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

CONFIDENTIAL

Sponsor code: SBP-9200-HBV-202
PRA code: SPB881EC-178881
EudraCT number: 2018-000607-16

Inarigivir/midazolam drug-drug interaction study

Investigational product	Inarigivir soproxil (further named inarigivir and formerly known as SB 9200)
Clinical phase	Phase 1 study
Indication to be studied	Not applicable

SPONSOR	Spring Bank Pharmaceuticals, Inc. 86 South Street Hopkinton, MA 01748 USA
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Version 1.0, 06 March 2018

This study will be performed in compliance with the principles of Good Clinical Practice.

AUTHORIZATION OF CLINICAL STUDY PROTOCOL

The Sponsor and the Contract Research Organization agree to conduct the study as outlined in this clinical study protocol. Any modification of the clinical study protocol must be agreed upon by the Sponsor and the Contract Research Organization and must be documented in writing.

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SYNOPSIS

Study 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

Note: Inarigivir soproxil will be further named inarigivir in this document and is formerly known as SB 9200.

Short Study Title
Inarigivir/midazolam drug-drug interaction study

Study Codes
Sponsor code : SBP-9200-HBV-202
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Principal Investigator
Jeroen van de Wetering, MD

Objectives
Primary
- To assess the effect of steady-state oral inarigivir on the single dose pharmacokinetics (PK) of oral midazolam in healthy subjects

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

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

Design and Treatments
This will be a Phase 1, single-center, open-label, fixed-sequence, drug-drug interaction 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)

Study Schedule

Screening	:	Between Day -28 and Day -1 (admission)
Confinement period	:	1 period in the clinic from Day -1 (admission) to approximately 24 hours after the last study drug administration on Day 19 (Day 20: day of discharge)
Follow-up	:	5 to 9 days after day of discharge (between Day 25 and Day 29)

Subjects

Sixteen healthy male or female subjects are planned to be dosed in the study.

Main Criteria for Inclusion

Gender	:	Male or female
Age	:	18-55 years, inclusive, at screening
Body mass index	:	18.0-30.0 kg/m^2, inclusive, at screening

Study Drug

Active medication

Active substance	:	Inarigivir soproxil
Activity	:	Antiviral agent
In development for	:	Hepatitis B virus infection
Strength	:	100 mg
Dosage form	:	Oral tablet
Manufacturer	:	Patheon, Inc., Mississauga, Ontario, Canada

Probe substrate

Active substance	:	Midazolam
Activity	:	Cytochrome P450 (CYP)3A4 substrate
Indication	:	Sleep disorder
Strength	:	1 mg/mL; a dose of 2 mg will be administered
Dosage form	:	Oral administration of intravenous solution formulation
Manufacturer	:	Will be commercially acquired

Variables

Pharmacokinetics : Plasma concentrations of inarigivir and its active metabolites Rp-SB 9000 and Sp-SB 9000, urine concentrations of Rp-SB 9000 and Sp-SB 9000, and plasma concentrations of midazolam and its metabolite 1′-OH midazolam will be determined. The following PK parameters will be estimated using noncompartmental analysis, as appropriate:

- Single dose plasma PK parameters: C_{max}, t_{max}, k_{el}, $t_{½}$, AUC_{0-t}, AUC_{0-inf}, $\%AUC_{extra}$, CL/F and V_z/F for inarigivir and midazolam; C_{max}, t_{max}, k_{el}, $t_{½}$, AUC_{0-t}, AUC_{0-inf}, $\%AUC_{extra}$, and metabolite to parent ratio for C_{max}, AUC_{0-t}, and AUC_{0-inf} for Rp-SB 9000, Sp-SB 9000 and 1′-OH midazolam
- Multiple dose plasma PK parameters (steady-state): C_{max}, t_{max} and $AUC_{0-\tau}$ (for inarigivir, Rp-SB 9000 and Sp-SB 9000) and R_{ac} (based on Rp-SB 9000 and Sp-SB 9000 C_{max} and AUC parameters)
- Single dose urine PK parameters: Ae_{0-t}, CL_R and fe (for Rp-SB 9000 and Sp-SB 9000)

Safety : Adverse events, clinical laboratory, vital signs and 12-lead electrocardiogram

Pharmacodynamics : Cytokine panel: serum levels of C-X-C motif chemokine 10 (also known as interferon gamma-induced protein 10), interleukin 6, tumor necrosis factor alpha, interferon (IFN)-α and IFN-γ
Cytokine RNA expression in peripheral blood mononuclear cells

Statistical Methods

Sample size calculation	:	Based on the intra-subject coefficient of variation of 19.7% found for AUC_{0-t} of midazolam in a previous interaction study 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.
PK parameters	:	Descriptive statistics for all relevant PK parameters: n, mean, standard deviation, minimum, median, maximum, geometric mean, and coefficient of variation; analysis of variance on midazolam and 1′-OH midazolam C_{max}, AUC_{0-t} and AUC_{0-inf} to determine the interaction between inarigivir (including metabolites) and midazolam
Safety parameters	:	Descriptive statistics
PD parameters	:	Descriptive statistics (including change from baseline)

Table 1 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		
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	

		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)**
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.

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.

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.

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)

TABLE OF CONTENTS

TABLE OF TABLES

LIST OF ABBREVIATIONS

ADL	Activities of daily living
AE	Adverse event
ALAT (ALT)	Alanine aminotransferase
aPTT	Activated partial thromboplastin time
ASAT (AST)	Aspartate aminotransferase
β-hCG	β-human chorionic gonadotropin
BMI	Body mass index
CA	Competent Authority
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CRO	Contract research organization
CSP	Clinical study protocol
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CTD	Clinical Trial Directive
CXCL10	C-X-C motif chemokine 10
CYP	Cytochrome P450
DNA	Deoxyribonucleic acid
DDI	Drug-drug interaction
dsRNA	Double-stranded RNA
EAPA	Europe, Asia-Pacific and Africa
ECG	Electrocardiogram
eCRF	Electronic case report form
EU	European Union
gamma-GT	Gamma glutamyl transferase
GCP	Good Clinical Practice
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IFN	Interferon
IL	Interleukin
INR	International normalized ratio
IP-10	Interferon gamma-induced protein 10
IV	Intravenous
LDH	Lactate dehydrogenase
LLOQ	Lower limit of quantitation
MAD	Multiple ascending dose
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MEB	Medicine Evaluation Board
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	No observed adverse effect level
OATP	Organic anion transporting polypeptide
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PD	Pharmacodynamic(s)
PI	Principal Investigator
PK	Pharmacokinetic(s)
PRA	PRA Health Sciences

PRA-EDS	PRA Early Development Services
PT	Prothrombin time
RIG-I	Retinoic acid inducible gene
RNA	Ribonucleic acid
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment-emergent adverse event
TNF-α	Tumor necrosis factor alpha
ULN	Upper limit of normal
WHO	World Health Organization
WMA	World Medical Association
WMO	Wet medisch-wetenschappelijk onderzoek met mensen (Medical research involving human subjects act)

Note: Definitions of pharmacokinetic (PK) parameters are provided in Section 3.5.3

1. INTRODUCTION

1.1 Background

Inarigivir soproxil (further named inarigivir and formerly known as SB 9200) is being developed as a monotherapy and in combination with approved and investigational antiviral agents for hepatitis B virus (HBV). Inarigivir is also highly active against hepatitis C virus (HCV), but no further clinical development is planned unless an unmet clinical need can be identified for combination with other direct acting antiviral agents.

HBV is a deoxyribonucleic acid (DNA) virus that leads to chronic hepatitis, cirrhosis and liver cancer. The World Health Organization (WHO) estimates that 257 million people worldwide were living with chronic hepatitis B in 2015[1] despite the presence and utilization of a highly effective vaccine. Current treatment involves lifelong viral suppression with oral nucleosides/nucleotides, which prevent HBV replication but do not cure the disease. HBV cure remains elusive and can only be achieved in 10% of the small number of patients who are willing or able to undergo a year of interferon (IFN) therapy. New agents that focus on the cure of HBV with viral clearance represent a significant unmet need.

1.2 Overview of the Investigational Product

Inarigivir is a dinucleotide prodrug, which is being developed as an oral antiviral agent for the treatment of chronic hepatitis infections in humans. *In vitro* and *in vivo* studies have established that inarigivir has antiviral activity against HBV and HCV ribonucleic acid (RNA) viruses.

