Statistical Analysis Plan

Sponsor:	Spring Bank Pharmaceuticals, Inc.
Protocol No:	SBP-9200-HBV-202
Protocol Title:	A PHASE 1, OPEN-LABEL, FIXED-SEQUENCE, DRUG-DRUG INTERACTION STUDY BETWEEN MULTIPLE ORAL DOSES OF INARIGIVIR SOPROXIL AND A SINGLE ORAL DOSE OF MIDAZOLAM IN HEALTHY SUBJECTS
PRA Project ID:	SPB881EC-178881
Version Date:	2-Jul-2018

1.0 Approvals

The undersigned have approved this Statistical Analysis Plan for use in this study.

Name of Sponsor Representative / Title:	Donald Mitchell / Vice President of Operations and Corporate Developments
Signature of Sponsor Representative / Date:	
Name of Author / Title:	Justyna Laba / Biostatistician Pharmaceutical Research Associates Group B.V., a PRA Health Sciences company
Signature of Author / Date:	

2.0 Table of Contents

3.0 Introduction

This Statistical Analysis Plan (SAP) describes the statistical methods that will be used during the analysis and reporting of data collected under Spring Bank Pharmaceuticals Inc. Protocol SBP-9200-HBV-202.

This SAP should be read in conjunction with the study protocol and electronic case report form (eCRF). This version of the plan has been developed using the protocol dated 30-Apr-2018 (including all amendments up to this protocol date) and the final eCRF(s) dated 31-May-2018.

An approved and signed SAP is a requirement for database lock.

This SAP only covers the results that will be processed by the PRA Early Development Services (EDS) Biostatistics Department.

PRA EDS will perform the pharmacokinetic (PK), pharmacodynamic (PD), and safety and tolerability evaluation.

This SAP supersedes the statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. Any post-hoc or unplanned analyses, or significant changes from the planned analysis in this SAP performed to provide results for inclusion in the clinical study report (CSR) but not included in this SAP, will be clearly identified in Section 9.8.2 of the CSR. Changes to planned analyses do not require an updated SAP but should be included in the CSR if significant.

4.0 Changes from Previous Version of Approved SAP

This is the first version of the SAP.

5.0 Study Objectives

5.1 Primary

- To assess the effect of steady-state oral inarigivir on the single dose PK of oral midazolam in healthy subjects.

5.2 Secondary

- To evaluate the safety and tolerability of a single oral dose of midazolam, a single oral dose of inarigivir, and multiple oral doses of inarigivir administered without and with a single oral dose of midazolam in healthy subjects
- To assess the PK of inarigivir after single and multiple oral doses in healthy subjects

5.3 Exploratory

- To evaluate the PD following single and multiple oral doses of inarigivir in healthy subjects

6.0 Study Design

This will be a Phase 1, single-center, open-label, fixed-sequence, drug-drug interaction (DDI) study in 16 healthy subjects to assess the effect of multiple doses of oral inarigivir on the single dose PK of oral midazolam. Also, the PK and PD of inarigivir after single and multiple oral doses, and the PK of midazolam after single oral doses will be assessed.

The following treatments are planned to be administered under fasted conditions:

- On Day 1, a single oral dose of 2 mg midazolam will be administered (Treatment A).

- On Day 3, a single oral dose of 400 mg inarigivir will be administered (Treatment B).
- From Day 6 to Day 18, a single oral dose of 400 mg inarigivir will be administered once daily each day (Treatment C).
- On Day 19, a single oral dose of 400 mg inarigivir will be co-administered with a single oral dose of 2 mg midazolam (Treatment D).

6.1 Sample Size Considerations

Based on the intra-subject coefficient of variation of 19.7% found for AUC_{0-t} of midazolam in a previous interaction study (Winter et al.) and assuming an expected geometric mean ratio (test/reference) of 1.00, a significance level of 5% (α=0.05) and a default no-effect boundary of 80-125%, a sample size of 14 subjects results in at least 80% power (calculated using SAS PROC POWER). Accounting for early-termination subjects, a sample size of 16 subjects is considered to be sufficient.

6.2 Randomization

This is a fixed sequence study in which all subjects get the same treatment. No randomization is required.

7.0 Overview of Planned Analysis

7.1 Changes from Protocol

There are no changes from the protocol.

7.2 Interim Analysis and Key Results

There will be no interim analyses or summaries of data provided prior to the delivery of the full set of post-lock tables, figures and listings (TFLs).

7.3 Final Analysis

Draft TFLs will be provided with the draft CSR. After Sponsor comments have been incorporated, the TFLs will be finalized and incorporated in the final CSR.

8.0 Data Review

8.1 Data Management

Data handling and transfer will take place under the PRA Data Management Plan for the study.

8.2 Acceptance of Data for Summarization

Programming of analysis datasets and TFLs may be ongoing during the data management of the study. However, programming of analysis datasets and TFLs will be completed and quality controlled (QC'd) after database lock. Only quality assured (QA'd) results released by the Safety Laboratory, Bioanalytical Laboratory, or other external data source will be used for the programming of analysis datasets and TFLs for the final CSR. Any data values requiring investigation or corrections that are identified while programming the analysis datasets and TFLs will be sent to the project Data Manager. If the issue affects the TFLs the Programmer or Statistician who identified the issue will follow it to resolution.

9.0 Definitions and General Analysis Methods

9.1 Analysis Data Presentation

9.1.1 Rounding

In listings, data will be presented with the same precision as the original data. Derived data will be rounded for presentation purposes.

Derived PK parameters, except T_{max}, will be reported with a precision of 3 significant digits or as integers when the value is >=1000. T_{max} values will be reported with 2 decimals.

For all summaries, all descriptive statistics will be presented with the same precision (number of decimals) as the data they are calculated from. Frequency percentages will be presented with 1 decimal.

P-values will be reported to 4 decimal places; p-value less than 0.0001 will be reported as p <0.0001.

9.1.2 Imputation

Except for the substitution of any PK concentrations below the lower limit of quantification (LLOQ) (see Section 16.2.1) and missing start or end date/times of Adverse Events (AEs) for the calculation of onset and duration (see Section 18.1.1), any missing data will not be imputed

9.1.3 Descriptive Statistics

Unless otherwise indicated, continuous variables will be summarized with the following descriptive statistics: n (number of observations), (arithmetic) mean, standard deviation (SD), minimum (min) value, median, and maximum (max) value. For PK data, the coefficient of variation (CV, geometric mean and coefficient of variation of the geometric mean (geoCV) will be presented additionally.

