Protocol Number: VONO-101

Official Title: An Open-Label, Fixed-Sequence, Clinical Drug Interaction Study to Evaluate the Time-Dependent Inhibition Potential of Vonoprazan on a Sensitive CYP3A4 Substrate, Midazolam, in Healthy Volunteers

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Phathom Pharmaceuticals, Inc.

VONO-101

An Open-Label, Fixed-Sequence, Clinical Drug Interaction Study to Evaluate the Time-Dependent Inhibition Potential of Vonoprazan on a Sensitive CYP3A4 Substrate, Midazolam, in Healthy Volunteers

23Oct2020

Final Statistical Analysis Plan

Version 1.0

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	Statistical Analysis Plan (SAP) Client Approval Form

Client:	**Phathom Pharmaceuticals, Inc.**
Protocol Number:	**VONO-101**
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List of Abbreviations

Abbreviation	Term
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration versus time curve
AUC_{0-24}	area under the plasma concentration versus time curve from time 0 to time 24 hours
AUC_{0-inf}	area under the plasma concentration versus time curve from time 0 extrapolated to infinity
AUC_{0-t}	area under the plasma concentration versus time curve from time 0 to the last quantifiable concentration
BID	twice daily
BLQ	below the limit of quantification
BMI	body mass index
CI	confidence interval
CL/F	apparent total body clearance
C_{max}	maximum observed plasma concentration
COVID-19	coronavirus disease 2019
CSR	clinical study report
C_{trough}	trough concentration
CV	coefficient of variation
CYP	cytochrome P450
DDI	drug-drug interaction
ECG	electrocardiogram
eCRF	electronic case report form
EE	erosive esophagitis
EOS	end of study
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GMR	geometric mean ratio
H^+, K^+-ATPase	hydrogen, potassium–adenosine triphosphatase
MedDRA	medical dictionary for regulatory activities
ICF	informed consent form
ICH	International Council for Harmonisation
IRB	institutional review board
LLOQ	lower limit of quantification
K_{el}	apparent terminal elimination rate constant
PCAB	potassium-competitive acid blocker
PK	pharmacokinetic(s)
PT	preferred term
PTE	pre-treatment event
QTcF	QT interval corrected for heart rate using Fridericia's formula
SAE	serious adverse event
SD	standard deviation
SOC	system organ class

$t_{1/2}$	terminal phase half-life
TEAE	treatment-emergent adverse event
T_{max}	time to maximum observed plasma concentration
V_d/F	apparent volume of distribution

1. Introduction

This document outlines the statistical methods to be implemented during the analysis of data collected within the scope of Phathom Pharmaceuticals, Inc., protocol VONO-101 (An Open-Label, Fixed-Sequence, Clinical Drug Interaction Study to Evaluate the Time-Dependent Inhibition Potential of Vonoprazan on a Sensitive CYP3A4 Substrate, Midazolam, in Healthy Volunteers), Amendment 1, Version 2.0, dated 04 September 2020. The purpose of this statistical analysis plan is to define the planned statistical analysis of the study data consistent with the study objectives.

Vonoprazan fumarate (TAK-438) belongs to a novel class of acid suppressants known as PCABs that suppress gastric acid secretion by competitively inhibiting gastric H^+, K^+-ATPase. It is metabolized mainly by hepatic drug-metabolizing enzyme CYP3A4 and partially by CYP2B6, CYP2C19, and CYP2D6 and is being developed by Phathom Pharmaceuticals, Inc. for healing of all grades of EE and relief of heartburn, maintenance of healing of all grades of EE and relief of heartburn, and treatment of *Helicobacter pylori* infection. Vonoprazan has been studied in a number of other acid-related diseases including gastric ulcer/duodenal ulcer healing and for the prevention of recurrence of a gastric or duodenal ulcer during nonsteroidal anti-inflammatory drugs or aspirin administration. Overall, vonoprazan has been well tolerated in healthy volunteers in Phase 1 studies, as well as in completed Phase 2 and 3 studies of Japanese, Asian, and Western subjects with acid-related diseases.