Inarigivir has a novel dual mechanism of action involving: (a) the activation of the host cytosolic protein - retinoic acid inducible gene (RIG-I) - involved in recognition of viral nucleic acids, which results in the stimulation of type I IFN production and induction of antiviral state in cells, and (b) direct inhibition of viral replication by blocking the access of the viral polymerase to the viral nucleic acid template; this is due to the interaction of inarigivir with the nucleotide-binding domain of RIG-I, which engages the viral RNA template. Given that inarigivir activates RIG-I, which is a cellular cytoplasmic protein involved in the detection of viral RNA in cells, it is unlikely to elicit resistance.

The regulation of IFN expression in the cells involves in part the activation of intracellular viral sensors such as RIG-I, which are pathogen recognition receptors. Inarigivir and the active moiety, SB 9000, cause the activation of RIG-I, and this mechanism may play an important role in its antiviral activity. RIG-I is a host cytosolic protein that recognizes double-stranded viral RNA (dsRNA) as a pathogen-associated molecular pattern (PAMP), blocks viral replication, and activates type 1 IFN production. RIG-I is a multimeric protein and consists of a C-terminal regulatory domain, two caspase activation and recruitment domains, as well as an ATPase domain. Viral dsRNA and 5'-triphosphate are 2 molecular patterns that enable RIG-I to discriminate viral RNA from host messenger RNA. RIG-I is known to recognize dsRNA structure as a PAMP present in HCV.

Inarigivir is the prodrug form of the active SB 9000 species. Inarigivir is a 60:40 mixture of two isomers designated as Rp and Sp. Upon oral administration, the inarigivir isomers are converted with similar efficiency to the corresponding active isomers Rp and Sp by esterases. *In vitro* studies using liver microsomes from different species, and *in vivo* studies in both rodents and woodchucks have demonstrated that SB 9000 is not subject to phase I metabolism or phase II conjugation reactions.

1.3 Nonclinical Summary

1.3.1 Pharmacodynamics

Inarigivir has been shown to be a potent inhibitor of HBV in *in vitro* assays against wild type and resistant variants of HBV, and was found to have synergistic antiviral activity when combined with other anti-HBV agents such as tenofovir and entecavir. Orally administered inarigivir has been shown to have potent antiviral activity in transgenic and woodchuck models of HBV including reduction in serum and hepatic viral DNA and viral antigens, as well as reduction in covalently closed circular DNA and viral RNA. Inarigivir has also been shown to have pan-genotypic antiviral activity against HCV including activity against resistant variants.

Inarigivir was evaluated in a battery of Good Laboratory Practice compliant safety pharmacology studies that assessed the central nervous system, cardiovascular system and pulmonary system. Oral administration of inarigivir produced no effects on cardiovascular function in Cynomolgus monkeys at doses up to and including 500 mg/kg, the highest dose tested. Likewise, oral administration of inarigivir, at doses up to and including 500 mg/kg, was not associated with any effects on pulmonary or neurobehavioral function in rats.

1.3.2 Pharmacokinetics

The PK of inarigivir have been characterized in *in vitro* studies in multiple species as well as *in vivo* studies in rats, rabbits, woodchucks and monkeys. In all species studied, a pattern of delayed plasma peaks of the active metabolites Rp SB 9000 and Sp SB 9000 was observed after oral dosing of inarigivir. In woodchucks, a pattern of delayed peak plasma levels was observed after intravenous (IV) dosing, confirming that enterohepatic cycling was the likely cause. Consistent with billiary excretion and reabsorption, *in vitro* transporter studies show that SB 9000 is a substrate for liver organic anion transporting polypeptide (OATP) 1A1 and OATP1A3 transporters located on the hepatocytes. It is common to observe high levels of OATP substrates in rat and human liver after oral dosing.[2] Indeed, SB 9000 has all of the characteristics common to OATP substrates.[2] It is an anion at physiological pH, it has a molecular weight between 400 and 600 g/mol and it has poor lipid solubility and high levels in the liver after oral dosing. The high liver levels of SB 9000 observed in rat liver relative to those in the plasma suggest that high liver levels of SB 9000 might also be expected in patients because the rat has been shown to be a highly predictive model of human OATP transport.[2] This paradigm is further supported by the observation that uptake of

SB 9000 into rat liver was a saturable process. Clinical trials will exclude concomitant medications that are taken up by OATP transport mechanisms.

Inarigivir and SB 9000 show no metabolic lability with liver metabolizing enzymes other than the conversion of inarigivir to SB900, and neither are not considered at risk as a drug-drug interaction victim. The compounds were evaluated *in vitro* for their potential as cytochrome P450 (CYP) 3A4 inhibitors or CYP inducers. Indeed, neither compound showed a potential to inhibit the standard battery of CYP enzymes. When hepatocytes were incubated with 0.1, 1 and 10 µM of inarigivir or SB 9000, inarigivir induced CYP1A2 mRNA levels by ≥2-fold at 1 and 10 µM, and induced CYP2B6 by <2-fold at all concentrations tested. SB 9000 induced CYP1A2 and CYP2B6 mRNA levels by ≥2-fold at 10 µM SB 9000; however, the maximum changes observed for CYP1A2 (3.3-fold increase) and CYP2B6 (2.5-fold increase) were only 18% and 10% of the positive controls, respectively. Therefore, there were no significant effects on either CYP1A2 or CYP2B6 as compared to their respective positive controls in those experiments. Inarigivir and SB 9000 induced CYP3A4 by >2-fold at all concentrations tested. The maximum change in CYP3A4 mRNA for 1 µM (6-fold increase) and 10 µM (13.4-fold increase) were 42% to 97% of the positive control, respectively. Both compounds, therefore, led to a dose-dependent increase in CYP3A4 gene expression over the 72-hour incubation period that was considered biologically relevant in comparison to the positive control.

Inarigivir and SB 9000 were also evaluated for their potential as substrates of the drug transporters. An *in vitro* study in LLC-MDR1 cells showed that apparent permeability of inarigivir and SB 9000 at 5 µM was low in parental and P-gp expressing cells, transport of these compounds across the cell was not polarized, and transport was unaffected by the P-gp inhibitor, GF120918. Therefore, inarigivir and SB 9000 are not considered P-gp substrates. An *in vitro* study was also done with sandwich cultured human hepatocytes to study the hepatobiliary transport of inarigivir and SB 9000. Based on this study, biliary excretion of SB 9000 (~30%) following incubation with 10 and 20 µM inarigivir was observed.

In rats, inarigivir was rapidly converted to its active form, SB 9000 (both isomers), such that only SB 9000 could be detected in plasma after either oral or IV dosing. A time course of SB 9000 levels after the last dose of a 3-month daily dosing regimen in rats demonstrated that SB 9000 was distributed to the liver with levels that were up to 20- to 40-fold higher than those in the plasma, but it was almost completely cleared from both the liver and plasma 24 hours post-dose. The plasma bioavailability of SB 9000 (oral plasma AUC compared with IV plasma AUC corrected for dose) was below 2% for rats and monkeys. However, consistent with a large first-pass uptake of SB 9000 into the liver from the portal blood, 58% of the SB 9000 administered after oral dosing was found in the rat liver compared with the systemic exposure after IV dosing (oral liver AUC compared with IV plasma AUC corrected for dose).

1.3.3 Toxicology

A 13-week repeat-dose study with a 4-week recovery period was initiated in Cynomolgus monkeys that were administered inarigivir daily at doses of 0, 15, 30, or 60 mg/kg/day for 13 weeks. Clinical chemistry alterations at 60 mg/kg/day which were characterized by increased gamma glutamyl transferase (gamma-GT) and triglycerides (both sexes), increased alanine aminotransferase (ALAT) (both sexes), increased aspartate aminotransferase (ASAT) (females), and increased globulin and decreased albumin/globulin ratio (males). The liver was the primary target organ and at doses of ≥30 mg/kg/day, there were dose-dependent findings of hepatocyte enlargement, hepatocyte vacuolar degeneration, mixed leukocyte infiltration/inflammation, and increased pigment and hypertrophy of Kupffer cells. Additional microscopic findings at 60 mg/kg/day included individual hepatocyte necrosis, increased mitotic figures, multinucleated hepatocytes, and bile duct hyperplasia. Following the recovery period, the findings at 60 mg/kg/day were reversing, although incomplete. No dose-limiting toxicity was observed over the course of the study. Doses up to and including 60 mg/kg/day for a period of 91 days were tolerated. The microscopic findings in the liver at the terminal and recovery necropsies, in conjunction with increased serum enzyme values and decreased serum albumin and fibrinogen values (changes generally associated with hepatocellular injury), were considered adverse in both sexes at ≥30 mg/kg/day. Accordingly, oral administration of inarigivir produced adverse effects in both sexes at ≥30 mg/kg/day; therefore, the no observed adverse effect level (NOAEL) was determined to be 15 mg/kg/day.

