STATISTICAL ANALYSIS PLAN

SPD422, Anagrelide Hydrochloride
PHASE 1

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

PROTOCOL IDENTIFIER: SPD422-113

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1.0	08MAY2019	Original final version

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ABBREVIATIONS

AE	adverse event
ANOVA	analysis of variance
AUC	area under the curve
$AUC_{0-\infty}$	area under the curve extrapolated to infinity
AUC_{0-t}	area under the curve from time 0 to the last time point of sample collection
AUC_{0-tau}	area under the curve from time 0 to the end of the dosing interval (24 hours post dose)
BLQ	below limit of quantitation
BMI	body mass index
CI	confidence interval
CL/F	total body clearance
C_{max}	maximum concentration
C_{min}	minimum concentration observed over the dosing interval
C_{trough}	Plasma concentration observed at the end of a dosing interval
CV	coefficient of variation
ECG	electrocardiogram
eCRF	electronic case report form
EM	extensive metabolizer
IM/PM	intermediate metabolizers/poor metabolizer
LLOQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
MR	metabolite ratio
PCI	potentially clinically important
PK	pharmacokinetic(s)
PT	preferred term
Q1	25th Percentile
Q3	75th Percentile
QD	once daily
QTcB	QT interval corrected for heart rate using Bazett's formula
QTcF	QT interval corrected for heart rate using Fridericia's formula
SAP	statistical analysis plan
SD	standard deviation
SOC	system organ class
t½	terminal half-life
TEAE	treatment-emergent adverse event
TFL	tables, figures, and listings
t_{max}	time of maximum observed concentration sampled during a dosing interval

V_z/F	volume of distribution associated with the terminal slope following extravascular administration
WHO	World Health Organization

1. INTRODUCTION

This statistical analysis plan (SAP) provides a technical and detailed elaboration of the statistical analyses of pharmacokinetic (PK) and safety data described in the original study protocol dated 04 Oct 2018. Specifications for tables, figures, and listings (TFLs) are contained in a separate document (SPD422-113 TFL Shells).

2. OBJECTIVES, ESTIMANDS, AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

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

2.1.2 Secondary Objective

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

2.2 Endpoints

2.2.1 Primary Endpoints

The primary endpoints will be maximum concentration (C_{max}) and area under the curve from time 0 to the last time point of sample collection (AUC_{0-t}) and area under the curve extrapolated to infinity ($AUC_{0-\infty}$) of anagrelide and 3-hydroxyanagrelide compared between anagrelide administered alone and anagrelide administered after omeprazole 40 mg once daily (QD) for 7 days.

