Official Title: A Safety,Tolerability, Pharmacokinetics And Efficacy Study Of Ro7049389 In: (1) Single- (With Or Without Food) And Multiple- (With Midazolam) Ascending Doses In Healthy Volunteers; (2) Patients Chronically Infected With Hepatitis B Virus (3) Patients With Chronic Hepatitis B

NCT Number: NCT02952924

Document Date: Protocol version 6: 21-May-2020

PROTOCOL

TITLE:	**A SAFETY, TOLERABILITY, PHARMACOKINETICS AND EFFICACY STUDY OF RO7049389 IN: (1) SINGLE- (WITH OR WITHOUT FOOD) AND MULTIPLE- (WITH MIDAZOLAM) ASCENDING DOSES IN HEALTHY VOLUNTEERS; (2) PATIENTS CHRONICALLY INFECTED WITH HEPATITIS B VIRUS (3) PATIENTS WITH CHRONIC HEPATITIS B**
PROTOCOL NUMBER:	YP39364
VERSION:	6
TEST PRODUCT:	RO7049389
SPONSOR:	F. Hoffmann-La Roche Ltd
DATE FINAL:	Version 1: 23 September 2016
DATE AMENDED:	Version 2: 12 January 2017 Version 3: 16 August 2017 Version 4: 02 January 2019 Version 5: 21 June 2019 Version 6: See electronic date stamp below

FINAL PROTOCOL APPROVAL

Date and Time (UTC)	Title	Approver's Name
21-May-2020 02:04:25	Company Signatory	[redacted]

PROTOCOL ACCEPTANCE FORM

TITLE:	**A SAFETY, TOLERABILITY, PHARMACOKINETICS AND EFFICACY STUDY OF RO7049389 IN: (1) SINGLE- (WITH OR WITHOUT FOOD) AND MULTIPLE- (WITH MIDAZOLAM) ASCENDING DOSES IN HEALTHY VOLUNTEERS; (2) PATIENTS CHRONICALLY INFECTED WITH HEPATITIS B VIRUS (3) PATIENTS WITH CHRONIC HEPATITIS B**
PROTOCOL NUMBER:	YP39364
VERSION NUMBER:	6
TEST PRODUCT:	RO7049389
SPONSOR:	F. Hoffmann-La Roche Ltd

I agree to conduct the study in accordance with the current protocol.

_____

Principal Investigator's Name (print)

_____ _____

Principal Investigator's Signature Date

Please keep the signed original form in your study files, and return a copy to your local study monitor.

PROTOCOL AMENDMENT, VERSION 6:
RATIONALE

Protocol YP39364 Version 5 has been amended to incorporate the following changes in Part 3:

- For patients **not** meeting the NUC Stopping Criteria (HBsAg < 100 IU/mL and HBV DNA < 20 IU/mL) at end of study treatment, the frequency of post-treatment follow-up visits has been reduced to every 8 weeks (Section 3.1.1.5., Table 5) because NUC therapy is expected to be continued in these patients, which poses no need for close monitoring every 2 or 4 weeks.

  As patients who do **not** meet NUC Stopping Criteria will continue with NUC therapy during follow-up, NUC will be provided or reimbursed by the Sponsor until 24 weeks post-treatment.

- The exclusion criterion 1 of Part 3 (Section 4.1.3.3.) has been adjusted to remove the text on male subjects with partners who are pregnant because the risk of drug exposure to a pregnant woman whose male partner is enrolled in this study became extremely low and negligible when the male study participant uses contraceptive measures as required by the inclusion criterion 9 (Section 4.1.2.3).

- The prohibited therapy and prohibited food sections (Sections 4.4.2. and 4.4.3.) have been updated for better clarity, and the examples of prohibited medicines have been listed in Appendix 16.

- It has been clarified in Section 3.1.1. (including Figure 2) and Sections 4.1. and 6.1. that Cohort C can be expanded to 30 patients based on emerging efficacy/safety data.

- Retreatment criteria for dose interruption due to hepatitis flares have been modified to take the baseline alanine transaminase (ALT) level and the decline of viral markers into consideration (Section 5.2.1.2.).

- Triplicate 12-lead ECG measurements have been changed to a single interpretable measurement per time-point (Section 4.5.1.4. and Appendix 7).

- Sample collection time points have been updated from HCV Ab or HCV RNA to include both HCV Ab and HCV RNA samplings at screening for eligibility assessment (Section 4.5.1.5. and Appendix 7).

- Collection time points for the following samples: HBV viral genotypes based on HBV RNA, HBV viral genotypes based on HBV DNA, viral resistance monitoring, HBV RNA, HBcrAg, and anti-HBc have been updated to Day −1 instead of during screening as these are not needed for the eligibility assessment (Appendix 7).

- Optional hospitalization for the Week 24 intensive PK has been included (Appendix 7).

- Guidance on how the study visits may be conducted to ensure subjects' safety while continuing to participate in the study during COVID-19, or similar epidemic, has been added as Appendix 17.

Additional minor changes have been made to improve clarity and consistency, including update of background sections of the protocol in line with recent changes to the Investigator Brochure.

Substantial new information appears in *Book Antiqua* italics. This amendment represents cumulative changes to the original protocol.

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PROTOCOL SYNOPSIS

TITLE:	**A SAFETY, TOLERABILITY, PHARMACOKINETICS AND EFFICACY STUDY OF RO7049389 IN: (1) SINGLE- (WITH OR WITHOUT FOOD) AND MULTIPLE- (WITH MIDAZOLAM) ASCENDING DOSES IN HEALTHY VOLUNTEERS; (2) PATIENTS CHRONICALLY INFECTED WITH HEPATITIS B VIRUS (3) PATIENTS WITH CHRONIC HEPATITIS B**
PROTOCOL NUMBER:	YP39364
VERSION:	6
TEST PRODUCT:	RO7049389
PHASE:	I/II
INDICATION:	HBV
SPONSOR:	F. Hoffmann-La Roche Ltd

OBJECTIVES

Objectives for Part 1

The **primary objectives** of Part 1 (healthy volunteers) of this study are as follows:

- To assess the safety and tolerability of single and multiple doses of RO7049389.
- To characterize the pharmacokinetics (PK) of RO7049389.

The **secondary objectives** of Part 1 of this study are as follows:

- To evaluate the effect of food on the PK of RO7049389 after a single dose administration.
- To evaluate the effect of multiple oral dosing of RO7049389 on CYP3A4 activity assessed by the PK of a single oral micro-dose of midazolam.

The **exploratory objectives** of Part 1 of this study are as follows:

- To screen for the presence of metabolites of RO7049389 in selected plasma and urine samples.
- To evaluate the effect of multiple oral dosing of RO7049389 on CYP3A4 activity assessed by plasma 4β-hydroxycholesterol/cholesterol ratio.

Objectives for Part 2

The **primary objectives** of Part 2 (patients chronically infected with HBV) of this study are as follows:

- To assess the safety and tolerability of 4 weeks of treatment with RO7049389.
- To investigate the antiviral effect (quantitative HBV DNA level) of 4 weeks of treatment with RO7049389.

The **secondary objective** of Part 2 of this study is:

- To investigate the plasma PK of RO7049389.

The **exploratory objectives** of Part 2 of this study are as follows:

- To investigate the response relationship between RO7049389 exposure and HBV DNA.
- To explore other viral response outcomes (e.g., quantitative HBeAg, loss of HBeAg, HBeAg seroconversion, quantitative HBsAg, loss of HBsAg, HBsAg seroconversion) after 4 weeks of treatment with RO7049389.
- Exploratory liver biomarkers will be examined.
- To investigate metabolites of RO7049389 in selected plasma samples.

Objectives for Part 3

The **primary objective** of Part 3 (patients with chronic hepatitis B) of this study is:

- To evaluate anti-viral effect (functional cure) of treatment with RO7049389 on top of Standard of Care (SoC).
    - Functional cure is defined as HBV DNA < lower limit of quantification (LLOQ, 20 IU/mL) with HBsAg loss (<0.05 IU/mL) at 24 weeks post-treatment.

The **secondary objectives** of Part 3 of this study are as follows:

- To further characterize the efficacy profile of RO7049389 on top of SoC over time, including HBsAg loss, HBsAg seroconversion, quantitative HBsAg change from baseline, HBeAg loss, HBeAg seroconversion, quantitative HBeAg change from baseline, HBV DNA < LLOQ, HBV DNA change from baseline in different proof of mechanism (POM) cohorts of Part 3 and in subgroups of baseline HBeAg +/- patients, or of baseline high / low HBsAg level patients.

- To assess other viral response outcomes (including HBV core-related antigen levels, anti-HBc antibody, quantitative HBV RNA, etc.) over time.
- To assess the ALT normalization in patients with baseline ALT elevation.
- To characterize the plasma PK profiles of RO7049389 and its metabolites.
- To assess the long-term safety and tolerability of RO7049389 on top of SoC (a NUC with or without Peg-IFN).
- To assess the PK interaction between NUCs and RO7049389.
- To assess *H*BV genotypic resistant variants associated with virologic failure.

The **exploratory objectives** of Part 3 of this study are as follows:

- To explore other viral response outcomes (i.e., total HBsAg) over time.
- To explore relationship between exposure and efficacy/safety response (e.g., HBsAg, HBV DNA, etc.).
- To explore safety, PK, and antiviral activities of RO7049389 in different ethnic subpopulations (e.g., Caucasian versus Asian).
- To assess effect of treatment on liver stiffness.
- To explore relationship between baseline disease characteristics (e.g., HBeAg status, viral load, HBV genotype based on DNA or RNA or historical data, etc.) and efficacy/safety response.
- To explore the effect of the genetic polymorphism of CYP3A4, UGT1A3 and OATP1B on the PK of RO7049389 by clinical genotyping.
- To explore the PK interaction among NUCs, Peg-INF and RO7049389.

STUDY DESIGN

Description of Study

Study YP39364 is a first-in-human study conducted in three parts. Parts 1 and 2 of the study are randomized sponsor-open, investigator-blinded, subject-blinded. Part 3 of the study is a non-randomized, non-controlled, open-label study.

Part 1 of the study will evaluate safety, tolerability, and PK of RO7049389 following oral administration of single (Parts 1a and 1b) or multiple (Part 1c) doses in healthy volunteers. The effect of food on the PK of RO7049389 will be evaluated within one of the SAD cohorts (Part 1b). Drug interaction with micro-dose midazolam will be assessed in all MAD cohorts (Part 1c). There will be no midazolam dosing in SAD cohorts (Part 1a and 1b). In Part 1 of the study, approximately 93 healthy volunteers will be enrolled. A Bayesian adaptive approach, i.e., Continuous Reassessment Method (CRM) will be used, alongside scientific and clinical judgment, to inform decisions about the dose of RO7049389 to be given to the next cohort during dose-escalation, as well as to identify the maximum tolerated dose (MTD).

Part 2 of the study will evaluate the safety, tolerability, PK and PD of RO7049389 following oral administration in patients chronically infected with HBV. In Part 2 of the study, approximately 35 patients will be enrolled.

Part 3 of the study is an open-label, adaptive, multi-arm trial designed to evaluate safety, tolerability, PK, PD, and efficacy of RO7049389 following oral administration on top of SoC (a NUC with/without Peg-IFN) in NUC-suppressed or treatment-naive chronic hepatitis B (CHB) patients. *Initially,* approximately 55 patients will be enrolled *in the first three cohorts of Part 3*. This part of the study is designed to be adaptive based on emerging data to enable *the increase of the* sample size of a*ny* cohort, and to open additional treatment cohorts to explore different treatment durations, dose regimens, or different combinations.

NUMBER OF STUDY SUBJECTS

The study may enroll up to a total of 93 healthy volunteers. Due to the adaptive nature of this study, the actual number of patients to be enrolled will be determined during the study:

- Up to 53 healthy volunteers in Part 1a of the study. The initial cohort will be of 5 healthy volunteers, with up to 6 subsequent cohorts of 8 healthy volunteers.
- Eight subjects in a dose-level in Part 1a will be asked to return for Part 1b of the study.
- Up to 40 healthy volunteers in Part 1c of the study. There will be up to 5 cohorts with 8 healthy volunteers in each.
- Up to 35 patients in Part 2. There will be up to 5 cohorts with 7 patients in each.
- Initially, approximately 55 patients will be enrolled in the first 3 cohorts in Part 3 with approximately 30 patients in POM Cohort A, 10 patients in POM Cohort B, and 15 patients in POM Cohort C. Based on emerging efficacy/safety data, the sample size in Cohort C can be increased to approximately 30 patients. In addition, additional treatment cohorts may be opened to explore different treatment durations, dose regimens, or different combinations.

Healthy volunteers or patients who drop out of the study for non-safety reasons may be replaced to ensure sufficient data to characterize the safety and PK profile, and to make a dose-escalation decision. Healthy volunteers or patients who withdraw from the study due to poor tolerability or due to study drug-related adverse events will not be replaced.

TARGET POPULATION

Part 1: SAD, Food Effect, and MAD in Healthy Volunteers

The study population consists of healthy male and female subjects, aged 18 to 60 years, inclusive.

Part 2: Chronic HBV-infected patients

The study population consists of male and female patients chronically infected with HBV, aged 18 to 60 years, inclusive, who are currently not on anti-HBV treatment (never treated or have not been on treatment within 6 months prior to randomization).

Part 3: Chronic Hepatitis B (CHB) patients

The study population consists of male and female NUC-suppressed or anti-HBV treatment-naïve CHB patients, aged 18 to 60 years, inclusive. Both HBeAg positive and negative patients will be enrolled, and at least 30% of patients enrolled in each cohort will be HBeAg positive. In order to evaluate PK, PD, and safety in different major ethnic subgroups (e.g., Caucasian versus Asian) in some cohorts, the Sponsor may postpone or stop enrolment of certain patient subgroups according to the actual enrollment situation to achieve suitable balance across the groups.

INCLUSION/EXCLUSION CRITERIA

Inclusion Criteria

Healthy volunteers/patients with HBV must meet the following criteria for study entry:

Part 1 – Healthy Volunteers only:

1. Healthy male and female subjects, 18 to 60 years of age, inclusive. Healthy status is defined by absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination including vital signs, 12-lead ECG, hematology, blood chemistry, serology and urinalysis.

2. A body mass index (BMI) between 18 to 30 kg/m^2 inclusive.
3. Female subjects must be either surgically sterile (by means of hysterectomy and/or bilateral oophorectomy) or post-menopausal for at least one year (defined as amenorrhea ≥12 consecutive months without another cause, and confirmed by follicle stimulating hormone level >35 mIU/mL).
4. For men: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive measures, and agreement to refrain from donating sperm, as defined below:
    a. With female partners of childbearing potential or pregnant female partners, men must remain abstinent or use a condom during the treatment period and for at least 28 days after the last dose of study drug to avoid exposing the embryo. Men must refrain from donating sperm during this same period.

   The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.
5. Informed of, willing and able to comply with all of the protocol requirements and the investigational nature of the study, and have signed an informed consent form (ICF) in accordance with institutional and regulatory requirements.

Part 2 – Chronic HBV-Infected Patients only:

1. Adult male and female patients, 18 to 60 years of age, inclusive.
2. A BMI between 18 to 30 kg/m^2 inclusive.
3. Chronic hepatitis B infection, defined as positive test for HBsAg for more than 6 months prior to randomization.
4. HBV DNA at screening ≥2 x 10^4 IU/mL for HBeAg positive patients, or $\geq 2 \times 10^3$ IU/mL for HBeAg negative patients.
5. ALT at screening ≤5 x upper limit of normal (ULN; both immune-tolerant and immune-active patients).
6. Screening laboratory values (hematology, chemistry [other than liver function test], urinalysis) obtained up to 28 days prior to first study treatment within acceptable range or judged to be not clinically significant by the Investigator and the Sponsor.
7. Liver biopsy, fibroscan or equivalent test obtained within the past 6 months demonstrating liver disease consistent with chronic HBV infection with absence of extensive bridging fibrosis and absence of cirrhosis (cutoff for fibroscan is liver stiffness measurement ≤8.5 kPa or Metavir fibrotic Stage <3, or other equivalent staging systems).
8. For men: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive measures, and agreement to refrain from donating sperm, as defined below:
    a. Men must remain abstinent or use a condom during the treatment period and for at least 28 days after the last dose of study drug to avoid exposing the embryo. Men must refrain from donating sperm during this same period.

   The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not

acceptable methods of contraception.

9. For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use non-hormonal contraceptive methods that result in a failure rate of <1% per year during the treatment period and for at least 3 months after the last dose of study drug
    a. A woman is considered to be of childbearing potential if she is post-menarcheal, has not reached a post-menopausal state (≥ 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).

    Examples of non-hormonal contraceptive methods with a failure rate of < 1% per year include male sterilization, and copper intrauterine devices.

    The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.
10. Informed of, willing and able to comply with all of the protocol requirements and the investigational nature of the study, and have signed an informed consent form (ICF) in accordance with institutional and regulatory requirements.

Part 3 – Chronic Hepatitis B Patients only:

1. Adult male and female patients, 18 to 60 years of age (inclusive) at the time of signing the informed consent form (ICF).
2. A BMI between 18 to 32 kg/m^2 inclusive.
3. Chronic hepatitis B infection, defined as positive test for HBsAg or HBV DNA (including qualitative, quantitative, and genotype testing) or positive HBeAg for more than 6 months prior to screening.
4. HBsAg >250 IU/mL at screening.
5. For Cohorts only enrolling NUC-suppressed CHB patients (e.g., POM Cohort A), patients must qualify for the following criteria:
    a. Patients treated with a single NUC (entecavir, tenofovir alafenamide, or tenofovir disoproxil fumarate) for ≥12 months. Patients must be on the same NUC therapy for at least 3 months before screening.
    b. At least one result showed HBV DNA <60 IU/mL at least 6 months prior to screening; and HBV DNA <20 IU/mL at screening by Roche Cobas assay.
    c. ALT ≤2 x ULN at screening and at Day -1 (can be checked by local lab result).
6. For Cohorts only enrolling anti-HBV treatment-naïve and immune-active patients (e.g., POM Cohort B and Cohort C), patients must qualify for the following criteria:
    a. Previous anti-HBV treatments for <30 days in total, and did not receive any anti-HBV treatments within 3 months prior to the first study dose.
    b. HBV DNA at screening ≥2 x 10^4 IU/mL for HBeAg positive patients, or ≥2 × 10^3 IU/mL for HBeAg negative patients.
    c. ALT at screening between 1–5 (exclusive) x ULN and ALT <5 x ULN at Day -1

(can be checked by local lab result).

7. Screening laboratory values (hematology, chemistry [other than liver function test], urinalysis) obtained up to 28 days prior to first study treatment within acceptable range or judged to be not clinically significant by the Investigator and the Medical Monitor.
8. Liver biopsy, Fibroscan, or equivalent test obtained within the past 6 months demonstrating liver disease consistent with chronic HBV infection with absence of extensive bridging fibrosis and absence of cirrhosis (cutoff for Fibroscan is liver stiffness measurement ≤8.5 kPa for treatment-naïve patients and ≤7.4 kPa for NUC-suppressed CHB patients, or Metavir fibrotic Stage <3, or other equivalent staging systems).
9. For men: agreement to remain abstinent (refrain from heterosexual intercourse) or agree to use contraceptive measures, and agree to refrain from donating sperm, as defined below:
    a. Men must remain abstinent or use a condom during the treatment period and for at least 6 months after the last dose of study drug to avoid exposing the embryo. Men must refrain from donating sperm during this same period.

    The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.
10. For women of childbearing potential: agree to use two methods of contraception, with at least one method considered as highly effective during the study and for at least 6 months after the last dose of study drug.
    a. A woman is considered to be of childbearing potential if she is post-menarcheal, has not reached a post-menopausal state (≥ 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).
    b. Contraceptive methods considered as highly effective (failure rate <1% per year when used consistently and correctly):

        - Combined (estrogen- and progestogen-containing) or progestogen-only hormonal contraception associated with inhibition of ovulation
        - intrauterine device (IUD)
        - intrauterine hormone-releasing system (IUS)
        - bilateral tubal occlusion
        - vasectomized partner
        - sexual abstinence*

        * Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of study treatment and at least 6 months after the last dose of study drug. In such case, there is no need to use two contraceptive methods. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not

acceptable methods of contraception.

c. Contraceptive methods NOT considered as highly effective (failure rate >1% per year):

- Progestogen-only oral hormonal contraception (where inhibition of ovulation is not the primary mode of action)

- Male or female condoms with or without spermicide

- Cap, diaphragm, or sponge with spermicide

11. Informed of, willing and able to comply with all of the protocol requirements and the investigational nature of the study, and have signed an informed consent form (ICF) in accordance with institutional and regulatory requirements.

Exclusion Criteria

Healthy volunteers/patients with HBV who meet any of the following criteria will be excluded from study entry:

Part 1 – Healthy Volunteers only:

1. Pregnant (positive pregnancy test) or lactating women, and male subjects with partners who are pregnant or lactating.
2. History or symptoms of any clinically significant gastrointestinal, renal, hepatic, broncho-pulmonary, neurological, psychiatric, cardio-vascular, endocrinological, hematological or allergic disease, metabolic disorder, cancer, or cirrhosis.
3. Personal history or family history of congenital long QT syndrome and/or cardiac sudden death.
4. History of Gilbert's syndrome.
5. History of having received or currently receiving any systemic anti-neoplastic (including radiation) or immune-modulatory treatment (including systemic oral or inhaled corticosteroids) $\leq$ 6 months prior to the first dose of study drug or the expectation that such treatment will be needed at any time during the study.
6. Subjects who have had significant acute infection, e.g., influenza, local infection, acute gastrointestinal symptoms, or any other clinically significant illness within two weeks of screening.
7. Any confirmed significant allergic reactions (urticaria or anaphylaxis) against any drug, or multiple drug allergies (non-active hay fever is acceptable).
8. Any clinically significant concomitant diseases or condition that could interfere with, or treatment of which might interfere with, the conduct of the study, or that would, in the opinion of the Investigator, pose an unacceptable risk to the subject in this study.
9. Confirmed (based on the average of 3 separate resting blood pressure measurements, properly measured with well-maintained equipment, after at least 10 minutes rest) systolic Blood Pressure greater than 140 or less than 90 mmHg, and diastolic BP greater than 90 or less than 50 mmHg at screening.
10. Clinically relevant ECG abnormalities on screening ECG e.g.:
    a. QTcF $>$ 450 msec or $<$ 300 msec

b. Notable resting bradycardia (HR < 45 bpm), or HR > 90 bpm
c. ECGs with documented machine errors in the interval duration assessments.
d. Evidence of atrial fibrillation, atrial flutter, complete bundle branch block, Wolf-Parkinson-White Syndrome, or cardiac pacemaker.

11. ECG with QRS and/or T-wave judged to be unfavorable for a consistently accurate QT measurement (e.g., neuromuscular artifact that cannot be readily eliminated, arrhythmias, indistinct QRS onset, low amplitude T-wave, merged T- and U-waves, prominent U-waves).
12. Creatinine Clearance (CrCl) ≤ 70 mL/min (using the Cockcroft-Gault formula).
13. Positive test at screening of any of the following: Hepatitis A (HAV IgM Ab), Hepatitis B (HBsAg), Hepatitis C (HCV RNA or HCV Ab) or human immunodeficiency virus (HIV Ab).
14. Any other clinically significant abnormalities in laboratory test results at screening. In the case of uncertain or questionable results, tests performed during screening may be repeated before randomization to confirm eligibility.
15. Participation in an investigational drug or device study within 90 days prior to screening or more than 4 times per year.
16. Donation or loss of blood over 500 mL within 3 months prior to screening.
17. Positive test for drugs of abuse (including recreational drugs) and/or positive alcohol test at screening or on Day -1.
18. History of drug and/or alcohol abuse or addiction.
19. History (within 3 months of screening) of alcohol consumption exceeding 2 standard drinks per day on average (1 standard drink = 10 grams of alcohol). Alcohol consumption will be prohibited at least 48 hours before screening, 48 hours before and 48 hours after each dose, and 48 hours before each scheduled visit.
20. Use of > 5 cigarettes or equivalent nicotine-containing product per day.
21. Taking any prescribed or over-the-counter (OTC) medications (including vitamins or herbal remedies) within 2 weeks of first dosing or within 5 times the elimination half-life of the medication prior to first dosing (whichever is longer). Occasional acetaminophen/paracetamol is allowed. Exceptions may be made on a case-by-case basis following discussion and agreement between the Investigator and the Sponsor.
22. Subjects under judicial supervision, guardianship or curatorship.
23. Medical or social conditions that would potentially interfere with the subject's ability to comply with the study visit schedule or the study assessments.
24. History of hypersensitivity to benzodiazepines or its formulation ingredients (for MAD-midazolam cohorts)
25. Acute narrow-angle glaucoma (for MAD-midazolam cohorts).

Part 2 – Chronic HBV-Infected Patients only:

1. Pregnant (positive pregnancy test) or lactating women, and male subjects with partners who are pregnant or lactating
2. History or other evidence of bleeding from esophageal varices.
3. Evidence of liver cirrhosis or decompensated liver disease such as ascites, esophageal or

gastric varices, splenomegaly, nodular liver, jaundice, hepatic encephalopathy.