Categorical data will be summarized with frequencies and percentages. Percentages by categories will be based on the number of subjects exposed within a treatment.

For categorical data the categories will be presented in the tables exactly as they appear in the CRF / Database. Categories will not be displayed for zero counts.

9.1.4 Pooling

Summary statistics will be calculated by treatment (day and time point, if applicable).

9.1.5 Unscheduled Measurements

Unscheduled measurements will be included in the listings. With the exception of unscheduled measurements used for baseline, unscheduled measurements will be excluded from the descriptive statistics and statistical analysis. However, unscheduled measurements that were performed immediately after the scheduled measurement, in case of a previous measurement error (e.g. equipment failure), will not be excluded from the analysis. In these cases, original erroneous measurement will be excluded from the analysis.

9.2 Analysis Data Definitions

9.2.1 Baseline Definition

For PD assessments baseline for post dose evaluations is defined as the last observation recorded before inarigivir administration on Day 3. For safety assessments baseline for post-dose evaluations is defined as the last observation recorded before first study drug administration on Day 1 (first Midazolam administration).

The last observation can be an unscheduled / repeated measurement.

9.2.2 Treatment/Subject Grouping

Label	Treatment
Study Drug	inarigivir, midazolam
Treatment	Treatment A: single oral dose of 2 mg midazolam Treatment B: single oral dose of 400 mg inarigivir Treatment C: oral dose of 400 mg inarigivir once daily for 13 days Treatment D: single oral dose of 400 mg inarigivir coadministered with 2 mg midazolam

9.2.3 Common Variable Derivations

Variable	Data Type	Definition/Calculation
Analysis Period	All	There are 4 analysis periods: Period 1 – treatment period A (from day -1 until the start of next period) Period 2 – treatment period B (from day 3 pre-dose until the start of next period) Period 3 – treatment period C (from day 6 pre-dose until the start of next period) Period 4 – treatment period D (from day 19 pre-dose until follow up)
Change from Baseline	All	Post-dose Observation minus Baseline Observation
Analysis Relative Day (Prior to Dose)	All	Date of Measurement minus Dose Date
Analysis Relative Day (Post Dose)	All	Date of Measurement minus Dose Date +1
Scheduled Time	All	Planned time of the assessment relative to the first dose date/time
Actual Time	PC	Actual time of the assessment relative to the first dose date/time
Relative Scheduled Time	PC	Planned time of the assessment relative to the dosing interval
Relative Actual Time	PC	Actual time of the assessment relative to the dosing interval
Sampling Time Deviation	PC	Actual Sampling time minus planned time within analysis period
Duration	AE	Stop date/time minus start date/time, presented in dd:hh:mm.

9.2.4 QC

The analysis datasets and the TFLs will be QC'd according to the general PRA EDS QC plan.

9.2.4.1 Critical Data

The QC plan requires datasets be classified as critical or non-critical. As the primary objective of this study is to assess the effect of steady-state oral inarigivir on the single dose PK of oral midazolam, the datasets considered critical are subject level and pharmacokinetic (ADSL, ADPC and ADPP).

9.2.5 ADaM Datasets and Metadata

The analysis datasets will be generated in accordance with Clinical Data Interchange Standard Consortium (CDISC) Analysis Data Model (ADaM) Version 2.1.

ADaM compliant datasets will be delivered to the sponsor. A define.xml file version 2 with the corresponding metadata will be included. Analysis results metadata are excluded. The define.xml-file and corresponding datasets will be delivered at the end of the study.

9.3 Software

The statistical analysis and reporting will be done using SAS® for Windows™ Version 9.4 or higher (SAS Institute, Inc.).

PK parameter calculations will primarily be done using Phoenix® WinNonlin® version 6.3 or higher (Pharsight, Inc.). Additional PK computations may be performed in SAS®.

9.4 Statistical Methods

9.4.1 Statistical Outlier Determination

No statistical outlier analysis is planned.

9.4.2 Predetermined Covariates and Prognostic Factors

There are no predetermined covariates or prognostic factors.

9.4.3 Hypothesis Testing

Unless otherwise stated all significance testing will be 2-sided at the significance level of 0.05.

9.5 TFL Layout

Report layout will be according to the PRA EDS – ICH E3 compliant – CSR Template. The layout of TFLs will be according to the PRA EDS standards.

No table shells will be provided. The TFLs will be provided in Adobe PDF format.

Format:

- Page size: <A4>
- Data in listings will be sorted by subject number, treatment, day and time point.
- Data In tables will be sorted by treatment, day and time point.
- Column titles will be in title case letters.
- All tables and listings will be in landscape format.
- The following treatment labels will be used in the TFLs:
  - Treatment A
  - Treatment B
  - Treatment C
  - Treatment D

10.0 Analysis Sets

Analyses	Safety Set	Pharmacokinetic Set	Pharmacodynamic Set
Disposition Summaries	✓		
Safety Assessments	✓		
Baseline Characteristics	✓		
Primary Analysis		✓	
PK Concentrations	✓		
PK Parameters		✓	
PD Parameters			✓

10.1 Safety Set

The safety set will consist of subjects who receive at least one dose of midazolam or inarigivir. This set will be used for the safety data summaries, baseline characteristic summaries, and PK concentration summaries.

10.2 Pharmacokinetic Set

The PK set will consist of subjects included in the safety set who have sufficient concentration-time data to calculate reliable estimates of the PK parameters. This set will be used for primary analysis.

10.3 Pharmacodynamic Set

The PD set will consist of all subjects included in the safety set for whom the PD data are considered sufficient and interpretable.

11.0 Subject Disposition

A listing containing the information for each subject completing the study and study withdrawal will be presented. The number and percentage of subjects enrolled, dosed, and members of each analysis set will be presented. The number and percentage of subjects who withdrew from the study prematurely and a breakdown of the corresponding reasons for withdrawal will also be presented.

12.0 Protocol Deviations and Violations

Protocol deviations/violations will be included in the CSR.

13.0 Demographic and Baseline Characteristics

13.1 Demographics

All demographic data as collected during the screening visit will be listed by subject.