2.2.2 Secondary Endpoints

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

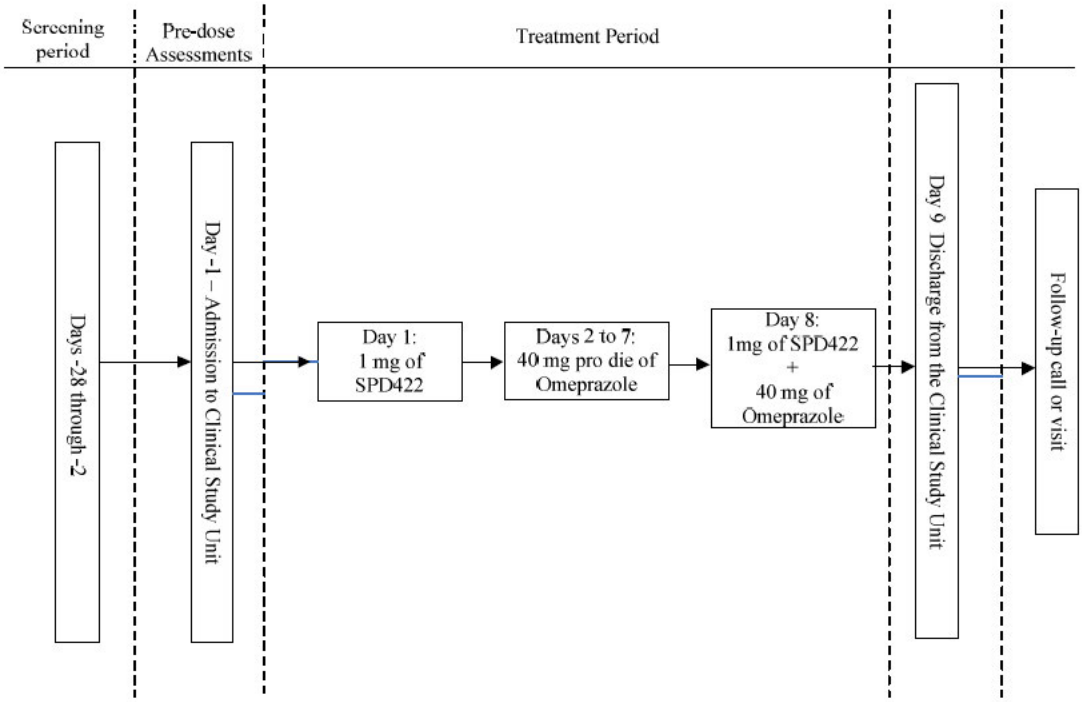
3. STUDY DESIGN

3.1 General Description

This is a Phase 1, open-label, single-sequence, non-randomized, multiple-dose, crossover PK study to assess the effect of a CYP1A2 inducer (omeprazole 40 mg QD) on the PK of anagrelide (1 mg) when administered concurrently in healthy subjects. Up to 35 healthy

male and female subjects, aged 18 to 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.

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 Randomization

Not applicable.

3.3 Blinding

Not applicable.

3.4 Sample Size and Power Considerations

Up to 35 healthy male and female subjects, aged 18 to 45 years will be screened to ensure enrollment and completion of 20 subjects. 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 (CI).

From a previous crossover study (SPD422-110), the within-subject coefficient variation (CV) 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 extensive metabolizer (EM) subjects and 6 to 7 CYP2C19 intermediate/poor metabolizer (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.

4. STATISTICAL ANALYSIS SETS

4.1 Enrolled Set

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.

4.2 Safety Set

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.

4.3 Pharmacokinetic Set

The PK Set consists of subjects who receive at least 1 dose of investigational product (anagrelide or omeprazole) and have at least 1 measurable post-dose plasma concentration. Each subject's data (eg, dosing records, AEs, sample collection records, etc.) will be reviewed for inclusion/exclusion from the descriptive statistics and statistical analysis on a case-by-case basis at the discretion of the Pharmacokineticist.

5. STUDY SUBJECTS

5.1 Disposition of Subjects

The number of subjects who were included in each defined analysis set (Enrolled, Safety, and PK) will be summarized overall.

The number and percentage of subjects who completed the study or prematurely discontinued will be presented overall, along with primary reasons for discontinuation, as recorded on the study completion/early termination page of the electronic case report form (eCRF). All enrolled subjects who prematurely discontinued the study will be listed in the subject disposition listing along with reasons for discontinuation.

Subject disposition summary and listings will be based on the Enrolled Set.

5.2 Demographic and Other Baseline Characteristics

Descriptive summaries of demographic and baseline characteristics will be generated for the Safety Set and the PK Set.

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. The summaries of demographic and baseline characteristics for the PK Set will be presented by the CYP2C19 metabolizer status (EMs and IMs/PMs) and overall.

The following demographic and baseline characteristics will be summarized in the following order in the tables: age (years), sex, ethnicity, race, weight (kg), height (cm), and body mass index (BMI, kg/m^2).

Continuous variables, such as age, weight, height, and BMI will be summarized using descriptive statistics including number of subjects, mean, standard deviation (SD), median, minimum, and maximum. Categorical variables such as sex, ethnicity and race will be summarized by reporting the number and percentage of subjects in each category. All summarized values will be taken from the latest available assessments at the Screening Visit.

Demographic and baseline characteristics will be listed for the Safety Set.

5.3 Medical History

Medical history will be collected at the Screening visit and will be coded using Medical Dictionary for Regulatory Activities (MedDRA) Version 21.1. A listing will be provided using the Safety Set.

Medical history will be summarized by system organ class (SOC) and preferred term (PT) for the Safety Set.

5.4 Prior Medications

Prior medications will be coded using the World Health Organization (WHO) Drug Dictionary Enhanced Version dated 01 September 2018.

Prior medication is defined as any medication with the start date prior to the date of first dose of investigational product.

Prior medication usage will be summarized by the number and proportion of subjects receiving each medication by PT and overall for the Safety Set. Multiple medication usage by a subject in the same category will be counted only once.

All prior medications will be listed for the Safety Set.

5.5 Concomitant Medications

Concomitant medications will be coded using the WHO Drug Dictionary Enhanced Version dated 01 September 2018.

Concomitant medication is defined as any medication with a start date prior to the date of the first dose of investigational product and continuing after the first dose of investigational product or with a start date between the dates of the first and last doses of investigational product, inclusive. Any medication with a start date after the date of the last dose of investigational product will not be considered a concomitant medication.