4. One or more of the following laboratory abnormalities at screening:
    a. Total serum bilirubin > 2.5 mg/dL (> 42.75 μmol/L). For patients with documented Gilbert's syndrome total bilirubin > 2.75 mg/dL (> 47 μmol/L).
    b. International normalized ratio (INR) > 1.5.
    c. Serum albumin < 3.0 g/dL (< 30 g/L).
    d. Platelet count < 140,000 cells/mm^3.
5. History or other evidence of a medical condition associated with chronic liver disease other than HBV infection (e.g., hemochromatosis, autoimmune hepatitis, alcoholic liver disease, toxin exposure, thalassemia, nonalcoholic steatohepatitis, etc.).
6. Documented history or other evidence of metabolic liver disease within one year of randomization.
7. Positive test for hepatitis A (IgM anti-HAV), hepatitis C, hepatitis D, or human immunodeficiency virus.
8. Expected to need systemic antiviral therapy other than that provided by the study at any time during their participation in the study, with the exception or oral therapy for HSV I or HSV II.
9. History of or suspicion of hepatocellular carcinoma or alpha-fetoprotein ≥ ULN at screening.
10. History of significant gastrointestinal disease (including but not limited to gastric ulcers).
11. History of clinically significant cardiovascular, endocrine, renal, ocular, pulmonary, psychiatric or neurological disease.
12. Evidence of an active or suspected cancer or a history of malignancy other than adequately treated basal cell carcinoma.
13. History of organ transplantation.
14. Previous or concurrent HBV treatments in the past 6 months.
15. History of capsid modulators treatment, including treatment in investigative trials
16. Participation in an investigational drug or device study within 30 days prior to randomization.
17. Taking any drugs or nutrients listed in prohibited medications and prohibited food sections.
18. Significant acute infection (e.g., influenza, local infection) or any other clinically significant illness within 2 weeks of randomization.
19. Clinically relevant ECG abnormalities on screening ECG.
20. Abnormal renal function including serum or plasma creatinine > ULN or calculated creatinine clearance < 70 mL/min using the Cockcroft Gault formula.
21. Donation or loss of blood over 500 mL within 3 months prior to randomization.
22. Administration of any blood product within 3 months prior to randomization.
23. History of alcohol abuse (consumption of more than 2 standard drinks per day on average; 1 standard drink = 10 grams of alcohol) and/or drug abuse within one year of randomization; positive test result for drugs of abuse or alcohol breath test at screening.

24. Subjects under judicial supervision, guardianship or curatorship.
25. Medical or social conditions that would potentially interfere with the subject's ability to comply with the study visit schedule or the study assessments.

Part 3 – Chronic Hepatitis B Patients only:

1. Pregnant (positive pregnancy test) or lactating women.
2. History or other evidence of bleeding from esophageal varices.
3. Evidence of liver cirrhosis or decompensated liver disease such as ascites, esophageal or gastric varices, splenomegaly, nodular liver, jaundice, hepatic encephalopathy.
4. One or more of the following laboratory abnormalities at screening:
    a. Total serum bilirubin > ULN (exception Gilbert's disease).
    b. International normalized ratio (INR) >1.1 ULN.
    c. Serum albumin <3.0 g/dL (<30 g/L).
    d. Platelet count <140,000 cells/mm^3
    e. Hemoglobin <12 g/dL (females) or <13 g/dL (males).
    f. White blood cell count <2500 cell/mm^3.
    g. Neutrophil count <1500 cell/mm^3 (<1200 cell/mm^3 if considered a physiological variant in a patient of African descent).
5. History or other evidence of a medical condition associated with chronic liver disease other than HBV infection (e.g., hemochromatosis, autoimmune hepatitis, alcoholic liver disease, toxin exposure, thalassemia, nonalcoholic steatohepatitis, etc.).
6. History of thyroid disease poorly controlled on prescribed medications or clinically relevant abnormal thyroid function tests (thyroid-stimulating hormone [TSH], free triiodothyronine [FT3], free thyroxin [FT4] at screening.
7. Documented history or other evidence of metabolic liver disease within one year of screening.
8. Positive test for hepatitis A (IgM anti-HAV), hepatitis C, hepatitis D, HEV or human immunodeficiency virus (HIV).
9. Expected to need systemic antiviral therapy other than that provided by the study at any time during their participation in the study, with the exception of oral therapy for HSV I or HSV II.
10. Diagnosed or suspected hepatocellular carcinoma as evidenced by screening alpha-fetoprotein (AFP) ≥100 ng/mL. If AFP >ULN, absence of mass/findings suspicious for HCC must be demonstrated by ultrasound or CT or MRI within the screening period.
11. History of significant gastrointestinal disease (including but not limited to gastric ulcers).
12. History of clinically significant cardiovascular, endocrine, renal, ocular, pulmonary, psychiatric, or neurological disease.
13. Evidence of an active or suspected cancer or a history of malignancy other than adequately treated basal cell carcinoma.
14. History of organ transplantation.
15. Participation in an investigational drug or device study within 30 days prior to screening.

16. Taking any drugs or nutrients listed in prohibited medications and prohibited food sections.
17. Significant acute infection (e.g., influenza, local infection) or any other clinically significant illness within 2 weeks of screening.
18. ECG at screening with clinically significant abnormalities, including QTcF interval (QT corrected using Fridericia's formula) ≥450 msec for males and ≥470 msec for females.
19. Abnormal renal function including serum or plasma creatinine > ULN or glomerular filtration rate (eGFR; using CDK-Epi equation) <60 mL/min.
20. Donation or loss of blood over 500 mL within 3 months prior to screening.
21. Administration of any blood product within 3 months prior to screening.
22. History of alcohol abuse (consumption of more than 2 standard drinks per day on average; 1 standard drink = 10 grams of alcohol) and/or drug abuse within one year of randomization; positive test result for drugs of abuse or alcohol breath test at screening.
23. Subjects under judicial supervision, guardianship, or curatorship.
24. Medical or social conditions that would potentially interfere with the subject's ability to comply with the study visit schedule or the study assessments.

LENGTH OF STUDY

The total duration of Part 1a (SAD) of the study will be approximately 8 weeks divided as follows:

- Screening: Up to 4 weeks.
- In Clinic period: Days –2 to 3 (may be extended at the discretion of the Investigator or at the request of the Sponsor based safety and emerging PK and/or PD data).
- Ambulatory visits: Days 4, 5
- Safety Follow-up: Days 8 ± 1 day and 29 ± 3 days.

The total duration of Part 1b (FE) of the study will be approximately 10 weeks divided as follows:

- Screening: Up to 4 weeks.
- In Clinic period: Days –2 to 3 and Days 15 to 18 (may be extended at the discretion of the Investigator or at the request of the Sponsor based safety and emerging PK and/or PD data).
- Ambulatory visits: Days 4, 5, 19, and 20.
- Safety Follow-up: Days 23 ± 1 day, and 44 ± 3 days.

The total duration of Part 1c (MAD) of the study will be approximately 10 weeks divided as follows:

- Screening: Up to 4 weeks.
- In Clinic period: Day -2 to Day 16 (may be extended at the discretion of the Investigator or at the request of the Sponsor based safety and emerging PK and/or PD data).
- Safety Follow-up: Days 21 ± 1 day and 42 ± 3 days.

The total duration of Part 2 (POM) of the study will be approximately 20 weeks divided as follows:

- Screening: Up to 4 weeks.
- Treatment period: 4 weeks.

- In Clinic period (optional): Day -1 to Day 2 and Day 27 to Day 29 (may be extended at the discretion of the Investigator or at the request of the Sponsor based safety and emerging PK and/or PD data).
- Ambulatory visits: Days -1, 1, 2, 3, 4, 8, 15, 22, 28, and 29.
- Safety Follow-up (Ambulatory): Days 35 (± 1 day), 56 (± 3 days), 84 (± 2 weeks), and 112 (± 2 weeks).

The total duration of Part 3 (POM) of the study will be approximately 76 weeks divided as follows:

- Screening: Up to 4 weeks.
- Treatment period: Up to 48 weeks, ambulatory visits:
    - 0–4 weeks: every 2 weeks.
    - 4–48 weeks: every 4 weeks.
- Post-treatment follow-up for 24 weeks, ambulatory visits:

  For patients who meet NUC Stopping Criteria (HBsAg < 100 IU/mL and HBV DNA *< 20 IU/mL) at end-of-treatment (EOT) visit:*
    - Post-EOT 0–12 weeks: every 2 weeks.
    - Post-EOT 12–24 weeks: every 4 weeks.

  For patients who do NOT meet NUC Stopping Criteria at EOT visit:
    - *Post-EOT 0-*24 weeks: every 8 weeks.

END OF STUDY

The end of the study is defined as the date when the last study subject last observation (LSLO) occurs. LSLO is expected to occur 24 weeks after the last dose is administered in Part 3.

OUTCOME MEASURES

SAFETY OUTCOME MEASURES

The safety outcome measures for this study are as follows:

- Incidence and severity of adverse events.
- Incidence of laboratory abnormalities, based on hematology, clinical chemistry, coagulation, and urinalysis test results, etc.
- ECGs.
- Vital signs including blood pressure, pulse rate and body temperature.

PHARMACOKINETIC OUTCOME MEASURES

The pharmacokinetic outcome measures for this study are as follows:

RO7049389 and its metabolites (M5, M6 and M11, if applicable) parameters in Parts 1a (SAD) and RO7049389 parameters in Part 1b (SAD and Food Effect):

- C_{max}: Maximum observed plasma concentration.
- T_{max}: Time to maximum observed plasma concentration.
- $AUC_{0\text{-last}}$: area under the plasma concentration versus time curve up to the last measurable concentration.
- $AUC_{0\text{-}\infty}$: Area under the plasma concentration versus time curve extrapolated to infinity;
- $T_{1/2}$: Apparent terminal phase half-life.
- CL/F: Apparent clearance after oral administration.
- Ae (Cumulative amount excreted unchanged in the urine) and CLR (Renal clearance, computed as Ae/AUC), if warranted.

RO7049389 and its metabolites (M5, M6 and M11, if applicable) parameters in Part 1c (MAD):

- C_{max}: Maximum observed plasma concentration.
- T_{max}: Time to maximum observed plasma concentration.
- $AUC_{0\text{-}\tau}$: Area under the plasma concentration-time curve for a dosing interval.
- C_{trough}: Trough plasma concentration.
- $T_{1/2}$: Apparent terminal phase half-life.
- Accumulation index of RO7049389 and its metabolites.
- Ae and CLR, if warranted.

Midazolam parameters in Part 1c (MAD):

- C_{max}: Maximum observed plasma concentration.
- T_{max}: Time to maximum observed plasma concentration.
- $AUC_{0\text{-last}}$: area under the plasma concentration versus time curve up to the last measurable concentration.
- $AUC_{0\text{-}\infty}$: Area under the plasma concentration versus time curve extrapolated to infinity.
- $AUC_{0\text{-}6h}$: Area under the plasma concentration versus time curve up to 6 hours post-dose.
- $T_{1/2}$: Apparent terminal phase half-life.
- CL/F: Apparent clearance after oral administration.

RO7049389 and its metabolites (M5, M6 and M11) parameters in Part 2 [POM] and Part 3 [RO7049389 on top of SoC]:

- C_{max}: Maximum observed plasma concentration.
- T_{max}: Time to maximum observed plasma concentration.
- $AUC_{0\text{-}\tau}$: Area under the plasma concentration-time curve for a dosing interval, if applicable.
- C_{trough}: Trough plasma concentration.
- $T_{1/2}$: Apparent terminal phase half-life, if applicable.
- Accumulation index of RO7049389 and its metabolites, if applicable.

NUC and Peg-IFN parameters in Part 3 (RO7049389 on top of SoC):

- C_{trough}: Trough plasma concentration.

PHARMACODYNAMIC OUTCOME MEASURES

Viral dynamic response outcomes will be measured in Part 2 and Part 3 of the study, and will include, but will not be limited to the following:

- Quantitative HBV DNA level (actual and change from baseline).
- HBsAg (qualitative and quantitative).
- HBeAg (qualitative and semi-quantitative).
- Anti-HBs antibody status (quantitative).
- Anti-HBe antibody status (qualitative).
- Anti-HBc antibody (qualitative).
- Quantitative Hepatitis B core related antigen (HBcrAg).
- Quantitative HBV RNA.

Outcomes of antiviral response will include:

- Quantitative HBsAg decline, loss of HBsAg, development of anti-HBs, HBsAg seroconversion (loss of HBsAg and presence of anti-HBs).
- Quantitative HBeAg decline, loss of HBeAg, development of anti-HBe, HBeAg seroconversion (loss of HBeAg and presence of anti-HBe).
- Quantitative HBV DNA decline, HBV DNA <LLOQ.
- Viral resistance *will be* monitor*ed* from Day −1 through follow-up.

EXPLORATORY OUTCOME MEASURES

Since the following measures are set to be exploratory, in some circumstances, these measures will only be tested in a subset of the patients or part of the visits:

In Part 1, endogenous substances levels will be investigated to assess the effect of multiple oral dosing or RO7049389 on CYP3A4 activity. Analysis will include plasma 4β-hydroxycholesterol/cholesterol ratio.

In Part 3:

- Changes of total HBsAg from baseline.
- Change of liver stiffness measurement by Fibroscan.

RESEARCH BIOSAMPLE REPOSITORY (RBR) SAMPLE COLLECTION

Specimens will be collected from study subjects who give specific consent to participate in this optional Research Biosample Repository.

INVESTIGATIONAL MEDICINAL PRODUCT(S)

Part 1 and Part 2:

All IMPs required for completion of this study (RO7049389 and matching placebo) will be provided by the Sponsor. Midazolam solution will be prepared by the site.

Part 3:

The IMPs required for completion of this study (RO7049389) will be provided by the Sponsor.

NON IMPs

All patients in Part 3 of the study will also be treated with SoC therapy (a NUC with or without Peg-IFN):

- NUC: For NUC-suppressed patients who have been on a NUC before entering the study, they should continue to take the same NUC per local label. If patients were taking generic NUC, they will be recommended to switch to the original drug after enrolling in the study. For anti-HBV treatment-naïve patients, the choice of entecavir, tenofovir alafenamide, or tenofovir disoproxil fumarate is at the discretion of treating physician and per local label. NUC will be provided *or* reimbursed by the Sponsor *until 24 weeks post treatment*.
- Peg-IFN (Pegylated interferon alfa): Patients who are required to use Peg-IFN, e.g., in POM Cohort C, will either be provided or reimbursed by the Sponsor for the duration of the study.

PROCEDURES

Schedules of Assessments (SoA) are provided in Appendices 1 – 8.

STATISTICAL METHODS

SAFETY ANALYSES

All healthy volunteers (Part 1) and patients (Parts 2 and 3) who have received at least one dose of the study drug, whether prematurely withdrawn from the study or not, will be included in the safety analysis.

PHARMACOKINETIC ANALYSES

Study subjects will be excluded from the pharmacokinetic analysis population if they significantly violate the inclusion or exclusion criteria, deviate significantly from the protocol or if data are unavailable or incomplete which may influence the pharmacokinetic analysis. Excluded cases will be documented together with the reason for exclusion. All decisions on exclusions from the analysis will be made prior to database closure.

PHARMACODYNAMIC ANALYSES

The analyses of viral dynamic response in Part 2 of the study will include all patients who were randomized and received at least one dose of study drug (RO7049389 or placebo). Patients will be analyzed according to the treatment group to which they were randomized.

The analyses of viral dynamic response in Part 3 of the study will include all patients who received at least one dose of RO7049389.

SAMPLE SIZE JUSTIFICATION

For Part 1 of the study, up to 93 healthy volunteers will be enrolled. This comprises up to 53 for Parts 1a and 1b, and up to 40 for Part 1c. For Part 2 of the study, up to 35 patients with chronic HBV-infection will be enrolled.

The number of study subjects to be randomized was chosen based on practical considerations and complies with standard safety review rules. With six study subjects receiving active drug at a dose level, there is an 82% chance to observe at least one adverse event that has an incidence rate of 25% in the population. The total sample size may increase adaptively based on the study findings for safety, PK or viral dynamic outcomes.

This sample size for POM (Part 2) is to support the assessment of viral dynamic response to the study drug treatment. When the sample size is 6, a one-sided 90% confidence interval for the

mean change from baseline of HBV DNA (Log_{10} IU/mL) will have an interval that extends no more than 0.904 from the observed mean assuming that the standard deviation is 1.5 and that the confidence interval is based on the t-statistic.

*Initially, a*pproximately 55 patients with Chronic Hepatitis B will be enrolled in the *first* 3 cohorts of Part 3 *with approximately 30 patients in* POM Cohort A, *10 patients in* POM Cohort B, and *15 patients in* POM Cohort C. *Based on emerging efficacy/safety data, the sample size in Cohort C can be increased to approximately 30 patients. In addition, additional treatment cohorts may be opened to explore different treatment durations, dose regimens, or different combinations.*

The sample size for Part 3 is intended to support the assessment of response rate of HBV DNA <LLOQ and HBsAg loss at 24 weeks after EOT. A sample size of 10, 15*,* or 30 would ensure that the lower 95% CI is above 5%, 9%, or 14%, respectively, if the underlying response rate is 30%, assuming binomial distribution.

INTERIM ANALYSIS

No interim analysis is planned for Part 1 and Part 2 of this study. Given the hypothesis-generating nature of this study, the Sponsor may choose to conduct interim efficacy analyses for Part 3 of the study. The decision to conduct an optional interim analysis and the timing of the analysis will be documented in the Sponsor's trial master file prior to the conduct of the interim analysis. The interim analysis will be performed and interpreted by Sponsor study team personnel.

LIST OF PROHIBITED MEDICATIONS

Healthy Volunteers

All medications (prescription and OTC) taken within 30 days of study screening will be recorded on the appropriate eCRF.

As a general rule, no concomitant medication will be permitted within 14 days prior to the first dosing or within 5 half-lives of the medication prior to the first dosing (whichever is longer), until the follow up visit (Day 8 for Part 1a, Day 23 for Part 1b, and Day 21 for Part 1c), with the exception of the cases listed as the Permitted Therapy.

Exceptions may be made on a case-by-case basis following discussion and agreement between the investigator and the sponsor after unless the rationale for exception is discussed and clearly documented.

Chronic HBV-infected patients in Part 2 and Chronic Hepatitis B Patients in Part 3

For all patients, the following are prohibited:

- Any systemic antiviral therapy other than that provided by the study at any time from 14 days before starting the study drug until the end of follow-up period, with the exception of oral therapy for HSV I or HSV II. Investigational drugs or herbal and other remedies being taken by the patient for possible or perceived effects against HBV are prohibited.
- Inducers of CYP3A enzyme (including but not limited to efavirenz, nevirapine, pioglitazone, rifampin, rifabutin, troglitazone, phenobarbital, phenytoin, carbamazepine, and St. John's wort) within 14 days or 5 half-lives, (whichever is longer) before the first administration of RO7049389 and while on study treatment.
- Inhibitors of CYP3A enzyme (including but not limited to indinavir, nelfinavir, clarithromycin, itraconazole, ketoconazole, nefazodone, ketoconazole, verapamil, suboxone, diltiazem, cimetidine, amiodarone, fluvoxamine, troleandomycin, voriconazole) within 7 days or 5 half-lives (whichever is longer) before the first administration of RO7049389 and while on treatment with RO7049389.
- Inducers of UDP-glucuronosyltransferase (UGT) enzymes (including but not limited to carbamazepine, nicotine) within 14 days or 5 half-lives, (whichever is longer) before the first administration of RO7049389 and while on study treatment.
- Inhibitors of UGT enzymes (including but not limited to atazanavir, gemfibrozil, indinavir, ketoconazole) within 7 days or 5 half-lives (whichever is longer) before the first administration of RO7049389 and while on treatment with RO7049389.

- Inhibitors of OATP transporters (including but not limited to cyclosporine, rifampicin, eltrombopag, lapatinib, lopinavir, ritonavir) within 7 days before the first administration of RO7049389 and while on treatment with RO7049389.
- Inhibitors of breast cancer resistance protein (BCRP) transporter (including but not limited to cyclosporine, elacridar, eltrombopag, gefitinib) within 7 days before the first administration of RO7049389 and while on treatment with RO7049389.
- The total daily dose of acetaminophen (paracetamol) should not exceed 1 g/day.
- Patients who have been on stable hormone replacement therapy for a period of at least 2 months prior to Screening will not be excluded from the study.
- Systemic immunosuppressive drugs, cytotoxic or chemotherapeutic agents, radiation therapy, anti-arrhythmics, ergot derivatives, oral/parenteral corticosteroids, or topical Class 1 and 2 steroids, probenecid, and bile acid binding resins while on study treatment.
- Any hormonal methods of contraception during the study treatment period and 28 days afterwards (only for patients enrolled into part 2).
- Midazolam data from part 1c indicated mild but not clinically significant inhibition of CYP3A enzyme by RO7049389. CYP3A4 substrates with narrow therapeutic range (including but not limited to alfentanil, cyclosporine, diegotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus) may need to be used with caution in Parts 2 and 3 of the study.
- For substrates of OATP1B (including but not limited to atrasentan, bosentan, ezetimibe, irinotecan, statins (e.g., atorvastatin, rosuvastatin, simvastatin, pitavastatin, pravastatin), repaglinide, rifampin, valsartan, olmesartan), the co-administration of RO7049389 is expected to result in mild (< 2 fold) increase in blood concentrations of the substrates. Therefore, use the lowest necessary dose, titrate the dose carefully, and monitor closely for substrate-associated adverse reactions.

Prohibited Food

Use of the following is prohibited during the study:

- Alcohol consumption is to be strongly discouraged in patients with chronic HBV infection 14 days before starting the study drug until 24 weeks after the last dose. During the study, patients should not consume more than 20 g of alcohol per day (or on average approximately 140 g of alcohol per week).
- In healthy volunteers, alcohol must not be consumed from 48 hours before screening, 48 hours before admission until completion of follow-up visit.
- Any nutrients known to modulate activity of CYP enzymes (e.g., grapefruit *juice* or Seville orange *juice*) will be prohibited within 3 days before Day -1 through the last dose of RO7049389.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AASLD	American Association for the Study of Liver Diseases
AE	Adverse event
Ae	Cumulative amount excreted unchanged in the urine
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APASL	Asian Pacific Association for the Study of the Liver
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the curve
AUC0-τ	Area under the plasma concentration-time curve for a dosing interval
BMI	Body mass index
BP	Blood Pressure
cccDNA	C*ovalently c*losed circular DNA
CHB	Chronic Hepatitis B
CL	Clearance
CL/F	Apparent clearance
CRM	Continuous reassessment method
CYP	Cytochrome P450
DAIDS	Division of AIDS
DDI	Drug-drug interaction
DILI	Drug-induced liver injury
DRF	Dose range finding
EASL	European Association for the Study of the Liver
EC	Ethics Committee
ECG	Electrocardiograms
eCRF	Electronic Case Report Form
EFD	Embryofetal development
EOT	End of Treatment
FDA	Food and Drug Administration
FT3	Free triiodothyronine
FT4	Free thyroxine
GLDH	Glutamate dehydrogenase

Abbreviation	Definition
GLP	Good Laboratory Practice
HAV	Hepatitis A virus
HBcrAg	Hepatitis B core-related antigen
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
hERG	human ether-à-go-go–related gene
HDV	Hepatitis D virus
HDL	High density lipoproteins
HEV	Hepatitis E virus
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IFN	Interferon
IM	Immunomodulatory
IMP	Investigational medicinal product
IND	Investigational New Drug (application)
INR	International normalized ratio
IRB	Institutional Review Board
IxRS	Interactive (voice/web) response system
LDL	Low density lipoproteins
LLOQ	lower limit of quantification
LPLV	Last patient, last visit
LSLO	Last study subject, last observation
MAD	Multiple-ascending Doses
MDR	Multi-drug resistance
MTD	Maximal tolerated dose
NOAEL	No observed adverse effect level
NUCs	Nucleos(t)ide analogues
OTC	Over the counter
PCR	Polymerase chain reaction
PD	Pharmacodynamic
Peg-IFN	Pegylated interferon
POM	Proof of mechanism

Abbreviation	Definition
PK	Pharmacokinetic
PT	Prothrombin time
QD	Once per day
QRS	QRS Complex
QT	QT Interval
QTc	QT corrected for heart rate
QTca	Rate corrected QT interval
QTcaT	Rate- and temperature-corrected QT interval
QTcF	QT corrected for heart rate using the Fridericia correction factor
RBC	Red Blood Cell
RBR	Research Biosample Repository
RNA	Ribonucleic acid
SAD	Single Ascending Dose
SAE	Serious Adverse Event
SoA	Schedule of Assessments
SoC	Standard of care
TSH	Thyroid-stimulating hormone
UGT	UDP glucuronosyltransferase
ULN	Upper limit of normal
US	United States
V	Volume
WBC	White Blood Cell

1. BACKGROUND AND RATIONALE

RO7049389, an inhibitor of hepatitis B virus (HBV) capsid assembly, is being developed for the treatment of patients with chronic HBV infection.

1.1 BACKGROUND ON HEPATITIS B VIRUS INFECTION

Hepatitis B virus (HBV) infection is a major cause of both acute hepatitis and chronic liver diseases, including cirrhosis and hepatocellular carcinoma. Approximately two billion people worldwide have serological evidence of past or present HBV infection. An estimated *887*,000 *deaths each year were caused by* the acute or chronic consequences of hepatitis B (WHO 2019).

Chronic HBV infection can be characterized by the persistence of high levels of HBV antigens (including hepatitis B e antigen [HBeAg] and hepatitis B surface antigen [HBsAg]). Disease progression is a dynamic process with several stages, during which chronic hepatitis B may be presented either as HBeAg-positive or HBeAg-negative hepatitis.