In summary, the nonclinical data to date show that inarigivir has significant antiviral activity, is well tolerated, produces no effects on cardiovascular function and preferentially distributes to the liver. Nonclinical toxicology studies in monkeys show effects in the liver that were dose dependent and either completely reversed or trended towards reversal. These overall findings provide a favorable risk-benefit ratio and indicate that monitoring of ALT and AST levels in patients will be important.

1.4 Clinical Experience

A completed Phase 1 study (SB12-9200-101) evaluated the safety, PK and pharmacodynamics of inarigivir in treatment naïve HCV infected adults. The study was conducted in 2 parts: 1) a single ascending dose (SAD) under fed or fasting conditions (Part A); 2) a placebo-controlled multiple ascending dose (MAD) under fasting conditions (Part B).

PK profiles of inarigivir and its metabolites were determined; increases in area under the plasma concentration-time curve from start to the time of the last quantifiable concentration post-dose (AUC_{0-t}) and maximum drug concentration (C_{max}) were found to be dose-proportional.

Inarigivir was well tolerated up to the highest dose level of 800 mg in the SAD part and up to the highest dose level of 900 mg daily for 7 days in the MAD part. The most frequently reported treatment-emergent adverse event (TEAE) following multiple dosing with inarigivir was headache (12 events reported by 10 subjects). Diarrhea,

nausea, ALT increase, AST increase and insomnia were reported for 2 subjects each. Headache was mild and self-limited. All ALT/AST elevations on treatment were less than 5 x the upper limit of normal (ULN), occurred after cessation of inarigivir and were deemed to be within the acceptable spontaneous fluctuation range that is common in HCV. No patient had hyperbilirubinemia.

The results on HCV RNA showed a similar viral kinetic response as that seen with IFN with approximately 30% of patients (6 of 22) having a greater than 0.9 log_{10} reduction in HCV RNA. The HCV RNA reduction was maximal at Day 3 and a comparison of non-responders (<0.5 log_{10} decline) to responders showed there was an associated increased in IFN stimulating gene 15 gene induction and IFN alpha. The responders demonstrated a PK profile with a greater AUC than the non-responder population.

The results of the Phase I study established a dose range for the Phase 2 study, generated a safety and PK profile of inarigivir as a monotherapy agent, and provided supportive data to advance inarigivir's clinical program as a part of a combination therapy in HBV, and HCV.

Based on the results from the Phase 1 Study SB12-9200-101, a 2-part Phase 2 study has been initiated to evaluate the safety, PK, and antiviral efficacy of inarigivir in subjects infected with chronic hepatitis B virus. Part A is being conducted as an ascending dose double-blind, placebo-controlled study. Part B will be an open-label, randomized combination therapy design study to evaluate safety, tolerability, and antiviral response of the chosen dose and regimen from Part A. Cohort 1 (25 mg inarigivir) and Cohort 2 (50 mg inarigivir) have completed dosing with no serious adverse events (SAEs) and antiviral activity confirmed as measured by a decline in HBV DNA.

A full description of the nonclinical and clinical studies with inarigivir can be found in the Investigator's Brochure (IB).[3]

1.5 Risk-Benefit Assessment

Overall, on the basis of the available nonclinical and clinical data, and prior knowledge, the risk-benefit profile of inarigivir is judged acceptable for the proposed clinical study.

1.6 Study Rationale

As described in Section 1.2, inarigivir and SB 9000 were evaluated for their potential effect to induce CYP-isoforms in cryopreserved human primary hepatocytes. The effects of 0.1, 1 and 10 uM of each compound were separately assessed on the expression of CYP1A2, CYP2B6, and CYP3A4. There were no significant effects on either CYP1A2 or CYP2B6 as compared to their respective positive controls in those experiments. However, incubation of either compound led to a dose-dependent increase in CYP3A4 gene expression over the 72-hour incubation period that was considered biologically relevant in comparison to the positive control.

Since many drugs are metabolized by CYP3A4, it is deemed relevant to perform an *in vivo* interaction study with a test drug that is a CYP3A4 substrate such as midazolam. The present study has therefore been designed to determine whether inarigivir impacts the single dose PK of midazolam.

Midazolam (a benzodiazepine used for induction and maintenance of anesthesia during surgical procedures and also for short term management of insomnia) is an ideal CYP3A4 probe because:

- It is entirely metabolized by CYP3A4 to 1 major metabolite (1′-OH midazolam).[4]
- It undergoes an intestinal and hepatic CYP3A4 first-pass effect (hepatic and intestinal extraction ratio of about 44% each).[5] The magnitude of the interaction is higher with drugs undergoing high CYP3A4 pre-systemic first-pass.
- It is entirely absorbed since almost 90% of an orally administered dose of [^{14}C]-midazolam is excreted in urine within 24 hours.[6]

2. OBJECTIVES

2.1 Primary

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

2.2 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

2.3 Exploratory

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

3. INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

3.1.1 Type of Study

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).

For each subject, the study will consist of:

- An eligibility screening period of a maximum of 28 days (Day -28 to Day -1).
- One study period from Day -1, the day of admission, to Day 20, the day of discharge.
- Admission to the clinical research center in the afternoon of Day -1.
- Administration of midazolam and inarigivir on the days as described above.
- Safety assessments, blood sampling and urine collection for PK purposes and blood sampling for PD purposes from Day -1 up to 24 hours after the last drug administration on Day 19.
- Discharge at 24 hours after the last study drug administration on Day 19 (Day 20: day of discharge).
- A follow-up visit 5 to 9 days after the day of discharge (between Day 25 and Day 29).

3.1.2 Screening Period

Subjects will report to the medical screening center for the eligibility screening (see Section 3.3 for inclusion and exclusion criteria) within 4 weeks prior to the first study drug administration.

Subjects will sign the study-specific informed consent form (ICF) prior to any study-specific screening procedures being performed. The written informed consent will be obtained for all subjects, regardless of their eligibility for the study; the signed ICFs will be retained and archived at PRA Health Sciences (PRA) and a copy will be provided to the subject.

Eligibility screening will consist of the assessments as presented in the schedule of assessments (Table 1).

3.1.3 Treatment Period

Subjects will be in the clinic for 1 treatment period. The subjects will be admitted to the clinical research center in the afternoon of Day -1, which is the day prior to Day 1, the day of the first drug administration.

Subjects will be discharged on Day 20 (24 hours after the last study drug administration on Day 19) after completion of the assessments.

Assessments during the treatment periods will be performed as presented in the schedule of assessments (Table 1).

3.1.4 Follow-up

The follow-up assessments will be performed 5 to 9 days after the day of discharge (between Day 25 and Day 29).

Assessments during follow-up will be performed as presented in the schedule of assessments (Table 1).

3.2 Discussion of Study Design

The fixed-sequence design was chosen to allow each subject to serve as his/her own control with respect to midazolam PK characteristics with and without inarigivir coadministration.

The Principal Investigator (PI) will take all the usual precautions necessary for studies at an early stage in the development of a new drug.

See Section 3.6.3 for sample size determination.

3.3 Selection of Study Population

Sixteen healthy male or female subjects are planned to be dosed in the study.

3.3.1 Inclusion Criteria

Note: Restrictions that apply to the period after admission are described in Section 3.4.8, except when they concern a statement of willingness.