Midazolam is an ultra–short-acting benzodiazepine used clinically for brief sedation and is commonly used as a sensitive CYP3A4 substrate in clinical drug interaction studies. After oral administration, midazolam is almost completely absorbed from the gastrointestinal tract. The mean $t_{1/2}$ of midazolam and 1-hydroxymidazolam, its major metabolite (mediated by CYP3A4), is approximately 3 hours in humans. The C_{max} is reached at about 1 hour after oral administration. Midazolam is contraindicated in patients with a known hypersensitivity to the drug or allergies to cherries or formulation excipients. Potential AEs are summarized in the midazolam prescribing information (Midazolam hydrochloride 2012).

This study is designed in accordance with the US FDA Guidance for Industry, Clinical Drug Interaction Studies - Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (DHHS 2020) to assess the effects of vonoprazan on the PK, safety, and tolerability of midazolam. This study will improve understanding of the interaction and resulting changes in exposure (if any) when a sensitive CYP3A4 substrate (midazolam) is given in combination with vonoprazan

2. Objectives

2.1. Primary Objectives

The primary objective of this study is to determine the time-dependent inhibition potential of repeated doses of oral vonoprazan on the PK of a single oral dose of midazolam, a sensitive CYP3A4 substrate, in healthy subjects.

2.2. Secondary Objectives

The secondary objectives of this study are:

- To assess the safety and tolerability of a single oral dose of midazolam alone or when coadministered with multiple oral doses of vonoprazan in healthy subjects.
- To evaluate the plasma PK of the metabolite 1-hydroxymidazolam in the presence of vonoprazan in healthy subjects

3. Investigational Plan

3.1. Overall Study Design and Plan

This is a Phase 1, open-label, fixed-sequence, clinical drug interaction study designed to assess the effect of vonoprazan on the PK of midazolam in healthy subjects.

The study will consist of a screening period, check-in, a treatment period, an EOS visit, and a follow-up telephone call. The duration of the study, excluding Screening and including the follow-up telephone call, is approximately 27 days. Subjects will be confined to the clinical unit from Day –1 until discharge on Day 11 (EOS visit). The treatment period will include administration of single oral doses of 2 mg of midazolam syrup on Day 1 and Day 9 and BID doses of 20 mg vonoprazan oral tablets on Days 2 through 10. A follow-up telephone call will occur on Day 25 (±2 days).

Subjects will fast overnight (nothing to eat or drink except water) for at least 8 hours before the morning dose administration. On Day 9, midazolam will be administered 1 hour after the vonoprazan morning dose administration. Subjects will continue fasting for 4 hours after dosing on Days 1 and 9. On vonoprazan-only days, food will be allowed 1 hour after the vonoprazan dose has been administered. Evening vonoprazan dosing should occur more than 1 hour before or after a meal.

Pharmacokinetic and safety endpoints will be evaluated in the study.

The schedule of events is presented in Appendix 12.1.

3.2. Study Endpoints

3.2.1. Pharmacokinetic Endpoints

The primary endpoints will be $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, and C_{max} of midazolam with and without vonoprazan.

The following PK parameters for midazolam and 1-hydroxymidazolam will be calculated as endpoints using standard noncompartmental methods:

- $AUC_{0\text{-}t}$
- $AUC_{0\text{-}inf}$
- C_{max}
- T_{max}
- K_{el}
- $t_{1/2}$
- CL/F for midazolam only
- V_d/F for midazolam only

Metabolite to parent ratios for C_{max} and $AUC_{0\text{-}inf}$ will also be reported. To assess attainment of steady state, C_{trough} concentrations will be reported for vonoprazan.

3.2.2. Safety Endpoints

Safety and tolerability endpoints will include monitoring and recording of AEs, clinical laboratory test results (hematology, serum chemistry, and urinalysis), vital sign measurements, 12-lead ECG results, and physical examination findings.

3.3. Treatments

Vonoprazan will be administrated as tablets. Midazolam will be administrated as syrup.

The vonoprazan 20 mg BID dose was selected because this is the highest dose being evaluated in clinical studies and approved for use, thus maximizing the possibility of identifying a DDI, in line with regulatory guidance. A single dose of 2 mg of midazolam was chosen because this is one of the most common dosing strategies for CYP3A drug interaction studies and is expected to provide adequate plasma concentrations to assess a drug interaction. Furthermore, this dose is anticipated to reduce the chance of excessive sedation in the presence of an interaction that could cause increased levels of midazolam.