Persistence of HBV infection is a consequence of the presence of covalently closed circular DNA (cccDNA) in the nuclei of infected hepatocytes. Despite the advancement in the understanding of HBV disease biology, eradication of cccDNA (defined as 'disease cure') may not be achievable for the foreseeable future. Nevertheless, the sustained clearance of HBsAg has been shown to be protective against disease progression and development of HBV complications including cirrhosis, liver failure and hepatocellular carcinoma. Accordingly, sustained HBsAg loss with or without seroconversion has been defined as 'functional cure', and is specified by current treatment guidelines as the *optimal* treatment endpoint. Achieving functional cure allows treatment cessation (EASL 2017) with low likelihood of virological relapse. Other clinically meaningful endpoints, irrespective of HBeAg status, are HBV DNA suppression and alanine aminotransferase (ALT) normalization, which indicate the virological and biochemical responses to therapies, respectively. For HBeAg-positive patients, HBeAg seroconversion is indicative of a better prognosis, including lower rates of cirrhosis and slower disease progression. For both HBeAg-positive and -negative CHB, sustained suppression of HBV replication is associated with biochemical remission, histological improvement and delayed disease progression. However, virological relapse is a major limitation of the currently approved therapies as they rarely result in *functional* cure (HBsAg loss).

Currently, there are two classes of standard of care (SoC) drugs available for the treatment of chronic hepatitis B (CHB): subcutaneously administered interferon (IFN) preparations and orally administered nucleos(t)ide analogues (NUCs). Although both SoC therapies can induce the loss of HBeAg with development of anti-HBe antibody (serological response) and the suppression of HBV DNA to an undetectable level by sensitive polymerase chain reaction (PCR) methods (virological response), and are able

to normalize liver transaminases levels (biochemical response), neither treatment achieves a high rate of $functional$ cure. HBsAg loss only occurs in approximately 3% of $patients$ after one year of treatment and in $<$15% after one to five years follow-up (EASL 2017). In addition, IFN-based therapies are associated with many side-effects, while NUCs frequently require prolonged or possibly life-long therapy, and some are associated with a high risk of viral resistance.

1.2 BACKGROUND ON RO7049389

RO7049389 is an inhibitor of HBV capsid assembly and belongs to the well-studied class of heteroaryldihydropyrimidine (HAP) compounds. This class of compounds induces formation of abnormal HBV core protein aggregates, which are subsequently recognized and depleted. Depleting functional core protein results in interruption of viral assembly and inhibition of HBV replication.

The HBV core protein is involved in multiple steps of the viral life cycle such as encapsidation of pre-genomic ribonucleic acid (pgRNA), subsequent initiation of reverse transcription, and is an important component of cccDNA mini-chromosome. Furthermore, literature suggests that the HBV core protein may play a role in suppressing host innate immune responses (Twu et al 1988; Fernandez et al 2003; Gruffaz et al 2013). Depletion of functional core protein may therefore facilitate host immune restoration. RO7049389 can therefore potentially provide anti-HBV benefits by both direct inhibition of viral replication and augmentation of host immune responses against the virus.

1.2.1 Previous Non-Clinical Studies

RO7049389 has shown potent antiviral activity through the induction of HBV core protein misassembly and subsequent degradation, and has a high degree of selectivity against HBV. RO7049389 demonstrated activity against most prevalent HBV genotypes (A, B, C, D) and against a panel of nucleos(t)ide analogue-resistant HBV variants tested in vitro.

RO7049389 showed additive or minor-to-moderate synergism in in vitro combination analyses with each of the following: entecavir, tenofovir, and IFN-α-2a (Roferon-A). In recombinant AAV-HBV-infected mice, orally administered RO7049389 was shown to reduce serum levels of HBV DNA, HBsAg, and HBeAg. Combination treatment of RO7049389 with the Roche TLR7 agonist RO7020531 in AAV-HBV mice resulted in HBsAg reduction to below the lower limit of quantification (LLOQ) in 5 of 7 animals by the end of the 6-week treatment. This reduction was sustained through the off-treatment follow-up period. Anti-hepatitis B surface antibody (anti-HBs) was detected in 3 of 7 mice treated with this combination. The anti-viral efficacy of RO7049389, either alone or in combination with Peg-IFN, was also demonstrated in an additional animal model of HBV-infected human liver chimeric mice.

Pharmacokinetic (PK) studies in rats, monkeys and mini-pigs showed a $moderate$ plasma clearance (~50% liver blood flow), moderate distribution (~body water volume) and

relatively short half-life ≤ 2 hours. Plasma exposures following oral administration at pharmacodynamic (PD) dose levels are moderate with ~20% oral bioavailability; at higher dose levels in 28-day toxicity studies, overall oral exposures were high, with male/female area under the curve (AUC) of 181000/362000 ng • h/mL at 250 mg/kg once per day (QD) in the rat and 111000/124000 ng • h/mL at 350 mg/kg QD in the mini-pig.

RO7049389 is stable in plasma of all species tested (including human) with unbound fraction in all species in the range of 4 to 12% unbound fraction with no excessive binding to erythrocytes.

Metabolism studies showed the disposition of RO7049389 to be mediated mainly by liver metabolism (oxidative via CYP3A4 as major and direct glucuronidation via UGT1A3 likely as minor); however, further contribution of biliary clearance cannot be excluded.

RO7049389 showed little or no induction potential for enzymes such as major cytochrome P450 (CYP) isoforms or UDP glucuronosyltransferases (UGTs) as well as various transporters, however, RO7049389 is a substrate of human multi-drug resistance (MDR)1 (P-glycoprotein [P-gp]) and of human liver organic anion-transporting polypeptide 1B1 (OATP1B1) and OATP1B3. RO7049389 shows little or no inhibition for all CYP isoforms, carboxyl esterases and aldehyde oxidase but it inhibited to various degrees MDR1, OAT3, BSEP, OATP1B3 and especially OATP1B1. Therefore, in vivo drug-drug interactions due to co-administration of respective transporter substrates cannot be excluded.

RO7049389 demonstrated no potential to cause time-dependent inhibition of all cytochrome P450 isoforms except for CYP3A4 where a mild inhibition was observed. Therefore, in vivo drug-drug interactions (DDIs) with co-administered drugs that are CYP3A4 substrates cannot be excluded.

Three metabolites of RO7049389 have been identified in human plasma that each exceed 10% of total drug-related material (M5, M6 and M11) based on steady state exposures. Metabolite M5 exceeds exposures of RO7049389. Based on the available PK evaluation of M5 and preliminary data for M6 and M11, accumulation of these metabolites is limited with twice daily or once daily repeated dosing. All three metabolites have been characterized for the inhibitory potential on drug metabolizing enzymes and do not appear to pose a concern for potential DDI.

There were no adverse test article-related effects in rats, monkeys or mini-pigs up to 1000 mg/kg/day in the single-ascending dose *or* the repeat-dose toxicity studies. The no-observed-adverse-effect level (NOAEL) was determined as the highest dose tested in each study: 14-day study 1000 mg/kg/day (AUC_{0-24} 546 µg • h/mL), 4-week study 250 mg/kg/day (AUC_{0-24} 272 µg • h/mL), and 26 week study 120 mg/kg/day (AUC_{0-24} 117 µg • h/mL) in rats; 15-day study 1000 mg/kg/day (AUC_{0-24} 575 µg • h/mL), 4-week

study 350 mg/kg/day (AUC_{0-24} 118 µg • h/mL), and 39-week study 250 mg/kg/day (AUC_{0-24} 73.4 µg • h/mL) in mini-pigs.

RO7049389 was neither mutagenic nor clastogenic in vitro or in vivo.

In definitive Good Laboratory Practice (GLP) reproductive and developmental toxicity studies, no adverse effects on embryofetal development (EFD) were observed in the rat at doses up to 75 mg/kg/day (AUC_{0-24} 70.2 µg • hr/mL) or in rabbits at the highest tested dose of 300 mg/kg/day (AUC_{0-24} 23.5 µg • hr/mL). In rats, increased early embryonic death and an increase in transient skeletal variations (bent scapulae and/or wavy ribs) were noted at 150 mg/kg/day (AUC_{0-24} 325 µg • hr/mL). Thus, the NOAEL for EFD was 75 mg/kg/day in the rat and 300 mg/kg/day in the rabbit. In a definitive (GLP) fertility study in male and female rats, no adverse effects on mating performance, fertility, or early embryonic development were noted up to the highest tested dose of 250 mg/kg/day.

Cardiovascular function was assessed in conscious telemetered minipigs in a pilot non-GLP study at doses of 100–1000 mg/kg and in a pivotal GLP-compliant study at 75, 150, and 250 mg/kg. In the pilot study, administration of 1000 mg/kg RO7049389 caused a core body temperature decrease of approximately 1.5°C and individually rate-corrected QT interval (QTca) increases of 40 ms. After rate and temperature correction, the rate- and temperature-corrected QT interval (QTcaT) increase was approximately 15–20 ms. The NOEL for cardiovascular parameters in the non-GLP pilot study was 300 mg/kg and in the pivotal GLP telemetry study was the highest dose evaluated of 250 mg/kg. Systemic concentrations at 4 hours after dosing were around 7 µg/mL in both sexes with corresponding free concentrations of around 800 ng/mL, which is more than 10-fold lower than the human ether-à-go-go–related gene (hERG) IC_{20}. *The hERG IC_{20} is about 20-fold higher than the maximum concentration of unbound RO7049389 at the C_{max} of the potential highest dose in clinical studies (600 mg BID).*

RO7049389 had no effect on neurobehavioral and respiratory function in rats at dose levels of up to 150 mg/kg.

An in vitro study indicated that RO7049389 had no phototoxicity potential.

Separate studies were performed with the major human metabolites M5, M6, and M11.

In vitro genotoxicity studies of M5, M6, and M11, and hERG assays of M5 did not reveal any new preclinical safety signals.

In a 2-week dose range finding (DRF) study with M5 administered to the rats, dose levels at ≥1000 mg/kg/day resulted in early mortalities and effects on body weight. The dose of 750 mg/kg/day was NOAEL (AUC_{0-24} 58.2 h • µg/mL).

A DRF EFD study in rats showed that administration of 375 or 750 mg/kg/day of M5 led to maternal toxicity leading to lower dose level selection for a subsequent GLP-compliant study. In the definitive GLP EFD study in rats, administration of M5 at 180 mg/kg/day led to failed implantation and/or early embryonic death and pregnancy failure in the majority of rats. The NOAEL for maternal and embryo fetal toxicity was 60 mg/kg/day (unbound M5 AUC_{0-24} 0.28 µg• h/mL, total M5 AUC_{0-24} 4.9 µg• h/mL). The exposure of unbound M5 is 3x and total M5 is 0.2x of those in humans given the recommended dose of RO7049389 (600 mg QD). There were no indications of teratogenicity at any dose level. -The unbound (free) M5 exposure is considered more relevant for risk assessment in human as generally pharmacological effects (on-target or off-target) are mediated by the unbound drug levels in plasma or tissue, and for M5 significant differences in plasma protein binding exists between species (FDA 2020).

See the RO7049389 Investigator's Brochure for further details on non-clinical studies.

1.2.2 Previous Clinical Studies

This study (YP39364) is the first-in-human study with RO7049389. At present, *Parts 1 and 2 have been completed and the Part 3 is ongoing. Two other* Phase I clinical studies with RO7049389: YP39406 and YP40218 have *also* been completed.

Overall, RO7049389 was safe and well tolerated across three studies in both HVs and patients. The safety profiles were consistent between HVs and patients, as well as between Asian and non-Asian HVs/patients. Additionally, there were no safety signals or dose-related trends in the ECGs, vital signs, or laboratory safety data (except for ALT elevations, which were only observed in patients).

1.2.2.1 Preliminary results of study YP39364

Preliminary results of *Parts 1 and 2 of* study YP39364 are as follows:

Part 1 of YP39364: Healthy Volunteers

- Forty-one healthy volunteers in the SAD part of the study received RO7049389 at dose levels ranging from 150 to 2500 mg or matching placebo. *Forty-*two healthy volunteers in the MAD part of the study were treated with twice-daily RO7049389 (dose ranging from 200 mg to 600 mg) for 14 days or 800 mg BID with a standard meal for 3 days (the cohort was prematurely terminated due to non-safety related reasons).
- *S*afety *data* review showed that both single doses and BID dosing were well tolerated in all healthy volunteers. Fifty-nine adverse events (AEs) were reported~~,~~ with 44 AEs in 30 of 62 HVs who received RO7049389 and 15 AEs in 9 of 21 HVs who received placebo. *The majority of* AEs were of mild intensity *and considered as not related to study drug*. All AEs resolved or *were* resolving. The most common AE was headache in both the SAD and MAD *treatment-active arms*. No severe AEs or serious adverse events (SAEs) were observed in any cohort and no AEs leading to

drug discontinuation were reported. There were no clinically significant changes in ECG parameters, vital signs, or laboratory safety test results.

Part 2 of YP39364: Patients with Chronic HBV infection

- In Part 2 of the study, 37 patients with chronic HBV infection received RO7049389 or matching placebo at doses of 200 or 400 mg BID with a standard meal, 600 mg QD,1000 mg QD or 200 mg QD fasted for 28 days in one of the five proof of mechanism (POM) Cohorts. Of these 37 patients, two patients (one in POM Cohort 2 and the other in POM Cohort 4) were discontinued due to non-safety reasons and were replaced.
- A total of 75 AEs were reported with 69 AEs in 19 of 31 patients who received RO7049389 and 6 AEs in 3 of 6 patients who received placebo. The majority of AEs in the treatment-active arms were of mild intensity with headache, ALT increased, AST increased, upper respiratory tract infection (URTI), and diarrhea being the most common AEs. All of the AEs resolved or were resolving at the time the data were extracted. Transient lab abnormalities (Grade 2-4 ALT/AST elevations) were observed in 6 of 31 patients who received RO7049389. There were no safety signals or dose related trends in the ECG parameters, vital signs, or laboratory safety data other than ALT/AST. No severe AEs or SAEs were reported in patients who received RO7049389. One pre-dose SAE (worsening of ALT/AST elevation) was reported in the placebo arm, and the patient was replaced.
- Preliminary efficacy data from the 35 patients (30 active: 5 placebo) who completed 28 days of treatment in Part 2 of the study showed robust declines in plasma HBV DNA in both HBeAg positive and negative patients with a median decline of 2.7, 3.2, 2.9, 3.2, and 3.0 $\log_{10}$ IU/mL from baseline in the five POM cohorts, respectively. Viral breakthrough was not observed during dosing; however, HBV DNA levels returned towards the baseline by 4 weeks post-treatment.

1.2.2.2 Results from Study YP39406

- YP39406 is a randomized, sponsor-open, investigator-blinded, subject-blinded, placebo-controlled, SAD and MAD study to investigate the safety, tolerability, and PK of RO7049389 in healthy Chinese volunteers. Safety data review showed that both single doses and BID dosing were well tolerated in all healthy volunteers. No SAEs or AEs leading to discontinuation were reported. No clinically significant changes in ECG parameters, vital signs, or laboratory safety test results were observed.
- Twenty-eight healthy volunteers in the SAD part of the study received RO7049389 or placebo at doses of 200 mg fasted, 400 mg fasted, and 600 mg fasted. Nine AEs were reported with 8 AEs in 4 of 22 HVs in the active arms and 1 AE in 1 of 6 HVs in the placebo arm. All AEs were of mild intensity and none of these AEs were considered to be related to study drug. All of these AEs resolved without treatment.
- Twenty-four healthy volunteers in the MAD part of the study received RO7049389 or placebo at a dose of 200mg BID and 400mg BID. There were 21 subjects who participated in both SAD and MAD parts of the study. Twenty-three AEs were

reported *with 19 AEs in 15 of 20 HVs who received RO7049389 and 4 AEs in 2 of 4 HVs who received placebo. A*ll AEs were of mild intensity. Thirteen AEs of *lip dry (*xerocheilia) *with 2 cases from the placebo arm and 11 cases from the active arm* were considered to be related to study drug *by the investigator*. All AEs resolved without treatment.

1.2.2.3 *R*esults from Study YP40218

- Study YP40218 is an open-label, fixed-sequence, two-period study to investigate the effect of RO7049389 on the pharmacokinetics of pitavastatin in healthy volunteers. Pitavastatin was administered orally to participants on Day 1 of Period 1 and Day 4 of Period 2. RO7049389 was administered at a dose of 800 mg BID for 6 days in Period 2.
- Eighteen healthy volunteers were enrolled in the study. Twelve AEs were reported in 7 out of 18 healthy volunteers. All AEs were of mild intensity except *for* one AE of vomiting, which also led to the discontinuation of the subject from the study. Nine AEs were considered related to the study treatment *by the investigator*. One AE of headache was considered to be related to both RO704389 and pitavastatin, while the remaining 8 AEs were considered related only to RO704389. All AEs resolved without treatment. No severe AEs or SAEs were observed. There were no clinically significant changes in ECG parameters, vital signs, or laboratory safety test results.

1.3 BACKGROUND ON MIDAZOLAM

As described in Section 1.2.1, preclinical studies conducted with RO7049389 could not exclude in vivo drug-drug interactions (DDIs) between the study drug and CYP3A4 substrates. Therefore, micro-doses of a clinically validated probe substrate of CYP3A4, Midazolam, will be used in this study to evaluate the DDIs of RO7049389 on CYP3A4 activity (EMA 2012). Midazolam is approved for use as a short-acting benzodiazepine and central nervous system depressant. For additional background, see the PI (Midazolam, DRUGDEX).

1.4 STUDY RATIONALE AND BENEFIT–RISK ASSESSMENT

1.4.1 Study Rationale

This is the first clinical study of RO7049389. The study comprises three parts.

Part 1 will be conducted in healthy volunteers to assess the safety, tolerability, and pharmacokinetics (PK) of orally administered single- and multiple-ascending doses (SAD and MAD). The solubility of RO7049389 in bio-relevant media is low and pH-dependent. However, physiologically-based pharmacokinetic (PBPK) modeling indicates that the absorption of RO7049389 is unlikely to be affected by food at the predicted therapeutic dose of 400 mg BID. To confirm this expectation and provide guidance with regard to food restriction for subsequent clinical trials, the effect of food on the PK of RO7049389 will be evaluated in one of the SAD cohorts.

In addition, the effect of multiple dosing of RO7049389 on the PK of a single oral micro-dose of midazolam (MDZ) will be evaluated in MAD cohorts. Micro-dosing of MDZ has been shown to be a safe and an effective means to probe the modulation effect of a compound on CYP3A4 activity (EMA 2012). It is being incorporated in this study to investigate the weak in vitro signal of time-dependent CYP3A4 inhibition observed with RO7049389. Studying a wide range of RO7049389 dose levels will provide a comprehensive understanding on the relationship between dose versus CYP3A4 activity and help to define the optimal dose-range for Phase II studies from the DDI perspective.

In the MAD cohorts, 4β-hydroxycholesterol/cholesterol ratio in plasma will be measured as an endogenous marker for hepatic CYP3A4 activity. The correlation between midazolam clearance and endogenous substances ratios will be explored in this study.

Part 2 of this study is a proof-of-mechanism (POM) evaluation investigating the relationship between systemic RO7049389 exposure and viral PD response in chronically HBV-infected patients who will receive study treatment for 28 days. In the Part 2 POM cohorts, antiviral effect of RO7049389 will be measured by the reduction of HBV DNA.

RO7049389 causes formation of abnormal capsid structure which is subsequently degraded, resulting in core protein depletion. Interference of capsid structure directly inhibits viral replication process. Therefore, HBV DNA reduction provides evidence of the mode-of-action (MoA) of a capsid inhibitor. In studies using mouse models of chronic HBV infection, significant reduction of HBV DNA (2-3 logs within 2 weeks) appeared to correlate with HBsAg and HBeAg decline at a later time-point. The first-in-patient, 28-days treatment in Part 2 is not expected to demonstrate HBsAg reduction within the planned 4-week dosing, and it is still unclear how the reduction in HBV DNA will be correlated with HBsAg reduction in patients with longer treatment duration. Nevertheless, it is hypothesized that in human, suppression of HBV DNA will also be the initial efficacy marker, followed by reduction in viral antigens. The aim of Part 2 of this study is to select a safe and effective dose as the basis for Part 3 of this study and future studies.

Part 3 of the study will assess the efficacy/safety of RO7049389 when administered in combination with SoC therapies (a NUC with/without Peg-IFN), for up to 48 weeks. The primary objective of Part 3 is to assess HBV DNA <LLOQ with HBsAg loss at 24 weeks post-treatment (i.e., rates of functional cure).

Currently two classes of SoC therapies have been approved for treatment of hepatitis B: Peg-IFN and NUCs. Current treatments reduce the risk of CHB sequelae but are associated with very low rates of functional cure (HBsAg loss rates generally not exceeding 3% after one year of therapy). Combinations of antiviral therapy that target different steps in the HBV life cycle to suppress viral replication with immune modulatory therapy to restore immune responses to HBV are more likely to be efficacious than

monotherapy to achieve sustained loss of HBsAg (functional cure). Thus, administration of RO7049389 in combination with NUC with/without Peg-IFN for 48 weeks may provide higher rates of functional cure than is observed with SoC therapies alone.

Interim data from Part 2 of this study has demonstrated that RO7049389 administration in HBV-infected patients is associated with a good safety and tolerability profile, as well as declines in HBV DNA levels (i.e., positive POM). The pre-clinical data in mice demonstrated that HBsAg and HBeAg reduction occurred only after maximal DNA suppression, which suggests the effect of RO7049389 on viral antigens requires a longer treatment duration than the 4 weeks explored in Part 2. Thus, Part 3 aims to evaluate the effect of RO7049389 after longer treatment duration of up to 48 weeks.

1.4.2 Benefit–Risk Assessment

Part 1 will be conducted in healthy volunteers, and no therapeutic benefit is anticipated for these subjects. Part 2 will involve patients chronically infected with HBV. The short-term nature of the proposed treatment is not expected to result in *functional* cure, as defined by HBsAg loss. However, the results from Part 2 will provide essential information for selecting suitable doses for future studies. Part 3 of the study will also involve CHB patients. The longer-duration treatment in combination with SoC aims to result in therapeutic benefit for participating subjects, including possibly higher functional cure rates than observed currently with SoC therapies (monotherapy with a NUC or Peg-IFN, or combination therapy with a NUC plus Peg-IFN).

Before initiation of Part 1, no prior clinical experience with RO7049389 existed. The evaluation of the potential risks of treatment and the specific tests, observations, and precautions required for clinical studies with RO7049389 were based on information from non-clinical toxicology and safety pharmacology studies. Safety and tolerability will be carefully assessed, and study subjects (referring to both healthy volunteers and patients in the protocol) will be closely monitored. Section 6 of the Investigator's Brochure (Guidance to the Investigator) summarizes key risk management activities to consider when dosing this novel compound.

Based on (i) the calculation of a safe starting-dose for the first-in-human study as recommended in the Food and Drug Administration (FDA) guidance (FDA 2005), and on (ii) exposure multiple calculations, the selected starting-dose of 150 mg is considered to be safe (please refer to Section 3.2.1 for details). The target exposure range of RO7049389 proposed within this study has been shown to be safe and well-tolerated in pre-clinical studies, and under a controlled setting, should provide a better understanding of clinical safety and tolerability to guide future clinical studies. The risks for an individual study subject due to ascending doses of RO7049389 or study-related procedures are considered to be minimal.

RO7049389 has shown to be safe and well tolerated when dosed for up to 4 weeks treatment in 30 CHB patients in Part 2 of this study. To support the long-term treatment (up to 48 weeks) of RO7049389 in Part 3, chronic toxicology studies including a 26-week study (120 mg/kg/day [AUC_{0-24} 117 µg • h/mL]) in rats and a 39-week study (250 mg/kg/day [AUC_{0-24} 73.4 µg • h/mL]) in mini-pigs have been completed. RO7049389 was neither mutagenic nor clastogenic in vitro or in vivo.

In Part 3 of the study, RO7049389 will be administered on top of SoC. According to the draft FDA guidance (FDA 2018), non-clinical combination studies of an investigational drug plus an approved drug are not recommended. Therefore, there is no combination toxicity study of RO7049389 on top of SoC therapy. However, long-term treatment with SoC therapies have shown a highly favorable safety profile. Safety and tolerability will be carefully assessed during the study by close monitoring.

2. OBJECTIVES

2.1 OBJECTIVES FOR PART 1

The **primary objectives** of Part 1 (healthy volunteers) of this study are as follows:

- To assess the safety and tolerability of single and multiple doses of RO7049389.
- To characterize the PK of RO7049389.

The **secondary objectives** of Part 1 of this study are as follows:

- To evaluate the effect of food on the PK of RO7049389 after a single dose administration.
- To evaluate the effect of multiple oral dosing of RO7049389 on CYP3A4 activity assessed by the PK of a single oral micro-dose of midazolam.

The **exploratory objectives** of Part 1 of this study are as follows:

- To screen for the presence of metabolites of RO7049389 in selected plasma and urine samples.
- To evaluate the effect of multiple oral dosing of RO7049389 on CYP3A4 activity assessed by plasma 4β-hydroxycholesterol/cholesterol ratio.

2.2 OBJECTIVES FOR PART 2

The **primary objectives** of Part 2 (patients chronically infected with HBV) of this study are as follows:

- To assess the safety and tolerability of 4 weeks of treatment with RO7049389.
- To investigate the antiviral effect (quantitative HBV DNA level) of 4 weeks of treatment with RO7049389.

The **secondary objective** of Part 2 of this study is:

- To investigate the plasma PK of RO7049389.

The **exploratory objectives** of Part 2 of this study are as follows:

- To investigate the response relationship between RO7049389 exposure and HBV DNA
- To explore other viral response outcomes (e.g., quantitative HBeAg, loss of HBeAg, HBeAg seroconversion, quantitative HBsAg, loss of HBsAg, HBsAg seroconversion) after 4 weeks of treatment with RO7049389.
- Exploratory liver biomarkers will be examined.
- To investigate metabolites of RO7049389 in selected plasma samples.

