qualifying, applicable, and covered studies according to external guidelines in a timely manner regardless of whether the outcomes are perceived as positive, neutral, or negative. Additionally, Shire adheres to external guidelines (eg, Good Publication Practices 2) when forming a publication steering committee. The purpose of the publication steering committee is to act as a non-commercial body that advises or decides on dissemination of scientific study data in accordance with the scope of this policy.

All publications relating to Shire products or projects must undergo appropriate technical and intellectual property review, with Shire agreement to publish prior to release of information. The review is aimed at protecting the sponsor's proprietary information existing either at the commencement of the study or generated during the study. To the extent permitted by the publisher and copyright law, the principal investigator will own (or share with other authors) the copyright on his/her publications. To the extent that the principal investigator has such sole, joint or shared rights, the principal investigator grants the sponsor a perpetual, irrevocable, royalty-free license to make and distribute copies of such publications.

The term "publication" refers to any public disclosure including original research articles, review articles, oral presentations, abstracts and posters at medical congresses, journal supplements, letters to the editor, invited lectures, opinion pieces, book chapters, electronic postings on medical/scientific websites, or other disclosure of the study results, in printed, electronic, oral or other form.

Subject to the terms of the paragraph below, the investigator shall have the right to publish the study results, and any background information provided by the sponsor that is necessary to include in any publication of study results, or necessary for other scholars to verify such study results. Notwithstanding the foregoing, no publication that incorporates the sponsor's confidential information shall be submitted for publication without the sponsor's prior written agreement to publish, and shall be given to the sponsor for review at least 60 days prior to submission for publication. If requested in writing by Shire, the institution and principal investigator shall withhold submission of such publication for up to an additional 60 days to allow for filing of a patent application.

If the study is part of a multicenter study, the first publication of the study results shall be made by the sponsor in conjunction with the sponsor's presentation of a joint, multicenter publication of the compiled and analyzed study results. If such a multicenter publication is not submitted to a journal for publication by the sponsor within an 18-month period after conclusion, abandonment, or termination of the study at all sites, or after the sponsor confirms there shall be no multicenter study publication of the study results, an investigator may individually publish the study results from the specific site in accordance with this section. The investigator must, however, acknowledge in the publication the limitations of the single-site data being presented.

Unless otherwise required by the journal in which the publication appears, or the forum in which it is made, authorship will comply with the International Committee of Medical Journal Editors (ICMJE) current standards. Participation as an investigator does not confer any rights to authorship of publications.

11. REFERENCES

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12. APPENDICES

APPENDIX 1 PROTOCOL HISTORY

Document	Date	Global/Country/Site Specific
Original Protocol	04 Oct 2018	Global