The following inclusion criteria must be met for a subject to be eligible for inclusion in the study:

1. Gender : male or female
2. Age : 18-55 years, inclusive, at screening
3. Body mass index (BMI) : 18.0-30.0 kg/m^2, inclusive, at screening
4. Status : healthy subjects
5. At screening, females must be non-pregnant and non-lactating, or of non-childbearing potential (either surgically sterilized or physiologically incapable

of becoming pregnant, or at least 1 year post-menopausal [amenorrhoea duration of 12 consecutive months]); non-pregnancy will be confirmed for all females by a serum pregnancy test conducted at screening, and a urine pregnancy test at each admission and at follow-up

6. Female subjects of childbearing potential, with a fertile male sexual partner, must agree to use adequate contraception from screening until 90 days after the follow-up visit. Adequate contraception is defined as using a non-hormonal intrauterine device combined with at least 1 of the following forms of contraception: a diaphragm or cervical cap, or a condom; please note that hormonal contraceptives are not allowed. Also, total abstinence, in accordance with the lifestyle of the subject, is acceptable
7. Male subjects, if not surgically sterilized, must agree to use adequate contraception and not donate sperm from admission to the clinical research center until 90 days after the follow-up visit. Adequate contraception for the male subject (and his female partner) is defined as using hormonal contraceptives or an intrauterine device, combined with at least 1 of the following forms of contraception: a diaphragm or cervical cap, or a condom. Also, total abstinence, in accordance with the lifestyle of the subject is acceptable
8. All prescribed medication, including hormonal contraceptives for female subjects, must have been stopped at least 30 days prior to admission to the clinical research center
9. All over-the-counter medication, vitamin preparations and other food supplements, or herbal medications (eg, St. John's Wort) must have been stopped at least 14 days prior to admission to the clinical research center. An exception is made for paracetamol, which is allowed up to admission to the clinical research center
10. Ability and willingness to abstain from alcohol, methylxanthine-containing beverages or food (coffee, tea, cola, chocolate, energy drinks) and grapefruit (juice) from 72 hours prior to admission to the clinical research center
11. Good physical and mental health on the basis of medical history, physical examination, clinical laboratory, electrocardiogram (ECG) and vital signs, as judged by the PI
12. Willing and able to sign the ICF

3.3.2 Exclusion Criteria

Note: Restrictions that apply to the period after admission are described in Section 3.4.8, except when they concern a statement of willingness.

A subject who meets any of the following exclusion criteria will not be eligible for inclusion in the study:

1. Employee of PRA or the Sponsor
2. History of relevant drug and/or food allergies
3. Using tobacco products within 60 days prior to the first drug administration
4. History of alcohol abuse or drug addiction (including soft drugs like cannabis products)

5. Positive drug and alcohol screen (opiates, methadone, cocaine, amphetamines [including ecstasy], cannabinoids, barbiturates, benzodiazepines, tricyclic antidepressants and alcohol) at screening and admission to the clinical research center
6. Average intake of more than 24 units of alcohol per week (1 unit of alcohol equals approximately 250 mL of beer, 100 mL of wine or 35 mL of spirits)
7. Positive screen for hepatitis B surface antigen (HBsAg), anti-HCV antibodies or anti-human immunodeficiency virus (HIV) 1 and 2 antibodies
8. Participation in a drug study within 60 days prior to the first drug administration in the current study. Participation in more than 4 other drug studies in the 12 months prior to the first drug administration in the current study
9. Donation or loss of more than 100 mL of blood within 60 days prior to the first drug administration. Donation or loss of more than 1.5 liters of blood (for male subjects) / more than 1.0 liters of blood (for female subjects) in the 10 months prior to the first drug administration in the current study
10. Significant and/or acute illness within 5 days prior to the first drug administration that may impact safety assessments, in the opinion of the PI

Please note that subjects should refrain from consumption of any foods containing poppy seeds within 48 hours (2 days) prior to screening and admission to the clinical research center to avoid false positive drug screen results. In addition, they should refrain from strenuous exercise within 96 hours (4 days) prior to screening and admission as this could result in abnormal clinical laboratory values.

3.3.3 Removal of Subjects from Assessment

Participation in the study is strictly voluntary. A subject has the right to withdraw from the study at any time for any reason.

The PI has the right to terminate participation of a subject for any of the following reasons: difficulties in obtaining blood samples, violation of the protocol, severe adverse events (AEs) or SAEs, or for any other reason relating to the subject's safety or integrity of the study data.

If a subject is withdrawn from the study, the Sponsor will be informed immediately. If there is a medical reason for withdrawal, the subject will remain under the supervision of the PI until satisfactory health has returned.

If a subject is withdrawn from the study for any reason, whether related to the study drug or not, or if a subject voluntarily withdraws before or after receiving the study drug, such subject will be considered an early-termination subject. If a subject is withdrawn for a reason related to the study drug, according to the judgment of the PI, the early-termination subject will not be replaced. If a subject does not complete the study for a reason not related to the study drug, the early-termination subject may be replaced after mutual agreement between the Sponsor and PRA.

The decision regarding the replacement of subjects will be documented.

PRA will make every effort to ensure that early-termination subjects who have received study drug complete the safety follow-up assessments.

3.4 Treatments

3.4.1 Treatments Administered

The treatments planned are described in Section 3.1.1.

3.4.2 Identity of Investigational Product

Active medication

Active substance	:	Inarigivir soproxil
Activity	:	Antiviral agent
In development for	:	HBV infection
Strength	:	100 mg
Dosage form	:	Oral tablet
Manufacturer	:	Patheon, Inc., Mississauga, Ontario, Canada

Probe substrate

Active substance	:	Midazolam
Activity	:	CYP3A4 substrate
Indication	:	Sleep disorder
Strength	:	1 mg/mL; a dose of 2 mg will be administered
Dosage form	:	Oral administration of IV solution formulation
Manufacturer	:	Will be commercially acquired

For details concerning drug storage and drug accountability see Appendix 8.1.

3.4.3 Method of Assigning Subjects to Treatment Groups

After obtaining informed consent, subjects will be screened according to the inclusion and exclusion criteria. Subjects who have met all eligibility criteria will receive a subject number upon inclusion in the study (subject numbers 001-016). They receive the subject number prior to dosing. The subject number will ensure identification throughout the study. Any replacement subjects will receive the number of the subject to be replaced, increased by 100 (eg, 101 replacement number for subject number 1), and will be administered the same treatments in the same order.

All subjects will be assigned to the same fixed treatment sequence.

Subjects who drop out or withdraw for any reason without completing all screening evaluations successfully, will be considered “screening failures“. Such subjects will not receive a subject number, and only applicable data will be entered in the electronic case report forms (eCRFs).

3.4.4 Selection of Doses in the Study

A Phase 1 study (SB12-9200-101) was completed in which inarigivir was administered to treatment naïve HCV infected adults as single ascending and multiple ascending

doses. Inarigivir was well tolerated up to the highest dose level of 800 mg in the SAD part and up to the highest dose level of 900 mg daily for 7 days in the MAD part. The most frequently reported TEAE following multiple dosing with inarigivir was headache (12 events reported by 10 subjects). Diarrhea, nausea, ALT increase, AST increase and insomnia were reported for 2 subjects each. Headache was mild and self-limited. All ALT/AST elevations on treatment were less than 5 x ULN, occurred after cessation of inarigivir and were deemed to be within the acceptable spontaneous fluctuation range that is common in HCV. No patient had hyperbilirubinemia.

A single oral dose of 2 mg midazolam will be administered on 2 occasions (alone and in combination with inarigivir). As the usual oral dose of midazolam for induction of sedation without anesthesia ranges between 7.5 and 15 mg, the selected dose of 2 mg should be safe and well tolerated with minimal pharmacodynamic effect. Additionally, an oral dose of 2 mg is recommended to investigate the maximal inhibitory effect in a DDI study with midazolam.[7]

3.4.5 Timing of Doses in the Study

The study drugs inarigivir and midazolam will be administered with the subject in the upright position. Each dosing day, study drug will be administered between 08:00 and 11:00 hours in the morning after an overnight fast of at least 10 hours. The inarigivir dose will be swallowed together with 240 mL tap water (room temperature) and should not be chewed. During fasting, no fluids are allowed except water; however, water is not allowed from 2 hours pre-dose until 1 hour post-dose (apart from the water taken with the dose as described above).

On Days 1, 3 and 19, fasting will continue for a period of 4 hours after drug administration, ie, until scheduled lunch. On other dosing days, breakfast will be given approximately 1 hour after study drug administration.