Treatments administered during the study will be denoted as below in this statistical analysis plan, as appropriate:

- Midazolam alone: from the time of single oral dose of 2 mg of midazolam syrup administered on Day 1 prior to (<) the time of BID doses of 20 mg vonoprazan oral tablets administered on Day 2

- Vonoprazan alone: from the time of BID doses of 20 mg vonoprazan oral tablets administered on Days 2 prior to (<) the time of single oral dose of 2 mg of midazolam syrup administered on Day 9
- Midazolam + vonoprazan: from the time of single oral dose of 2 mg of midazolam syrup administered on Day 9 and onward

4. General Statistical Considerations

All statistical analyses will be performed using SAS® software (SAS Institute Inc., Cary, North Carolina) Version 9.4 or higher. All tables and data listings will appear in landscape format, and all figures in portrait format, employing Courier New 9-point black font.

All data collected on the eCRFs will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings but not included in the calculation of summary statistics.

For categorical variables, frequencies and percentages will be presented. Continuous variables will be summarized using descriptive statistics (number of subjects, mean, median, SD, minimum, and maximum).

Baseline is defined as the last non-missing assessment (including repeated and unscheduled assessments) prior to study treatment on Day 1 unless otherwise stated. If there are repeated assessments at a time point, the first non-missing assessment will be included in the summary tables.

Unscheduled results will not be included in the summary tables except for determining baseline or when the initial scheduled assessment is unavailable, but will be presented in data listings.

Study days will be calculated relative to the first dose of study drug, i.e., the single oral dose of 2 mg of midazolam syrup administered on Day 1, as:

- Study Day = Assessment Date – Date of Midazolam on Day 1 + 1, if the assessment date is on or after the date of midazolam on Day 1; or
- Study Day = Assessment Date – Date of Midazolam on Day 1, if the assessment date is prior to the date of midazolam on Day 1.

4.1. Sample Size

The sample size (N = 16 evaluable subjects) for this study is based on consideration of the precision of the estimate of GMRs of PK parameters of midazolam with and without vonoprazan. To evaluate the effect of vonoprazan on the PK of midazolam, there is an 80% probability that the 90% CI will be within 81.4% and 122.9% of the point estimate of the GMR of midazolam PK parameters with and without vonoprazan. This calculation

assumes that midazolam PK parameters are log-normally distributed with an intrasubject CV no greater than 30% (Mueller et all 2017).

4.2. Randomization, Stratification, and Blinding

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

4.3. Analysis Population

4.3.1. Pharmacokinetic Population

The PK population will include subjects who receive at least 1 dose of study drug and have sufficient concentration data to support accurate estimation of at least 1 PK parameter. Subjects who experience vomiting within 2 times the median T_{max} after midazolam dosing will be excluded from the PK analysis.

4.3.2. Safety Population

The safety population will include all subjects who receive at least 1 dose of study drug.

5. Subject Disposition

5.1. Disposition

A summary table reflecting the number and percentage of subjects included in the Safety and PK populations and who completed the study will be presented overall. This table will also present the number and percentage of subjects who discontinued from the study, both overall and according to reasons for discontinuation from the study. Safety population will be used.

In addition, a summary table reflecting the number and percentage of subjects who signed the informed consent form (ICF), who screen failed, and who were administered study drug will be presented. This table will also present the number and percentage of each reason for screen failure. All subjects who signed the ICF will be included.

Subject disposition will be presented in data listings. Analysis populations will be presented in a separate data listing.

5.2. Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the protocol. An important deviation (sometimes referred to as a major or significant deviation) is a subset of protocol deviations that leads to a subject being discontinued from the study or significantly affects the subject's rights, safety, or well-being and/or the completeness, accuracy, and reliability of the study data. An important deviation can include nonadherence to inclusion or exclusion criteria or nonadherence to FDA regulations or ICH E6(R2) guidelines.

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. The investigator will be notified of deviations in writing by the monitor. The IRB should be notified of all protocol deviations, if appropriate, in a timely manner.

Important protocol deviations will be summarized in a summary table overall for the safety population. All protocol deviations will be presented in a data listing.

6. Demographics and Baseline Characteristics

6.1. Demographics

Demographics and baseline characteristics, including age (years), sex, race, ethnicity, fertility status (not applicable for males), height (cm), weight (kg), and body mass index (BMI) (kg/m^2) will be summarized for the Safety Population in a summary table. Height, weight, and BMI will be taken from the screening visit.

The above subject demographic characteristics for subjects in the safety population will be presented in a data listing.