Concomitant medication usage will be summarized by the number and proportion of subjects receiving each medication by PT, by treatment group and overall, for the Safety Set. Multiple medication usage by a subject in the same category will be counted only once. Concomitant medications will be assigned to the treatment(s) with which they are concomitant. A medication is concomitant with a treatment if the subject received that medication any time between the time when that treatment was received and when the next treatment was received, or in the case of the last treatment, until the end of the

follow-up period. Therefore, a medication may be concomitant with more than one treatment.

All concomitant medications will be listed for the Safety Set.

5.6 Exposure to Investigational Product

Exposure to investigational product for the Safety Set will be summarized in terms of treatment duration, which is calculated as the number of days from the date of first dose of investigational product taken to the date of the last dose of investigational product taken, inclusively. Descriptive statistics (number of subjects, mean, standard deviation, minimum, median, and maximum) will be presented to describe the exposure to investigational product.

A listing will be created by subject number and visit giving the date and time of dose administration for the Safety Set.

5.7 Measurements of Treatment Compliance

This is a study in which the investigational products will be administered from Day 1 to Day 8 at the study site only (in-house confinement treatment period). All compliance related data will be included in the exposure data listing. No separate treatment compliance summary or data listing will be provided.

5.8 Protocol Deviations

Protocol deviations will be recorded by the site separately from the clinical database. Protocol deviations will be classified as major or minor per the agreed protocol deviation management plan. The Shire study team will review the protocol deviations and their classification throughout the study and before database lock.

Decisions of the review will include categorization of major and minor protocol deviations.

Confirmed major and minor protocol deviations will be documented in the protocol deviation tracker for the study. Major/minor protocol deviations will be summarized by category for the Safety Set. Major/minor protocol deviations will be listed for the Enrolled Set.

6. EFFICACY ANALYSES

Not applicable.

7. SAFETY ANALYSIS

The safety analysis will be performed using the Safety Set. Safety parameters include AEs, clinical laboratory results, vital signs, and ECG results. For each safety parameter, the last value collected prior to dosing on Day 1 will be used as baseline for all analyses of that safety parameter.

7.1 Adverse Events

Adverse events will be coded using MedDRA Version 21.1.

An AE (classified by PT) will be considered a treatment-emergent AE (TEAE) if it starts on or after dosing on Day 1, or if it starts before dosing on Day 1 but increases in severity on or after dosing on Day 1 through the end of the study. If more than 1 AE with the same PT is reported before dosing on Day 1, then the AE with the greatest severity will be used as the benchmark for comparison to the AEs occurring after dosing on Day 1 under the PT.

Treatment most recently taken when the AE occurred will be used for the summaries:

- Any AE that started (or increased in severity) on or after the administration of anagrelide on Day 1, but before the administration of omeprazole on Day 2 will be assigned to anagrelide alone;

- Any AE that started (or increased in severity) on or after the administration of omeprazole on Day 2, but before the administration of anagrelide on Day 8 will be assigned to omeprazole alone;

- Any AE that started (or increased in severity) on or after the administration of anagrelide on Day 8 will be assigned to anagrelide with omeprazole.

An overall summary of the number of subjects with TEAEs as well as the number of events will be presented by treatment group, including the number and percentage of subjects with any TEAEs, serious TEAEs, TEAEs related to investigational product, serious TEAEs related to investigational product, TEAEs leading to discontinuation of investigational product, serious TEAEs leading to discontinuation of investigational product, TEAEs leading to discontinuation from study, serious TEAEs leading to discontinuation from study, severe TEAEs, TEAEs leading to hospitalization, and TEAEs leading to death.

The number and percentage of subjects reporting TEAEs, as well as the number of events, in each treatment group and overall will be tabulated by SOC and PT, and by SOC, PT, and maximum severity. The TEAEs considered related to investigational product will also be summarized by SOC and PT. If more than 1 AE occurs with the same PT, after the same treatment for the same subject, then the subject will be counted only once for that treatment group and PT using the most severe and most related occurrence for the summarization by severity and by relationship to investigational product. Summaries by SOC and PT will present SOC sorted alphabetically and PT within SOC by descending incidence.

The TEAEs and related TEAEs will be summarized by PT by descending frequency. Serious TEAEs, TEAEs leading to discontinuation of investigational product and serious TEAEs leading to death, will be summarized by SOC, PT, and treatment group.

7.2 Clinical Laboratory Data

Descriptive statistics for clinical laboratory values (in SI units) and changes from baseline at each assessment time point as well as shift tables from baseline to each visit for quantitative variables will be presented by treatment group for the following clinical laboratory parameters:

Hematology Hemoglobin, hematocrit, red blood cells, platelet count, white blood cell count – total and differential, total neutrophils (absolute), eosinophils (absolute), monocytes (absolute), basophils (absolute) and lymphocytes (absolute).