When not fasting, non-caffeinated fluids are allowed ad libitum.

Dosing for each individual subject will be at around the same time (± 15 min) on each dosing day.

On Days 1, 3 and 19, subjects will not lie down for 4 hours after drug administration, except when required by assessments that need to be performed.

Administration of the study drug will be supervised by the PI or authorized designee. After drug administration a mouth and hand inspection will take place.

3.4.6 Meals During the Study

A fasting period of at least 4 hours is required before obtaining clinical laboratory samples at all time points.

There are no special requirements related to food and beverage intake other than described in Sections 3.4.5 and 3.4.8. When not fasting, meals and snacks (such as

decaffeinated coffee, herbal tea, fruit, biscuits) will be provided according to PRA standard operating procedures (SOPs). A light supper will be provided on the evening before those days where fasting is required until lunch-time; a snack will be provided on the evening before those days where fasting is required until breakfast.

3.4.7 Blinding

This is an open-label study.

3.4.8 Concomitant Medication and Other Restrictions During the Study

Note: Restrictions that apply to the period before admission are described in Sections 3.3.1 and 3.3.2.

The use of all prescribed medication is not allowed from admission to the clinical research center up to follow-up. The use of all over-the-counter medication, vitamin preparations and other food supplements, or herbal medications (eg St. John's Wort) is not allowed from admission to the clinical research center up to follow-up. An exception is made for paracetamol: from admission onwards, the PI may permit a limited amount of paracetamol for the treatment of headache or any other pain. Other medication to treat AEs may only be prescribed if deemed necessary by the PI. If medication is used, the name of the drug, the dose and dosage regimen will be recorded in the eCRF.

The use of alcohol, methylxanthine-containing beverages or food (coffee, tea, cola, chocolate, energy drinks), grapefruit (juice) and tobacco products is not allowed during the stay in the clinical research center.

Strenuous exercise is not allowed during the stay in the clinical research center.

Male subjects, if not surgically sterilized, are required to use adequate contraception and not donate sperm from admission to the clinical research center until 90 days after the follow-up visit. Adequate contraception for the male subject (and his female partner) is defined as using hormonal contraceptives or an intrauterine device, combined with at least 1 of the following forms of contraception: a diaphragm or cervical cap, or a condom. Also, total abstinence, in accordance with the lifestyle of the subject is acceptable.

Female subjects of childbearing potential, with a fertile male sexual partner, are required to use adequate contraception from screening until 90 days after the follow-up visit. Adequate contraception is defined as using a non-hormonal intrauterine device combined with at least 1 of the following forms of contraception: a diaphragm or cervical cap, or a condom; please note that hormonal contraceptives are not allowed. Also, total abstinence, in accordance with the lifestyle of the subject, is acceptable.

3.4.9 Treatment Compliance

Study drug will be administered in the clinical research center. To ensure treatment compliance, administration of the study drug will be supervised by the PI or authorized

designee. Compliance will be further confirmed by bioanalytical assessment of inarigivir and midazolam in plasma samples (see Section 3.5.4).

The exact times of study drug administration and the number of units administered will be recorded in the eCRF.

3.5 Pharmacokinetic, Safety and Pharmacodynamic Variables

3.5.1 Pharmacokinetic, Safety and Pharmacodynamic Measurements Assessed and Schedule of Assessments

The schedule of assessments is presented in Table 1.

3.5.1.1 Pharmacokinetic Measurements

3.5.1.1.1 Blood Sampling

At the time points defined in the schedule of assessments, blood samples of 4 mL each will be taken for the analysis of inarigivir and its metabolites Rp-SB 9000 and Sp-SB 9000 in plasma samples. At the time points defined in the schedule of assessments, blood samples of 1 mL each will be taken for the analysis of midazolam and its metabolite 1′-OH midazolam in plasma samples. The blood samples will be taken via an indwelling IV catheter or by direct venipuncture. Details on sample collection, sample aliquoting, sample handling, sample storage and sample shipping will be described in the laboratory manual prepared by PRA.

3.5.1.1.2 Urine Collection

During the intervals defined in the schedule of assessments, urine will be collected for the analysis of metabolites Rp-SB 9000 and Sp-SB 9000. The subjects will be instructed to empty their bladders completely before study drug administration and at the end of each collection interval. A blank urine sample will be collected within 12 hours prior to the first study drug administration. The exact times of urine collection and the urine weight of the entire interval will be recorded in the eCRF. Details on sample collection, sample aliquoting, sample handling, sample storage and sample shipping will be described in the laboratory manual prepared by PRA.

3.5.1.2 Safety and Tolerability Measurements

Safety and tolerability assessments will consist of AEs, clinical laboratory, vital signs, 12-lead ECG and physical examination. Assessments will be performed in accordance with the schedule of assessments.

3.5.1.2.1 Adverse Events

Adverse events will be recorded from admission until completion of the follow-up visit. Any clinically significant observations in results of clinical laboratory, 12-lead ECGs, vital signs or physical examinations will be recorded as AEs.

A TEAE is defined as any event not present prior to the first administration of the study drug or any event already present that worsens in either severity or frequency following exposure to the study drug.

An AE which occurs prior to the first administration of the study drug will be considered a pre-treatment AE.

At several time points before and after drug administration, subjects will be asked non-leading questions to determine the occurrence of AEs. Subjects will be asked in general terms about any AEs at regular intervals during the study. In addition, all AEs reported spontaneously during the course of the study will be recorded.

All answers will be interpreted by the PI using the Medical Dictionary for Regulatory Activities (MedDRA; most recent version) for AEs and will be recorded in the eCRF.

The severity of the AEs will be rated as “mild”, “moderate”, “severe”, “life-threatening” or “death”, and the relationship between the AEs and the study drug will be indicated as “none”, “unlikely”, “possibly”, “likely” or ‘’definitely”. Adverse events that will be assessed as “possibly”, “likely” or ‘’definitely” will be considered to be related to the study drug whereas AEs that will be assessed as “none” or “unlikely” will be considered not to be related to the study drug. Details on the rating of the severity of the AEs and relationship to the study treatment are given in Appendix 8.2.

3.5.1.2.2 Clinical Laboratory

Blood and urine samples for clinical laboratory assessments will be collected according to PRA SOPs.
The following parameters will be measured:

- Clinical chemistry (serum quantitatively):
  total bilirubin, alkaline phosphatase, gamma-GT, ASAT, ALAT, lactate dehydrogenase (LDH), creatinine, urea, total protein, glucose, inorganic phosphate, sodium, potassium, calcium and chloride
- Hematology (blood quantitatively):
  leukocytes, erythrocytes, hemoglobin, hematocrit, thrombocytes, partial automated differentiation: lymphocytes, monocytes, eosinophils, basophils, neutrophils, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)
- Coagulation (blood quantitatively):
  prothrombin time (PT) (reported in seconds and as international normalized ratio [INR]), activated partial thromboplastin time (aPTT), fibrinogen
- Urinalysis (urine qualitatively):
  hemoglobin, urobilinogen, ketones, glucose and protein
- Serology:
  HBsAg, anti-HCV and anti-HIV
- Drug and alcohol screen:
  opiates, methadone, cocaine, amphetamines (including ecstasy), cannabinoids, barbiturates, benzodiazepines, tricyclic antidepressants and alcohol
- Pregnancy test (females only):
  β-human chorionic gonadotropin (β-hCG) in serum/urine

In case of unexplained or unexpected clinical laboratory test values, the tests will be repeated as soon as possible and followed up until the results have returned to the normal range and/or an adequate explanation for the abnormality is found. The clinical laboratory will clearly mark all laboratory test values that are outside the normal range and the PI will indicate which of these deviations are clinically significant. These clinically significant deviating laboratory results will then be recorded as AEs and the relationship to the treatment will be indicated (see also Appendix 8.2).

3.5.1.2.3 Vital Signs

Systolic and diastolic blood pressure and pulse will be recorded after the subject has been resting for at least 5 minutes in the supine position. These assessments will be made using an automated device. Body temperature and respiratory rate will be measured subsequently.