Subject demographics will be summarized descriptively. The summary will include the subjects' age (in years), gender, race, ethnicity, weight (in kg), height (in cm), and BMI (Body Mass Index) (in kg/m^2). Demographics will be summarized for the safety and PK.

13.2 Medical History

Medical history will be listed.

13.3 Other Baseline Characteristics

Drug and alcohol screen: The results of urine drug screen and urine alcohol test at screening and admission will be listed.

Serology: The results of serology (Hepatitis B surface antigen [HBsAg], anti-HCV and anti-HIV) at screening will be listed.

Pregnancy test (females only): The results of serum pregnancy test at screening and urine pregnancy test at admission will be listed. Non-compliance to in- or exclusion criteria (if any) will be listed

14.0 Concomitant Medications

Concomitant medication will be listed. Medications with an end date prior to the first dose of study drug (midazolam dose on Day 1) will be considered prior medications and will be noted in the listing. If a partial date allows a medication to be considered concomitant it will be categorized as such.

15.0 Treatment Compliance and Exposure

Exposure data will be listed by subject.

16.0 Pharmacokinetic Analyses

16.1 Pharmacokinetic Variables

PK concentrations will be collected in plasma and urine. The analysis of inarigivir and its metabolites Rp-SB 9000 and Sp-SB 9000 in plasma, metabolites Rp-SB 9000 and Sp-SB 9000 in urine, and midazolam and its metabolite 1′-OH midazolam in plasma will be performed at Q2 Solutions (New York, USA) using validated liquid chromatography-mass spectrometry/mass spectrometry methods.

16.1.1 Plasma Variables

16.1.1.1 Concentrations

- Plasma concentrations of inarigivir
- Plasma concentrations of metabolite Rp-SB 9000
- Plasma concentrations of metabolite Sp-SB 9000
- Plasma concentrations of midazolam
- Plasma concentrations of metabolite 1'-OH midazolam

16.1.1.2 Parameters

- PK Parameters for inarigivir, Rp-SB 9000 and Sp-SB 9000 as defined in Table 1
- PK Parameters for midazolam and 1'-OH midazolam as defined in Table 1

Table 1: Plasma Parameters

Param-eter	Description	Midazolam ,1'-OH Midazolam Day 1	Midazolam ,1'-OH Midazolam Day 19	Inarigivir, Rp-SB 9000, Sp-SB 9000 following single dose on Day 3	Inarigivir, Rp-SB 9000, Sp-SB 9000 following multiple doses on Day 19	SAS Program-ming Notes
C_{max}	Maximum plasma concentration. Observed peak analyte concentration obtained directly from the experimental data without interpolation, expressed in concentration units	X	X	X	X	Cmax from WNL
T_{max}	Time to maximum plasma concentration. First observed time to reach peak analyte concentration obtained directly from the experimental data without interpolation, expressed in time units.	X	X	X	X	Tmax from WNL
C_{min}	Minimum plasma concentration during dosing interval in steady state				X	In SAS
AUC_{0-t}	Area under the plasmaconcentration-time curve (time 0 to time of last quantifiable concentration).	X	X	X		AUClast from WNL
AUC_{0-inf}	Area under the plasma concentration-time curve (time 0 to infinity). Percent extrapolation less than or equal to 20% is required to obtain a reliable AUC0-inf.	X	X	X		AUCINF_obs from WNL If AUC_%Extrap_obs >20% will be excluded from descriptive statistics.
$\%AUC_{extra}$	percentage of the AUC0-inf that is due to the extrapolation	X	X	X		%AUCext = ([AUC0-inf – AUC0-t]/AUC0-inf) * 100
AUC_{0-tau}	Area under the plasma concentration-time curve over the dosing interval (time 0 to 24hr).				X	AUC0-tau from WNL where tau is equal to 24hr.
RAUC	Ratio for AUC0-tau of Day 19 vs AUC0-inf of Day 3				X Rp-SB 9000 and Sp-SB 9000 only	RAUC = AUC0-tau steady-state/AUC0-inf single dose
RC_{max}	Ratio for				X	RCmax = Cmax steady-

Param-eter	Description	Midazolam ,1'-OH Midazolam Day 1	Midazolam ,1'-OH Midazolam Day 19	Inarigivir, Rp-SB 9000, Sp-SB 9000 following single dose on Day 3	Inarigivir, Rp-SB 9000, Sp-SB 9000 following multiple doses on Day 19	SAS Program-ming Notes
	Cmax of Day 19 vs Cmax of Day 3				Rp-SB 9000 and Sp-SB 9000 only	state/Cmax single dose
kel	Terminal phase rate constant calculated by linear regression of the terminal log-linear portion of the concentration vs. time curve. Linear regression of at least three points and an r^2 adjusted greater than 0.80 are required to obtain a reliable kel.	X	X	X		Lambda_z from WNL If Rsqadj ≤ .80 then parameter will be flagged, and excluded from descriptive statistics.
t1/2	Terminal phase half-life expressed in time units	X	X	X		HL_Lambda_z from WNL
CL/F	Apparent oral clearance	X (midazolam only)		X (inarigivir only)	X (inarigivir only)	CL = Dose/AUC0-inf or AUC0-tau
Vd/F	Apparent volume of distribution	X (midazolam only)		X (inarigivir only)	X (inarigivir only)	Vd/F = (CL/F)/kel
MR_AUC$_{(0-t)}$	Ratio of metabolite AUC0-t to parent AUC0-t, corrected for molecular weight.			X (Rp-SB 9000 and Sp-SB 9000 only)		In SAS
MR_AUC$_{(INF)}$	Ratio of metabolite AUC0-inf to parent AUC0-inf, corrected for molecular weight.	X (1'OH-midazolam only)	X (1'OH-midazolam only)	X (Rp-SB 9000 and Sp-SB 9000 only)		In SAS
MR_AUC$_{0-tau}$	Ratio of metabolite AUC0-tau to parent AUC0-tau, corrected for molecular weight.				X (Rp-SB 9000 and Sp-SB 9000 only)	In SAS
MR_C$_{max}$	Ratio of metabolite Cmax to parent Cmax, corrected for molecular weight.	X (1'OH-midazolam only)	X (1'OH-midazolam only)	X (Rp-SB 9000 and Sp-SB 9000 only)	X (Rp-SB 9000 and Sp-SB 9000 only)	In SAS

Note: AUCs will be calculated using linear up / log down, expressed in units of concentration x time.