Biochemistry Sodium, potassium, glucose, blood urea nitrogen, creatinine, calcium, chloride, phosphorus, total protein, total CO2 (bicarbonate), albumin, aspartate transaminase, alanine transaminase, gamma glutamyl transferase, alkaline phosphatase, total bilirubin, and uric acid.

Urinalysis pH, glucose, protein, blood, ketones, bilirubin, nitrites, leukocyte esterase, and specific gravity.

Clinical laboratory test values are potentially clinically important (PCI) if they meet either the low or high PCI criteria listed in Table 1. The number and percentage of subjects with post-baseline PCI values will be tabulated overall. The percentages will be calculated relative to the number of subjects with available baseline values and at least 1 post-baseline assessment. The numerator is the total number of subjects with at least

1 post-baseline PCI value. A supportive listing of subjects with post-baseline PCI values will be provided including the subject number, baseline, and post-baseline values.

Table 1: Criteria for Potentially Clinically Important Laboratory Tests

Parameter	Classification	Criteria: SI Units (Conventional Units)
Hematology		
Hemoglobin	LOW and DECREASE	<100 g/L (10g/dL) AND Decrease of ≥ 20 g/L (2.0 g/dL) from baseline value
	HIGH	>200 g/L (20g/dL)
Hematocrit	LOW and DECREASE	≤0.6 × LLN AND Decrease of ≥0.06 L/L (6.0%) from baseline value
	HIGH	>1.3 × ULN
Red blood cells count	LOW	$<3 \times 10^{12}$/L
	HIGH	$>7.5 \times 10^{12}$/L
Platelet count	LOW	$<0.6 \times$ LLN OR $<100 \times 10^9$/L (100×10^3/μL)
	HIGH	$>1.5 \times$ ULN OR $>500 \times 10^9$/L (500×10^3/μL)
White blood cell count	LOW	$<0.5 \times$ LLN OR $<3.0 \times 10^9$/L (3×10^3/μL)
	HIGH	$>2 \times$ ULN OR $>16.0 \times 10^9$/L (16×10^3/μL)
Neutrophils	LOW	$<1.5 \times 10^9$/L (1.5×10^3/μL) OR < 40%
	HIGH	$>6.2 \times 10^9$/L (6.2×10^3/μL) OR > 70 %
Eosinophils	HIGH	$>0.5 \times 10^9$/L (0.5×10^3/μL) OR > 10.0%
Monocytes	HIGH	$>1.1 \times 10^9$/L (1.1×10^3/μL) OR >11 %
Basophils	HIGH	$>0.2 \times 10^9$/L (0.2×10^3/μL) OR > 2%
Lymphocytes	LOW	$<0.8 \times 10^9$/L (0.8×10^3/μL) OR < 22 %
	HIGH	$> 4.0 \times 10^9$/L (4.0×10^3/μL) OR > 44 %
Biochemistry		
Sodium	LOW	>5 mmol/L (5 mEq/L) below LLN
	HIGH	>5 mmol/L (5 mEq/L) above ULN
Potassium	LOW and DECREASE	Below LLN AND Decrease of >0.5 mmol/L (0.5 mEq/L) from baseline value
	HIGH and INCREASE	Above ULN AND Increase of > 0.5 mmol/L (0.5 mEq/L) from baseline value
Glucose (fasting)	LOW	≤4.2 mmol/L
	HIGH	≥6.7 mmol/L
Blood urea nitrogen	HIGH	>1.5 × ULN
Creatinine	HIGH and INCREASE	>150 μmol/L AND Increase > 30% from baseline value
Calcium	LOW and DECREASE	Below LLN AND Decrease of ≥0.25 mmol/L (1.0 mg/dL) from baseline value