2.3 OBJECTIVES FOR PART 3

The **primary objective** of Part 3 (patients with chronic hepatitis B) of this study is:

- To evaluate anti-viral effect (functional cure) of treatment with RO7049389 on top of Standard of Care (SoC).
    - Functional cure is defined as HBV DNA < lower limit of quantification (LLOQ, 20 IU/mL) with HBsAg loss (<0.05 IU/mL) at 24 weeks post-treatment.

The **secondary objectives** of Part 3 of this study are as follows:

- To further characterize the efficacy profile of RO7049389 on top of SoC over time, including HBsAg loss, HBsAg seroconversion, quantitative HBsAg change from baseline, HBeAg loss, HBeAg seroconversion, quantitative HBeAg change from baseline, HBV DNA < LLOQ, HBV DNA change from baseline in different proof of mechanism (POM) cohorts of Part 3 and in subgroups of baseline HBeAg +/- patients, or of baseline high / low HBsAg level patients.
- To assess other viral response outcomes (including HBV core-related antigen levels, anti-HBc antibody, quantitative HBV RNA, etc.) over time.
- To assess the ALT normalization in patients with baseline ALT elevation.
- To characterize the plasma PK profiles of RO7049389 and its metabolites.
- To assess the long-term safety and tolerability of RO7049389 on top of SoC (a NUC with or without Peg-IFN).
- To assess the PK interaction between NUCs and RO7049389.
- To assess HBV genotypic resistant variants associated with virologic failure.

The **exploratory objectives** of Part 3 of this study are as follows:

- To explore other viral response outcomes (i.e., total HBsAg) over time.

- To explore relationship between exposure and efficacy/safety response (e.g., HBsAg, HBV DNA, etc.).
- To explore safety, PK, and antiviral activities of RO7049389 in different ethnic subpopulations (e.g., Caucasian versus Asian).
- To assess effect of treatment on liver stiffness.
- To explore relationship between baseline disease characteristics (e.g., HBeAg status, viral load, HBV genotype based on DNA or RNA or historical data, etc.) and efficacy/safety response.
- To explore the effect of the genetic polymorphism of CYP3A4, UGT1A3 and OATP1B on the PK of RO7049389 by clinical genotyping.
- To explore the PK interaction among NUCs, Peg-INF and RO7049389.

3. STUDY DESIGN

3.1 DESCRIPTION OF STUDY

3.1.1 Overview of Study Design

Study YP39364 is a first-in-human study comprised of three parts.

Part 1 of the study will evaluate safety, tolerability, and PK of RO7049389 following oral administration of single (Parts 1a and 1b, Figure 1) or multiple (Part 1c) doses in healthy volunteers. The effect of food on the PK of RO7049389 will be evaluated within one of the SAD cohorts (Part 1b). Drug interaction with micro-dose midazolam will be assessed in all MAD cohorts (Part 1c). There will be no midazolam dosing in SAD cohorts (Part 1a and 1b). In Part 1 of the study, approximately 93 healthy volunteers will be enrolled. A Bayesian adaptive approach, i.e., Continuous Reassessment Method (CRM) will be used, alongside scientific and clinical judgment, to inform decisions about the dose of RO7049389 to be given to the next cohort during dose-escalation, as well as to identify the maximum tolerated dose (MTD).

Part 2 of the study will evaluate the safety, tolerability, PK and PD of RO7049389 following oral administration in patients chronically infected with HBV (Figure 1). In Part 2 of the study, approximately 35 patients will be enrolled.

Part 3 of the study will evaluate the safety, tolerability, PK, PD, and efficacy of RO7049389 following oral administration on top of SoC therapies (a NUC with/without Peg-IFN) in NUC-suppressed or treatment-naive CHB patients (Figure 2). Initially, approximately 55 patients *are planned i*n the *first* three cohorts of Part 3. *Based on emerging efficacy/safety data, the sample size in Cohort C can be increased to approximately 30 patients. In addition, additional treatment cohorts may be opened to explore different treatment durations, dose regimens, or different combinations.*

Figure 1 Study Design (Parts 1 & 2)

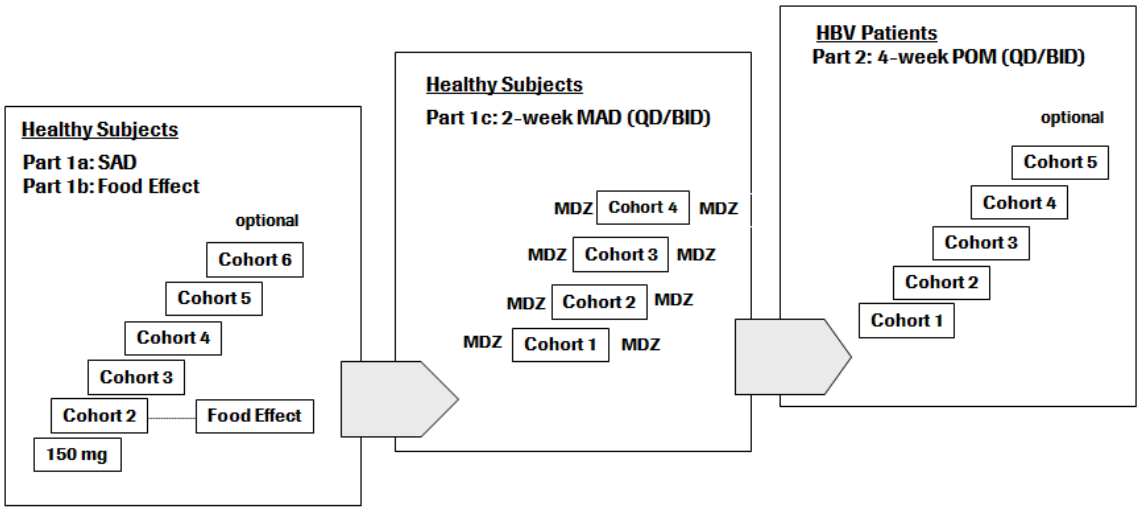
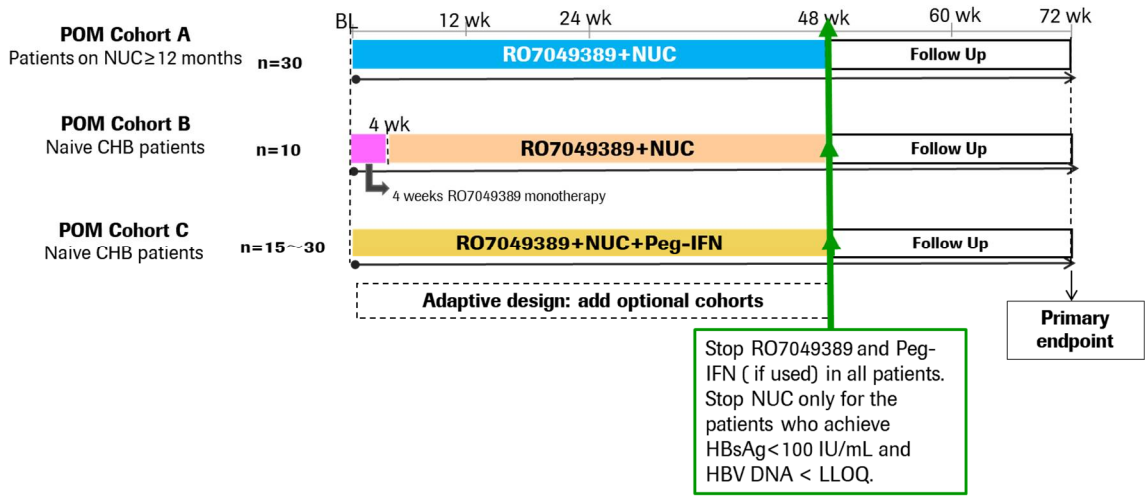


Figure 2 Study Design (Part 3)



If there are no safety and PK concerns during the dose-escalation, the planned study procedures are as follows:

- Cohort 1 in the SAD arm of the study (Part 1a) will receive a single-dose of 150 mg (see Section 3.1.2 for rationale). Doses for subsequent cohorts will be defined by an adaptive approach based on the safety and PK data in previously-dosed healthy subjects (refer to Section 3.2.1 for details).
- The effect of food on the PK of RO7049389 will be evaluated within one of the SAD cohorts (Part 1b). The food-effect cohort will be selected such that the expected exposure of RO7049389 will be efficacious, based on the safety and PK data from previous SAD cohorts, and estimated efficacious target from non-clinical data.

- Cohort 1 in the MAD arm of the study (Part 1c) will start when exposure in the SAD dose level has reached 0.5-fold or higher of the predicted efficacious exposure. The starting dose of the MAD arm of the study will be chosen such that the expected steady-state exposure of RO7049389 will be 0.5-fold of the expected efficacious exposure or higher, but less than, or equal to, the exposure that has been explored and well-tolerated in previous SAD cohort(s).
- Cohort 1 in Part 2 of the study will start when steady-state exposure in the MAD dose level has reached 0.5-fold or higher of the projected efficacious exposure. The starting dose of Part 2 of the study will be chosen such that the expected steady-state exposure of RO7049389 will be less than, or equal to, the exposure that has been explored and well-tolerated in the previous MAD cohort(s).
- If MTD in SAD part of the study is reached at an exposure level which is lower than 0.5 -fold of the estimated efficacious exposure, MAD will start at a dose level less than MTD in SAD.
- If MTD in MAD part of the study is reached at an exposure level which is lower than 0.5-fold of the estimated efficacious exposure, POM will start at a dose level less than or equal to MTD in MAD.
- Doses and dosing regimens in the cohorts of Part 3 will be selected based on accumulated PK, PD, efficacy and safety data from this study or other related studies, as well as exposure and safety data from chronic GLP tox studies.

If any safety or PK concerns emerge during the study, the planned cohorts may be adjusted. Details are described in the relevant sections of the protocol. Details on efficacious target from non-clinical data are described in Section 3.2.1.

3.1.1.1 Part 1a: Single-Ascending Dose (SAD) in Healthy Subjects

Part 1a is a randomized, sponsor-open, investigator-blinded, subject-blinded, placebo-controlled, adaptive single-ascending dose study. This part of the study consists of approximately 5 planned dose levels with four or five healthy volunteers in each. An additional 1-2 cohorts may be enrolled to evaluate additional doses as needed based on emerging data. Up to two back-up subjects can undergo the preparatory activities in order to replace participants who withdraw from the study for non-safety reasons.

The first cohort of the SAD arm of the study will be split into two groups. Initially, two healthy volunteers will be dosed, one with RO7049389, and one with placebo. Following acceptable safety assessment of the first two healthy volunteers by the Investigator, and at least 24 hours after the initial administration, three more healthy volunteers will be dosed, two with RO7049389, and one with placebo.

For subsequent cohorts in Part 1a, a minimum of 4 HVs will be enrolled in a 3:1 ratio to receive RO7049389 and placebo. The sample size may be increased to 8, with 6 volunteers receiving RO7049389 and 2 receiving placebo at dose levels at or above the projected efficacious exposure.

The doses in this part will be administered under fasted condition (see Section 4.3.2 for detail).

Healthy volunteers enrolling for Part 1a of the study, and who do not go on to participate in Part 1b, will be in the study for approximately 8 weeks, as follows (and in Table 1):

Eight healthy volunteers from Part 1a will go on to participate in Part 1b of the study, described below.

Table 1 High-level Overview of Study Procedure: Part 1a (SAD)

Activities	Screening		In-Clinic Period			Ambulatory Visit		Follow-up Visit[a]	Follow-up Call[a]
						↓	↓		
Day	-28	-3	-2	1	3	4	5	8 (±1)	29 (±3)
Study Drug				▲					

[a] the healthy subjects who will participate in Part 1b (FE) will not have follow-up in Part 1a

3.1.1.2 Part 1b: Food Effect in Healthy Subjects

Part 1b is a sponsor-open, investigator-blinded, subject-blinded, placebo-controlled, two-period (fasted, fed) sequential food-effect study in healthy volunteers. Part 1b will include a minimum of 8 healthy volunteers from one of the dose levels of Part 1a, 6 of whom will have received RO7049389, and 2 of whom will have received placebo. In the first period of the Part 1b, up to 2 back-up subjects can undergo the preparatory activities in order to replace participants who withdraw from the study for non-safety reasons. The dose at which food-effect will be assessed is that which is projected to be efficacious based on emerging PK data from previous cohort. The fed dose will occur when:

- The safety and available PK data through 24 hours post-dose from the fasted dosing were reviewed.
- The fasted dose was considered well-tolerated and there is agreement to continue to the next dose escalation.
- The selected dose level is agreed by both the Sponsor and the Investigator.
- The allocation of RO7049389 or placebo and the dose administered will be the same as that which was administered in the fasted state.
- The eight healthy volunteers from Part 1a will be asked to return to the study unit on Day 15 after the initial dose of RO7049389.

Volunteers will be administered a single-dose of RO7049389, or placebo, on Day 16 in the fed state. ‘Fed state’ will be after eating the standard US FDA-recommended high-fat and high-calorie breakfast. Specific details on the content of the high-fat meal are provided in Appendix 9.

Healthy volunteers enrolling for Part 1b of the study will be in the study for approximately 10 weeks, from screening in Part 1a, through study completion in Part 1b, as follows (and in Table 2):

Table 2 High-level Overview of Study Procedure: Part 1b (FE)

Activities	Screening	In-Clinic Period	Ambulatory Visit	Follow-up Visit	Follow-up Call
			↓ ↓		
Day	N/A	15 16 17 18	19 20	23 (±1)	44 (±3)
Study Drug		▲			

3.1.1.3 Part 1c: Multiple-Ascending Dose (MAD) in Healthy Subjects

Part 1c is a randomized, sponsor-open, investigator-blinded, subject-blinded, placebo-controlled, adaptive, multiple-ascending dose study. Up to 5 cohorts of 8 healthy volunteers will be enrolled. In each cohort, 6 healthy volunteers will be randomized to receive RO7049389, and 2 will be randomized to receive placebo. The dose and dosing regimen of MAD cohort 1 would be determined based on PK and safety data from available SAD cohorts. Based on emerging PK and safety evaluation from initial cohorts, additional cohorts may be enrolled to receive a higher or lower dose, or a different regimen. Up to 2 back-up subjects can undergo the preparatory activities in order to replace participants who withdraw from the study for non-safety reasons. Potential drug interaction with micro-dose midazolam will be assessed in all MAD cohorts.

Healthy volunteers in the first MAD cohort will be dosed with RO7049389 for 13 days (QD or BID regimen, depending on available PK and safety data). On Day 14, only one dose in the morning will be given regardless of the dosing regimen. A single oral dose of midazolam solution (100 μg) will be administered on Day -1 and Day 14 (see Section 4.3.1.2).

Healthy volunteers enrolling for Part 1c of the study will be in the study for approximately 10 weeks from screening through to study completion as follows (see also Table 3):

Table 3 High-level Overview of Study Procedure: Part 1c (MAD)

Activities	Screening	In-Clinic Period	Follow-up Visit	Follow-up Call
Day	-28 -3	-2 -1 1 2 … 13 14 15 16	21 (±1)	42 (±3)
Midazolam		△ △		
Study Drug[a]		▲ ▲ … ▲ ▲ ▲ ▲ … ▲		

[a] If PK data from SAD cohorts support QD regimen, subjects will be dosed for two weeks once daily

3.1.1.4 Part 2: Proof-of-Mechanism (POM) in Patients with Chronic HBV Infection

Part 2 is a multi-center randomized, sponsor-open, investigator-blinded, subject-blinded, placebo-controlled, adaptive multiple-dose study in chronically HBV-infected patients. Five cohorts are planned. In each cohort, six patients will be randomized to receive RO7040389, and one will be randomized to receive placebo. Based on emerging PK and safety evaluation from previous cohorts, additional cohorts may be enrolled based on emerging data and development needs. Due to the adaptive nature of the study, the actual number of dose levels will be determined during the study. Up to two back-up subjects can undergo the preparatory activities in order to replace participants who withdraw from the study for non-safety reasons.

Patients will be dosed twice daily with RO7049389 for 27 days. On Day 28, only one dose in the morning will be given. Depending on the PK and safety evaluation from available data, a QD regimen may be used instead of BID dosing. Patients will have the option of two brief in-clinic stays, as described in the SoA (see Appendix 5).

Patients enrolling for Part 2 of the study will be in the study for approximately 20 weeks from screening through to study completion as follows (see also Table 4):

Table 4 High-level Overview of Study Procedure: Part 2 (POM)

Activities	Screening	Ambulatory Visit[b]												Follow-up Visits			
Day	-28	-2	-1	1	2	3	4	…	8	…	15	…	22	…	28	29	35 (±1 Day)	56 (±3 Days)	84 (±2 Weeks)	112 (±2 Weeks)
Study Drug[a]				▲	▲	▲	▲	…	▲	…	▲	…	▲	…	▲					
				▲	▲	▲	▲	…	▲	…	▲	…	▲	…						

a. If PK data from previous dose cohorts support QD regimen, subjects will be dosed for 28 days once daily
b. Patients will have an option to be housed in the clinical unit for two nights from Day -1 to Day 2 and for three nights from Day 27 to Day 30.

3.1.1.5 Part 3: Proof-of-Mechanism (POM) in Patients with Chronic Hepatitis B

Part 3 is a multi-center, open-label, non-controlled, adaptive, multi-arm study in CHB patients. *Initially,* three cohorts (A-C) are planned *to enroll approximately 30 patients i*n POM Cohort A, *10 patients in POM Cohort* B, and *15 to 30 patients in POM Cohort* C to receive RO7049389 on top of SoC therapies.

Both HBeAg positive and negative patients will be enrolled*, and at least* 30% of patients enrolled in each cohort will be HBeAg positive. In order to evaluate pharmacokinetics, pharmacodynamics and safety in different ethnic subgroups, the Sponsor may postpone or stop enrolment of certain patient subgroups to achieve suitable balance *across* the groups.

Due to the adaptive and exploratory nature of the study, the sample size of existing cohorts can be increased *and* additional cohorts of different treatment regimens or treatment durations can be started, based on emerging clinical data.

- POM Cohort A will enroll NUC-suppressed CHB patients. Patients will receive RO7049389 on top of a NUC for 48 weeks. RO7049389 will be administered at a dose determined from study Part 2. NUC therapy (entecavir, tenofovir alafenamide, or tenofovir disoproxil fumarate as per investigators decision) will be administrated as per local label or guidelines.
- POM Cohort B will enroll treatment-naïve immune-active CHB patients. Patients will first receive RO7049389 only for 4 weeks to compare PK, safety, and its effect on HBV DNA declining in different ethnic subgroups when RO7049389 is used alone. Then a NUC will be added on for another 44 weeks. RO7049389 will be administered at a dose determined from study Part 2. NUC therapy (entecavir, tenofovir alafenamide, or tenofovir disoproxil fumarate as per investigators decision) will be administered as per local label or guidelines.
- POM Cohort C will enroll treatment-naïve immune-active CHB patients. Patients will receive RO7049389 + NUC + Peg-IFN therapy for 48 weeks. RO7049389 will be administered at a dose regimen determined from study Part 2. NUC (entecavir, tenofovir alafenamide, or tenofovir disoproxil fumarate as per investigators decision) and Peg-IFN therapy will be administered as per local label or guidelines. For Peg-IFN therapy, there are stopping rules recommended in major guidelines (2018 AASLD, 2017 EASL, 2015 APASL guidelines). These criteria were developed based on Peg-IFN monotherapy data and may or may not be applicable to this investigational combination (NUC + RO7049389 + Peg-IFN). Investigators may refer to these stopping rules to individualize Peg-IFN treatment duration in this study. For patients who stop Peg-IFN early due to intolerability or meeting some stopping criteria, NUC and RO7049389 should be continued till 48 weeks. The reasons of stopping Peg-IFN and length of treatment duration should be clearly recorded on CRF.

At the end of study treatment (EOT) visit, stop RO7049389 and Peg-IFN (if used), but NUC therapy is expected to be continued as Standard of Care (SoC) with the exception of patients who achieve NUC Stopping Criteria: HBsAg < 100 IU/mL and HBV DNA < LLOQ *(20 IU/mL)* at EOT visit. NUC should be continued until central lab results from EOT visit become available and NUC Stopping Criteria are met.

The sample size of individual cohorts range from 10 to 30, and may be increased to 30 subjects if considered warranted by emerging efficacy/safety data. In addition, based on emerging clinical data, additional cohorts may be enrolled to explore alternative combination regimens (e.g., shorter treatment duration of 12 or 24 weeks).

Patients enrolling for Part 3 of the study will be in the study for approximately 76 weeks from screening to study treatment and follow up as shown in Table 5. The screening

period will be up to 4 weeks. The treatment period will be for a maximum of 48 weeks followed by 24 weeks Follow-up period.

Table 5 High-level Overview of Study Procedure: Part 3 (POM)

	Screening		Treatment period				Follow-up period		Early Termination/ Study Drug Discontinuation
Protocol Activity	Day -28 to -2	Day -1	Day 1	2w ±3d	4w ±3d	8-48w ±6d (Every 4 weeks)	56-72w ±6d (Every 8 weeks)		
							50-60w±3d (Every 2 weeks) [a]	64-72w±6d (Every 4 weeks) [a]	
Dispense RO7049389 *and/*or provide SoC			x		x	x			
Safety evaluation			x	x	x	x	x	x	x
Efficacy assessment			x	x	x	x	x	x	x

a. Only applicable to participants who meet NUC Stopping Criteria at the EOT visit.

3.1.2 Dose-Related Decision Criteria

Dose-related decisions will be made jointly by the Sponsor's Clinical Team and the Investigator(s). Doses may be repeated, reduced or escalated based on emerging safety, tolerability, PK data and/or observations on viral dynamic response (Part 2 only) at each dose-level. The dose-escalation decision will be submitted to Ethics Committee (EC) and/or Health Authorities (HA) per local requirement.

The decision to escalate to the next dose-level or to add an additional cohort at a previously-studied dose will be based primarily on safety and tolerability data, and secondarily on available PK data, at the previous dosage level. In addition, all available safety, tolerability, and PK data from the previous dose-level(s) will be reviewed.

The decision to escalate to the next dose level will be made following review of the following data from the completed cohort:

- All available safety information, including adverse events (AEs), electrocardiograms (ECGs), vital signs, clinical laboratory test results collected up to 48 hours post-dose (Part 1a) or post-last dose (Part 1c and Part 2).
- Adequate PK data over at least 24 hours post-dose (Part 1a) or at steady-state (Part 1c and Part 2) to characterize the PK and predict exposure in the following cohort(s).
    - Pre-clinical data suggest above-proportional increases in exposure. Hence, PK data is needed for dose escalation.
- Available viral dynamic response data will also be considered in dose-decisions in Part 2 of the study.

Dose-escalation will continue according to the protocol until the MTD is identified, or the systemic exposure reaches the exposures observed at the NOAEL in mini-pig from the GLP toxicity studies (AUC_{ss} of 117,000 ng • h/mL and C_{max} of 18588 ng/mL). Potential

exploration of exposure beyond the NOAEL in the mini-pig will not occur unless approval is received from the Ethics Committees of the study sites.

Safety and tolerability data from all 5 subjects in SAD Cohort 1 will be required for the dose-escalation decision of Cohort 2. Except for SAD Cohort 1, evaluable safety and tolerability data from a minimum of 4 subjects in SAD cohorts, 6 subjects in MAD cohorts, and 5 patients in POM cohorts will be required in order to make the decision to escalate to the next dose.

3.1.2.1 Part 1a: SAD

A dose may not be escalated to more than 3-fold increase in $AUC_{0-\infty}$ from one cohort to the next, except in the case where the observed exposure of SAD Cohort 1 in Part 1a of the study is substantially below the predicted values. Given the uncertainty of the predicted exposure of RO7049389, PK data from SAD Cohort 1 will be examined after completion of dosing of all subjects from the cohort. In case the observed exposure of this cohort is substantially below the predicted values, the dose-level for SAD Cohort 2 may be adjusted to reach the originally-expected exposure level for the starting-dose. The dose-escalation thereafter will follow an adaptive approach. This procedure will limit the number of subjects going through the study procedures at a non-informative low-dose.

3.1.2.2 Part 1c: MAD

The dose-range and dosing regimen in MAD cohorts in Part 1c of the study will be determined based on available safety, tolerability, and PK data from the available SAD cohorts. The mean predicted steady-state $AUC_{0-\tau}$ and C_{max} values of the highest MAD dose will not substantially exceed the mean $AUC_{0-\infty}$ and C_{max} values for the highest safe and well-tolerated single-dose studied in the SAD cohorts.

A dose may not be escalated to more than 3-fold increase in $AUC_{0-\tau}$ from one cohort to the next. Dose-escalation will continue according to the study design until the MTD is identified or the systemic exposure reaches the exposure which is around 10-fold above the projected efficacious exposure or the exposure attained at NOAEL in the mini-pig. The MTD is defined by the incidence of dose-limiting events (DLEs). A DLE will be defined as any treatment-related adverse reaction per investigators assessment (e.g., adverse event, laboratory abnormality, change in vital signs, ECG) that would prevent another drug administration at the same dose level in a given study subject.

3.1.2.3 Part 2: POM

Doses and dosing regimen in Part 2 of the study will be determined based on available safety, tolerability, and PK data and also guided by adaptive approach with consideration of predictive models for safety and viral dynamic response as a function of exposure. Based on the PK and safety evaluation of the initial and previous dose cohorts,

subsequent cohorts may be enrolled to receive a higher or lower dose or different regimen.