6.2. Medical History

Each verbatim term collected in the eCRF will be mapped to a preferred term (PT) and system organ class (SOC) using the medical dictionary for regulatory activities (MedDRA), Version to be delineated in the CSR.

The medical history data will be presented in a data listing.

6.3. Inclusion and Exclusion Criteria

Admission criteria deviations are defined as any violation of protocol-defined inclusion/exclusion criteria.

Admission criteria deviations will be presented in a data listing for the safety population.

7. Treatments and Medications

7.1. Prior and Concomitant Medications

Prior medications will be defined as medications that stopped before the first dose of study drug. Information regarding prior medications taken by the subject within the 30 days before signing the ICF will be recorded in the subject's eCRF.

Concomitant medications will be defined as medications that started after the date of the first dose of study drug up to the subject's last visit. Medications that started before the first dose of study drug and are ongoing after the first dose of study drug will be considered as both prior and concomitant.

Prior and concomitant medications will be coded using the latest version of the World Health Organization Drug Dictionary.

Prior and concomitant medications will be presented in a data listing.

7.2. Medical or Surgical Treatment Procedures

Medical or surgical treatment procedures performed during the study will be presented in a data listing.

7.3. Study Treatments

Study drugs administration will be presented in a data listing. Fasting data will be presented in a separate data listing.

8. Safety Analysis

All safety analyses will be based on the safety population unless otherwise stated.

8.1. Adverse Events

A PTE is defined as any untoward medical occurrence that has occurred prior to administration of any study drug in a clinical investigation subject who has signed ICF to participate in a study; it does not necessarily have to have a causal relationship with study participation.

An AE is defined as any untoward medical occurrence in a subject enrolled in this study regardless of its causal relationship to study drug. An AE can therefore be an unfavorable sign or symptom, or a disease temporally associated with the use of study drug.

A TEAE is defined as any event that occurs after the first dose of study drug or any event at baseline that worsens in either intensity or frequency after the first dose of study drug.

Adverse events will be coded by PT and SOC using the latest version of the MedDRA.

All AE summaries will be limited to TEAEs and will include the number and percentage of subjects experiencing the specific AE category and the number of occurrences of the events, and will be presented by treatment (midazolam alone, vonoprazan alone, and midazolam + vonoprazan) and overall. For summary purpose, an event will be assigned to the last treatment the subject received when the event occurred. If a subject reported multiple AEs, the subject will be counted once at the specific summarization level using the most related occurrence for by relationship summary or the most severe occurrence for the by severity summary.

All AE data will be presented in a data listing.

8.1.1. Incidence of Adverse Events

An overall AE summary will be generated presenting the frequency and percentage of subjects and the number of AEs for the following for the safety population:

- Any TEAE
- Any TEAE for each severity level
- Any treatment-related TEAE
- Any treatment-related TEAE for each severity level
- Any serious adverse event (SAE)
- Any treatment-related SAE
- Any TEAE leading to early study discontinuation

Events with missing relationship will be considered as treatment-related.

The occurrence of TEAEs using MedDRA SOC and PTs will be summarized in a separate summary table.

8.1.2. Relationship of Adverse Events to Study Drug

The relationship of all AEs and SAEs with midazolam alone, vonoprazan alone, or midazolam/vonoprazan together will be assessed separately by the investigator as not related or related.

Treatment-emergent AEs will be summarized by SOC, PT, and relationship. A missing category will be added if the relationship is missing. At each level of subject summarization, a subject will be classified according to the closest relationship if the subject reported one or more events.

8.1.3. Severity of Adverse Event

The severity (or intensity) of an AE refers to the extent to which it affects the subject's daily activities and will be classified as mild, moderate, or severe.

A summary of TEAEs will be presented by SOC, PT, and severity. A missing category will be added if the severity is missing. At each level of subject summarization, a subject will be classified according to the highest severity if the subject reported one or more events.

8.1.4. Serious Adverse Events

An SAE is defined as any untoward medical occurrence at any dose that meets one of the following criteria:

- Results in death.
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly/birth defect.

- Is an important medical event

All SAEs, including deaths, will be presented in a data listing.

8.1.5. Treatment-Emergent Adverse Events Leading to Study Discontinuation

Treatment-emergent AEs leading to study discontinuation will be presented in a data listing.