Parameter	**Classification**	**Criteria: SI Units (Conventional Units)**
	HIGH and INCREASE	Above ULN AND Increase of ≥ 0.25 mmol/L (1.0 mg/dL) from baseline value
Phosphorus	LOW	>0.162 mmol/L (0.5 mg/dL) below LLN
	HIGH	>0.162 mmol/L (0.5 mg/dL) above ULN
Total protein	LOW and DECREASE	Below LLN AND Decrease of ≥20 g/L (2.0 g/dL) from baseline value
	HIGH and INCREASE	Above ULN AND Increase of ≥20 g/L (2.0 g/dL) from baseline value
Albumin	LOW and DECREASE	Below LLN AND Decrease of ≥10 g/L (1.0 g/dL) from baseline value
	HIGH and INCREASE	Above ULN AND Increase of ≥10 g/L (1.0 g/dL) from baseline value
Aspartate transaminase	HIGH	>2 × ULN
Alanine transaminase	HIGH	>2 × ULN
Gamma glutamyl transferase	HIGH	>1.5 × ULN
Alkaline phosphatase	HIGH	>1.5 × ULN
Total bilirubin	HIGH	>1.5 × ULN
Uric acid (with normal diet)	LOW and DECREASE	Below LLN AND Decrease of >0.119 mmol/L (2.0 mg/dL) from baseline value
Triiodothyronine	LOW	<0.922 nmol/L
	HIGH	>2.765 nmol/L
Thyroxine	LOW	<57.92 nmol/L
	HIGH	>140.28 nmol/L
Thyroid stimulating hormone	LOW	<0.5 µU/L
	HIGH	>5.0 µU/L
Uric acid (with normal diet)	HIGH and INCREASE	Above ULN AND Increase of >0.119 mmol/L (2.0 mg/dL) from baseline value
Urinalysis		
Glucose	HIGH	≥1+
Protein	HIGH	≥2+
Blood	HIGH	≥2+
Ketones	HIGH	≥2+
Bilirubin	HIGH	Positive
Nitrites	HIGH	Positive
Leukocyte esterase	HIGH	Positive

Abbreviations: LLN=lower limit of normal value provided by the laboratory, ULN=upper limit of normal value provided by the laboratory

All laboratory data will be listed for the Safety Set.

7.3 Vital Signs

Descriptive statistics for vital signs (systolic and diastolic blood pressure and pulse rate) and their changes from baseline at each post-baseline visit and at end of study will be presented by treatment group.

Vital sign values will be considered PCI if they meet both the observed value criteria and the change from baseline criteria listed in Table 2. The number and percentage of subjects with PCI post-baseline values will be tabulated by time point and treatment group. The percentages will be calculated relative to the number of subjects with baseline and available assessment at that time point in that treatment group. The numerator is the total number of subjects with at least 1 PCI post-baseline vital sign value at that time point in that treatment group. A supportive listing of subjects with post-baseline PCI values will be provided including the subject number, baseline, and post-baseline PCI values.

Table 2: Criteria for Potentially Clinically Important Vital Signs

Parameter	Classification	Criteria
Sitting and Supine		
Systolic BP (mmHg)	LOW and DECREASE	≤90 and decrease of ≥20 from baseline value
	HIGH and INCREASE	≥140 and increase of ≥20 from baseline value
Diastolic BP (mmHg)	LOW and DECREASE	≤50 and decrease of ≥15 from baseline value
	HIGH and INCREASE	≥90 and increase of ≥15 from baseline value
Pulse rate (bpm)	LOW and DECREASE	≤45 and decrease of >15 from baseline value
	HIGH and INCREASE	≥100 and increase of >15 from baseline value
Temperature (regardless of method)	LOW	<35°C or <95°F
	HIGH	>38.3°C or >100.9°F
Respiratory rate (breaths/min)	LOW	<10
	HIGH	>25

Abbreviations: BP=blood pressure, bpm=beats per minute.

All vital signs data will be listed for the Safety Set.

7.4 Electrocardiogram (ECG)

Descriptive statistics for ECG variables (heart rate, PR, RR, QRS, QT, and QTc intervals) and their changes from baseline at each time point will be presented by treatment group. QTc interval will be calculated using both Bazett ($QTcB=QT/(RR)^{1/2}$) and Fridericia ($QTcF=QT/(RR)^{1/3}$) corrections; and if RR is not available, it will be replaced with 60/(heart rate) in the correction formula. The ECG interpretation at each time point will be summarized by treatment group. A shift table from baseline to each time point for qualitative ECG results will be presented by treatment group.

Electrocardiogram variable values will be considered PCI if they meet or exceed the upper limit values listed in Table 3. The number and percentage of subjects with post-baseline PCI values will be tabulated by treatment and time point. The percentages will be calculated relative to the number of subjects with available non-PCI baseline and available assessment at that time point in that treatment group. The numerator is the total number of subjects with at least 1 PCI post-baseline ECG value at that time point in that treatment group. A listing of all subjects with post-baseline PCI value will be provided including the subject number, baseline, and post-baseline PCI values.

Table 3: Criteria for Potentially Clinically Important ECG Values

Parameter	**Classification**	**Criteria**
Overall evaluation	ABNORMAL	Overall evaluation is ABNORMAL
Rhythm	NON-SINUS RHYTHM	Rhythm is not SINUS
Heart rate (bpm)	LOW and DECREASE	≤45 and decrease of >15 from baseline value
	HIGH and INCREASE	≥100 and increase of >15 from baseline value
PR interval (msec)	HIGH and INCREASE	≥200 and increase of ≥20 from baseline value
QRS interval (msec)	HIGH	≥120
QTc interval (men) (msec)*	HIGH	>430 and increase from baseline value >30
QTc interval (women) (msec)*	HIGH	>450 and increase from baseline value >30

Abbreviations: bpm=beats per minute; QTc=QT interval corrected.
*Values noted refer to both Bazett's (QTcB) and Fridericia's (QTcF) formula

7.5 Other Safety Data

No other safety assessments/variables are planned for this study.