3.5.1.2.4 Electrocardiogram

A standard 12-lead ECG will be recorded after the subject has been resting for at least 5 minutes in the supine position. The ECG will be recorded using an ECG machine equipped with computer based interval measurements. The following will be recorded: heart rate, PR-interval, QRS-duration, QT-interval, QTc-interval (Fridericia's) and the interpretation of the ECG profile by the PI.

3.5.1.2.5 Physical Examination

Physical examination will be performed according to PRA SOPs. In addition, body weight and height will be measured according to PRA SOPs.

3.5.1.3 Pharmacodynamic Measurements

Cytokine panel

At the time points defined in the schedule of assessments, blood samples of 4 mL each will be taken for the analysis of the cytokines C-X-C motif chemokine 10 (CXCL10; also known as IFN gamma-induced protein 10 [IP-10]), interleukin (IL)-6, tumor necrosis factor alpha (TNF-α), IFN-α and IFN-γ in serum. The blood samples will be taken via an indwelling IV catheter or by direct venipuncture. Details on sample collection, sample aliquoting, sample handling, sample storage and sample shipping will be described in the laboratory manual prepared by PRA.

Peripheral blood mononuclear cells (PBMCs)

At the time points defined in the schedule of assessments, blood samples of 16 mL each will be taken for the collection of PBMCs. RNA will be extracted from these PBMCs for potential future analysis of cytokine RNA expression. The blood samples will be taken via an indwelling IV catheter or by direct venipuncture. Details on sample collection, sample aliquoting, sample handling, sample storage and sample shipping will be described in the laboratory manual prepared by PRA.

3.5.1.4 Pharmacogenomic Measurements

One blood sample of 4 mL will be collected per subject for potential future genotyping of DNA sequence variants to explore potential relationships with PK/PD and or

tolerability. The sample is optional for all subjects; subjects who do not consent to collection of this sample will not be excluded from participation in this study provided all other inclusion and exclusion criteria have been met. The blood sample will be double coded (one code at PRA and one code at the Sponsor) and the sample will be kept until 15 years after completion of the study. The blood sample will be taken via an indwelling IV catheter or by direct venipuncture. Details on sample collection, sample aliquoting, sample handling, sample storage and sample shipping will be described in the laboratory manual prepared by PRA.

3.5.1.5 Total of Blood Volume

Table 2 presents the number and volume of blood samples and the total volume of blood that will be collected throughout the study per subject.

If deemed necessary by the PI or the Sponsor, the number and/or volume of blood samples per assessment in each study part may be increased as long as the total volume of blood drawn for a subject does not surpass 500 mL (except when extra blood samples need to be taken for safety reasons).

Table 2 Number and Volume of Blood Samples and Total Blood Volume Collected per Subject

Assessment	Maximum # samples	Volume of blood per sample (mL)	Total volume of blood (mL)
PK			
- Inarigivir and its metabolites	42	4	168
- midazolam and 1′-OH midazolam	28	1	28
PD			
- Cytokine panel	14	4	56
- PBMCs	7	16	112
Genotyping	1	4	4
Clinical chemistry	11	3.5	38.5
Hematology	11	3	33
Coagulation	11	4.5	49.5
Serology	1	5	5
Total volume of blood drawn			494

3.5.2 Appropriateness of Measurements

The assessments, which will be made in this study are standard, and generally recognized as reliable, accurate and relevant.

3.5.2.1 Timing of Assessments

For PK, pre-dose samples will be obtained between waking up and dosing. For pre-dose (trough) samples during multiple dosing, a 5% time window since the last dose is allowed, but the sample must be taken before the next dose. Post-dose samples up to 20 minutes post-dose will be obtained with a time window of ± 1 minute.

Thereafter, post-dose samples will be obtained with time margins of ± 5% of the time that has passed since (last) dosing. The ±5% time window also applies to the start and end times of urine collection intervals and in addition to the total duration of each consecutive collection interval.

For PD, pre-dose samples will be obtained between waking up and dosing. Post-dose samples up to 40 minutes post-dose will be obtained with a time window of ±2 minutes. Thereafter, post-dose samples will be obtained with time margins of ±5% of the time that has passed since (last) dosing.

For safety assessments, pre-dose assessments will be performed between waking up and dosing. For safety assessments up to 2.5 hours post-dose a time window of ±15 minutes is allowed. Thereafter, serial post-dose assessments (eg, multiple assessments within any given day) will be performed with time margins of ±10% of the time that has passed since (last) dosing.

In the event assessments are planned for the same scheme time, the order of the assessments should be arranged in such a way that PK blood sampling will be done after the ECG and vital signs recordings have been conducted, with PK blood sampling exactly on time.

3.5.3 Pharmacokinetic, Safety and Pharmacodynamic Variables

3.5.3.1 Pharmacokinetic Variables

PK variables will be the plasma concentrations of inarigivir and its metabolites Rp-SB 9000 and Sp-SB 9000, urine concentrations of Rp-SB 9000 and Sp-SB 9000, and plasma concentrations of midazolam and its metabolite 1′-OH midazolam and their PK parameters. The plasma and urine PK parameters to be determined or calculated using non-compartmental analysis include but are not limited to the parameters as given in Table 3 and Table 4, respectively. A complete list of PK parameters will be provided in the Statistical Analysis Plan (SAP).

Table 3 Plasma Pharmacokinetic Parameters

Parameter	Inarigivir and midazolam following a single dose	Rp-SB 9000, Sp-SB 9000 and 1′-OH midazolam following a single dose	Inarigivir, Rp-SB 9000 and Sp-SB 9000 following multiple doses	Description
C_{max}	X	X	X	Maximum observed plasma concentration
t_{max}	X	X	X	Time to attain maximum observed plasma concentration
AUC_{0-t}	X	X		Area under the plasma concentration-time curve up to time t, where t is the last point with concentrations above the lower limit of quantitation (LLOQ)
AUC_{0-inf}	X	X		Area under the plasma concentration-time curve from time 0 to infinity calculated as: $AUC_{0-inf} = AUC_{0-t} + C_{last}/k_{el}$, where C_{last} is the last measurable plasma concentration
$\%AUC_{extra}$	X	X		Percentage of estimated part for the calculation of AUC_{0-inf} $((AUC_{0-inf}-AUC_{0-t})/AUC_{0-inf})*100\%$
$AUC_{0-\tau}$			X	Area under the plasma concentration-time curve over a dosing interval τ
k_{el}	X	X		Terminal elimination rate constant
$t_{1/2}$	X	X		Terminal elimination half-life, calculated as $0.693/k_{el}$
R_{ac}			X (Rp-SB 9000 and Sp-SB 9000 only)	Accumulation ratio for C_{max} of Day 19 versus C_{max} of Day 3 and $AUC_{0-\tau}$ of Day 19 versus AUC_{0-inf} of Day 3
CL/F	X			Apparent oral clearance, calculated as dose/AUC_{0-inf}
V_z/F	X			Apparent volume of distribution at terminal phase
MR		X		Metabolite to parent ratio for C_{max}, AUC_{0-t}, and AUC_{0-inf}

Table 4 Urine Pharmacokinetic Parameters

Parameter	Rp-SB 9000 and Sp-SB 9000 following single doses	Description
Ae_{0-t}	X	Amount excreted up to time t, where t is the last point with concentrations above the LLOQ
CL_R	X	Renal clearance
fe	X	Fraction of the dose administered excreted in urine (%)

3.5.3.2 Safety Variables

The safety variables will be as follows:

- AEs
- Clinical laboratory
- Vital signs
- ECG

3.5.3.3 Pharmacodynamic Variables

The PD variables will be the serum levels of the cytokines CXCL10, IL-6, TNF-α, IFN-α and IFN-γ and cytokine RNA expression in PBMCs.

3.5.4 Drug Concentration Measurements

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 Q^2 Solutions (New York, USA) using validated liquid chromatography-mass spectrometry/mass spectrometry methods. The Bioanalytical Report for the determinations will be included in the clinical study report (CSR).

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

3.6 Statistical Procedures and Determination of Sample Size

3.6.1 Analysis Sets

3.6.1.1 Pharmacokinetic Set

All subjects who have received at least a single dose of midazolam or inarigivir and provided sufficient bioanalytical assessment results to calculate reliable estimates of the PK parameters.

3.6.1.2 Safety Set

All subjects who have received at least 1 dose of study drug.