16.1.2 Urine Variables

16.1.2.1 Amounts Excreted

- Amount of Rp-SB 9000 excreted in urine (Ae)
  - Calculated as the urine volume times the urine concentration for each interval.
- Amount of Sp-SB 9000 excreted in urine
  - Calculated as the urine volume times the urine concentration for each interval.

16.1.2.2 Parameters

- PK Parameters for Rp-SB 9000 and Sp-SB 9000 as defined in table 2

Table 2: Urine Parameters

Parameter	Description	Rp-SB 9000 and Sp-SB 9000 Day 3	SAS Programming Notes
Ae0-t (urine)	Total amount excreted into urine to time t, obtained by adding the amounts excreted over each collection interval.	X	Summation t1-tn(Concentration (ng/mL)ti-t2*volume(mL)t1-t2)
fe (urine)	Fraction (%) of the administered dose excreted into urine fe = (Ae0-t (urine) / Dose) * 100 (after correction for molecular weight).	X	Ae0-t/XXmg*100
CLr	Renal clearance	X	Quotient of the cumulative urinary excretion of the drug up to some fixed time t where Ae0-t and AUC are taken over the largest common interval in which plasma and urine concentrations are both quantifiable. Ae0-t and AUC0-t. CLr=Ae0-t/AUC0-t

16.2 Pharmacokinetic Summaries

16.2.1 Pharmacokinetic Concentrations

Individual plasma concentration data for inarigivir, Rp-SB 9000, Sp-SB 9000, midazolam and 1'-OH midazolam will be presented together with descriptive statistics by treatment, day and scheduled time point

Plasma concentrations below the quantifiable limit (BQL) will be set to ½ LLOQ in the computation of mean concentration values. If over ½ the subjects in a given cell have values BQL then the descriptive statistics will not be presented and will instead display as BQL for the mean and minimum. With the exception of maximum all other statistics will be missing.

Linear and semi-logarithmic plots of the geometric mean plasma concentrations by scheduled sampling time will be provided. The following plots will be presented:

- Midazolam geometric mean plasma concentrations for Treatment A (midazolam alone on Day 1) and Treatment D (midazolam + inarigivir on Day 19)

- 1'-OH midazolam geometric mean plasma concentrations for Treatment A (midazolam alone on Day 1) and Treatment D (midazolam + inarigivir on Day 19)
- Inarigivir geometric mean plasma concentrations for Treatment B (single dose of inarigivir on Day 3) and for Treatment D (midazolam + inarigivir on day 19)
- Rp-SB 9000 geometric mean plasma concentrations for Treatment B (single dose of inarigivir on Day 3) and for Treatment D (midazolam + inarigivir on day 19)
- Sp-SB 9000 geometric mean plasma concentrations for Treatment B (single dose of inarigivir on Day 3) and for Treatment D (midazolam + inarigivir on day 19)

These plots will show time in hours. The plots will match the summary table results and will not have an observation at a given time point if more than half of the subjects have values BQL.

Linear and semi-logarithmic plots of the combined individual plasma concentrations by scheduled sampling time for each treatment, day and analyte separately will be provided. These plots will show time in hours.

Linear and semi-logarithmic plots of the individual plasma concentrations by actual sampling time will be provided by subject. The following plots will be presented for each subject:

- Midazolam plasma concentrations for Treatment A (midazolam alone on Day 1) and Treatment D (midazolam + inarigivir on Day 19)
- 1'-OH midazolam plasma concentrations for Treatment A (midazolam alone on Day 1) and Treatment D (midazolam + inarigivir on Day 19)
- Inarigivir plasma concentrations for Treatment B (single dose of inarigivir on Day 3) and for Treatment D (midazolam + inarigivir on day 19)
- Rp-SB 9000 plasma concentrations for Treatment B (single dose of inarigivir on Day 3) and for Treatment D (midazolam + inarigivir on day 19)
- Sp-SB 9000 plasma concentrations for Treatment B (single dose of inarigivir on Day 3) and for Treatment D (midazolam + inarigivir on day 19)

These plots will show time in hours. Individual plots will use the BQL handling procedure described below for "Pharmacokinetic Parameters".

A plot of geometric mean of the Rp-SB 9000 and Sp-SB-9000 trough plasma concentrations over time (using only the trough plasma levels on days 8-20) will also be presented.

Time deviations from planned dosing and comments will be listed.

16.2.2 Pharmacokinetic Amounts Excreted

Descriptive statistics (number of subjects, arithmetic mean, standard deviation, CV, median, minimum, and maximum) will be used to summarize the urine amounts excreted for Rp-SB 9000 and Sp-SB 9000 at each scheduled time interval.

All urinary Rp-SB 9000 and Sp-SB 9000 concentrations and volumes per interval and derived urinary excretion and cumulative excretion per interval will be listed.

16.2.3 Pharmacokinetic Parameters

PK parameters for inarigivir, Rp-SB 9000, Sp-SB 9000, midazolam and 1'-OH midazolam will be estimated using non-compartmental methods with WinNonlin®.

The plasma PK parameters will be estimated from the concentration-time profiles. In estimating the PK parameters, BQL values at the beginning of the profile will be set to zero. BQL values that occur after the first quantifiable point will be considered missing. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times. If the actual time or dose time is missing, the scheduled time may be substituted in order to calculate the PK parameter.

Individual plasma PK parameters will be presented together with descriptive statistics by treatment and day. Subjects excluded from PK set will be flagged and excluded from descriptive statistics. For tmax, only median, min and max will be presented.

The points to be included in the kel range will be determined by the pharmacokineticist after inspection of the semi-log concentration-time profiles. At least 3 points will be required to be used. The C_{max} data point will not be included. Parameters based on $r^2_{adjusted}$ below 0.80 or $\%AUC_{extra}$ above 20% will be excluded from descriptive statistics.

Individual urine PK parameters will be presented together with descriptive statistics. Subjects excluded from the PK analysis set will be flagged and excluded from calculation of descriptive statistics.