8.2. Clinical Laboratory Evaluations

The following clinical laboratory assessments will be performed:

Hematology	Hematocrit, hemoglobin, red blood cell count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, red blood cell distribution width, leukocytes and leukocyte differential (basophils, eosinophils, lymphocytes, monocytes, neutrophils), and platelet count
Serum Chemistry	ALT, albumin, alkaline phosphatase, AST, bilirubin (total and direct), blood urea nitrogen, calcium, carbon dioxide, chloride, cholesterol (total, high-density lipoprotein, and calculated low-density lipoprotein), creatine kinase, creatinine, gamma-glutamyltransferase, globulin, glucose (fasting), lactate dehydrogenase, magnesium, phosphorus, potassium, sodium, total protein, triglycerides (fasting), and uric acid
Urinalysis	Appearance, bilirubin, color, glucose, ketones, leukocyte esterase, reflex microscopy (performed if dipstick is positive for protein or the blood value is 1+ or greater; includes bacteria, casts, crystals, epithelial cells, red blood cells, and white blood cells), nitrites, occult blood, pH, protein, specific gravity, turbidity, and urobilinogen
Serology	Hepatitis B surface antigen, hepatitis C virus antibody, and human immunodeficiency virus type 1 and 2 antibodies (Screening only)
Other analyses	All subjects: urine drug screen (alcohol, amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine metabolites, cotinine, methamphetamines, methylenedioxymethamphetamine, and opiates [including heroin, codeine, and oxycodone]) All subjects: COVID-19 screening All female subjects: pregnancy test (human chorionic

gonadotropin [serum or urine, per site practice])

Female subjects with at least 12 months of amenorrhea: FSH

Hematology, serum chemistry, and urinalysis laboratory tests will be performed at Screening, Check-in, before morning dose on Days 6 and 8, on EOS visit, and at early termination if applicable. Serology and FSH will be performed at Screening only. COVID-19 screening test will be performed at Screening, Check-in, and on Day 7. Urine drug screen tests will be performed at Screening and Check-in. Pregnancy test will be performed at Screening, Check-in, on EOS visit, and at early termination if applicable.

Summary tables presenting observed values and changes from baseline overall at each visit using descriptive statistics (number of subjects, mean, SD, median, minimum, and maximum) will be presented for hematology, serum chemistry, and urinalysis laboratory tests with numeric values.

Shift from baseline in hematology, serum chemistry, and urinalysis laboratory tests results will be summarized using the frequency count and percentage of subjects in each category (low, normal, and high for quantitative results; normal and abnormal for qualitative results). Percentages will be based on the number of subjects with both non-missing baseline and relevant post-baseline results.

Early termination visit will be summarized in the EOS visit in the tables.

All clinical laboratory test results will be presented in the data listings. Laboratory values that are below the lower limit or above the upper limit of the reference range will be flagged in the listings and the listing will include the investigator's assessment for clinical significance.

8.3. Vital Sign Measurements

Vital signs will be obtained after the subject has been in the seated position for at least 5 minutes at Screening and Check-in; on Days 1 and 9 within 60 minutes prior to midazolam dosing and at 0.5, 1, 2, 4, 8, 12, and 24 hours following midazolam dosing; on Days 3 through 8 before the morning dose of vonoprazan; on EOS visit, and at early termination visit if applicable. Vital signs will include systolic and diastolic blood pressure, pulse rate, respiratory rate, and tympanic temperature.

Height and weight will be measured and BMI will be calculated at Screening. Only weight will be measured at Check-in and Day 11.

Actual values and change from baseline for vital signs measurement (systolic and diastolic blood pressure, pulse rate, respiratory rate, and tympanic temperature) will be summarized

overall at each time point using descriptive statistics (number of subjects, mean, SD, median, minimum, and maximum) in a summary table.

Early termination visit will be summarized in the EOS visit in the table.

All vital sign measurements, including weight measured at all visits, and height and BMI performed at unscheduled visits, will be presented in a data listing.

8.4. Physical Examination

A full physical examination will be performed at Screening. Full physical examination includes, at minimum, assessment of skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, cardiovascular system, abdomen, lymph nodes, and musculoskeletal system/extremities. A brief physical examination will be performed at Check-in and EOS visit, and at early termination visit if applicable. Brief physical examination includes, at minimum, assessment of skin, lungs, cardiovascular system, and abdomen (liver and spleen). Interim physical examinations may be performed at the discretion of the investigator, if necessary, to evaluate AEs or clinical laboratory abnormalities.