3.6.1.3 Pharmacodynamic Set

All subjects belonging to the safety set and for whom the PD data are considered to be sufficient and interpretable.

3.6.2 Statistical and Analytical Plan for Pharmacokinetic, Safety and Pharmacodynamic Evaluation

A SAP will be generated by the Biostatistics Department of PRA; the SAP will be finalized prior to database lock. Full details of the analysis to be performed will be included in the SAP.

Any deviation from the SAP will be reported in the Section "Changes in Planned Analysis" in the CSR.

3.6.2.1 Pharmacokinetic Evaluation

The PK parameters and their statistical evaluation will be included in the CSR of this study.

All data will be summarized using descriptive statistics (n, mean, standard deviation, minimum, median, maximum, geometric mean, and coefficient of variation) and will be listed and summarized in tabular and/or graphical form.

A mixed-effects model with subject as a random effect and treatment as a fixed effect will be performed on the following natural logarithm (ln)-transformed PK parameters of midazolam and 1′-OH midazolam: C_{max}, AUC_{0-t} and AUC_{0-inf}. The point estimates will be calculated for each parameter listed above as the geometric mean ratio of Day 19 (coadministration of midazolam + inarigivir) relative to Day 1 (midazolam alone), with corresponding 90% confidence intervals (CIs). The absence of interaction will be concluded if these CIs are included in the bioequivalence range (80-125%).

3.6.2.2 Evaluation of Safety and Tolerability

Safety and tolerability will be assessed through AEs, clinical laboratory, vital signs, ECGs and physical examination findings, and any other parameter that is relevant for safety assessment.

3.6.2.2.1 Adverse Events

A listing of all individual AEs will be provided. Summary tables of TEAEs will be presented by system organ class based on the MedDRA terminology list (preferred terms): 1 containing the number of TEAEs (frequency of occurrence, number of subjects experiencing the event) by treatment and 1 containing the number of drug-related TEAEs (frequency of occurrence, number of subjects experiencing the event) per treatment. Additional tables of total counts by treatment and relationship and by treatment and severity will be created.

3.6.2.2.2 Clinical Laboratory

Clinical laboratory data will be listed and accompanied by an indication if the parameter is outside the reference range. A summary of all data outside the reference range of

the clinical laboratory parameters will be provided. Clinical laboratory data will be presented descriptively, where applicable.

3.6.2.2.3 Vital Signs and Electrocardiograms

Vital signs and ECG parameters will be listed and they will be presented descriptively, where applicable.

3.6.2.2.4 Physical Examination

Changes from baseline for physical examination will be described and listed.

3.6.2.3 Pharmacodynamic Evaluation

The PD parameters and their statistical evaluation will be included in the CSR of this study.

All data, including change from baseline, will be summarized using descriptive statistics and will be listed and summarized in tabular and/or graphical form.

3.6.3 Determination of Sample Size

Based on the intra-subject coefficient of variation of 19.7% found for AUC_{0-t} of midazolam in a previous interaction study[8] 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.

3.7 Data Quality Assurance

The study may be audited by the Quality Assurance Department at PRA to assess adherence to the clinical study protocol (CSP) and Quality System. During the conduct of the study, process-related audits may be performed. An audit certificate will be provided in the appendices of the final CSR outlining any audits and other related activities performed.

The clinical research site will be monitored by the study monitor to ensure correct performance of the study procedures and assure that the study will be conducted according to the relevant regulatory requirements. The eCRF entries will be verified with the source documentation, if applicable (in some cases there are no source pages, therefore verification is not necessary).

Regulatory authorities, the Independent Ethics Committee (IEC) and/or the Sponsor's clinical quality assurance group may request access to all source documents, eCRFs, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the PI, who must provide support at all times for these activities.

Quality control principles will be applied throughout the performance of this study. Review procedures will be followed at PRA for all documents that are generated in relation with the study.

An explanation will be given for all missing, unused and spurious data in the relevant sections of the CSR.

4. ETHICS

4.1 Independent Ethics Committee

The CSP and the ICFs will be submitted for review and approval by the IEC of the foundation "Beoordeling Ethiek Biomedisch Onderzoek" (English translation: "Evaluation of Ethics in Biomedical Research") (Stationsstraat 9, 9401 KV Assen, The Netherlands) prior to the eligibility screening. The composition of the IEC is in accordance with the recommendations of the WHO, the International Council for Harmonisation (ICH) E6 Guideline for Good Clinical Practice (GCP)[9], and the European Union (EU) Clinical Trial Directive (CTD) (Directive 2001/20/EC)[10] (see below).

PRA will keep the IEC informed about the progress of the study. All changes in research activities and all unanticipated problems involving risks to human subjects will be immediately reported to the IEC. In accordance with Section 10, Subsection 1, of the Dutch law on Medical Research in Human Subjects (WMO, revised December 2015)[11], PRA will inform the subjects and the IEC if anything occurs on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal, or if further recruitment of subjects in the study has been put on hold for that reason, whichever occurs first. The study may be suspended pending further review by the IEC, except insofar as suspension would jeopardize the subjects' health. PRA will take care that all subjects are kept informed.

No changes will be made in the study without IEC approval, except when required to eliminate apparent immediate hazards to human subjects.

Notification of the end of the study will be sent by PRA to the Competent Authority (CA) in The Netherlands and to the IEC within 90 days after completion of follow-up for the last subject. In case a study is temporarily halted, PRA will notify the IEC immediately, including the reason for this. In case a study is ended prematurely, PRA will notify the IEC and the CA in The Netherlands within 15 days, including the reasons for the premature termination. A summary of the results of the study will be sent by PRA to the CA and the IEC within 1 year after the end of the study.

4.2 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origin in the World Medical Association (WMA) Declaration of Helsinki, adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and subsequent amendments.[12]

This study is also designed to comply with ICH E6 Guideline for GCP (EMA/CHMP/ICH/135/1995)[9], and the EU CTD Directive 2001/20/EC[10], as incorporated into Dutch Law.[11]

ICH adopted guidelines and other relevant international guidelines, recommendations and requirements will be taken into account as comprehensively as possible, as long as they do not violate Dutch law.

The PI will be responsible for the care of the subjects throughout the study. If the PI is not present at the clinical site, he will leave instructions for the staff and a telephone number where he can be reached.

The PI will be responsible for the medical follow-up of the subjects.

If a subject refuses to follow the instructions of the PI, the latter is released from any legal responsibility.

4.3 Subject Information and Consent

All subjects will be informed verbally and in writing regarding the objectives, procedures and risks of study participation. The subjects will have to sign the Dutch or English version of the ICF before any study-related procedures are started. The ICF contains information about the objectives of the study, about the procedures followed during the study and about the risks and restrictions of the study, with special reference to possible side effects of the medication and potential interactions. In addition, insurance coverage provided during the study is explained. The elements addressed in the ICF are according to the ICH E6 Guideline for GCP (CPMP/ICH/135/95).[9]

4.4 Privacy

All personal details will be treated as confidential by the PI and staff at PRA and handling of personal data will be in compliance with the Dutch Data Protection Act. Personal details are stored in databases that have been registered with the Dutch Data Protection Authority.

5. STUDY ADMINISTRATIVE STRUCTURE

5.1 Distribution of Activities

Preparation of study drug

Inarigivir will be provided by the Sponsor. Midazolam will be obtained by the PRA Pharmacy.

Laboratory assessments

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 Q^2 Solutions (New York, USA).

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

The analysis of clinical laboratory samples and the extraction of RNA from PBMCs will be performed at the PRA Clinical Laboratory.

eCRF design

The eCRF design will be performed with the computer program Oracle Clinical (Oracle, Redwood Shores, Redwood City, CA, USA) by the Database Programming Department of PRA.

Data management

Data management will be performed with the computer programs Oracle Clinical (Oracle, Redwood Shores, Redwood City, CA, USA), SAS® (SAS Institute Inc., Cary, NC, USA) and EXACT (Kinship EXACT™, Kinship technologies, a technology subsidiary of PRA) by the Data Management Department of PRA.

Statistics

A SAP will be provided by the Biostatistics Department of PRA. The safety analysis and the statistical evaluation of PK and PD parameters will be conducted by the Biostatistics Department of PRA. Statistical analysis will be performed with the computer program SAS® (SAS Institute Inc., Cary, NC, USA). Pharmacokinetic parameters will be calculated using Phoenix® WinNonlin® (Certara, Princeton, NJ, USA). Additional PK computations can also be performed in SAS for Windows® (SAS Institute Inc., Cary, NC, USA).