The individual values and geometric mean of midazolam and 1-OH midazolam C_{max}, AUC_{0-t} and AUC_{0-inf} will be presented graphically versus treatment with intra-subject lines indicating which values are from the same subject.

16.2.3.1 Drug-Drug Interaction

Primary analysis will be performed on all subjects belonging to PK set. The effect of steady state inarigivir on the PK of midazolam and 1'-OH-miazolam will be assessed. The effect of inarigivir on the natural log-transformed C_{max}, AUC_{0-t} and AUC_{0-inf} will be assessed with a linear mixed effects model. Treatment will be used as fixed effect and subject will be used as a random effect. Point estimates for the means and corresponding 90% confidence intervals for the differences in means between the two treatments (Treatment D versus Treatment A) will be obtained from the linear mixed effects model and exponentiated to obtain geometric means, geometric mean ratios and respective 90% confidence intervals on the original scale. The absence of interaction will be concluded if these CIs are included in the bioequivalence range (80-125%).

17.0 Pharmacodynamic Analysis

The analysis of the cytokine panel will be conducted at MLM Medical Labs GmbH (Moenchengladbach, Germany).

17.1 Pharmacodynamic Variables

Exploratory PD endpoints will summarize changes in serum levels of the cytokine panel after single dose (Day 3 before and after dosing) and multiple doses of inarigivir (predose on day 11, 18, 19, after dosing on Day 18).

17.1.1 PD Variables

Serum levels of:

- C-X-C motif chemokine 10 (CXCL10; also known as IFN gamma-induced protein 10 [IP-10])
- interleukin (IL)-6
- IL-12
- tumor necrosis factor alpha (TNF-α)
- IFN-α
- IFN-γ

17.2 Pharmacodynamic Summaries

17.2.1 Serum Levels Cytokine Panel

Serum levels below the BQL will be set to zero in the computation of mean concentration values (and changes from baseline). Descriptive statistics (number of subjects, mean, standard deviation, CV, median,

min, and max) will be used to summarize the serum levels and changes from baseline (absolute values) by treatment and day. If over half the subjects in a given cell have values BQL then only min and max will be presented.

Changes from baseline (absolute change) will be graphically displayed by treatment.

Linear plots of the arithmetic mean change from baseline by scheduled sampling time will be provided for each analyte separately and will present data from Day 3 (Treatment B, single dose of inarigivir) and Day 18 (Treatment C, daily inarigivir administration from day 6) together.

Linear plots of the combined individual change from baseline (absolute change) profiles by actual sampling time will be provided by analyte and treatment.

All individual subject PD serum levels, and changes from baseline (absolute change) will be listed together with the descriptive statistics.

18.0 Safety Analyses

18.1 Safety Variables

The following safety variables will be summarized:

- Adverse Events (AEs)
- Vital Signs
  - Supine Blood Pressure
    - Systolic Blood Pressure (SBP)
    - Diastolic Blood Pressure (DBP)
  - Pulse rate
  - Body temperature
  - Respiratory rate
- Electrocardiograms (ECG)
  - Heart Rate
  - PR Interval
  - QRS-Duration
  - QT Interval
  - QTc (Fridericia's) Interval
- Clinical Laboratory Evaluations
  - Clinical Chemistry
  - Hematology
  - Urinalysis
  - Coagulation
- Physical Examination

18.1.1 Adverse Events

All AE summaries will include only treatment emergent adverse events. A Treatment Emergent Adverse Event (TEAE) is defined as any event not present prior to the first administration of the study drug or any event present that worsens in either severity or frequency following exposure of the study drug.

TEAEs occurring following dosing in a specific analysis period but before first dosing in the next analysis period will be attributed to that specific analysis period. If the time is missing for an AE on a dosing day then the AE will be attributed to the treatment given on that day.

A breakdown of the number of adverse events and number and percentage of subjects reporting each AE, categorized by body system and preferred term coded according to the latest Medical Dictionary for Regulatory Activities (MedDRA), will be presented by treatment and overall. Counting will be done by event and subject; subjects will only be counted once within each body system or preferred term. This summary

will be provided for all TEAEs and for related TEAEs. Relationship to study drug recorded in the eCRF as 'possibly', 'likely' or 'definitely' will be categorized as related to study drug, while eCRF relationship categories 'none' and 'unlikely' will be considered not related.

A summary of events reported, categorized by relationship (categories: related, not related) and severity (categories: mild, moderate, severe, life-threatening, death), will also be provided by treatment and overall. Subjects with multiple events will be counted under the category of their most severe or most related event.

A listing of adverse events leading to study discontinuation will be provided.

Summary tables will present the counts in descending order by system organ class and preferred term (within a system organ class) based on the number of subjects experiencing the event in the total group.

All adverse events (including non-treatment-emergent events) recorded on the eCRF will be listed.

The following missing data will be imputed as defined (for calculations only / will not be presented):

- Missing AE start and / or end times for the calculation of onset and duration will be assumed to be at 00:01 for a start time and 23:59 for end times
- Missing AE severity or relationship will be assumed to be severe or related, respectively
- Missing AE start times for the determination of treatment emergence will be assumed to occur after treatment unless partial date documents the AE as happening prior to treatment
- Missing AE start times for the determination of treatment assignment will be assumed to occur after treatment on the recorded date one minute after dosing
- Missing AE start date will be assumed to be after treatment for the determination of TEAE but will not be attributed to a specific treatment

18.1.2 Deaths and Serious Adverse Events

A listing of deaths and other serious adverse events (SAE) will be provided by subject.

18.1.3 Laboratory Data

Clinical laboratory data will be presented using units from the study data tabulation model (SDTM) Controlled Terminology.

All laboratory data will be listed (observed values and changes from baseline), including laboratory variables not listed in the protocol. An indication if the parameter is outside of the reference range will be included in the listing. A separate listing, including out-of-range values will also be provided. Reference ranges will be used directly from the clinical laboratory. A separate listing with comments will be provided, if applicable.

Descriptive statistics summarizing continuous laboratory results of clinical chemistry, hematology and coagulation (observed and derived changes from baseline) by treatment, day and scheduled time will be provided.

18.1.4 Vital Signs

All vital signs measurements and derived changes from baseline will be listed.

Vital signs parameters and corresponding changes from baseline will be summarized by treatment, day and scheduled time using descriptive statistics.