Physical examination results will only be recorded in the source document; therefore, tables or listings are not planned for physical examinations

8.5. Electrocardiogram

Single 12-lead ECG recordings will be made after the subject has been in the supine position for at least 5 minutes at Screening and Check-in, on Days 1 and 9 within 30 minutes before midazolam dosing, on EOS visit, and at early termination visit if applicable. Measurements of the following intervals will be reported: RR interval, PR interval, QRS width, QT interval, and QTcF.

Actual values and changes from baseline for 12-lead ECG results will be summarized overall at each visit using descriptive statistics (number of subjects, mean, SD, median, minimum, and maximum).

Early termination visit will be summarized in the EOS visit in the table.

9. Pharmacokinetics

9.1. Data Handling

All summaries and analyses of the PK data of midazolam, 1-hydroxymidazolam and vonoprazan will be based on the Pharmacokinetic Population defined in Section 4.3.

The following procedures will be used for plasma concentrations data of midazolam, 1-hydroxymidazolam and vonoprazan below the LLOQ and missing values:

- Concentration values that are BLQ will be reported as provided by the bioanalytical data in the PK data listings.
- Concentration values that are BLQ will be treated as zero for the calculation of summary statistics (eg, mean, SD, etc.) at individual time points.
- Mean concentrations will be reported as BLQ if all concentration values are BLQ, and SD and CV will be reported as not applicable.
- For calculation of PK parameters, BLQ values will be treated as zero with the exception that a BLQ value between 2 quantifiable concentrations will be set as missing. Missing concentrations will be treated as missing from the PK parameter calculations. If consecutive BLQ concentrations are followed by quantifiable concentrations in the terminal phase, those concentrations after BLQ concentrations will be treated as missing.
- Missing concentration values will not be imputed.

Plasma concentrations of midazolam, 1-hydroxymidazolam and vonoprazan will be displayed using the data format as provided in the bioanalytical report. Plasma pharmacokinetic parameters will be reported to 3 significant figures.

9.2. Plasma Concentrations

Blood samples will be collected during treatment period to measure plasma concentrations of vonoprazan, midazolam and 1-hydroxymidazolam as follows:

Analyte	Matrix	Day	Scheduled Time Point (h)
Midazolam and 1-hydroxymidazolam	Plasma	1	Predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16 h post dose
		2	24 h postdose
		9	Predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16 h post dose
		10	24 and 36 h postdose
		11	48 h postdose
Vonoprazan	Plasma	2 through 10	Predose
		11	No scheduled time

Plasma samples will be analyzed for midazolam, 1-hydroxymidazolam and vonoprazan via Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS) using validated assays. Specifics of analytical methodology will be provided in a separate report.

A subject listing of all plasma concentration-time data following study treatment will be presented by treatment, subject, and scheduled sample collection time. Plasma

concentration data of midazolam, 1-hydroxymidazolam and vonoprazan will be summarized by nominal time point using descriptive statistics: the number of observations (n), arithmetic mean, standard deviation (SD), coefficient of variation (CV), geometric mean, geometric CV, median, minimum and maximum.

Individual subject plasma concentration versus actual time data of midazolam, 1-hydroxymidazolam and vonoprazan will be presented graphically by each treatment on linear and semilogarithmic scales. The individual semilogarithmic plots will include the regression line used for estimation of K_{el}. Mean concentration versus nominal time of midazolam, 1-hydroxymidazolam and vonoprazan will also be provided by treatment on linear and semilogarithmic scales.

9.3. Pharmacokinetic Parameters

The principal PK parameters describing the PK of midazolam and 1-hydroxymidazolam for each subject will be derived from plasma versus time data using noncompartmental analysis by Phoenix® WinNonlin® (Certara USA Inc., New Jersey) version 8.0 or higher.