CSR writing

The CSR, structured in accordance with the guideline ‘Structure and Content of Clinical Study Reports - ICH E3’[13], will be written by PRA.

5.2 Documentation

5.2.1 Archiving

All documents concerning the study will be kept on file in the Central Archives of PRA for at least 15 years after conduct of the study. The Sponsor will receive the completed eCRFs (upon request, as PDF file).

5.2.2 Recording of Data in Source Documents and CRFs

Wherever possible, all data will be entered directly into the eCRFs. In some cases source documents will be used.

A Data Management Plan will be written by the Data Management Department of PRA, which will be finalized prior to performing any data validation. An appendix to the data management plan (source identification list) will identify any data to be recorded directly in the eCRF (ie, no prior written or electronic record of data), and which data should be considered source data.

6. CONFIDENTIALITY AND PUBLICATION POLICY

By signing this CSP, the PI reaffirms to the Sponsor that he will maintain in confidence all information furnished to him, or resulting from this study. The PI will only divulge such information as may be necessary to the IEC, the members of the staff and the subjects who are involved in this study.

All relevant aspects regarding publication will be part of the contract (or similar document) between the Sponsor and PRA.

7. REFERENCES

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9. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2), November 2016. Guideline for Good Clinical Practice E6(R2) (EMA/CHMP/ICH/135/1995), December 2016.
10. Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.
11. Medical Research in Human Subjects Act (WMO, Wet Medisch-Wetenschappelijk Onderzoek met mensen), revision December 2015
12. WMA Declaration of Helsinki (18th WMA General Assembly 1964), revised at 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013.
13. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E3: Structure and Content of Clinical Study Reports (CPMP/ICH/137/95), November 1995.
14. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, Note for Guidance on Clinical Safety Data Management, June 1995.

8. APPENDICES

8.1 Drug Accountability

Upon receipt of the study drug, it will be inspected and counted by the responsible pharmacist. If necessary, all study drug will be re-packed per dosing occasion, and labeled according to PRA SOPs.

The study drug will be kept in the PRA Pharmacy or in a locked and secured storage facility accessible to the pharmacist and the pharmacy assistant only.

The responsible pharmacist will keep an inventory. This will include a description of the formulation and the quantity of study drug received for the study and a record of what is dispensed, to whom and when.

On termination of the study the responsible pharmacist will conduct a final inventory of the study drug supply and will record the results of this inventory in the Drug Accountability Form. Unused study drug will be returned to the Sponsor at the end of the study or will be locally destroyed according to PRA standard procedures.

8.2 Adverse Events and Serious Adverse Events Evaluation and Reporting

8.2.1 Adverse Events

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. AE definitions will be followed as stated in the 'Note for Guidance on clinical safety data management: definitions and standards for expedited reporting' (ICH topic E2A).[14]

All AEs reported by the subjects or apparent from their physical appearance during the clinical study will be reported on the AE eCRF page.

The severity of AEs will be graded using the most current version of the Common Terminology Criteria for Adverse Events (CTCAE) 5-point scale:

- **Mild (Grade I)**: Asymptomatic or mild symptoms: clinical or diagnostic observations only; intervention not indicated.
- **Moderate (Grade II)**: Minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).
- **Severe (Grade III)**: Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- **Life-threatening (Grade IV)**: Life-threatening consequences; urgent intervention indicated.
- **Death (Grade V)**: Death related to AE.

If an AE has multiple aspects, the aspect with the highest severity will be graded.

It is emphasized that the term severe is a measure of severity; thus, a severe AE is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

In addition, clinically significant changes in physical examination findings and abnormal objective test findings (eg, laboratory, x-ray, ECG) should also be recorded as AEs. Test findings and physical examination findings can result in AEs if they:

- Are associated with accompanying symptoms, and/or
- Require additional diagnostic testing or medical/surgical intervention, and/or
- Lead to a change in study dosing or discontinuation from the study; result in the addition of significant additional concomitant drug treatment or other therapy, and/or
- Lead to any of the outcomes included in the definition of an SAE, and/or
- Are considered to be an AE by the PI or Sponsor.

Reporting as an AE should not be triggered by:

- Merely repeating an abnormal test or
- Any abnormal test result that is determined to be an error.

The relationship of any AE to the study drug will be assessed and graded on a 5-point scale: none, unlikely, possibly, likely or definitely.

Relationship between use of study drug and AE (Causality)					
AE (is)	**None**	**Unlikely**	**Possibly**	**Likely**	**Definitely**
Clearly the result of an external factor	Yes	No	No	No	No
Probably/possibly the result of another factor	No	Yes	Yes	No	No
Has a chronological relationship with the time of study drug administration and/or represents a known reaction to study drug	No	No	Yes	Yes	Yes
Disappears or decreases after discontinuation of the study drug	NA	NA	NA	Yes	Yes
Recurs on renewed administration (re-challenge)	No	No	NA	NA	Yes or NA*

* A re-challenge is not required; if done, re-challenge would be expected to be positive

NA Not applicable

8.2.2 Serious Adverse Events

An SAE is any untoward medical occurrence that, on the basis of medical and scientific judgment:

- Results in death, or
- Is life-threatening (this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe), or
- Requires inpatient hospitalization for a medical reason or prolongation of existing hospitalization (this refers to hospital admission required for treatment of the AE). (Note: this does not include confinement in, for example, a respite unit; a skilled nursing unit; rehabilitation facility; the clinical research center; or confinement due to planned or unplanned reason unrelated to study), or
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

SAEs will be collected from admission until the final study visit. SAEs that are related to the investigational drug and continue beyond the normal collection period (ie, are ongoing at the time a subject exits the study) will be followed until resolution or until stabilized with sequelae. SAEs that begin after the subject's participation in the study is complete, but that the PI considers to be related to study drug, may be reported at any time.

The PI or clinical site personnel must notify the Sponsor's Chief Medical Officer, the 3rd Party Drug Safety CRO and the PRA Drug Safety Center Europe, Asia-Pacific and Africa (EAPA) of all SAEs, regardless of relationship to the investigational drug, within 24 hours of clinical site personnel becoming aware of the event. The PI will provide the initial notification by sending a completed "SAE Notification Form", which must include the PI's assessment of the relationship of the event to investigational drug, and must be signed by the PI.

In addition, notification is sent by PRA to the IEC, and the subject's general practitioner.

Follow-up information, or new information regarding an ongoing SAE, must be provided promptly to the Sponsor's Chief Medical Officer, the 3rd Party Drug Safety CRO and the PRA Drug Safety Center EAPA.

All SAE reports should be sent to the contacts provided on page 3: SAE Contact Information.

8.2.3 Suspected Unexpected Serious Adverse Reactions

An SAE that is also an unexpected adverse drug reaction is called a Suspected Unexpected Serious Adverse Reaction (SUSAR). Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (eg, IB for an unapproved investigational medicinal product or the Summary of Product Characteristics for an authorized medicinal product).

The Sponsor or its representative (eg, PRA if agreed to before start of the study) will urgently report the following SUSARs to the IEC:

- SUSARs that have arisen in the current clinical study that was assessed by the IEC
- SUSARs that have arisen in other clinical studies of the same Sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the current clinical study that was assessed by the IEC

The Sponsor will urgently report all SUSARs to the CA and the Medicine Evaluation Board (MEB) of the country where this study is being conducted and to the CAs in other Member States, as applicable.

SUSARs that have already been reported to the European Medicines Agency Eudravigilance database do not have to be reported again to the CA and the MEB because they have direct access to the Eudravigilance database.

Expedited reporting will occur no later than 15 calendar days after the Sponsor or its representative has first knowledge of the adverse reactions. For fatal or life-threatening cases the term will be a maximum of 7 calendar days for a preliminary report with another 8 days for completion of the report.

8.2.4 Follow-up of Adverse Events

Follow-up of AEs will continue until resolution, stabilization or death. In case of ongoing AEs at the moment of database closure, the data obtained at the moment of database closure will be used in the statistical analysis. The follow-up of the AE will be documented in the source documents and will be described in the final CSR only if considered relevant by the PI.