8. PHARMACOKINETIC ANALYSIS

8.1 Drug Concentration

Blood samples will be drawn from each subject during this study for the determination of plasma concentration of anagrelide, 3-hydroxy-anagrelide, and RL603. Serial blood samples will be collected on Day 1 and Day 8 for PK analysis at predose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 8, and 12 hours after administration of the investigational product. Plasma concentrations of anagrelide and its metabolites will be measured using a validated analytical method.

Blood samples will be drawn from each subject during this study for the determination of plasma concentration of omeprazole. Omeprazole PK sample collections on Days 6 and

Day 7 will be taken at pre-dose only. Serial blood samples will be collected on treatment on Day 8 for PK analysis at predose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 8, 12, and 24 hours after administration of the investigational product. Plasma concentrations of omeprazole will be measured using a validated analytical method.

8.2 Handling Below Limit of Quantitation Values

The following procedures will be used for plasma concentrations of anagrelide and its metabolites and omeprazole data below the lower limit of quantification (LLOQ):

- Samples that are below limit of quantification (BLQ) are reported as <LLOQ on the data listings, where LLOQ is replaced by the actual value for LLOQ for specific PK assay.
- Samples that are BLQ are treated as zero in the calculation of summary statistics (eg, mean, SD, etc.) for the plasma concentrations at individual time points. Geometric mean will be set to missing where zero values exist.
- Mean concentrations are reported as zero if all values are BLQ or zero, and no other descriptive statistics are reported. If the calculated mean (± SD) concentration is less than the LLOQ, the value will be reported as calculated. The mean values derived using these conventions will be used to create the mean plasma concentration versus time plots.
- For calculation of area under the curve (AUC), BLQ values are set equal to zero in the dataset loaded into Phoenix® WinNonlin® (Certara USA, Inc, Princeton, NJ) for PK analysis. WinNonlin® uses the zero values that occur before the first time point with a concentration greater than LLOQ. All BLQ values following the first measurable concentration will be set to "missing" in the dataset loaded into WinNonlin®.
- Missing values will not be imputed.

8.3 Pharmacokinetic Parameters

Pharmacokinetic parameters will be determined on Day 1 and Day 8 for anagrelide and its metabolites and on Day 8 for omeprazole from the individual plasma concentration versus actual time data. Pharmacokinetic parameters will be estimated by non-compartmental analysis, as deemed appropriate, using Phoenix WinNonlin (Certara USA, Inc., Princeton, NJ) Version 8.0 or higher and all calculations will be based on the actual time since dose.

The PK parameters for anagrelide, 3-hydroxy-anagrelide and RL603 with and without omeprazole administration on Day 1 and Day 8, and PK parameters for omeprazole (and its metabolite) will include, but may not be limited to:

Parameter	Description
$AUC_{0-\infty}$	Area under the curve extrapolated to infinity, calculated using the observed value of the last non-zero concentration, calculated using the linear up/log down trapezoidal rule (anagrelide and metabolites)
AUC_{0-t}	Area under the curve from time 0 to the last time point of sample collection, calculated using the linear up/ log down trapezoidal rule (anagrelide and metabolites)
AUC_{0-tau}	Area under the curve from time 0 to the end of the dosing interval (24 hours post dose), calculated using the linear up/log down trapezoidal rule (omeprazole only)
C_{max}	Maximum concentration
C_{min}	Minimum concentration observed over the dosing interval (omeprazole only)
C_{trough}	Plasma concentration observed at the end of a dosing interval (collected before the next administration [predose concentration at Day 6, Day 7 and Day 8]) (omeprazole only)
t_{max}	Time of maximum observed concentration sampled during a dosing interval
t½	Terminal half-life, calculated as $\ln(2)/\lambda_z$
CL/F	Total body clearance, calculated as: Dose/$AUC_{0-\infty}$ (anagrelide only) and Dose/AUC_{0-tau} (omeprazole only)
V_z/F	Volume of distribution associated with the terminal slope following extravascular administration, calculated as: CL/F / λ_z (anagrelide and omeprazole only)
MRAUC	Metabolite ratio (anagrelide metabolites only), calculated as: $AUC_{0-\infty}$ (metabolite) / $AUC_{0-\infty}$ (parent); molecular weight adjustment needed for metabolites.
MRC_{max}	Metabolite ratio (anagrelide metabolites only), calculated as: C_{max} (metabolite) / C_{max} (parent); molecular weight adjustment needed for metabolites.