The following plasma PK parameters will be determined using the actual sampling times for midazolam and 1-hydroxymidazolam:

C_{max}	The maximum observed plasma concentration
T_{max}	The time at which C_{max} occurs
AUC_{0-t}	AUC from time zero to time t, the last quantifiable time point
AUC_{0-inf}	AUC from time zero extrapolated to infinity, calculated per the formula: $AUC_{0-inf} = AUC_{0-t} + C_{last} / K_{el}$,, where C_{last} is the concentration of the last quantifiable timepoint sample and K_{el} is the first order rate constant of the terminal phase
K_{el}	Apparent elimination rate constant (slope of the natural log of concentration vs time curve)
$t_{1/2}$	Apparent elimination half-life, calculated as $t_{1/2} = \ln 2/K_{el}$
CL/F	Apparent oral clearance (midazolam only)
V_d/F	Apparent volume of distribution (midazolam only)
MR_{Cmax}	Metabolite-to-parent C_{max} ratio, adjusted for molecular weight
$MR_{AUC0-inf}$	Metabolite-to-parent AUC_{0-inf} ratio, adjusted for molecular weight

Area under the plasma concentration versus time curve values will be calculated by numeric integration using the trapezoidal rule with linear up and log down interpolation. The number of data points used to calculate K_{el} must be ≥ 3 (not including C_{max}). Parameters that utilize K_{el} for determination (K_{el}, $t_{1/2}$ and AUC_{0-inf}) can be excluded from summaries and statistical analysis if the parameter estimates are not judged reliable. The criteria for any exclusions will be reported. Additional parameters, such as partial AUCs (e.g., AUC_{0-24}), can be reported and analyzed as needed.

Plasma PK parameters will be summarized by analyte and treatment using descriptive statistics: the n, arithmetic mean, SD, CV, median, minimum, maximum, and geometric mean and geometric CV. Time to C_{max} will be summarized by n, median, minimum, and maximum only.

9.4. Pharmacokinetic Statistical Analysis

To assess the effect of vonoprazan on the PK of midazolam, a linear mixed model with treatment as a fixed effect and subject as a random effect will be performed on the natural log-transformed values of AUC_{0-t}, AUC_{0-inf}, and C_{max} for midazolam and 1-hydroxymidazolam. The point estimates and the associated 90% CI for the ratio (midazolam + vonoprazan versus midazolam alone) will be constructed as the antilog of the confidence limits of the mean difference. No adjustment will be made for multiplicity. The absence of an effect of vonoprazan on the PK of midazolam and its metabolite will be concluded if the 90% CIs of the ratios are contained in the interval of 80% to 125%. A forest plot will be generated to display the point estimates and CIs for the ratio of midazolam + vonoprazan versus midazolam alone.

Non-parametric methods will be used to examine median differences in T_{max} for midazolam and 1-hydroxymidazolam between treatments. The 90% CIs of the median difference will be calculated from Hodges-Lehman estimate, and p-value will be produced from Wilcoxon signed rank test.

In addition, a linear mixed model with treatment as a fixed effect and subject as a random effect will be performed on the natural log-transformed values of AUC_{0-inf} and C_{max} for the midazolam metabolite-to-parent ratios for midazolam + vonoprazan versus midazolam alone.

10. Interim Analysis

No formal interim analyses will be performed in this study.

11. References

Midazolam hydrochloride (midazolam hydrochloride syrup) [prescribing information]. Roxane Laboratories, Inc. Columbus (OH); 2012. 22 p.

Mueller SC, Majcher-Peszynska J, Wacke R, et al. Within and between subject variability of oral midazolam pharmacokinetic parameters as surrogate for global CYP3A function in female and male volunteers [abstract] Clin Ther. 2017;39(8 Suppl):e29.

12. Appendices

12.1. Schedule of Study Procedures

Phase	Screening	Check-in	Treatment Period										EOS/ET	Follow-up
Procedure[a] / Day	–28 to –2	–1	1	2	3	4	5	6	7	8	9	10	11	25 (±2 days)
Admission to clinic		X												
Discharge from clinic[b]													X	
Telephone call														X
Informed consent	X													
Demographics	X													
Serology[c]	X													
COVID-19 screening	X	X							X					
Serum FSH[d]	X													
Inclusion/exclusion criteria	X	X												
Medical history	X	X												
Height, weight, and BMI[e]	X	X											X	
Physical examination[f]	X	X											X	
Vital sign measurements[g]	X	X	X	X	X	X	X	X	X	X	X	X	X	
12-lead ECG[h]	X	X	X								X		X	
Clinical laboratory testing[i]	X	X						X		X			X	
Urine drug/alcohol/cotinine screen[j]	X	X												
Pregnancy test[k]	X	X											X	
Guidance on avoidance of pregnancy	X	X											X	
Midazolam administration[l]			X								X			
Vonoprazan administration (BID)				X	X	X	X	X	X	X	X	X		
Midazolam PK sample collection[m]			X	X							X	X	X	
Vonoprazan PK sample collection[n]				X	X	X	X	X	X	X	X	X	X	
Fasting/nonfasting periods[o]		X	X	X	X	X	X	X	X	X	X	X	X	
PTEs[p]	X	X												
AEs[q]			◄						X					►
Prior/concomitant medications	◄							X						►