3.1.2.4 Part 3: POM

Doses and dosing regimens in Part 3 of the study will be determined based on accumulated safety, tolerability, PK, and PD data from this study, as well as the other related studies. The total daily dose will not exceed 800 mg while 1000 mg QD and 400 mg BID have been demonstrated to be safe and well tolerated in Part 2. For parent drug, the projected mean unbound exposure in Part 3 should not be higher (safety margin ≥1.0) than the NOAEL mean unbound exposure in animals (including the 39-week study in mini-pigs). For the metabolites, the projected mean unbound exposure in Part 3 should not be substantially higher (metabolites safety margin ≥ 0.7) than the NOAEL mean unbound exposure in animals (including the 39-week study in mini-pigs).

3.1.3 Dose-Escalation Stopping Criteria

Planned dose-escalation will be stopped if one of the following circumstances occurs in healthy volunteers or patients treated with RO7049389, unless it is obvious that the occurrence is not related to the administration of study drug.

At a given dose-level, more than one third of subjects on active drug experience:

- Severe or clinically significant (as defined by the Investigator) RO7049389-related AEs of the same character.
- Clinically significant RO7049389-related laboratory abnormality of the same character.
- Clinically significant RO7049389-related changes in vital signs or ECGs of the same character (e.g., QT corrected for heart rate using the Fridericia correction factor (QTcF) > 500 msec, or > 60 msec longer than the pre-dose baseline, within the first 48 hours post-dose).
- It is predicted that further dose-escalation will not result in a further increase in plasma exposure.
- Systemic exposure (AUC or C_{max}) reaches the NOAEL after single-dose or at steady-state after repeated dosing. Potential exploration of exposure beyond the NOAEL in mini-pig will not occur unless approval is received from the Ethics Committees.
- Other findings (regardless of the incidence rates) that, at the joint discretion of the Sponsor and the Investigator, indicate dose-escalation should be halted.

In case the dose-escalation is stopped, lower doses could be investigated, or a dose could be repeated in the subsequent cohorts by mutual agreement between the Sponsor and Investigator.

For discontinuation from study drug for an individual study subject, see Section 4.6.1.

3.1.4 End of Study

The end of the study is defined as the date when the last study subject last observation (LSLO) occurs. LSLO is expected to occur 24 weeks after the last dose is administered in Part 3.

3.2 RATIONALE FOR STUDY DESIGN

The rationale for the design of study YP39364 is given in Section 1.4.1.

3.2.1 Rationale for Dosage Selection

Dose selection in all parts of the study will be guided by adaptive approach. A continual re-assessment method (CRM), with control for the probability of over-dosing, based on occurrence of dose-limiting event (DLE) will be used to inform the decision about the dose of RO7049389 to be given to the next cohort during dose escalation.

3.2.1.1 Part 1a: SAD

Starting-dose selection is pharmacology-driven and supported by non-clinical safety data. $C_{average}$ of 290 ng/mL total plasma concentration is considered efficacious and projected to provide two-log DNA reduction based on mouse models of chronic HBV infection. The proposed starting dose of 150 mg is predicted to provide total plasma $C_{average}$ concentration of 55 ng/mL, which is around one-fifth of that which is expected to be efficacious in humans.

The 4-week GLP-toxicology studies defined the NOAEL as 250 mg/kg/day and 350 mg/kg/day in rats and mini-pigs, respectively. These doses convert to human equivalent doses (HED) of 40 mg/kg and 318 mg/kg, respectively. Using the lowest HED of 40 mg/kg from the rats NOAEL calculation and a safety factor of 10, the maximum recommended starting-dose (MRSD) for a 60 kg individual is 240 mg. The starting-dose of 150 mg proposed in this study is approximately 1.6-fold lower than the MRSD and is one fifth of the expected efficacious target (400 mg BID).

The human PK of RO7049389 was predicted using physiologically-based pharmacokinetic modelling. Based on hepatocytes data, hepatic metabolic plasma clearance in human was predicted to be 7.6 mL/min/kg. The prediction of RO7049389 absorption was based on solubility, permeability, and pH solubility profile. The volume of distribution in man was estimated to be 0.9 L/kg. Based on a predicted human clearance of 7.6 mL/min/kg, volume of distribution of 0.9 L/kg, and a bioavailability of 28%, a single-dose of 150 mg is expected to lead to a $AUC_{0-\infty}$ of 1331 ng • h/mL and C_{max} of 169 ng/mL. These predicted exposures are approximately 88- and 110-times lower than the systemic exposures observed at NOAEL in mini-pig from GLP toxicity study (AUC_{ss} of 117,000 ng • h/mL and C_{max} of 18588 ng/mL).

In summary, a single-dose of 150 mg is considered to be a safe starting-dose for this entry-into-human (EIH) study. The dose-range proposed for this part of the study is

150 mg to MTD, or until the systemic exposure reaches the exposure attained at NOAEL in mini-pig.

3.2.1.2 Part 1b: Food Effect

The purpose of Part 1b of the study is to determine dosing instructions with regard to food for Part 1c and Part 2 of the study, as well as the future clinical studies. A dose in the middle of the planned dose-range will be selected in attempt to generate data that can reasonably cover the entire dose-range, including potential dose-levels in Part 1c and Part 2.

3.2.1.3 Part 1c: MAD

Based on single-dose RO7049389 safety and PK data, dose-range and dosing regimen for Part 1c cohorts will be determined. The dose-range in MAD cohorts in Part 1c of the study will be determined based on available safety, tolerability, and PK data from the SAD cohorts in Part 1a. The mean predicted steady-state $AUC_{0-\tau}$ and C_{max} values of the highest MAD dose will not substantially exceed the mean $AUC_{0-\infty}$ and C_{max} values for the highest safe and well-tolerated single-dose studied in the SAD cohorts.

3.2.1.4 Part 2: POM

In addition to PK and tolerability in chronically-infected patients, Part 2 of the study will investigate the short-term effect of RO7049389 on the viral dynamics in humans during 4 weeks of treatment at different dose-levels. The response profiles and the relationship between the viral dynamics and the exposure of RO7049389 will be characterized. These findings, in combination with the tolerability and safety observations in patients, will be used to support further clinical studies, such as dose-selection for subsequent clinical studies.

The starting-dose of Part 2 (POM study) will be a dose that has been studied and considered to be well-tolerated in Part 1c (MAD). Subsequent doses can be higher or lower than the starting-dose, and will be selected using PK, tolerability/safety, and viral dynamic response data.

The highest dose level for this study will not markedly exceed the observed mean exposures of RO7049389 from the highest dose level considered to be well-tolerated in healthy subjects. Predictions on the drug exposure will be based on the PK of RO7049389 from the cumulative data and analyses through the study.

3.2.1.5 Part 3: POM

Part 3 of the study will investigate the efficacy/safety of RO7049389 when administered on top of SoC therapies for up to 48 weeks. The doses and dosing regimen in Part 3 will be determined based on accumulated data from this study and related studies, but the total daily dose will not exceed 800 mg while 1000 mg QD and 400 mg BID have been demonstrated to be safe and well tolerated in Part 2. For parent drug, the projected mean unbound exposure in Part 3 should not be higher (safety margin ≥1.0) than the

NOAEL mean unbound exposure in animals (including the 39-week study in mini-pigs). For the metabolites, the projected mean unbound exposure in Part 3 should not be substantially higher (metabolites safety margin ≥0.7) than the NOAEL mean unbound exposure in animals (including the 39-week study in mini-pigs). Based on the PK, efficacy, and safety evaluation of the initial cohorts (A-C), subsequent cohorts may be added to receive a higher or lower dose or different regimen.

3.2.2 Rationale for Assessing Midazolam Pharmacokinetics

An important aspect of drug development is to predict clinically-relevant drug-drug interactions. Based on in vitro data, RO7049389 is a weak CYP3A time-dependent inhibitor. This study will assess the effect of RO7049389 on CYP3A4 inhibition as measured by midazolam systemic exposure at all the dose levels tested in the MAD cohorts. At the early stage of drug development, it is often challenging to choose a therapeutically-relevant dose to assess drug interaction potentials. Studying a wide range of RO7049389 dose levels will provide a comprehensive understanding on the dose versus CYP3A4 inhibition relationship and help to define the optimal dose-range for Phase II studies from the DDI perspective.

Midazolam is a short-acting drug in the benzodiazepine class. It is selected because it is exclusively metabolized by CYP3A4, it is not a substrate of P-glycoprotein and it has been validated in a number of studies. It is a recommended 'sensitive' model substrate for CYP3A4 by Regulatory Agencies and is the most widely-used CYP3A4 probe substrate across the literature (EMA, 2012). As the majority of co-medications in the targeted patient population are administered by the oral route, the oral administration of midazolam is selected, which will reveal the net effect of possible intestine and liver CYP3A4 inhibition.

In humans, it has been shown that oral midazolam PK is linear in a range of 0.1 μg to 3 mg (30,000-fold). Therefore, PK from nano- and microgram doses of midazolam can reliably predict its PK at milligrams dose-level, which are often used in clinical drug interaction studies (Halama et al., 2013). Also, it is reported that the folds of CYP3A4 inhibition detected by micro-dose of midazolam were similar to those by therapeutic dose of midazolam, suggesting this unobstructive micro-dose approach can be used to assess CYP3A4 activity as an alternative to the regular approach with milligrams dose level of midazolam.

The recommended oral dose of midazolam for pre-operative sedation, anxiolysis, and amnesia in pediatric patients is 0.25 to 0.5 mg/kg with a maximum dose of 20 mg (Midazolam, DRUGDEX). The dose-selection for midazolam in this study (100 μg) is far lower than the therapeutic dose-levels and considered micro-dose according to the ICH guidance (ICH guideline M3).

Giving 100 µg of midazolam to the study participant is not expected to affect the safety assessment of RO7049389 when given together on Day 14. The estimate of average CYP3A4 half-life ranges from 26 to 140 hours (Yang et al., 2008). The 14-day treatment duration of RO7049389 allows the achievement of approximate steady-state of CYP3A4 induction and maximizes the possibility of demonstrating an interaction with a CYP3A4 substrate.

3.2.3 Rationale for Measuring 4β-Hydroxycholesterol/Cholesterol Ratio

In the MAD cohorts, in Part 1c of the study, 4β-hydroxycholesterol/cholesterol ratio in plasma will be measured as an endogenous marker for hepatic CYP3A4 inhibition. 4β-hydroxycholesterol is a metabolite formed by CYP3A4 and CYP3A5-catalyzed metabolism of cholesterol (Björkhem-Bergman et al., 2013).

The ratio of metabolite to parent will be calculated to adjust for between-subject differences in cholesterol levels. This ratio has been used to assess CYP3A4 activity in humans and has been shown to have concordance with the midazolam method. Unlike midazolam, which is extensively metabolized by the intestinal and liver CYP3A4, the metabolism of cholesterol in the intestine is not expected to be significant. Hence, the magnitude of the induction determined by oral midazolam can be greater than by 4β-hydroxycholesterol/cholesterol as it mostly reflects liver CYP3A4 only.

Correlation between midazolam clearance and endogenous substances ratios will be explored in this study. If CYP3A4 activity needs be monitored for future RO7049389 clinical studies, the 4β-hydroxycholesterol/cholesterol ratio, instead of midazolam, can be conveniently employed in such studies.

3.2.4 Rationale for Study Population

3.2.4.1 Part 1: SAD and MAD in Healthy Volunteers

The Part 1 section of the study will be conducted in healthy male and female volunteers of non-childbearing potential, 18 to 60 years of age. The absence of confounding diseases and co-medications in healthy volunteers allows for a clearer and more consistent assessment of drug disposition and safety profile.

3.2.4.2 Part 2: Patients with Chronic HBV infection

Part 2 (POM) of the study will be conducted in patients with chronic HBV infection, who are HBV treatment-naïve, or have stopped HBV treatment for more than 6 months. Both HBeAg-positive (immune-tolerant/immune-active) and HBeAg-negative patients can be enrolled in the study. All the enrolled patients will have a HBV DNA level at minimal $\geq 2 \times 10^4$ IU/mL for HBeAg positive patients, or $\geq 2 \times 10^3$ IU/mL for HBeAg-negative patients. This will allow for the detection of antiviral activity, given that HBV DNA reduction is the direct evidence of primary MoA of RO7049389. The dynamics of HBV DNA readout will be used for dose-selection for a Phase II study in patients with

chronic HBV infection. The PK and safety of RO7049389 will also be evaluated in this population.

3.2.4.3 Part 3: Patients with Chronic Hepatitis B

Part 3 of the study will be conducted in both NUC-suppressed and treatment-naïve CHB patients and will include both HBeAg positive and negative CHB patients.

It was shown that minimizing the HBV viral load by antivirals increases the responsiveness of HBV specific T-cells which are hypo-responsive in cases of persistent HBV infection, and that HBV DNA suppression is an essential requirement for the functional reconstitution of anti-HBV T cell responses (both CD4 and CD8) (Boni et al 1998; Boni et al 2001; Boni et al 2003). RO7049389 will contribute to combination regimens with one or more antiviral agents with different mode of actions or immune-enhancer (e.g., IFN), with the aim of inducing a functional cure. Therefore, treatment with NUCs should provide a good basis for addition of RO7049389 and increase the possibility of functional cure.

POM Cohort B and C will enroll treatment-naïve and immune-active CHB patients. These patients are indicated to initiate SoC antiviral therapy according to the current major HBV guideline (2018 AASLD, 2017 EASL, 2015 APASL guidelines). Elevated ALT and HBV DNA levels will allow for the characterization of antiviral activity (on both HBV DNA and HBsAg) of RO7049389 on top of SoC. In addition, in the immune-active phase, the host immune response to HBV is increased. HBeAg/HBsAg seroconversion may occur in this phase. Therefore, an exploration of antiviral effects in treatment naïve and immune-active patients will bring value in scientific understanding of RO7049389 on top of SoC.

3.2.5 Rationale for Biomarker Assessments

RO7049389 is an inhibitor of HBV capsid assembly. The molecule induces formation of abnormal HBV core protein aggregates, which are subsequently recognized and depleted. Depleting functional core protein results in interruption of viral assembly, inhibition of HBV replication, and reduction of HBV DNA. Blood samples for quantitative or qualitative determination of HBV DNA and other viral parameters will be collected at the time-points indicated in the SoA.

3.3 OUTCOME MEASURES

3.3.1 Safety Outcome Measures

The safety outcome measures for this study are as follows:

- Incidence and severity of adverse events.
- Incidence of laboratory abnormalities, based on hematology, clinical chemistry, coagulation, and urinalysis test results, etc.
- ECGs.

- Vital signs including blood pressure (BP), pulse rate and body temperature.

3.3.2 Pharmacokinetic Outcome Measures

Blood and urine samples will be collected to evaluate the PK, as specified in the SoA. RO7049389 plasma and urine concentrations will be measured by specific validated methods. PK parameters will be estimated using standard non-compartmental methods for RO7049389.

RO7049389 and its metabolites (M5, M6 and M11, if applicable) parameters in Parts 1a (SAD) and RO7049389 parameters in Part 1b (SAD and Food Effect):

- C_{max}: Maximum observed plasma concentration.
- T_{max}: Time to maximum observed plasma concentration.
- $AUC_{0\text{-}last}$: area under the plasma concentration versus time curve up to the last measurable concentration.
- $AUC_{0\text{-}\infty}$: Area under the plasma concentration versus time curve extrapolated to infinity.
- $T_{1/2}$: Apparent terminal phase half-life.
- CL/F: Apparent clearance after oral administration.
- Ae (Cumulative amount excreted unchanged in the urine) and CLR (Renal clearance, computed as Ae/AUC), if warranted.

RO7049389 and its metabolites (M5, M6 and M11, if applicable) parameters in Part 1c (MAD):

- C_{max}: Maximum observed plasma concentration.
- T_{max}: Time to maximum observed plasma concentration.
- $AUC_{0\text{-}\tau}$: Area under the plasma concentration-time curve for a dosing interval.
- C_{trough}: Trough plasma concentration.
- $T_{1/2}$: Apparent terminal phase half-life.
- Accumulation index of RO7049389 and its metabolites.
- Ae and CLR, if warranted.

Midazolam parameters in Part 1c (MAD):

- C_{max}: Maximum observed plasma concentration.
- T_{max}: Time to maximum observed plasma concentration.
- $AUC_{0\text{-}last}$: area under the plasma concentration versus time curve up to the last measurable concentration.
- $AUC_{0\text{-}\infty}$: Area under the plasma concentration versus time curve extrapolated to infinity.

- $AUC_{0\text{-}6h}$: Area under the plasma concentration versus time curve up to 6 hours post-dose.
- $T_{1/2}$: Apparent terminal phase half-life.
- CL/F: Apparent clearance after oral administration.

RO7049389 and its metabolites (M5, M6 and M11) parameters in Part 2 (POM) and Part 3 [RO7049389 on top of SoC]:

- C_{max}: Maximum observed plasma concentration.
- T_{max}: Time to maximum observed plasma concentration.
- $AUC_{0\text{-}\tau}$: Area under the plasma concentration-time curve for a dosing interval, if applicable.
- C_{trough}: Trough plasma concentration.
- $T_{1/2}$: Apparent terminal phase half-life, if applicable.
- Accumulation index of RO7049389 and its metabolites, if applicable.

NUC and Peg-IFN parameters in Part 3 (RO7049389 on top of SoC):

- C_{trough}: Trough plasma concentration.

3.3.3 Viral Dynamic Response Measures

Blood samples for quantitative and qualitative determination of viral dynamic response measurements will be collected at the time-points indicated in the Schedule of Assessment tables and as detailed in Section 4.5.1.9. Viral dynamic response measures will be measured only in Part 2 and Part 3 of the study, and will include, but will not be limited to the following:

- Quantitative HBV DNA level (actual and change from baseline).
- HBsAg (qualitative and quantitative).
- HBeAg (qualitative and semi-quantitative).
- Anti-HBs antibody status (quantitative).
- Anti-HBe antibody status (qualitative).
- Anti-HBc antibody (qualitative).
- Quantitative Hepatitis B core related antigen (HBcrAg).
- Quantitative HBV RNA.
- Quantitative HBsAg decline, loss of HBsAg, development of anti-HBs, HBsAg seroconversion (loss of HBsAg and presence of anti-HBs).
- Quantitative HBeAg decline, loss of HBeAg, development of anti-HBe, HBeAg seroconversion (loss of HBeAg and presence of anti-HBe).
- Quantitative HBV DNA decline, HBV DNA <LLOQ.

- Viral resistance will be monitored from Day −1 through follow-up, see Section 4.5.1.10.

3.3.4 Exploratory Outcome Measures

Since the following measures are set to be exploratory, in some circumstances, these measures will only be tested in a subset of the patients or part of the visits:

In Part 1, endogenous substances levels will be investigated to assess the effect of multiple oral dosing or RO7049389 on CYP3A4 activity. Analysis will include plasma 4β-hydroxycholesterol/cholesterol ratio.

In Part 3:

- Changes of total HBsAg from baseline.
- Change of liver stiffness measurement by Fibroscan.

4. MATERIALS AND METHODS

4.1 STUDY POPULATION

The study may enroll up to a total of 93 healthy volunteers. Due to the adaptive and exploratory nature of this study, the actual number of patients will be determined during the study:

- Up to 53 healthy volunteers in Part 1a of the study. The initial cohort will be of 5 healthy volunteers, with up to 6 subsequent cohorts of 8 healthy volunteers.
- Eight subjects in a dose-level in Part 1a will be asked to return for Part 1b of the study.
- Up to 40 healthy volunteers in Part 1c of the study. There will be up to 5 cohorts with 8 healthy volunteers in each.
- Up to 35 patients in Part 2. There will be up to 5 cohorts with 7 patients in each.
- Initially, approximately 55 patients will be enrolled in the first 3 cohorts in Part 3 with approximately 30 patients in POM Cohort A, 10 patients in POM Cohort B, and 15 patients in POM Cohort C. Based on emerging efficacy/safety data, the sample size in Cohort C can be increased to approximately 30 patients. In addition, additional treatment cohorts may be opened to explore different treatment durations, dose regimens, or different combinations.

Healthy volunteers or patients who drop out of the study treatment for non-safety reasons may be replaced to ensure sufficient data to characterize the safety and PK profile, and to make a dose-escalation decision. Healthy volunteers or patients who withdraw from the study due to poor tolerability or due to study drug-related adverse events will not be replaced.

4.1.1 Recruitment Procedures

Healthy volunteers and patients will be identified for potential recruitment using pre-screening enrollment logs, Independent Ethics Committee (IEC)/Institutional Review Board (IRB) approved newspaper/radio/television/Social Network Service/campus poster advertisements, mailing lists and other distributable documents, prior to consenting to take part in this study.

4.1.2 Inclusion Criteria

Healthy volunteers/patients with HBV must meet the following criteria for study entry:

4.1.2.1 Part 1 – Healthy Volunteers only:

1. Healthy male and female subjects, 18 to 60 years of age, inclusive. Healthy status is defined by absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination including vital signs, 12-lead ECG, hematology, blood chemistry, serology and urinalysis.
2. A body mass index (BMI) between 18 to 30 kg/m^2 inclusive.
3. Female subjects must be either surgically sterile (by means of hysterectomy and/or bilateral oophorectomy) or post-menopausal for at least one year (defined as amenorrhea ≥ 12 consecutive months without another cause, and confirmed by follicle-stimulating hormone level > 35 mIU/mL).
4. For men: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive measures, and agreement to refrain from donating sperm, as defined below:

   a. With female partners of childbearing potential or pregnant female partners, men must remain abstinent or use a condom during the treatment period and for at least 28 days after the last dose of study drug to avoid exposing the embryo. Men must refrain from donating sperm during this same period.

   The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.
5. Informed of, willing and able to comply with all of the protocol requirements and the investigational nature of the study, and have signed an informed consent form (ICF) in accordance with institutional and regulatory requirements.

4.1.2.2 Part 2 – Chronic HBV-Infected Patients only:

1. Adult male and female patients, 18 to 60 years of age, inclusive.
2. A BMI between 18 to 30 kg/m^2 inclusive.
3. Chronic hepatitis B infection, defined as positive test for HBsAg for more than 6 months prior to randomization.
4. HBV DNA at screening $\geq 2 \times 10^4$ IU/mL for HBeAg positive patients, or $\geq 2 \times 10^3$ IU/mL for HBeAg negative patients.

5. ALT at screening $\leq 5 \times$ upper limit of normal (ULN; both-immune tolerant and immune-active patients).
6. Screening laboratory values (hematology, chemistry [other than liver function test], urinalysis) obtained up to 28 days prior to first study treatment within acceptable range or judged to be not clinically significant by the Investigator and the Sponsor.
7. Liver biopsy, fibroscan or equivalent test obtained within the past 6 months demonstrating liver disease consistent with chronic HBV infection with absence of extensive bridging fibrosis and absence of cirrhosis (cutoff for fibroscan is liver stiffness measurement ≤8.5 kPa or Metavir fibrotic Stage <3, or other equivalent staging systems).
8. For men: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive measures, and agreement to refrain from donating sperm, as defined below:
    a. Men must remain abstinent or use a condom during the treatment period and for at least 28 days after the last dose of study drug to avoid exposing the embryo. Men must refrain from donating sperm during this same period.

    The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.
9. For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use non-hormonal contraceptive methods that result in a failure rate of $<$ 1% per year during the treatment period and for at least 3 months after the last dose of study drug.
    a. A woman is considered to be of childbearing potential if she is post-menarcheal, has not reached a post-menopausal state ($\geq$ 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).

    Examples of non-hormonal contraceptive methods with a failure rate of $<$ 1% per year include male sterilization, and copper intrauterine devices.

    The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.
10. Informed of, willing and able to comply with all of the protocol requirements and the investigational nature of the study, and have signed an informed consent form (ICF) in accordance with institutional and regulatory requirements.

4.1.2.3 Part 3 – Chronic Hepatitis B Patients only:

1. Adult male and female patients, 18 to 60 years of age (inclusive) at the time of signing the informed consent form (ICF).
2. A BMI between 18 to 32 kg/m^2 inclusive.

3. Chronic hepatitis B infection, defined as positive test for HBsAg or HBV DNA (including qualitative, quantitative, and genotype testing) or positive HBeAg for more than 6 months prior to screening.
4. HBsAg >250 IU/mL at screening.
5. For Cohorts only enrolling NUC-suppressed CHB patients (e.g., POM Cohort A), patients must qualify for the following criteria:
    a. Patients treated with a single NUC (entecavir, tenofovir alafenamide, or tenofovir disoproxil fumarate) for ≥12 months. Patients must be on the same NUC therapy for at least 3 months before screening.
    b. At least one result showed HBV DNA <60 IU/mL at least 6 months prior to screening; and HBV DNA <20 IU/mL at screening by Roche Cobas assay.
    c. ALT ≤2 x ULN at screening and at Day -1 (can be checked by local lab result).
6. For Cohorts only enrolling anti-HBV treatment-naïve and immune-active patients (e.g., POM Cohort B and Cohort C), patients must qualify for the following criteria:
    a. Previous anti-HBV treatments for <30 days in total, and did not receive any anti-HBV treatments within 3 months prior to the first study dose.
    b. HBV DNA at screening ≥2 x 10^4 IU/mL for HBeAg positive patients, or ≥2 x 10^3 IU/mL for HBeAg negative patients.
    c. ALT at screening between 1–5 (exclusive) x ULN and ALT <5 x ULN at Day -1 (can be checked by local lab result).
7. Screening laboratory values (hematology, chemistry [other than liver function test], urinalysis) obtained up to 28 days prior to first study treatment within acceptable range or judged to be not clinically significant by the Investigator and the Medical Monitor.
8. Liver biopsy, Fibroscan, or equivalent test obtained within the past 6 months demonstrating liver disease consistent with chronic HBV infection with absence of extensive bridging fibrosis and absence of cirrhosis (cutoff for Fibroscan is liver stiffness measurement ≤8.5 kPa for treatment-naïve patients and ≤7.4 kPa for NUC-suppressed CHB patients, or Metavir fibrotic Stage <3, or other equivalent staging systems).
9. For men: agreement to remain abstinent (refrain from heterosexual intercourse) or agree to use contraceptive measures, and agree to refrain from donating sperm, as defined below:
    a. Men must remain abstinent or use a condom during the treatment period and for at least 6 months after the last dose of study drug to avoid exposing the embryo. Men must refrain from donating sperm during this same period.