The primary PK parameters will include the C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ for anagrelide and its metabolites, 3-OH-anagrelide (BCH24426) (active) and RL603 (inactive).

8.4 Statistical Analysis of Pharmacokinetic Data

Individual anagrelide, 3-hydroxy-anagrelide, RL603, and omeprazole concentrations will be listed by subject, treatment, day, and time and summarized by treatment, day, and time based on the PK Set.

The following descriptive statistics of plasma concentration summary will be provided: number of subjects, arithmetic mean, geometric mean, SD, arithmetic CV%, geometric CV%, 25th Percentile (Q1), 75th Percentile (Q3), median, minimum, and maximum.

The mean and individual plasma anagrelide, 3-hydroxy-anagrelide, and RL603, and omeprazole concentrations versus time profiles by treatment will be presented in figures on both linear and semi-logarithmic scales. Mean (±SD) and individual anagrelide, 3-hydroxy-anagrelide, RL603, and omeprazole plasma concentrations versus time profiles will be presented using nominal time based on the PK Set.

As well as being summarized and presented graphically for all subjects combined, anagrelide, 3-hydroxy-anagrelide, and RL603 concentrations will also be summarized and presented graphically by CYP2C19 metabolizer status (EM and IM/PM). Refer to Section 5.2 for CYP2C19 metabolizer status criteria.

Individual anagrelide, 3-hydroxy-anagrelide, RL603, and omeprazole PK parameters will be listed by subject, treatment, and day, and summarized by treatment and day based on the PK Set with the following descriptive statistics: n, arithmetic mean, arithmetic SD, 95% CI of the mean, CV%, median, Q1, Q3, minimum, maximum, geometric mean, 95% CI of the geometric mean, and geometric CV%.

As well as being performed for all subjects combined, anagrelide, 3-hydroxy-anagrelide, and RL603 parameters will also be analyzed by CYP2C19 metabolizer status (EM and IM/PM).

A linear mixed model using a 2-factor analysis of variance (ANOVA) for a single-sequence crossover design with fixed factors for treatment and subject will be used to compare the log-transformed PK parameters between the 2 treatments (anagrelide with omeprazole versus anagrelide alone). The magnitude of the effect of omeprazole on the PK profile of anagrelide and its metabolites will be evaluated by the point estimate and ninety percent (90%) CIs for the treatment difference on the log-transformed parameters back-calculated to the original scale for the comparisons of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. The geometric mean ratios of the PK parameters comparing anagrelide with omeprazole to anagrelide alone and the corresponding 90% CIs will be calculated for (1) all subjects, (2) EMs, and (3) IMs/PMs.

9. OTHER ANALYSES

No other analyses are planned for this study.

10. INTERIM ANALYSIS/ DATA MONITORING COMMITTEE

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

11. DATA HANDLING CONVENTIONS

11.1 General Data Reporting Conventions

Continuous variables will be summarized using the following descriptive statistics: number of subjects, mean, median, standard deviation, Q1, Q3, minimum, maximum. Categorical and count variables will be summarized by the number of subjects and the percent of subjects in each category.

See Shire TFL Standards for rules on the number of decimal places to present data and *p*-values.

11.2 Definition of Baseline

Baseline for all safety analyses is defined as the last observed value for the safety assessment prior to taking the first dose of investigational product on Day 1 (based on dates or date/times).

11.3 Repeated or Unscheduled Assessments of Safety Parameters

If a subject has repeated assessments before the start of investigational product, then the results from the final assessment made prior to the start of investigational product will be used as baseline. If end of study assessments are repeated or unscheduled, the last post-baseline assessment will be used as the end of study assessment for generating descriptive statistics. However, all post-baseline assessments will be used for PCI value determination and all assessments will be presented in the data listings.

11.4 Handling of Missing, Unused, and Spurious Data

11.4.1 Missing Date Information for Prior or Concomitant Medications (Therapies/Procedures)

For prior or concomitant medications, incomplete (ie, partially missing) start date and/or stop date will be imputed. When the start date and the stop date are both incomplete for a subject, impute the start date first.

11.4.1.1 Incomplete Start Date

The following rules will be applied to impute the missing numerical fields. If the stop date is complete and the imputed start date is after the stop date, then the start date will be imputed using the stop date.