Notes:

[a] When procedures overlap or occur at the same time point, all blood draws should follow vital signs or ECGs, and PK sampling should be timed to occur last and as close to the scheduled time window as possible.
[b] Discharge following last study assessment.
[c] Serology testing will include hepatitis B surface antigen, hepatitis C virus antibody, and human immunodeficiency virus type 1 and 2 antibodies.
[d] Females with at least 12 months of amenorrhea should have a serum FSH test performed at Screening to confirm postmenopausal status.
[e] Height and weight will be measured and BMI will be calculated at Screening. Only weight will be measured at Check-in and Day 11.
[f] A full physical examination will be performed at Screening (at minimum, assessment of skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, cardiovascular system, abdomen, lymph nodes, and musculoskeletal system/extremities). A brief physical examination will be performed at Check-in and Day 11 (at minimum, assessment of skin, lungs, cardiovascular system, and abdomen [liver and spleen]). Interim physical examinations may be performed at the discretion of the investigator, if necessary, to evaluate AEs or clinical laboratory abnormalities.
[g] Vital signs will be measured after the subject has been in the seated position for at least 5 minutes at Screening and Check-in; on Days 1 and 9 within 60 minutes prior to midazolam dosing and at 0.5, 1, 2, 4, 8, 12, and 24 hours following midazolam dosing; on Days 3 through 8 before the morning dose of vonoprazan; and on Day 11. Vital signs will include systolic and diastolic blood pressure, pulse rate, respiratory rate, and tympanic temperature.
[h] Single 12-lead ECG recordings will be made after the subject has been in the supine position for at least 5 minutes at Screening and Check-in, on Days 1 and 9 within 30 minutes before midazolam dosing, and on Day 11. A single repeat measurement is permitted at Screening for eligibility determination. Measurements of the following intervals will be reported: RR interval, PR interval, QRS width, QT interval, and QTcF. Assessments should include comments on whether the tracings are normal or abnormal (if abnormal, whether clinically significant or not clinically significant); rhythm; the presence of arrhythmia or conduction defects; morphology; any evidence of myocardial infarction; and ST-segment, T-wave, and U-wave abnormalities.
[i] Clinical laboratory testing will occur at Screening, Check-in, before morning dosing on Days 6 and 8, and on Day 11. Testing includes liver function tests: ALT, AST, and total and direct bilirubin. A complete list of assessments is provided in Section 6.2.2 of the protocol. Blood and urine samples will be collected under fasting conditions and prepared per the clinic's standard procedures.
[j] Urine drug/alcohol/cotinine screen will occur at Screening and Check-in per the clinic's standard procedures.
[k] All females will have a pregnancy test performed at Screening, Check-in, and Day 11.
[l] The time of midazolam dosing will be called "0" hour. Doses of midazolam and vonoprazan will be administered with 240 mL of room temperature water. Subjects will maintain an upright (ie, seated or standing) position for at least 4 hours after dosing.
[m] Blood samples for PK analysis of midazolam and 1-hydroxymidazolam in plasma will be collected within 30 minutes prior to midazolam dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours following midazolam dosing on Day 1 and within 30 minutes prior to midazolam dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, and 48 hours following midazolam dosing on Day 9.
[n] Blood samples for PK analysis of vonoprazan in plasma will be collected within 30 minutes prior to administration of vonoprazan on Days 2 through 10 and on Day 11 for the assessment of steady state.
[o] During fasting periods, subjects should have nothing to eat or drink except water from 8 hours prior to study drug dosing. On Days 1 and 9, subjects will continue to fast until 4 hours after dosing. Water is permitted as desired except for the period 1 hour before and 1 hour after administration of study drug (other than as permitted for study drug dosing). During non-fasting periods, subjects should receive standardized meals per the clinic's standard procedures.
[p] Collection of PTEs will start after the subject has signed the ICF.

(q) Adverse events will be assessed from the time of midazolam dosing until the follow-up visit and should be followed until they are resolved, stable, or judged by the investigator to be not clinically significant.