    The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.

10. For women of childbearing potential: agree to use two methods of contraception, with at least one method considered as highly effective during the study and for at least 6 months after the last dose of study drug.

    a. A woman is considered to be of childbearing potential if she is post-menarcheal, has not reached a post-menopausal state (≥ 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).

    b. Contraceptive methods considered as highly effective (failure rate <1% per year when used consistently and correctly):

        - Combined (estrogen- and progestogen-containing) or progestogen-only hormonal contraception associated with inhibition of ovulation
        - intrauterine device (IUD)
        - intrauterine hormone-releasing system (IUS)
        - bilateral tubal occlusion
        - vasectomized partner
        - sexual abstinence*

            * Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of study treatment and at least 6 months after the last dose of study drug. In such case, there is no need to use two contraceptive methods. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.

    c. Contraceptive methods NOT considered as highly effective (failure rate >1% per year):

        - Progestogen-only oral hormonal contraception (where inhibition of ovulation is not the primary mode of action)
        - Male or female condoms with or without spermicide
        - Cap, diaphragm, or sponge with spermicide

11. Informed of, willing and able to comply with all of the protocol requirements and the investigational nature of the study, and have signed an informed consent form (ICF) in accordance with institutional and regulatory requirements.

4.1.3 Exclusion Criteria

Healthy volunteers/patients with HBV who meet any of the following criteria will be excluded from study entry:

4.1.3.1 Part 1 – Healthy Volunteers only:

1. Pregnant (positive pregnancy test) or lactating women, and male subjects with partners who are pregnant or lactating.

2. History or symptoms of any clinically significant gastrointestinal, renal, hepatic, broncho-pulmonary, neurological, psychiatric, cardio-vascular, endocrinological, hematological or allergic disease, metabolic disorder, cancer, or cirrhosis.
3. Personal history or family history of congenital long QT syndrome and/or cardiac sudden death.
4. History of Gilbert's syndrome.
5. History of having received or currently receiving any systemic anti-neoplastic (including radiation) or immune-modulatory treatment (including systemic oral or inhaled corticosteroids) $\leq$ 6 months prior to the first dose of study drug or the expectation that such treatment will be needed at any time during the study.
6. Subjects who have had significant acute infection, e.g., influenza, local infection, acute gastrointestinal symptoms, or any other clinically significant illness within two weeks of dose administration.
7. Any confirmed significant allergic reactions (urticaria or anaphylaxis) against any drug, or multiple drug allergies (non-active hay fever is acceptable).
8. Any clinically significant concomitant diseases or condition that could interfere with, or treatment of which might interfere with, the conduct of the study, or that would, in the opinion of the Investigator, pose an unacceptable risk to the subject in this study.
9. Confirmed (based on the average of 3 separate resting blood pressure measurements, properly measured with well-maintained equipment, after at least 10 minutes rest) systolic Blood Pressure greater than 140 or less than 90 mmHg, and diastolic BP greater than 90 or less than 50 mmHg at screening.
10. Clinically relevant ECG abnormalities on screening ECG e.g.:
    a. QTcF $>$ 450 msec or $<$ 300 msec
    b. Notable resting bradycardia (HR $<$ 45 bpm), or HR $>$ 90 bpm
    c. ECGs with documented machine errors in the interval duration assessments.
    d. Evidence of atrial fibrillation, atrial flutter, complete bundle branch block, Wolf-Parkinson-White Syndrome, or cardiac pacemaker.
11. ECG with QRS and/or T-wave judged to be unfavorable for a consistently accurate QT measurement (e.g., neuromuscular artifact that cannot be readily eliminated, arrhythmias, indistinct QRS onset, low amplitude T-wave, merged T- and U-waves, prominent U-waves).
12. Creatinine Clearance (CrCl) $\leq$ 70 mL/min (using the Cockcroft-Gault formula [Appendix 10]).
13. Positive test at screening of any of the following: Hepatitis A (HAV IgM Ab), Hepatitis B (HBsAg), Hepatitis C (HCV RNA or HCV Ab) or human immunodeficiency virus (HIV Ab).
14. Any other clinically significant abnormalities in laboratory test results at screening. In the case of uncertain or questionable results, tests performed during screening may be repeated before randomization to confirm eligibility.

15. Participation in an investigational drug or device study within 90 days prior to screening or more than 4 times per year.
16. Donation or loss of blood over 500 mL within 3 months prior to screening.
17. Positive test for drugs of abuse (including recreational drugs) and/or positive alcohol test at screening or on Day -1.
18. History of drug and/or alcohol abuse or addiction.
19. History (within 3 months of screening) of alcohol consumption exceeding 2 standard drinks per day on average (1 standard drink = 10 grams of alcohol). Alcohol consumption will be prohibited at least 48 hours before screening, 48 hours before and 48 hours after each dose, and 48 hours before each scheduled visit.
20. Use of > 5 cigarettes or equivalent nicotine-containing product per day.
21. Taking any prescribed or over-the-counter (OTC) medications (including vitamins or herbal remedies) within 2 weeks of first dosing or within 5 times the elimination half-life of the medication prior to first dosing (whichever is longer; see also Section 4.4.2). Occasional acetaminophen/paracetamol is allowed (see also Section 4.4.1). Exceptions may be made on a case-by-case basis following discussion and agreement between the Investigator and the Sponsor.
22. Subjects under judicial supervision, guardianship or curatorship.
23. Medical or social conditions that would potentially interfere with the subject's ability to comply with the study visit schedule or the study assessments.
24. History of hypersensitivity to benzodiazepines or its formulation ingredients (for MAD-midazolam cohorts)
25. Acute narrow-angle glaucoma (for MAD-midazolam cohorts).

4.1.3.2 Part 2 – Chronic HBV-Infected Patients only:

1. Pregnant (positive pregnancy test) or lactating women, and male subjects with partners who are pregnant or lactating
2. History or other evidence of bleeding from esophageal varices.
3. Evidence of liver cirrhosis or decompensated liver disease such as ascites, esophageal or gastric varices, splenomegaly, nodular liver, jaundice, hepatic encephalopathy.
4. One or more of the following laboratory abnormalities at screening:
    a. Total serum bilirubin > 2.5 mg/dL (> 42.75 μmol/L). For patients with documented Gilbert's syndrome total bilirubin > 2.75 mg/dL (> 47 μmol/L).
    b. International normalized ratio (INR) > 1.5.
    c. Serum albumin < 3.0 g/dL (< 30 g/L).
    d. Platelet count < 140,000 cells/mm^3.
5. History or other evidence of a medical condition associated with chronic liver disease other than HBV infection (e.g., hemochromatosis, autoimmune hepatitis,

alcoholic liver disease, toxin exposure, thalassemia, nonalcoholic steatohepatitis, etc.).

6. Documented history or other evidence of metabolic liver disease within one year of randomization.
7. Positive test for hepatitis A (IgM anti-HAV), hepatitis C, hepatitis D, or human immunodeficiency virus.
8. Expected to need systemic antiviral therapy other than that provided by the study at any time during their participation in the study, with the exception or oral therapy for HSV I or HSV II.
9. History of or suspicion of hepatocellular carcinoma or alpha-fetoprotein $\geq$ ULN at screening.
10. History of significant gastrointestinal disease (including but not limited to gastric ulcers).
11. History of clinically significant cardiovascular, endocrine, renal, ocular, pulmonary, psychiatric or neurological disease.
12. Evidence of an active or suspected cancer or a history of malignancy other than adequately treated basal cell carcinoma.
13. History of organ transplantation.
14. Previous or concurrent HBV treatments in the past 6 months.
15. History of capsid modulators treatment, including treatment in investigative trials
16. Participation in an investigational drug or device study within 30 days prior to randomization.
17. Taking any drugs or nutrients listed in prohibited medications and prohibited food sections (see also Section 4.4.3).
18. Significant acute infection (e.g., influenza, local infection) or any other clinically significant illness within 2 weeks of randomization.
19. Clinically relevant ECG abnormalities on screening ECG.
20. Abnormal renal function including serum or plasma creatinine > ULN or calculated creatinine clearance $<$ 70 mL/min using the Cockcroft Gault formula (Appendix 10).
21. Donation or loss of blood over 500 mL within 3 months prior to randomization.
22. Administration of any blood product within 3 months prior to randomization.
23. History of alcohol abuse (consumption of more than 2 standard drinks per day on average; 1 standard drink = 10 grams of alcohol) and/or drug abuse within one year of randomization; positive test result for drugs of abuse or alcohol breath test at screening.
24. Subjects under judicial supervision, guardianship or curatorship.
25. Medical or social conditions that would potentially interfere with the subject's ability to comply with the study visit schedule or the study assessments.

4.1.3.3 Part 3 - Chronic Hepatitis B Patients only:

1. Pregnant (positive pregnancy test) or lactating women.
2. History or other evidence of bleeding from esophageal varices.
3. Evidence of liver cirrhosis or decompensated liver disease such as ascites, esophageal or gastric varices, splenomegaly, nodular liver, jaundice, hepatic encephalopathy.
4. One or more of the following laboratory abnormalities at screening:
    a. Total serum bilirubin > ULN (exception Gilbert's disease).
    b. International normalized ratio (INR) >1.1 ULN.
    c. Serum albumin <3.0 g/dL (<30 g/L).
    d. Platelet count <140,000 cells/mm^3
    e. Hemoglobin <12 g/dL (females) or <13 g/dL (males).
    f. White blood cell count <2500 cell/mm^3.
    g. Neutrophil count <1500 cell/mm^3 (<1200 cell/mm^3 if considered a physiological variant in a patient of African descent).
5. History or other evidence of a medical condition associated with chronic liver disease other than HBV infection (e.g., hemochromatosis, autoimmune hepatitis, alcoholic liver disease, toxin exposure, thalassemia, nonalcoholic steatohepatitis, etc.).
6. History of thyroid disease poorly controlled on prescribed medications or clinically relevant abnormal thyroid function tests (thyroid-stimulating hormone [TSH], free triiodothyronine [FT3], free thyroxin [FT4] at screening.
7. Documented history or other evidence of metabolic liver disease within one year of screening.
8. Positive test for hepatitis A (IgM anti-HAV), hepatitis C, hepatitis D, HEV or human immunodeficiency virus (HIV).
9. Expected to need systemic antiviral therapy other than that provided by the study at any time during their participation in the study, with the exception of oral therapy for HSV I or HSV II.
10. Diagnosed or suspected hepatocellular carcinoma as evidenced by screening alpha-fetoprotein (AFP) ≥100 ng/mL. If AFP >ULN, absence of mass/findings suspicious for HCC must be demonstrated by ultrasound or CT or MRI within the screening period.
11. History of significant gastrointestinal disease (including but not limited to gastric ulcers).
12. History of clinically significant cardiovascular, endocrine, renal, ocular, pulmonary, psychiatric, or neurological disease.
13. Evidence of an active or suspected cancer or a history of malignancy other than adequately treated basal cell carcinoma.

14. History of organ transplantation.
15. Participation in an investigational drug or device study within 30 days prior to screening.
16. Taking any drugs or nutrients listed in prohibited medications and prohibited food sections.
17. Significant acute infection (e.g., influenza, local infection) or any other clinically significant illness within 2 weeks of screening.
18. ECG at screening with clinically significant abnormalities, including QTcF interval (QT corrected using Fridericia's formula) ≥450 msec for males and ≥470 msec for females.
19. Abnormal renal function including serum or plasma creatinine > ULN or glomerular filtration rate (eGFR; using CKD-Epi equation) <60 mL/min (Appendix 10).
20. Donation or loss of blood over 500 mL within 3 months prior to screening.
21. Administration of any blood product within 3 months prior to screening.
22. History of alcohol abuse (consumption of more than 2 standard drinks per day on average; 1 standard drink = 10 grams of alcohol) and/or drug abuse within one year of randomization; positive test result for drugs of abuse or alcohol breath test at screening.
23. Subjects under judicial supervision, guardianship, or curatorship.
24. Medical or social conditions that would potentially interfere with the subject's ability to comply with the study visit schedule or the study assessments.

4.2 METHOD OF TREATMENT ASSIGNMENT AND BLINDING

Randomization numbers for healthy volunteers will be generated by independent representatives of the Sponsor, or its designee, for Part 1 of the study. The patient randomization numbers for Part 2 (POM) will be generated by the IxRS (Interactive voice/web response system), according to specifications provided by the Sponsor to the external randomization vendor. Part 3 of the study is a non-randomized, non-controlled, open-label study.

Parts 1 and 2 of the study are investigator-blinded and subject-blinded. In order to minimize bias in reporting, collecting, and interpreting safety data, healthy volunteers, patients, Investigator(s), and all individuals in direct contact with study subjects at the investigative site will remain blinded until the completion of the study, except the pharmacist handling the study drug distribution in Part 1.

Part 1 and Part 2 of this study are sponsor-open. At the Sponsor side, the Clinical Pharmacologists, the Study Pharmacometrician, the Study Clinical Pharmacology Scientist, the Study Biostatistician, the Statistical Programmer/Data Acquisition Specialist/Clinical Data Programmer, the IxRS service provider, the individuals responsible for metabolite identification, and the individual responsible for PK sample

bioanalysis will be unblinded to treatment assignment for each of Parts 1a (SAD), 1c (MAD) and Part 2 (POM) of the study. Other members of the sponsor staff may be unblinded at the Clinical Pharmacologist's discretion, if this is considered necessary to optimize data analysis and dose-escalation decision-making.

Before each of the dose-escalation decisions, the individual safety, tolerability, available PK data, and viral dynamic response (Part 2 only) will be reviewed in a blinded fashion by the Investigator and the Sponsor's clinical team. The Investigator may also be unblinded in mutual agreement with the Clinical Pharmacologist if treatment assignment knowledge is considered critical to allow study progression, and/or is deemed important to define future risk management activities. Unblinding can occur as soon as the Investigator(s) and the responsible Roche scientists have reviewed the data, even if the data have not been entered onto the Case Report Forms (CRFs). If required, unblinded data (individual as well as at group level) may also be presented to the Drug Safety Committee or other experts of the Sponsor.

As per Health Authority reporting requirements, the Sponsor will break the treatment code for all unexpected serious adverse events (SAE; see Section 5.1.2) that are considered by the Investigator to be related to study drug.

The Principal Investigator will receive a set of sealed treatment codes for Parts 1a (SAD) and 1c (MAD). These will have the form of sealed envelopes or scratch codes. If the identity of the test medication needs to be known in order to manage the subject's condition (e.g., in the case of a serious adverse event), the treatment code for that subject may be broken.

At the final monitoring visit, the unused code labels will be counted and checked and a statement to the effect that all are intact (or not as the case may be) will be made by the Monitor; this statement will be included or referred to in the final study report. All code labels will be returned to Roche.

For Part 2 (POM), the Investigator will be able to break the treatment code by contacting the IxRS. Treatment codes should not be broken except in emergency situations. If the Investigator wishes to know the identity of the study drug for any other reason, he or she should contact the Medical Monitor directly before the code is broken, if possible. The Investigator should document and provide an explanation for any premature unblinding (e.g., accidental unblinding, unblinding due to a serious adverse event).

Whenever disclosure of the identity of the test medication is necessary, adequate procedures will be in place to ensure integrity of the data. Any unblinding will be documented in the study report with date, reason for identifying the drug and the name(s) and role(s) in the study of the person(s) unblinded.

For Part 3, the IxRS is used to register patients to one of the three open label cohorts. Additionally, IxRS is used to dispense RO7049389 for patients to provide them with sufficient tablets for that time until the next visit. NUC and Peg-IFN will be provided or reimbursed as non-IMP by the Sponsor.

4.3 STUDY TREATMENT

The following drugs which will be used in the study are marketed drugs and will not be considered as IMPs, midazolam; entecavir, tenofovir alafenamide, or tenofovir disoproxil fumarate; and peginterferon alfa (Peg-IFN).

4.3.1 Formulation, Packaging, and Handling

4.3.1.1 RO7049389 and Placebo

RO7049389 and matching placebo (IMP, Placebo only in Parts 1 and 2) to be used in the study will be provided by the Sponsor.

The clinical formulation is a film-coated tablet for oral administration.

Two different formulations have been developed to date:

The first formulation is a *reddish-brown* film-coated tablet consisting of RO7049389 and the inactive ingredients hypromellose, microcrystalline cellulose, croscarmellose sodium, silicon dioxide, mannitol, sodium stearylfumarate. *T*he coating mixture consists of hypromellose, lactose monohydrate, macrogol, red iron oxide, titanium dioxide, black iron oxide, *and* yellow iron oxide. All excipients used in the formulation are compendial (Ph. Eur. and/or USP/NF) grade.

The film-coated tablets are available containing 50 mg, 100 mg, 200 mg, and 500 mg of RO7049389. A matching-placebo, in terms of size, shape and color, has also been developed for clinical usage. Placebo film-coated tablets have been manufactured containing the inactive ingredients isomalt, microcrystalline cellulose, sodium stearylfumarate, croscarmellose sodium*,* and the same coating mixture, but no active substance.

This clinical formulation of RO7049389 should be stored under the recommended storage conditions: “Store at 2-8°C, protect from light and moisture.”

The second formulation has been manufactured as a *white* film-coated tablet consisting of RO7049389 with dose strength 200 mg and the inactive ingredients hypromellose, microcrystalline cellulose, croscarmellose sodium, silicon dioxide, mannitol, and sodium stearylfumarate. The coating mixture consists of hypromellose, lactose monohydrate, macrogol and titanium dioxide. All excipients used in the formulation are compendial (Ph. Eur. and/or USP) grade.

This clinical formulation of RO7049389 should be stored under the recommended storage conditions: “Do not store above 25 ℃, protect from light and moisture.”

For information on the shelf life of RO7049389 film-coated tablets and the matching placebo, see the packaging.

Study drug packaging will be overseen by the Roche clinical trial supplies department and bear a label with the identification required by local law, the protocol number, drug identification and dosage. The packaging and labeling of the study drug will be in accordance with Roche standard and local regulations.

Upon arrival of investigational products at the site, site personnel should check them for damage and verify proper identity, quantity, integrity of seals and temperature conditions, and report any deviations or product complaints to the Monitor upon discovery.

For further details, see the Investigator’s Brochure.

4.3.1.2 Midazolam (Part 1 only)

The study site pharmacy will prepare a midazolam stock solution of 50 μg/mL from commercially-available midazolam. Two mL of 50 μg/mL stock solution will be administered to subjects in the MAD part of study giving a dose of 100 μg of midazolam.

4.3.1.3 SoC (NUC and Peg-IFN)

NUC (entecavir, tenofovir alafenamide, or tenofovir disoproxil fumarate) and Peg-IFN will be used in Part 3 of the study for an authorized indication, and follow the general warnings and precautions as per local label. Both NUC and Peg-IFN are marketed drugs and will not be considered as investigational medicinal products (IMPs). Peg-IFN and NUC will be provided or reimbursed by the Sponsor *until 24 weeks post-treatment*.

4.3.2 Dosage, Administration and Compliance

Active or placebo (placebo in Parts 1 and 2 only) doses will be administered orally to the study subjects with approximately 240 mL of still water at room temperature. An additional amount of water up to 100 mL could be given to assist dose administration only if needed. *For Parts 1 and 2, t*he dose in mg, the date and time of dosing, and the exact volume of water should be recorded on the eCRF (the exact volume of water will only be recorded during the in-clinic period). *For Part 3, the daily dose in mg, the start and end dates of dosing, plus the date and time of the most recent dosing prior to PK sample collection should be recorded on the eCRF.* The starting-dose of 150 mg will be administered as three 50 mg tablets. All other doses will be administered as the appropriate combination of 50 mg, *100 mg,* 200 mg, and 500 mg tablets.

The qualified individual responsible for dispensing the study drug will prepare the correct dose according to the randomization schedule. This individual will write the date dispensed and study subject’s number and initials on the study drug vial label and on the

Drug Accountability Record. This individual will also record the study drug batch or lot number received by each study subject during the study.

Guidelines for dosage modification and treatment interruption or discontinuation are provided in Sections 3.1.2, 3.1.3, and 4.6.1.1.

4.3.2.1 Part 1a: SAD

RO7049389, or matching-placebo, will be administered orally to healthy volunteers by investigational staff during the morning of Day 1, after an overnight fast of at least 8 hours. Healthy volunteers will not eat for at least 4 hours after the dose is administered. Water will be allowed until one hour prior to dosing, and from one hour post-dosing. Approximately 4-5 hours after dosing, healthy subjects will be served lunch.

4.3.2.2 Part 1b: Food Effect

Healthy volunteers will fast overnight for at least 8 hours prior to Day 16. On the morning of Day 16, a high-fat breakfast (see Appendix 9) will be served to healthy volunteers, which should be consumed within 30 minutes. RO7049389 or placebo will then be administered at the same dose as Day 1 in Part 1a of the study.

4.3.2.3 Part 1c: MAD

RO7049389, or matching-placebo, will be administered orally to healthy volunteers by investigational staff from Day 1 to Day 13 twice daily, and on Day 14 once in the morning. A light breakfast will be served 30 minutes after morning dosing. Lunch will be served approximately 4-5 hours post-morning-dose. The second daily dose will be administered in the evening, approximately 12 hours after the morning dose. Food will be restricted 2 hours prior to, and 1 hour post, the evening dosing.

If QD dosing is required, RO7049389, or matching-placebo, will be administered orally in the morning, and a light breakfast will be served 30 minutes after dosing.

Timing of meals relative to dosing may be changed by the results of the food effect investigation. For example, if food has a marked negative effect on absorption of RO7049389, then, for the morning dose healthy volunteers must fast overnight (at least 8 hours) and will not eat for 2 hours after the morning dose is administered. If food has no marked effect on bioavailability, then the morning dose will be administered with a light breakfast and the evening dose 12 hours later with, or shortly after, dinner. An optional evening snack will be allowed, provided that the fasting period is ensured before the following morning assessments.

The dose-range for RO7049389 MAD cohorts will be determined based on available PK, safety and tolerability data from the SAD arm of the study (Part 1a).

Midazolam

In MAD cohorts, a single oral dose of 100 μg midazolam solution will be administered orally to all subjects on Day -1 and Day 14. The Day -1 dosing will occur at least 24 hours prior to the first dose event of RO7049389 on Day 1. The fasting requirement prior to dosing on Day -1 and Day 14 will be the same as for RO7049389. The Day 14 dosing will occur at the same time when subjects take the last dose of RO7049389. When administered alone, midazolam will be taken with 240 mL of water. When co-administration occurs, midazolam will be taken with ~100 mL of water, whereupon RO7049389 will be taken with the rest of 140 mL of water. Lunch will be given approximately 4-5 hours after dosing.

4.3.2.4 Part 2: POM

If BID dosing is required, RO7049389, or matching-placebo, will be administered twice daily from Day 1 to Day 27, and once on Day 28 in the morning. The second daily dose will be administered in the evening, approximately 12 hours after the morning dose. During the in-house study periods (optional) and ambulatory visits, study drug will be administered by investigational staff. During out-patient study periods, study drug will be self-administered by patients following the guidance provided by investigational staff.

During the in-house period, a light breakfast will be served 30 minutes before the morning dosing. Lunch will be served approximately 4-5 hours post-dose. Dinner will be served 30 minutes before evening dosing if BID.

During the out-patient period, the medication is recommended to be taken within 30 min of completing a meal. Patients will be advised to take evening dose 12 hours apart from the morning dose and to avoid high fat meal.

If QD dosing is required, RO7049389, or matching-placebo, will be administered orally in the morning, and a light breakfast will be served 30 minutes before dosing during the in-house period. During the out-patient period, the medication is recommended to be taken within 30 min of completing a meal.

Food restrictions may be changed based on the results from the food effect arm of the study (Part 1b) study. If it is determined that food significantly affects RO7049389 absorption, other dietary instructions (e.g., meals 2 hours prior to, and 1 hour post, both morning and evening doses) may be implemented.

4.3.2.5 Part 3 of the study

RO7049389 administration

RO7049389 will be administered orally to patients every day from Day 1 to *the actual EOT visit at* week 48. The dosing regimen will be defined prior to dosing of the first patient in each cohort.

If BID dosing is required, the first dose will be administered in the morning and the second dose will be administered in the evening. If QD dosing is required, RO7049389 will be administered once daily. RO7049389 should be administered at approximately the same time of each day. Dietary instructions may be implemented, fasted state is defined as at least 2 hours after a meal and 2 hours before the next meal; with meal is defined as within 30 minutes of completing a meal.

RO7049389 can be given together with NUC or separately.

During the in-house study periods (optional) and ambulatory visits, study drug will be administered by investigational staff. During out-patient study periods, study drug will be self-administered by patients following the guidance provided by investigational staff.

Patients will be asked to record their intake of RO7049389 at home via the patient diary.

NUC administration

NUC will be administered per local label (see the local prescribing information for entecavir, tenofovir alafenamide, or tenofovir disoproxil fumarate for more details). NUC will be provided or reimbursed by Sponsor. NUC administration during the study, including the follow-up period, should be captured in the site documentation and eCRF. This should include information about any missing doses during treatment and follow-up period. Patients will be asked to record their intake of NUCs at home via the patient diary.