11.4.1.1.1 Missing Day and Month

- If the year of the incomplete start date is the same as the year of the date of the first dose of investigational product, then the day and month of the date of the first dose of investigational product will be assigned to the missing fields.
- If the year of the incomplete start date is before the year of the date of the first dose of investigational product, then December 31 will be assigned to the missing fields.
- If the year of the incomplete start date is after the year of the date of the first dose of investigational product, then 01 January will be assigned to the missing fields.

11.4.1.1.2 Missing Month Only

- The day will be treated as missing and both month and day will be replaced according to the above procedure.

11.4.1.1.3 Missing Day Only

- If the month and year of the incomplete start date are the same as the month and year of the date of the first dose of investigational product, then the day of the date of the first dose of investigational product will be assigned to the missing day
- If either the year is before the year of the date of the first dose of investigational product or if both years are the same, but the month is before the month of the date of the first dose of investigational product, then the last day of the month will be assigned to the missing day
- If either the year is after the year of the date of the first dose of investigational product or if both years are the same, but the month is after the month of the date of the first dose of investigational product, then the first day of the month will be assigned to the missing day.

11.4.1.2 Incomplete Stop Date

The following rules will be applied to impute the missing numerical fields.

11.4.1.2.1 Missing Day and Month

- If the year of the incomplete stop date is the same as the year as of the date of the last dose of investigational product, then the day and month of the date of the last dose of investigational product will be assigned to the missing fields.
- If the year of the incomplete stop date is before the year of the date of the last dose of investigational product, then 31 December will be assigned to the missing fields.

- If the year of the incomplete stop date is after the year of the date of the last dose of investigational product, then 01 January will be assigned to the missing fields.

11.4.1.2.2 Missing Month Only

- The day will be treated as missing and both month and day will be replaced according to the above procedure.

11.4.1.2.3 Missing Day Only

- If the month and year of the incomplete stop date are the same as the month and year of the date of the last dose of investigational product, then the day of the date of the last dose of investigational product will be assigned to the missing day
- If either the year is before the year of the date of the last dose of investigational product or if both years are the same, but the month is before the month of the date of the last dose of investigational product, then the last day of the month will be assigned to the missing day
- If either the year is after the year of the last dose of investigational product or if both years are the same, but the month is after the month of the date of the last dose of investigational product, then the first day of the month will be assigned to the missing day.

11.4.2 Missing Date Information for Adverse Events

For AEs with partial start dates, non-missing date parts will be used to determine if the AE is treatment-emergent or not. If a determination cannot be made using the non-missing date parts as to when the AE occurred relative to study drug administration, eg, AE start year and month are the same as the year and month of the first dose of investigational product, then the AE will be classified as treatment-emergent.

To facilitate categorization of AEs as treatment-emergent, imputation of dates can be used. For AEs, the default is to only impute incomplete (ie, partially missing) start dates. Incomplete stop dates may also be imputed when calculation of the duration of an AE is required per the protocol. If imputation of an incomplete stop date is required, and both the start date and the stop date are incomplete for a subject, impute the start date first.

11.4.2.1 Incomplete Start Date

Follow the same rules as in Section 11.4.1.1.

11.4.2.2 Incomplete Stop Date

Follow the same rules as in Section 11.4.1.2.

11.4.3 Missing Severity Assessment for Adverse Events

If severity is missing for an AE starting prior to the date of the first dose of investigational product, then a severity of "Mild" will be assigned. If the severity is missing for an AE starting on or after the date of the first dose of investigational product, then a severity of "Severe" will be assigned. The imputed values for severity assessment will be used for incidence summaries, while both the actual and the imputed values will be used in data listings.

11.4.4 Missing Relationship to Investigational Product for Adverse Events

If the relationship to investigational product is missing for an AE starting on or after the date of the first dose of investigational product, a causality of "Related" will be assigned. The imputed values for relationship to investigational product will be used for incidence summaries, while both the actual and the imputed values will be presented in data listings.

11.4.5 Character Values of Clinical Laboratory Variables

If the reported value of a clinical laboratory variable cannot be used in a statistical analysis due to, for example, that a character string is reported for a numerical variable. The appropriately determined coded value will be used in the statistical analysis. However, the actual values as reported in the database will be presented in data listings.

12. ANALYSIS SOFTWARE

Statistical analyses will be performed using Version 9.4 (or newer) of SAS® on a suitably qualified environment.

Phoenix WinNonlin (Certara USA, Inc., Princeton, NJ) Version 8.0 (or higher) will be used for calculation of PK parameters.

13. CHANGES TO ANALYSIS SPECIFIED IN PROTOCOL

Not applicable.

14. REFERENCES

Not applicable.

15. APPENDICES

15.1 Schedule of Activities

Table 4: 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
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; 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 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 5: 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 hours		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 6: 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 hours		X	X		X	X		X	X
24 hours			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.