18.1.5 Electrocardiograms

All ECG parameters (including changes from baseline) and the corresponding abnormalities and physicians conclusions will be listed by subject.

Descriptive statistics will be provided to summarize ECG parameters (observed and changes from baseline) by treatment, day and scheduled time.

18.1.6 Physical Examination

The findings and changes during the study will be listed.

19.0 References

SAS Institute, Inc., SAS® Version 9.4 software, Cary, NC.

Clinical Study Protocol. A PHASE 1, OPEN-LABEL, FIXED-SEQUENCE, DRUG-DRUG INTERACTION STUDY BETWEEN MULTIPLE ORAL DOSES OF INARIGIVIR SOPROXIL AND A SINGLE ORAL DOSE OF MIDAZOLAM IN HEALTHY SUBJECTS. Version 3.0, Final, 30 Apr 2018.

Winter H, Egizi E, Erondu N, Ginsberg A, Rouse DJ, Severynse-Stevens D, Pauli E, Everitt D, 2013. Evaluation of Pharmacokinetic Interaction between PA-824 and Midazolam in Healthy Adult Subjects. Antimicrob Agents Chemother 2013 Aug; 57(8): 3699-3703.

Appendix 1: Glossary of Abbreviations

Glossary of Abbreviations:	
ADaM	Analysis data model
AE	Adverse event
ANOVA	Analysis of variance
BMI	Body mass index
BQL	Below the quantifiable limit
CDISC	Clinical Data Interchange Standard Consortium
CSR	Clinical study report
CV	Coefficient of variation
CXCL10	C-X-C Motif Chemokine 10
DBP	Diastolic Blood Pressure
DDI	Drug-Drug Interaction
ECG	Electrocardiogram
eCRF	Electronic case report form
EDS	Early Development Services
geoCV	Coefficient of Variation of the Geometric Mean
ICH	The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IL	Interleukin
IP-10	IFN gamma-induced protein 10
LLOQ	Lower limit of quantification
max	Maximum Value
MedDRA	Medical Dictionary for Regulatory Activities
min	Minimum Value
PD	Pharmacodynamics
PK	Pharmacokinetics
QA'd	Quality assured
QC'd	Quality controlled
SAP	Statistical analysis plan
SAE	Serious adverse event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SDTM	Study data tabulation model
TEAE	Treatment-emergent adverse event

TFL(s)	Tables, figures and listings
WNL	WinNonlin

Appendix 2: Schedule of Assessments

		Treatment A (Midazolam)				Treatment B (inarigivir single dose)				Treatment C (inarigivir multiple dose)	Treatment D (inarigivir and midazolam)			
Visit	**Screening**	**Pre-Treatment**		**Treatment**		**Pre-Treatment**	**Treatment**			**Treatment**	**Pre-Treatment**	**Treatment**		**Follow-up**
Study Day	**-28 to -1**	**-1**	**1 (Pre-dose)**	**1**	**2**	**3 (Pre-dose)**	**3**	**4**	**5**	**6-18**	**19 (Pre-dose)**	**19**	**20**	**5 to 9 days after day of discharge (Day 25 to Day 29)**
Confinement		X	X	X	X	X	X	X	X	X	X	X	X	
Ambulatory	X													X
Admission		X												
Discharge													X	
Informed consent	X													
Medical history	X													
Demographics	X													
Physical examination	X													X
Body weight and height (including BMI calculation)	X													
Serology[1]	X													
Drug and alcohol screen	X	X												
Serum pregnancy test (females only)	X													
Urine pregnancy test (females only)		X												X
Clinical chemistry, hematology and coagulation[2]	X	X			X	X		X		X	X		X	X
Urinalysis	X	X												X
12-lead ECG[3]	X		X		X	X		X		X	X		X	X
Vital signs[4]	X		X		X	X		X		X	X		X	X
Eligibility check	X	X	X											
Randomization			X											
Midazolam administration[5]				X								X		
Inarigivir administration[6]							X			X		X		

		Treatment A (Midazolam)				Treatment B (inarigivir single dose)				Treatment C (inarigivir multiple dose)	Treatment D (inarigivir and midazolam)			
Visit	**Screening**	**Pre-Treatment**		**Treatment**		**Pre-Treatment**	**Treatment**			**Treatment**	**Pre-Treatment**	**Treatment**		**Follow-up**
Study Day	**-28 to -1**	**-1**	**1 (Pre-dose)**	**1**	**2**	**3 (Pre-dose)**	**3**	**4**	**5**	**6-18**	**19 (Pre-dose)**	**19**	**20**	**5 to 9 days after day of discharge (Day 25 to Day 29)**
Blood sampling for PK: midazolam and metabolite[7]			X	X							X	X		
Blood sampling for PK: inarigivir and its metabolites Rp-SB 9000 and Sp-SB 9000[8]						X	X	X	X	X	X	X	X	
Urine collection for PK: metabolites Rp-SB 9000 and Sp-SB 9000[9]						X	X	X	X					
Blood sampling for PD: cytokine panel[10]						X	X	X	X	X	X			
Blood sampling for PD: PBMCs[11]						X	X	X		X				
Blood sampling for pharmacogenomics[12]			X											
Previous and concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse event monitoring		X	X	X	X	X	X	X	X	X	X	X	X	X

1 Serology at screening will consist of HBsAg, anti-HCV and anti-HIV 1 and 2.

2 Clinical chemistry, hematology and coagulation: at screening, at admission on Day -1 and at 24 hours after dosing on Day 1, at pre-dose on Day 3 and at 24 hours after dosing on Day 3, at pre-dose on Days 6, 11, 15 and 19, at 24 hours after dosing on Day 19, and at follow-up.

3 12-lead ECG: at screening, at pre-dose on Day 1, at 24 hours after dosing on Day 1, at pre-dose on Day 3 and at 24 hours after dosing on Day 3, at pre-dose on Days 6, 11, 15 and 19, at 24 hours after dosing on Day 19, and at follow-up.

4 Vital signs (supine systolic and diastolic blood pressure, pulse, body temperature and respiratory rate): at screening, at pre-dose on Day 1, at 24 hours after dosing on Day 1, at pre-dose on Day 3 and at 24 hours after dosing on Day 3, at pre-dose on Days 6, 11, 15 and 19, at 24 hours after dosing on Day 19, and at follow-up.