For further information on the NUCs used in this study, please follow local prescribing information.

Peg-IFN administration

Peg-IFN (Peginterferon alfa) will be administered in POM Cohort C for 48 weeks per local label. Peg-IFN will either be provided or reimbursed by the Sponsor for the duration of the study. For patients who are unable to tolerate the dosing scheme of Peg-IFN, dose adjustments and interruptions are permitted in order to keep the patient on study treatment. It is highly recommended that dose reductions/interruptions and side-effect management per local labels or guidelines should be implemented in the study. However, in the short-term management of an individual patient, investigators may use their medical judgments in the best interest of the patient. These changes and adverse events must be recorded on eCRF. If Peg-IFN was interrupted or discontinued permanently, patients can continue to be treated with RO7049389 and NUC until the end of the treatment period. Patients will be asked to record their weekly Peg-IFN self-injection at home via the patient diary.

For further information on the Peg-IFN, please follow local prescription information.

4.3.3 Investigational Medicinal Product Accountability

All investigational medicinal products (IMPs) required for completion of this study (RO7049389 or matching placebo) will be provided by the Sponsor. In Part 1, midazolam solution is prepared by the site.

The investigational site will acknowledge receipt of IMPs, to confirm the shipment condition and content. Any damaged shipments will be replaced.

The Investigator(s) is responsible for the control of drugs under investigation. Adequate records of the receipt (e.g., Drug Receipt Record) and disposition (e.g., Drug Dispensing Log) of the study drug must be maintained. The Drug Dispensing Log must be kept current and should contain the following information:

- The identification of the study subject to whom the study drug was dispensed (for example study subject initials and date of birth).
- The date(s), quantity of the study drug dispensed to the study subject.
- The identification of the person who dispensed the drug.
- All records and drug supplies must be available for inspection by the Clinical Trial Monitor at every monitoring visit.

IMPs will either be disposed of at the study site according to the study site's institutional standard operating procedure or returned to the Sponsor with the appropriate documentation. The site's method of IMP destruction must be agreed upon by the Sponsor. Local or institutional regulations may require immediate destruction of used investigational medicinal product for safety reasons. In these cases, it may be acceptable for investigational study site staff to destroy dispensed investigational product before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned, destroyed and provided that adequate storage and integrity of drug has been confirmed.

The site must obtain written authorization from the Sponsor before any IMP is destroyed, and IMP destruction must be documented on the appropriate form.

Written documentation of destruction must contain the following:

- Identity of investigational product(s) destroyed
- Quantity of investigational product(s) destroyed
- Date of destruction
- Method of destruction
- Name and signature of responsible person [or company] who destroyed investigational product[s].

Accurate records of all IMPs received at, dispensed from, returned to, and disposed of by the study site should be recorded on the Drug Inventory Log.

4.3.4 Non-Investigational Medicinal Product Accountability

NUC and Peg-IFN are considered Non-IMP. NUC or Peg-IFN required for completion of this study will be provided or reimbursed by Sponsor.

The Investigator is responsible for providing and checking compliance of NUC and Peg-IFN during the study. The Drug Prescribe/Dispensing Log must be kept current and should contain the following information:

- The identification of the study subject to whom the NUC or Peg-IFN was prescribed (for example study subject initials and date of birth).
- The date(s), quantity of the NIMP prescribed or dispensed to the study subject.
- The identification of the person who prescribed or dispensed the drug.
- All records and drug supplies must be available for inspection by the Clinical Trial Monitor at every monitoring visit.

4.3.5 Post-Trial Access to RO7049389

Currently, the Sponsor does not have any plans to provide RO7049389 to the patients after the end of the study, or when patients discontinue or have been withdrawn from the study. The Sponsor will evaluate whether to continue providing RO7049389 to patients after the main study is over, in accordance with the Roche Global Policy on Continued Access to Investigational Medicinal Product, available at the following website: http://www.roche.com/policy_continued_access_to_investigational_medicines.pdf

4.3.6 Post-*T*rial *A*ccess to SoC

NUC or Peg-IFN are marketed drugs and commercially available. After *completion of* study *participation*, no SoC medication or drug reimbursement is provided as part of this protocol. The investigator is responsible for ensuring that consideration has been given to the post-study care of the patient's medical condition.

4.4 CONCOMITANT THERAPY AND FOOD

4.4.1 Permitted Therapy

4.4.1.1 Healthy Volunteers

As a general rule, no concomitant medication (including herbal products and vitamins) (see exclusion criteria) will be permitted, unless the rationale for exception is discussed and clearly documented between the Investigator(s), the Medical and Safety Monitor, and the Roche Clinical Pharmacologist.

The following medications are permitted:

- Medications used to treat AEs may only be prescribed after consultation with the Sponsor (with the exception of acetaminophen/paracetamol), unless there is a medical need to ensure the well-being of the subject that should not be delayed. All therapy and/or medication administered to manage adverse events should be recorded on the Adverse Event eCRF.
- Hormone replacement therapy (HRT): permitted if initiated at least 2 months prior to study start.
- Occasional use of acetaminophen (up to 1 g per day, not to exceed 3 g/5 days) or ibuprofen (up to 400 mg per day) will be permitted. During the period of confinement to the clinical research unit, subjects will be restricted from the use of acetaminophen/paracetamol and other non-prescription medications beginning 4 hours prior to dosing through 4 hours after dosing unless deemed necessary to treat an Adverse Event (AE) by the Investigator.

4.4.1.2 Chronically HBV-Infected Patients in Part 2 and Chronic Hepatitis B Patients in Part 3

Concomitant therapy includes any medication, e.g., prescription drugs, OTC drugs, approved dietary and herbal supplements, nutritional supplements and any non-medication interventions (e.g., individual psychotherapy, cognitive behavioral therapy, smoking cessation therapy, and rehabilitative therapy) used by a patient from 4 weeks prior to the first dosing through the study completion. All concomitant medications should be reported to the Investigator and recorded on the Concomitant Medications eCRF.

4.4.2 Prohibited Therapy

4.4.2.1 Healthy Volunteers

All medications (prescription and OTC) taken within 30 days of study screening will be recorded on the appropriate eCRF.

As a general rule, no concomitant medication will be permitted within 14 days prior to the first dosing or within 5 half-lives of the medication prior to the first dosing (whichever is longer), until the follow up visit (Day 8 for Part 1a, Day 23 for Part 1b, and Day 21 for Part 1c), with the exception of the cases listed in Section 4.4.1.

Exceptions may be made on a case-by-case basis following discussion and agreement between the investigator and the sponsor after unless the rationale for exception is discussed and clearly documented.

4.4.2.2 Chronic HBV-infected Patients in Part 2 and Chronic Hepatitis B Patients in Part 3

For patients who take NUC or Peg-IFN during the study, please follow the local prescription information to guide co-administration drug or prohibited therapy, if applicable. For all patients, the following are prohibited *(for examples of drugs, see Appendix 16*):

- Any systemic antiviral therapy other than that provided by the study at any time from 14 days before starting the study drug until the end of follow-up period, with the exception of oral therapy for HSV I or HSV II. Investigational drugs or herbal and other remedies being taken by the patient for possible or perceived effects against HBV are prohibited.
- Inducers of CYP3A enzyme within 14 days or 5 half-lives (whichever is longer) before the first administration of RO7049389 and while on study treatment with RO7049389.
- Inhibitors of CYP3A enzyme within 7 days or 5 half-lives (whichever is longer) before the first administration of RO7049389 and while on treatment with RO7049389.
- Inducers of UDP-glucuronosyltransferase (UGT) enzymes within 14 days or 5 half-lives (whichever is longer) before the first administration of RO7049389 and while on study treatment.
- Inhibitors of UGT enzymes within 7 days or 5 half-lives (whichever is longer) before the first administration of RO7049389 and while on treatment with RO7049389.
- Inhibitors of OATP transporters within 7 days before the first administration of RO7049389 and while on treatment with RO7049389.
- Inhibitors of breast cancer resistance protein (BCRP) transporter within 7 days before the first administration of RO7049389 and while on treatment with RO7049389.
- The total daily dose of acetaminophen (paracetamol) should not exceed 1 g/day.
- Patients who have been on stable hormone replacement therapy for a period of at least 2 months prior to Screening will not be excluded from the study.
- Systemic immunosuppressive drugs, cytotoxic or chemotherapeutic agents, radiation therapy anti-arrhythmics, ergot derivatives, oral/parenteral corticosteroids, or topical Class 1 and 2 steroids, probenecid, and bile acid binding resins while on study treatment.
- Any hormonal methods of contraception during the study treatment period and 28 days afterwards (only for patients enrolled into part 2).
- Midazolam data from part 1c indicated mild but not clinically significant inhibition of CYP3A enzyme by RO7049389. CYP3A4 substrates with narrow therapeutic range may need to be used with caution in Parts 2 and 3 of the study.
- For substrates of OATP1B, the co-administration of RO7049389 is expected to result in mild (< 2 fold) increase in blood concentrations of the substrates. Therefore, use the lowest necessary dose, titrate the dose carefully, and monitor closely for substrate-associated adverse reactions.

Exceptions to the above-mentioned concomitant medications will be made if the rationale is discussed and documented between the Investigator and the Medical Monitors.

4.4.3 Prohibited Food

Use of the following is prohibited:

- Alcohol consumption is to be strongly discouraged in patients with chronic HBV infection 14 days before starting the study drug until 24 weeks after the last dose. During the study, patients should not consume more than 20 g of alcohol per day (or on average approximately 140 g of alcohol per week; 1 standard drink = 10 grams of alcohol, for further guidance see Appendix 14). Patients will be queried on a regular basis concerning their alcohol consumption and appropriate comments concerning this intake will be recorded on the eCRF.
- In healthy volunteers, alcohol must not be consumed from 48 hours before screening, 48 hours before admission until completion of follow-up visit.
- Any nutrients known to modulate activity of CYP enzymes (e.g., grapefruit *juice* or Seville orange *juice*) will be prohibited within 3 days before Day -1 through the last dose of RO7049389.

4.4.4 Dietary and Special Requirements

In addition to the meal restrictions described in Section 4.4.3, the following requirements will also be applied in all substudies:

- Laboratory safety assessments should be conducted after study subjects have been fasted for a minimum of 8 hours (4h for screening and follow-up).
- The excessive consumption of fluids (greater than 3 liters per day) should be avoided (only for Part 1).
- On the days of a study visit caffeine (i.e., beverage, chocolate, or supplements) should not be consumed from 48 hours prior to study drug administration until discharge from the clinic (only for Part 1).
- No strenuous exercise is permitted during the study from 96 hours before admission until completion of the follow-up visit (only for Part 1).

Meals will be similar in composition and time of administration across all cohorts during the in-house period in Part 1 with the exception of those healthy subjects participating in the food effect substudy, where breakfast on Day 16 will be a meal with a high-fat breakfast.

The use of tobacco is not permitted during the study for healthy subjects.

4.5 STUDY ASSESSMENTS

4.5.1 Description of Study Assessments

All examinations listed below will be performed according to the schedule of assessments outlined in Appendix 1 to Appendix 8.

All time-points when several assessments coincide, the following sequence should be followed with the PK blood sample to be taken at the nominal time-point:

- Urine collection
- ECG recordings
- Vital signs
- PK, viral dynamic, safety blood sampling
- Administer meal (Part 1b only)
- Study drug administration

4.5.1.1 Medical History and Demographic Data

Medical history includes clinically significant diseases, reproductive status, smoking history, use of alcohol and drugs of abuse, and all medications (e.g., prescription drugs, OTC drugs, herbal or homeopathic remedies, nutritional supplements) used by the study subjects within 30 days prior to the screening visit.

For the patients with chronic HBV infection, the detailed HBV history will be documented, which will include date of HBV diagnosis, mode of HBV infection (if known), HBV genotype (if known), all previous HBV treatments and outcomes of treatments (if any), occurrence of nucleos(t)ide analogue resistance (if any), previous evaluations for cirrhosis, dates/outcomes of liver biopsies (if any).

Demographic data will include age, sex, and self‑reported race/ethnicity.

4.5.1.2 Physical Examinations

A complete physical examination should include an evaluation of the head, eyes, ears, nose, throat, neck and lymph nodes, and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurological systems. A genitourinary examination may be performed in case of evocative symptoms at the Investigator discretion.

Any abnormality identified at baseline (prior to dosing) should be recorded on the General Medical History and Baseline Conditions eCRF.

At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations should be performed at the discretion of the Investigator. Changes from baseline abnormalities should be recorded in study subject’s notes. New or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF.

Body weight will be recorded at screening and during study. Height will only be recorded at screening. BMI will be calculated at screening.

4.5.1.3 Vital Signs

Blood pressure, pulse rate and body temperature (tympanic or oral) will be recorded at the time points specified in Overall Schedule of Assessments (Part 1a and 1b Appendix 1, Part 1c Appendix 3, Part 2 Appendix 5, and Part 3 Appendix 7) and Detailed Schedule of Assessments (Part 1a and 1b Appendix 2, Part 1c Appendix 4, Part 2 Appendix 6, and Part 3 Appendix 8).

Blood pressure and pulse rate should be obtained in a quiet room at a comfortable temperature, with the study subject's arm unconstrained by clothing or other material. All measurements will be obtained from the same arm and, with the same cuff size, using a well-calibrated automatic instrument with a digital readout, throughout the study.

Blood pressure and pulse rate will be performed in triplicate (*interval between measurements* can be as short as 20 sec to 1 min) after the subject has rested in *a* supine position for at least 5 minutes. The mean of three consecutive replicates will be used as the value for the defined time-point.

4.5.1.4 Electrocardiograms

12-Lead ECG

ECGs will be collected after the study subject has been in a supine position for at least 10 minutes. At the specified time-points, 12-lead ECG*s* will be obtained in triplicate, i.e., three consecutive interpretable 12-lead ECGs within a 3-5-minute interval, *for Parts 1 and 2. For Part 3, a single interpretable 12-lead ECG will be taken. R*ecordings should be taken for any unscheduled ECG. *All ECG measurements should be recorded in the eCRF.*

All ECG recordings must be performed using a standard digital high-quality, high-fidelity ECG machine equipped with computer-based interval measurements. Automated ECG intervals (PR [PQ], QRS, QT, QTcF [to be derived in eCRF]) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented. T-wave information will be captured as normal or abnormal, U-wave information will be captured in two categories: absent/normal or abnormal.

For safety monitoring purposes, the Investigator must review, sign and date all ECG tracings either by wet signature or electronically. Paper or electronic ECG tracings must be appropriately kept by the study center and must fulfill all applicable archiving requirements. The ECG intervals and interpretation will be recorded on the eCRF or may be sent electronically. If considered appropriate by Roche, ECGs may be analyzed retrospectively at a central laboratory.

The following are requirements for ECG assessments:

1. Digital ECG recordings, storage and analysis.

2. *Tripilcate/One* useful recording(s) must be collected without artefacts, per time-point.
3. Body position should also be consistently maintained for each ECG performed. In particular, changes in HR should be avoided. The absence of any environmental distractions (TV, radio, conversation) during the pre-ECG rest and the ECG recording in the clinic must be emphasized.
4. Avoid ECG recordings within 3 hours after meals if possible (it is accepted that this is not possible after the breakfast and lunch for some relevant cohorts).
5. Strictly match timing and conditions of ECG recording to baseline. Conditions to be standardized include food intake, activity level, stressors, and room temperature.
6. If possible, the same machine, brand and model, should be used for the same study subject throughout the study.
7. ECGs should be 12-lead, recorded at 25 mm/sec for at least 10 seconds.
8. In some cases, it may be appropriate to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality.
9. ECG machines should have periodic calibration and service records (minimum once a year).
10. If any QT / QT corrected for heart rate (QTc) values $>$ 500 msec or increases from pre-dose on Day 1 QTc $>$ 60 msec (as provided by the machine), the site should repeat the ECG within the next 5 minutes and notify the Sponsor. If confirmed, ECG recordings should be repeated at least hourly until two successive ECGs show QTc values below the threshold value that triggered the repeated measurement.

ECG Holter

Continuous 12-lead ECG recordings (Holter Monitor) will be obtained as specified in the SoA in Part 1a (SAD) and Part 1c (MAD) only (see SoAs; Appendix 1 to Appendix 4). A central ECG laboratory will extract triplicate ECG measurements from the continuous recordings within $\pm$ 10 minutes of the pre-defined ECG time-points, specified in the SoA, to evaluate heart rate, ECG intervals and wave form analysis. The results of these analyses will be available prior to dose-escalation.

Healthy subjects should be at rest and in a supine position for $\geq$ 10 minutes prior to and remain in a supine position for $\geq$ 10 minutes after the specified ECG extraction time-points. Other time-points may also be retrospectively assessed in the event of clinical observations or findings which require further investigation.

While the healthy subjects are on Holter ECG monitoring, the absence of any environmental distractions (e.g., television, radio, conversation, or phone calls) during the pre- and post-ECG rest period, and at every ECG time-point must be emphasized. In particular, activities known to cause changes in heart rate should be avoided.

The timings of assessments may be amended or the number of assessments increased during study conduct on the basis of emerging data in order to allow for optimal characterization of the effect profile.

4.5.1.5 Laboratory Assessments

The samples listed below will be collected at the time-points indicated in the SoA.

- Hematology: Hemoglobin, hematocrit, total white blood cell (WBC) count, differential WBC count (basophils, eosinophils, lymphocytes, monocytes, and neutrophils), platelet count, erythrocytes count (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and reticulocyte counts.
- Coagulation: prothrombin time *(PT)*, international normalized ratio (INR), activated *partial* thromboplastin time (aPTT).
- Blood Chemistry: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and indirect bilirubin, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) (at screening only), creatinine phosphokinase (at screening only), total protein, albumin, urea, creatinine, CrCl (at screening only, using the Cockcroft-Gault formula in Part 1 and Part 2 of *the* study), eGFR (CKD-EPI formula in Part 3 of the study [Appendix 10]), uric acid, total cholesterol, low density lipoproteins (LDL) *cholesterol*, high density lipoproteins (HDL) *cholesterol*, triglycerides, fasting glucose, sodium, chloride, potassium, calcium, phosphorus, bile acids.
- Urinalysis: A midstream, clean-catch urine specimen will be collected for dipstick analysis of protein, blood, glucose, leucocyte *esterase,* and pH. If the dipstick result is 2+ or greater for blood, protein or leukocytes, urine will be sent to the laboratory for microscopy. If there is an explanation for the positive dipstick result, e.g., menses, it should be recorded, and there is no need to perform microscopy. Urine color may be evaluated from urinalysis or urine PK samples if considered necessary.
- Drugs of abuse will be measured in urine: cannabinoids, amphetamines, methamphetamines, opiates, methadone, cocaine, benzodiazepines, and barbiturates.
- Alcohol: Alcohol Breath test.
- Viral markers:
    - Healthy subjects: Hepatitis A (HAV IgM Ab), Hepatitis B (HBsAg), Hepatitis C (HCV RNA or HCV Ab), human immunodeficiency virus (HIV-1 and HIV-2 Ab).
    - Patients with chronic HBV infection: Hepatitis A (HAV IgM Ab), Hepatitis C (HCV RNA *and* HCV Ab), Hepatitis E (HEV IgM Ab and IgG Ab), human immunodeficiency virus (HIV-1 and HIV-2 Ab), Hepatitis D (HDV Ab). For HBV-specific viral assessment in patients, please refer to Section 4.5.1.9.
- Pregnancy Test: Serum or plasma beta-human chorionic gonadotropin (β-HCG) at screening, urine on all other occasions (females only).

- Hormones: Follicle-stimulating hormone (females only to confirm post-menopausal status*, performed at screening only*).
- Alpha fetoprotein (at screening only for patients with chronic HBV infection).
- Thyroid function test: FT3, FT4, TSH (Part 3).
- Potential early liver injury biomarkers including, but not limited to *glutamate dehydrogenase (*GLDH*)* (Part 1c, Part 2, and Part 3).

Normal ranges for the study laboratory parameters must be supplied to the Sponsor before the study starts. Laboratory safety tests shall be collected at time-points specified in the Schedule of Assessments (Part 1a and 1b Appendix 1, Part 1c Appendix 3, Part 2 Appendix 5, and Part 3 Appendix 7).

Additional blood or urine samples may be taken at the discretion of the Investigator if the results of any test fall outside the reference ranges, or clinical symptoms necessitate additional testing to monitor study subject's safety. Where the clinical significance of abnormal lab results is considered uncertain, screening lab tests may be repeated before randomization to confirm eligibility. If there is an alternative explanation for a positive urine or blood test for drugs of abuse, e.g., previous occasional intake of a medication or food containing for example codeine, benzodiazepines or opiates, the test could be repeated to confirm washout.

In the event of unexplained abnormal clinically significant laboratory test values, the tests should be repeated immediately and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found. Results of clinical laboratory testing will be recorded on the eCRF or be received as electronically produced laboratory reports submitted directly from the local (Part 1) or central (Part 2) laboratory.

4.5.1.6 Liver Imaging Assessments

FibroScans will be conducted during Part 3 of the study at the time points indicated in Appendix 7 to monitor the change in liver stiffness. Liver stiffness measure using FibroScan has been widely accepted *in the assessment and* stag*ing of* liver fibrosis.

Abdominal hepatic ultrasound will be conducted during Part 3 of the study at the time points indicated in Appendix 7. Patients with chronic hepatitis B are at risk of disease progression to liver cirrhosis or developing HCC. Abdominal hepatic ultrasound is current practice to monitor disease progress and HCC surveillance in patients with chronic hepatitis B.

4.5.1.7 Pharmacokinetic Assessments

Blood and urine samples will be collected to evaluate the PK as specified in the SoA (see - Overall Schedule of Assessments (Part 1a and 1b Appendix 1, Part 1c Appendix 3, Part 2 Appendix 5, and Part 3 Appendix 7) and Detailed Schedule of

Assessments (Part 1a and 1b Appendix 2, Part 1c Appendix 4, Part 2 Appendix 6, and Part 3 Appendix 8).

When the PK assessment is scheduled for the same nominal time as another scheduled assessment, the PK blood samples should be taken as close as possible to the scheduled time.

Blood samples will be collected from all cohorts. Urine samples will be collected from all cohorts in Part 1a and Part 1c. No urine PK samples will be collected during the food effect dosing period (Part 1b), Part 2 or Part 3. Aliquots of the urine sample will be collected and stored for analysis. The volume of each urine sample at each interval will be measured by the site staff and recorded in CRF.

The actual date and time of each blood sample collection, the start and end date and time for each urine sample collection will be recorded in CRF.

A decision to stop PK sampling earlier or to collect more samples than currently proposed scheduled times will be based on the PK profile of the study drug. Timing of PK sampling may change based on emerging PK results after agreement with the Sponsor and the Investigator.

A PK sample will also be collected following occurrence of a dose-limiting event or serious adverse event.

Plasma and urine concentrations of RO7049389 will be measured by specific and validated liquid chromatography tandem mass chromatography (LC-MS/MS) methods conducted in a GLP compliance environment.

PK parameters will be estimated using standard non-compartmental methods for RO7049389.

PK samples will be destroyed no later than 2 years after the date of final closure of the clinical database, unless regulatory authorities require specimens to be maintained for a longer time period.

For sampling procedures, storage conditions, and shipment instructions, see the separate laboratory manual.

4.5.1.8 Exploratory Assessments

In Part 1, urine and plasma samples collected will be qualitatively and/or quantitatively analyzed for some of the metabolites of RO7049389 with the use of non-validated or validated methods. Endogenous substances levels will be investigated to assess the effect of multiple oral dosing of RO7049389 on CYP3A4 activity. Analysis will include plasma 4β-hydroxycholesterol/cholesterol ratio.

In Part 3, in order to avoid a potential loss of sensitivity of HBsAg detection due to the formation of HBsAg/anti-HBs complexes, an assay is being developed by the Sponsor to measure total HBsAg independently of the putative formation of complexes.

To explore relationship between baseline disease characteristics (e.g., HBeAg status, viral load, HBV genotype) and efficacy /safety response, *for NUC-suppressed patients (e.g., in POM Cohort A),* HBV genotyping will be attempted based on HBV RNA.

To explore the effect of the genetic polymorphism of CYP3A4, UGT1A3 and OATP1B on the PK of RO7049389, clinical genotyping will be performed.

These samples will be destroyed no later than 5 years after the date of final closure of the clinical database, unless regulatory authorities require specimens to be maintained for a longer time period.

4.5.1.9 HBV-Specific Viral Assessments

HBV-specific viral assessments will be performed only in patients in Part 2 and Part 3 of the study.

Blood sample collection time-points are provided in the SoA; also see viral dynamic *response* measures in Section 3.3.3. Samples for laboratory tests as specified below will be sent to one or several central laboratories or to the Sponsor for analysis. Instruction manuals and supply kits will be provided for all central laboratory assessments.

The collected samples will be destroyed no later than 5 years after the date of final closure of the clinical database, unless regulatory authorities require specimens to be maintained for a longer time period.

Blood samples for *quantitative and qualitative determination of* viral dynamic *response including* HBV DNA, HBsAg, HBeAg, anti-HBs*,* anti-HBe, HBV RNA, *HBcrAg, anti-HBc, as well as* viral resistance monitoring, viral genotypes based on DNA or RNA, *and* total HBsAg, etc. will be collected as detailed in the SoA.