5 On Day 1 and Day 19, a single oral dose of midazolam will be administered. *On Day 19, midazolam will be administered together with inarigivir (within a maximum of 10 minutes).*

6 On Day 3, a single oral dose of inarigivir will be administered; from Day 6 to Day 18, a single oral dose of inarigivir will be administered each day; on Day 19: a single oral dose of inarigivir will be administered. *On Day 19, midazolam will be administered together with inarigivir (within a maximum of 10 minutes).*

7 Blood sampling for PK of midazolam and metabolite in plasma: at pre-dose on Days 1 and 19, and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 hours after dosing on Days 1 and 19.

8 Blood sampling for PK of inarigivir and its metabolites Rp-SB 9000 and Sp-SB 9000 in plasma: at pre-dose on Day 3 and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24 and 48 hours after dosing on Day 3, at pre-dose on Days 8, 9, 10, 12, 13, 14, 16, 17, 18 and 19, and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16 and 24 hours after dosing on Day 19.

		Treatment A (Midazolam)				Treatment B (inarigivir single dose)				Treatment C (inarigivir multiple dose)	Treatment D (inarigivir and midazolam)			
Visit	**Screening**	**Pre-Treatment**		**Treatment**		**Pre-Treatment**	**Treatment**			**Treatment**	**Pre-Treatment**	**Treatment**		**Follow-up**
Study Day	**-28 to -1**	**-1**	**1 (Pre-dose)**	**1**	**2**	**3 (Pre-dose)**	**3**	**4**	**5**	**6-18**	**19 (Pre-dose)**	**19**	**20**	**5 to 9 days after day of discharge (Day 25 to Day 29)**

9 Urine collection for PK of metabolites Rp-SB 9000 and Sp-SB 9000: at pre-dose on Day 3 (within 12 hours prior to dosing) and over 0-6, 6-12, 12-24, 24-36 and 36-48 hour collection intervals after dosing on Day 3.

10 Blood sampling for PD (cytokine panel) in serum: at pre-dose on Day 3 and at 2, 4, 6, 12, 24 and 48 hours after dosing on Day 3, at pre-dose on Days 11 and 18, at 2, 4, 6 and 12 hours after dosing on Day 18, and at pre-dose on Day 19.

11 Blood sampling for PD (PBMCs): at pre-dose on Day 3 and at 2, 6 and 24 hours after dosing on Day 3, and at pre-dose on Day 18 and at 4 and 12 hours after dosing on Day 18.

12 Blood sampling for pharmacogenomics is optional for all subjects. The blood sample will be taken at pre-dose on Day 1.

BMI: body mass index; ECG: electrocardiogram; HBsAg: hepatitis B surface antigen; HCV: hepatitis C virus; HIV: human immunodeficiency virus; PBMC: peripheral blood mononuclear cell; PD: pharmacodynamic(s); PK: pharmacokinetic(s)

Appendix 3: List of In-Text Outputs

List of CSR In-Text Outputs:		
Output	**Title**	**Population Set**
Table 1	Subject Demographics and Characteristics	Safety
Figure 1	Geometric Mean Midazolam Plasma Concentrations by Treatment versus Time (Days 1 and 19)	PK
Figure 2	Geometric Mean 1-OH Midazolam Plasma Concentrations by Treatment versus Time (Days 1 and 19)	PK
Figure 3	Geometric Mean Inarigivir Concentrations by Treatment versus Time (Days 3 and 19)	PK
Figure 4	Geometric Mean Rp-SB 9000 Concentrations by Treatment versus Time (Days 3 and 19)	PK
Figure 5	Geometric Mean Sp-SB 9000 Concentrations by Treatment versus Time (Days 3 and 19)	PK
Table 2	Summary of Midazolam and 1-OH Midazolam PK Parameters	PK
Table 3	Summary of Inarigivir, Rp-SB 9000 and Sp-SB 9000 PK Parameters	
Table 4	Summary of Analysis on PK Drug-Drug interaction	PK
Table 5	Summary of All TEAEs by SOC and PT	Safety
Table 6	Summary of TEAEs by Treatment, Relationship and Severity	Safety

Appendix 4: List of End of Text Outputs

List of End of Text Tables and Figures:		
Output	**Title**	**Population Set**
Section 15.1 – Disposition and Demographic Data		
Table 15.1.1	Summary of Subject Disposition	Safety
Table 15.1.2.1	Summary of Demographics	Safety
Table 15.1.2.2	Summary of Demographics	PK
Section 15.2 – Pharmacokinetic Data		
Table 15.2.1	Individual and Summary Statistics of Midazolam and 1'OH-Midazolam Plasma Concentrations by Treatment	Safety
Table 15.2.2	Individual and Summary Statistics of Inarigivir, RP-SB 9000 and Sp-SB 9000 Plasma Concentrations by Treatment	Safety
Table 15.2.3	Individual and Summary Statistics of Midazolam and 1'OH-Midazolam Plasma PK Parameters by Treatment	PK
Table 15.2.4	Individual and Summary Statistics of Inarigivir, RP-SB 9000 and Sp-SB 9000 Plasma PK Parameters by Treatment	PK
Table 15.2.5	Statistical Assessment of Drug-interaction on Midazolam and 1'-OH Midazolam PK Parameters	PK
Figure 15.2.6	Geometric Mean Midazolam Plasma Concentrations by Treatment versus Time (Days 1 and 19) (Linear and Semi-Log Scale)	PK
Figure 15.2.7	Geometric Mean 1'OH-Midazolam Plasma Concentrations by Treatment versus Time (Days 1 and 19) (Linear and Semi-Log Scale)	PK
Figure 15.2.8	Geometric Mean Inarigivir Concentrations by Treatment versus Time (Days 3 and 19) (Linear and Semi-Log Scale)	PK
Figure 15.2.9	Geometric Mean Rp-SB 9000 Concentrations by Treatment versus Time (Days 3 and 19) (Linear and Semi-Log Scale)	PK
Figure 15.2.10	Geometric Mean Sp-SB 9000 Concentrations by Treatment versus Time (Days 3 and 19) (Linear and Semi-Log Scale)	PK

Figure 15.2.11	Combined Individual Midazolam and 1'-OH Midazolam Concentrations versus Time by Analyte, Treatment and Day (Linear and Semi-Log Scale)	Safety
Figure 15.2.12	Combined Individual Inarigivir, Rp-SB 9000 and Sp-SB 9000 Concentrations versus Time by Analyte, Treatment and Day (Linear and Semi-Log Scale)	Safety
Figure 15.2.13	Individual Midazolam Concentrations by Treatment versus Time (Days 1 and 19) (Linear and Semi-Log Scale)	Safety
Figure 15.2.14	Individual 1'-OH Midazolam Concentrations by Treatment versus Time (Days 1 and 19) (Linear and Semi-Log Scale)	Safety
Figure 15.2.15	Individual Inarigivir Concentrations by Treatment versus Time (Days 3 and 19) (Linear and Semi-Log Scale)	Safety
Figure 15.2.16	Individual Rp-SB 9000 Concentrations by Treatment versus Time (Days 3 and 19) (Linear and Semi-Log Scale)	Safety
Figure 15.2.17	Individual Sp-SB 9000 Concentrations by Treatment versus Time (Days 3 and 19) (Linear and Semi-Log Scale)	Safety
Figure 15.2.18	Rp-SB 9000 Geometric Mean Trough Plasma Concentrations over Time (Trough Plasma Levels on Days 8-20)	PK
Figure 15.2.19	Sp-SB 9000 Geometric Mean Trough Plasma Concentrations over Time (Trough Plasma Levels on Days 8-20)	PK
Figure 15.2.20	Midazolam Individual PK Parameters and Geometric Mean versus Treatment (with Intra-subject Lines)	PK
Figure 15.2.21	1-OH Midazolam Individual PK Parameters and Geometric Mean versus Treatment (with Intra-subject Lines)	PK
Table 15.2.22	Individual Values and Summary Statistics of Rp-SB 9000 and Sp-SB 9000 Amounts Excreted in Urine by Collection Interval	Safety
Table 15.2.23	Individual Values and Summary Statistics of Rp-SB 9000 and Sp-SB 9000 Urine PK Parameters	
Section 15.3 – Pharmacodynamic Data		
Table 15.3.1	Individual Values and Summary Statistics of Cytokine Panel Serum Levels	PD

Figure 15.3.2	Arithmetic Mean of Cytokine Panel Changes from Baseline in Serum by Analyte (Days 3 and 18)	PD
Figure 15.3.3	Combined Individual Changes from Baseline of Cytokine Panel in Serum by Analyte, Day and Treatment	PD
Section 15.4 – Safety Data		
15.4.1 Adverse Events		
Table 15.4.1.1	Summary of Treatment Emergent Adverse Events by System Organ Class and Preferred Term	Safety
Table 15.4.1.2	Summary of Related Treatment Emergent Adverse Events by System Organ Class and Preferred Term	Safety
Table 15.4.1.3	Summary of Treatment Emergent Adverse Events by Relationship to Study Drug and Severity	Safety
15.4.2 Listing of Deaths and Other Serious Adverse Events		
Table 15.4.2.1	Listing of Deaths and Other Serious Adverse Events	Safety
Table 15.4.3	Not part of TFL – Reserved for Narratives in CSR	
15.4.4 Clinical Laboratory		
Table 15.4.4.1	Listing of Out-of-Range Laboratory Values	Safety
Table 15.4.4.2	Summary of Clinical Laboratory Data - Clinical Chemistry	Safety
Table 15.4.4.3	Summary of Clinical Laboratory Data - Hematology	Safety
Table 15.4.4.4	Summary of Clinical Laboratory Data - Coagulation	Safety
15.4.5 Other Safety Parameters		
Table 15.4.5.1	Summary of Vital Signs	Safety
Table 15.4.5.2	Summary of 12-Lead Electrocardiogram	Safety

List of End of Text Listings:	
Output	**Title**
Section 16.2.1 – Disposition	

Listing 16.2.1	Subject Disposition
Listing 16.2.2	Not part of TFL – Reserved for protocol deviations in CSR
Section 16.2.3 – Excluded Subjects	
Listing 16.2.3	Analysis Sets
Section 16.2.4 – Demographics and Baseline Characteristics	
Listing 16.2.4.1	Subject Demographics
Listing 16.2.4.2	Medical History
Listing 16.2.4.3	Prior and Concomitant Medications
Listing 16.2.4.4	Drug and Alcohol Screen
Listing 16.2.4.5	Pregnancy Test
Listing 16.2.4.6	Serology
Section 16.2.5 - Compliance	
Listing 16.2.5.1	Study Dates
Listing 16.2.5.2	Deviations from Inclusion/Exclusion Criteria
Listing 16.2.5.3	Study Drug Administration
Section 16.2.6 – Response Data	
Listing 16.2.6.1	Individual Pharmacokinetic Plasma Concentrations Sample Time Deviations and Comments
Listing 16.2.6.2	Rp-SB 9000 and Sp-SB 9000 Concentrations in Urine, Volumes per Interval and Derived Amounts Excreted
Listing 16.2.6.3	Pharmacodynamic Serum Levels – Comments (if Applicable)
Section 16.2.7 – Adverse Events Data	
Listing 16.2.7.1	Adverse Events
Listing 16.2.7.2	Adverse Events Leading to Withdrawal
Section 16.2.8 – Laboratory Data	
Listing 16.2.8.1	Clinical Laboratory Results – Hematology
Listing 16.2.8.2	Clinical Laboratory Results – Chemistry
Listing 16.2.8.3	Clinical Laboratory Results – Urinalysis
Listing 16.2.8.4	Clinical Laboratory Results – Coagulation
Listing 16.2.8.5	Clinical Laboratory Results – Comments (if Applicable)
Section 16.2.9 Other Safety Data	
Listing 16.2.9.1	Vital Signs
Listing 16.2.9.2	12-Lead Electrocardiogram Results
Listing 16.2.9.3	Physical Examination

Other Appendix Outputs:	
Output	**Title**
Appendix 16.1.7	Randomization
Appendix 16.1.9.2	Statistical Appendices

20.0 Document History

Version Date	Modified/Reviewed By	Brief Summary of Changes (if created from a template, include template code)
22-May-2018	JKL	First draft
1-Jun-2018	HHK, PK	Review biostatistics and MW
2-Jul-2018	JKL	Finalized after sponsor review (no comments)