4.5.1.10 Viral Resistance Monitoring

Blood samples will be collected as detailed in the SoA to monitor for the development of drug resistance. Sequencing (e.g., ultra-deep sequencing) of the HBV genome for viral resistance monitoring may be performed on all patients *with HBV DNA > LLOQ at baseline, and on those patients* who experienced sub-optimal response or confirmed virological breakthrough (defined as a confirmed quantifiable HBV DNA level in patients who previously achieved HBV DNA below the LLOQ or a confirmed increase in HBV DNA level of more than one $\log_{10}$ [10-fold] over nadir while receiving RO7049389*)*. Subjects, who did not experience virological breakthrough while receiving RO7049389 and/or NUC, but showed a quantifiable HBV DNA level at or post the end of dosing may also be included in *resistance testing*.

Resistance testing may be done at baseline (Day -1), at the end of treatment (or at Study Drug Discontinuation/Early Termination) and at the end of the follow-up period, as well as at other visits, e.g., in the case that emerging mutations potentially linked to resistance will be found in the HBV genome.

These samples will be destroyed no later than 5 years after the date of final closure of the clinical database, unless regulatory authorities require specimens to be maintained for a longer time period.

4.5.1.11 Clinical Genotyping (Drug Metabolizing Enzymes and Transporters)

A blood sample will be taken as detailed in the SoA for DNA extraction from all subjects if feasible. If, however, the genetic blood sample is not collected during the scheduled visit, it may be collected at any time during the conduct of the clinical study. The clinical genotyping DNA samples are collected to explore whether genetic polymorphism of drug metabolizing enzymes (e.g., but not limited to UGT, CYP3A) and drug transporters (e.g., but not limited to OATP) can be related to the PK profile of RO7049389. For Part 3, only the genetic polymorphism of CYP3A4, UGT1A3, and OATP1B will be analyzed.

The specimen will be destroyed immediately after analysis and the results checked. Data arising from clinical genotyping will be subjected to the confidentiality standards described in Section 8.4.

4.5.1.12 Samples for Research Biosample Repository

Overview of the Research Biosample Repository

The Roche Research Biosample Repository (RBR) is a centrally administered group of facilities for the long-term storage of human biologic specimens, including body fluids, solid tissues, and derivatives thereof (e.g., DNA, RNA, proteins, peptides). The collection, storage and analysis of these specimens will facilitate the rational design of new pharmaceutical agents and the development of diagnostic tests, which may allow for individualized drug therapy for patients in the future.

Specimens will be collected from study subjects who give specific consent to participate in this optional Research Biosample Repository. Collected specimens will be used to achieve the following objectives:

- To study the association of biomarkers with efficacy, adverse events, or disease progression.
- To increase knowledge and understanding of disease biology.
- To study drug response, including drug effects and the processes of drug absorption and disposition (only for Part 1 and Part 2).
- To develop biomarker or diagnostic assays and establish the performance characteristics of these assays (only for Part 1 and Part 2).

Approval by the Institutional Review Board or Ethics Committee

Sampling for the RBR is contingent upon the review and approval of the exploratory research and the RBR portion of the Informed Consent Form by each site's Institutional Review Board or Ethics Committee (IRB/EC) and, if applicable, an appropriate regulatory body. If a site has not been granted approval for RBR sampling, this section of the protocol will not be applicable at that site.

Sample Collection

The following samples will be collected for identification of dynamic (non-inherited) biomarkers:

- Serum to assess protein biomarkers including, but not limited to proteomics.
- Plasma to assess viral nucleic acids including, but not limited to sequencing, splice variants.
- Whole blood for RNA extraction to assess biomarkers including, but not limited to gene expression profiling.

The following samples will be collected for identification of genetic (inherited) biomarkers:

- Whole blood for DNA extraction to assess biomarkers including, but not limited to single-nucleotide polymorphisms (SNPs).

For all samples, dates of consent and specimen collection should be recorded on the associated RBR page of the eCRF. For sampling procedures, storage conditions, and shipment instructions, see the separate laboratory manual.

RBR specimens will be stored and used until no longer needed or until they are exhausted. The Research Biosample Repository storage period will be in accordance with the IRB/EC‑approved Informed Consent Form and applicable laws (e.g., Health Authority requirements).

The repository specimens will be subject to the confidentiality standards (as described under Confidentiality and in Section 8.4).

Confidentiality

Data generated from RBR specimens must be available for inspection upon request by representatives of national and local Health Authorities, and Roche Monitors, representatives, and collaborators, as appropriate.

Study subject’s medical information associated with RBR specimens is confidential and may only be disclosed to third parties as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the study subject, unless permitted or required by law.

Data derived from RBR specimen analysis on individual study subject's will generally not be provided to study investigators unless a request for research use is granted. The aggregate results of any conducted research will be available in accordance with the effective Roche policy on study data publication.

Genetic research data and associated clinical data may be shared with researchers who are not participating in the study or submitted to government or other health research databases for broad sharing with other researchers. Patients will not be identified by name or any other personally identifying information. Given the complexity and exploratory nature of these analyses, genetic data and analyses will not be shared with investigators or patients unless required by law.

Any inventions and resulting patents, improvements, and/or know-how originating from the use of the RBR specimen data will become and remain the exclusive and unburdened property of Roche, except where agreed otherwise.

Consent to Participate in the Research Biosample Repository

The Informed Consent Form will contain a separate section that addresses participation in the RBR. The Investigator or authorized designee will explain to each subject the objectives, methods, and potential hazards of participation in the RBR. Study subjects will be told that they are free to refuse to participate and may withdraw their specimens at any time and for any reason during the storage period. A separate, specific signature will be required to document a subject's agreement to provide optional RBR specimens. Study subjects who decline to participate will not provide a separate signature.

The Investigator should document whether or not the study subject has given consent to participate by completing the RBR Sample Informed Consent eCRF.

In the event of death or loss of competence of a subject who is participating in the Research, the participant's specimens and data will continue to be used as part of the RBR.

Withdrawal from the Research Biosample Repository

Study subject who give consent to provide specimens for the RBR have the right to withdraw their specimens at any time for any reason. If a study subject wishes to withdraw consent to the testing of his or her specimens, the Investigator must inform the Medical Monitor in writing of the study subject's wishes using the RBR Withdrawal Form and, if the trial is ongoing, must enter the date of withdrawal on the Research Biosample Repository Withdrawal of Informed Consent eCRF. The study subject will be provided with instructions on how to withdraw consent after the trial is closed. A study subject 's withdrawal from Study YP39364 does not, by itself, constitute withdrawal of specimens from the RBR. Likewise, a study subject's withdrawal from the RBR does not constitute withdrawal from Study YP39364. Data already generated before time of withdrawal of consent to Research Biosample Repository will still be used.

Monitoring and Oversight

Specimens collected for the Research Biosample Repository will be tracked in a manner consistent with Good Clinical Practice by a quality-controlled, auditable, and appropriately validated laboratory information management system, to ensure compliance with data confidentiality as well as adherence to authorized use of specimens as specified in this protocol and in the Informed Consent Form. Roche monitors and auditors will have direct access to appropriate parts of records relating to study subject participation in Research Biosample Repository for the purposes of verifying the data provided to Roche. The site will permit monitoring, audits, IRB/EC review, and Health Authority inspections by providing direct access to source data and documents related to the samples.

4.5.2 Timing of Study Assessments

4.5.2.1 Screening and Pretreatment Assessments

Written informed consent for participation in the study must be obtained before performing any study‑specific screening tests or evaluations. Informed Consent Forms for enrolled study subjects and for study subjects who are not subsequently enrolled will be maintained at the study site.

All screening and pre-treatment assessments must be completed and reviewed to confirm that study subjects meet all eligibility criteria. The Investigator will maintain a screening log to record details of all study subjects screened and to confirm eligibility or record reasons for screening failure.

An Eligibility Screening Form documenting the investigator's assessment of each screened study subject with regard to the protocol's inclusion and exclusion criteria is to be completed by the Investigator and kept at the investigational site.

Screening assessments will be performed within 28 days prior to dosing, unless otherwise specified. If a subject fails an inclusion/exclusion criterion due to a transient and non-clinically significant condition at screening, the Investigator may repeat the relevant screening assessment(s) within the 28-day screening period. If the subject fails a second time they will be classed as a screen failure and cannot be re-screened.

Re-screening is allowed for subjects who were screened in the study and met study inclusion/exclusion criteria but failed to be dosing within 28 days after the start of screening period because the recruitment into a cohort was suspended, or a stratum cap in a cohort was reached. In order to re-screen such a subject, all inclusion and exclusion criteria should be re-evaluated and all applicable screening assessments repeated if done more than 28 days before dosing. There is no need to repeat alpha-fetoprotein test if done for the study in central laboratory within 6 months before re-screening.

4.5.2.2 Assessments During Treatment

Under no circumstances will study subjects who enroll in this study and have completed treatment as specified, be permitted to be allocated a new randomization number and re-enroll in the same part of the study.

All assessments must be performed as per SoA (see - Overall Schedule of Assessments (Part 1a and 1b Appendix 1, Part 1c Appendix 3, Part 2 Appendix 5, and Part 3 Appendix 7) and Detailed Schedule of Assessments (Part 1a and 1b Appendix 2, Part 1c Appendix 4, Part 2 Appendix 6, and Part 3 Appendix 8).

4.5.2.3 Follow-Up Assessments

All study subjects who complete the last dose administration or discontinue from the study *treatment* early will be asked to return to the clinic and complete *follow-up* assessments as specified in the SoA *unless the study subjects refuse to do so*. The follow-up visit at 112 days post-first dose (for Part 2) or 72 weeks post-first dose (for Part 3) will be reported as the study completion visit in eCRF.

4.5.2.4 Assessments at *Unscheduled Visits* and *Early Termination*

If an unscheduled visit is required to ensure safety of study subjects, necessary assessments will be undertaken at the discretion of the Investigator. All unscheduled assessments should be reported in eCRF.

Unscheduled local laboratory tests may be ordered per Investigator's discretion and may be used for the individual management of the HV/patient. A duplicate sample *is recommended to* simultaneously be sent to the central laboratory for analysis.

In case of early termination of a subject in Part 1c, a blood sample for PK assessment of RO7049389 should be collected at the time of discontinuation. In case of premature study drug discontinuation in Part 2 and Part 3, a patient should be called *to complete* an *"early termination"* visit *within 7 days after the last dose of study drug or at the earliest possible time after "early termination" occurs,* and assessments should be performed as listed in the SoA (*Part 2* Appendix 5 *and Part 3 Appendix 7*) under "Early Termination/Study Drug Discontinuation", including physical examination, vital signs, ECG, safety labs, HBV parameters*,* and *RO7049389* PK assessment*, etc*.

4.6 HEALTHY VOLUNTEER/PATIENT, STUDY, AND SITE DISCONTINUATION

4.6.1 Healthy Volunteer/Patient Discontinuation

The Investigator has the right to discontinue a study subject from RO7049389 or withdraw a study subject from the study at any time. In addition, study subjects have the right to voluntarily discontinue study drug or withdraw from the study at any time for any reason. Reasons for discontinuation of study drug or withdrawal from the study may include, but are not limited to, the following:

- Study subject withdrawal of consent at any time.
- Any medical condition that the Investigator or Sponsor determines may jeopardize the study subject's safety if he or she continues in the study.
- Investigator or Sponsor determines it is in the best interest of the study subject.
- Patient non-compliance.

4.6.1.1 Discontinuation from Study Drug

4.6.1.1.1 Discontinuation of Study Treatment(s) Because of Safety Issues

All study subjects: individual study subjects must discontinue RO7049389/placebo if they experience any of the following:

- Clinically significant RO7049389-related changes in safety parameters that are considered not acceptable by the Investigator and/or the Sponsor
- Poor gastrointestinal (GI) tolerability that is considered to affect the study subject's well-being and/or the PK evaluation.
- In cases of suspected DILI, please follow the management plan in Section 5.2.1.1.

Part 2 (Patients with chronic HBV infection) and Part 3 (Patients with Chronic Hepatitis B):

If patients experience a Hepatitis flare, please follow the management plan in Section 5.2.1.2. Other than hepatitis flare, individual patients must discontinue RO7049389/placebo if they experience any of the following:

- Any other confirmed (within 48-72 hours) Grade 4 laboratory abnormality, including Grade 4 hematology, deemed clinically significant (based on Investigator's assessment).
- Any sign of development of liver cirrhosis (or liver imaging indicating cirrhosis) or decompensation (e.g., ascites, variceal hemorrhage, Child-Pugh Class B or C clinical classification).

After the initial event, subsequent monitoring and treatment plan should be discussed with the Medical Monitor.

4.6.1.1.2 Discontinuation of Study Treatment(s) Because of Virological Breakthrough

For both Part 2 and Part 3, virological breakthrough will be defined as either a) a confirmed quantifiable HBV DNA level in a patient who previously achieved HBV DNA suppression below the assay LLOQ, or b) a confirmed increase in HBV DNA of more than 1 $\log_{10}$ (10-fold) IU/mL over the nadir achieved during the study treatment period. Blood samples taken at the time of virological breakthrough will be tested for evidence of treatment-emergent drug resistance.

A timely confirmation of the initial HBV DNA (>LLOQ) result will be sought to meet the definition of virological breakthrough: the patient will be recalled for retesting, aiming to complete the confirmation visit within 2 weeks of the initial sample whenever possible. During the confirmation visit, there will be a thorough evaluation of adherence and factors that may be affecting compliance with treatment, and support for improved adherence will be offered as per routine clinical practice. At this visit, a blood sample will be collected to test for HBV DNA and plasma drug levels. If the HBV DNA load is confirmed, the sample will also be tested for antiviral drug resistance using genotypic and where feasible phenotypic testing.

The results of these assessments and investigations will be used to guide management. The general approach in cases of *confirmed* virological breakthrough *while on CpAM treatment and not caused by poor compliance with treatment* will be to discontinue the investigational study medications. NUC therapy is expected to be continued with any adjustment to be informed by the patient's treatment history and assessment of adherence, HBV DNA levels, and any available result of drug resistance testing.

4.6.1.1.3 Other Causes of Discontinuation of Study Treatment(s)

All healthy volunteers and patients have the right to withdraw from the study at any time for any reason.

The investigators have the right to withdraw study subject from the study in the event of intercurrent illness, adverse events, for administrative or other reasons. The Sponsor should be informed of study subjects' discontinuations from the study or from the study drug.

Healthy volunteers or patients who withdraw from the study prematurely will be asked to return to the clinic for a*n "early termination"* visit *with*in 7 days after the last dose of study drug *or at the earliest possible time after "early termination" occurs (see section 4.5.2.4)*. The primary reason for premature study drug discontinuation should be documented on the appropriate eCRF.

4.6.1.2 Withdrawal from Study

Every effort should be made to obtain information on study subjects who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate eCRF.

Healthy volunteers and patients will not be followed for any reason after consent has been withdrawn.

When a patient voluntarily withdraws from the study, or is withdrawn by the Investigator, samples collected until the date of withdrawal will be analyzed, unless patient specifically requests for these to be discarded or local laws require their immediate destruction. A patient's withdrawal from Study YP39364 does not, by itself, constitute withdrawal of specimens donated to the Research Biosample Repository.

Healthy volunteers and patients who withdraw from the study for safety reasons will not be replaced. Healthy volunteers and patients who withdraw from the study for other reasons will be replaced (see section 4.1).

4.6.2 Study and Site Discontinuation

The Sponsor has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of adverse events in this or other studies indicates a potential health hazard to study subjects.
- Study subject enrollment is unsatisfactory.

The Sponsor will notify the Investigator and Health Authorities if the study is placed on hold, or if the Sponsor decides to discontinue the study or development program.

The Sponsor has the right to replace a site at any time. Reasons for replacing a site may include, but are not limited to, the following:

- Excessively slow recruitment
- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with the International Conference on Harmonisation (ICH) guideline for Good Clinical Practice.

5. ASSESSMENT OF SAFETY

5.1 SAFETY PARAMETERS AND DEFINITIONS

Safety assessments will consist of monitoring and recording adverse events, including serious adverse events and non-serious adverse events of special interest; measurement of protocol‑specified safety laboratory assessments; measurement of

protocol-specified vital signs, ECGs; and other protocol-specified tests that are deemed critical to the safety evaluation of the study.

Certain types of events require immediate reporting to the Sponsor, as outlined in Section 5.4.

5.1.1 Adverse Events

According to the ICH guideline for Good Clinical Practice, an adverse event is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, regardless of causal attribution. An adverse event can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition), except as described in Section 5.3.5.9.
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline.
- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug.
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies).

5.1.2 Serious Adverse Events (Immediately Reportable to the Sponsor)

A serious adverse event is any adverse event that meets any of the following criteria:

- Fatal (i.e., the adverse event actually causes or leads to death).
- Life-threatening (i.e., the adverse event, in the view of the Investigator, places the patient at immediate risk of death).
- This does not include any adverse event that had it occurred in a more severe form or was allowed to continue might have caused death.
- Requires or prolongs inpatient hospitalization (see Section 5.3.5.10).
- Results in persistent or significant disability/incapacity (i.e., the adverse event results in substantial disruption of the healthy volunteer/patient's ability to conduct normal life functions).
- Congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drug.

- Significant medical event in the Investigator's judgment (e.g., may jeopardize the healthy volunteer/patient or may require medical/surgical intervention to prevent one of the outcomes listed above).

The terms “severe” and “serious” are not synonymous. Severity refers to the intensity of an adverse event (rated as mild, moderate, or severe, or Grade 1-5 according to a pre-defined grading criteria (see Section 5.3.3); the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Severity and seriousness need to be independently assessed for each adverse event recorded on the eCRF.

Serious adverse events are required to be reported by the Investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2 for reporting instructions).

5.1.3 Non-Serious Adverse Events of Special Interest (Immediately Reportable to the Sponsor)

Non-serious adverse events of special interest are required to be reported by the Investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2 for reporting instructions). Adverse events of special interest for this study include the following:

- Cases of elevated ALT or AST in combination with either elevated bilirubin or clinical jaundice, as defined in Section 5.3.5.6.
- Suspected transmission of an infectious agent by the study drug, as defined below:
    - Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected.

5.2 SAFETY PLAN

5.2.1 Management of Specific Adverse Events

5.2.1.1 Potential Drug-Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be considered to be reported as SAEs, and discontinuation of all study medications (RO7049389/placebo, NUC, or Peg-IFN, where applicable) must be considered.

In this study, potential drug-induced liver injury is defined as follows:

For healthy volunteers (Part 1): ALT >3 × ULN accompanied by total bilirubin >2 × ULN

For CHB patients (Part 2 and Part 3): ALT >3 × baseline* **AND** >3 × ULN, accompanied by total bilirubin >2 × ULN

*The threshold of ALT >3 × baseline will be capped at 10× ULN.

AND

No other immediately apparent possible causes of ALT elevation and hyperbilirubinemia, including, but not limited to, acute viral hepatitis, cholestasis, pre-existing hepatic disease excluding HCV/HEV or the administration of other drug(s), herbal medications or substances known to be hepatotoxic.

5.2.1.2 Hepatitis flare

Hepatitis flares (abrupt elevation of serum ALT above normal) are known to occur spontaneously in patients with CHB during the course of disease, and/or during anti-viral therapy. Transient hepatitis flares are not always harmful and may portend immune clearance of infected hepatocytes. Hepatitis flares may be due to direct DILI, drug-induced immune-mediated hepatitis, the underlying disease (incomplete viral suppression), or immune clearance of infected hepatocytes (successful viral suppression and accompanying restitution of the host immune response). Isolated ALT elevations of 2-3 times the baseline value that are self-limited may reflect a desirable treatment-related immunologic response rather than drug toxicity. However, progressive increases, increases inconsistent with the time course of a “flare” or ALT elevations associated with increases in bilirubin with or without alkaline phosphatase rise may primarily be considered adverse.

There is no consistent definition of the ALT threshold for hepatitis flares. In this study, for all on-treatment or off-treatment ALT/AST elevation cases, more frequent monitoring of liver tests should be performed based on medical judgment. Other causes of ALT elevations should be investigated. For patients with ALT >5 × ULN, the following management plan is recommended:

Confirmation and investigation of reasons

- Schedule the patient to return to the clinic as soon as possible (ideally within 3 days after initial laboratory results were drawn).
- During the visit, a thorough clinical assessment of the subject will be performed.
- The following liver tests should be conducted: including but not limited to, serum ALT and AST, alkaline phosphatase (ALP), total and *in*direct bilirubin, International Normalized Ratio [INR]/prothrombin time [PT], serum albumin and liver ultrasound.
- *A PK sample (unscheduled) for RO7049389 should also be collected.*

- Investigate the patient for potential etiologies of the laboratory changes. At the discretion of investigators, the following testing can be conducted, such as plasma HBV DNA, serology for HBV, HDV, HAV IgM, HCV and HEV.

Monitoring frequency during on-treatment and off-treatment periods

- ALT >10 × ULN (or rise rapidly i.e., >2-fold increase within 48 hours): Repeat liver test twice weekly until ALT <*10* × ULN.
- ALT 5–10 × ULN: Repeat liver test every week until ALT returns to below 5 × ULN.

Management during on-treatment period

- Hepatitis flare with increasing HBV DNA: please follow Section 4.6.1.1.2 "Discontinuation of Study Treatment(s) Because of Virological Breakthrough".
- Isolated ALT >5 × ULN with preserved hepatic function (e.g., no clinically significant changes in ALP, total and direct bilirubin, INR/PT, and albumin):
    1. ALT 5-10 × ULN: Continue study treatment and keep monitoring; after up to 8 weeks monitoring, if ALT has not returned to <2 × ULN *or to the baseline level*, and there is no decline of viral markers (e.g., HBsAg, HB*eAg*, HBcrAg etc.), consider to discontinue RO7049389 and Peg-IFN permanently. NUC therapy can be continued or changed at the discretion of the investigator and applicable CHB guideline.
    2. ALT >10 × ULN (or rise rapidly i.e., >2-fold increase within 48 hours): Consider interruption of RO7049389 and/or Peg-IFN; after up to 8 weeks interruption, if ALT has not returned to < 2 × ULN *or to the baseline level, and there is no decline of viral markers (e.g., HBsAg, HBeAg, HBcrAg etc.), consider to* discontinue RO7049389 and Peg-IFN *permanently.* NUC therapy can be continued or changed at the discretion of the investigator and applicable CHB guideline. If ALT returns to <2 × ULN, re-introduce RO7049389 from 200 mg QD, and gradually increase to the determined dose regimen for the respective cohort at the investigator's discretion.
- ALT >5 × ULN accompanied by declining liver synthetic and excretory functions (total bilirubin >2 × ULN, or albumin <3.0 g/dL, or INR >1.5) or other signs of hepatic impairment (severe fatigue, nausea, vomiting, right upper quadrant pain):
    1. RO7049389 and Peg-IFN will be permanently discontinued.
    2. NUC therapy can be continued or changed at the discretion of the investigator and applicable CHB guideline.

Management during off-treatment period

- If ALT>2 × ULN is accompanied by confirmed virological relapse (HBV DNA >2,000 IU/mL in HBeAg negative patients, or HBV DNA >20,000 IU/mL in HBeAg positive patients). NUC treatment may be restarted at the discretion of the investigator and applicable CHB guideline.

The Investigator should aim to exclude development of decompensated liver disease. Patients who develop signs of decompensated liver disease (e.g., ascites, variceal hemorrhage, Child-Pugh Class B or C clinical classification [Appendix 11]) should discontinue study treatment (Section 4.6.1.1). Patients who develop flares should be monitored more closely with additional unscheduled visits and laboratory assessments.

No dose modification of RO7049389/placebo for safety reasons is expected in the study. At the discretion of the Investigator, study treatment can be discontinued.

5.3 METHODS AND TIMING FOR CAPTURING AND ASSESSING SAFETY PARAMETERS

The Investigator is responsible for ensuring that all adverse events (see Section 5.1.1 for definition) are recorded on the Adverse Event eCRF and reported to the Sponsor in accordance with instructions provided in this section and in Sections 5.4, 5.5, and 5.6.

For each adverse event recorded on the Adverse Event eCRF, the Investigator will make an assessment of seriousness (see Section 5.1.2 for seriousness criteria), severity (see Section 5.3.3), and causality (see Section 5.3.4).

5.3.1 Adverse Event Reporting Period

Investigators will seek information on adverse events at each study subject contact. All adverse events, whether reported by the study subject or noted by study personnel, will be recorded in the study subject's medical record. Adverse events will then be reported on the Adverse Event eCRF as follows:

After informed consent has been obtained **but prior to initiation of study drug**, only serious adverse events caused by a protocol-mandated intervention should be reported (e.g., serious adverse events related to invasive procedures such as biopsies). Any other adverse event should not be reported.

After initiation of study drug, all adverse events, regardless of relationship to study drug, will be reported until 84 days in Part 2 of the study or until 24 weeks *after the last dose of study drug* in Part 3 of the study.

After a period of 84 days (Part 2) or 24 weeks from the last dose of study drug *(Part 3)*, investigators should report any deaths, serious adverse events, or other adverse events of concern that are believed to be related to prior treatment with study drug (see Section 5.6).

5.3.2 Eliciting Adverse Event Information

A consistent methodology of non-directive questioning should be adopted for eliciting adverse event information at all healthy volunteer/patient evaluation time-points. Examples of non-directive questions include the following:

"How have you felt since your last clinic visit?"

"Have you had any new or changed health problems since you were last here?"

5.3.3 Assessment of Severity of Adverse Events

Table 6 provides guidance for assessing adverse event severity in Part 1 and Part 2.

Table 6 Adverse Event Severity Grading Scale (For Part 1 and Part 2)

Severity	Description
Mild	Discomfort noticed, but no disruption of normal daily activity
Moderate	Discomfort sufficient to reduce or affect normal daily activity
Severe	Incapacitating with inability to work or to perform normal daily activity

Note: Regardless of severity, some events may also meet seriousness criteria. Refer to definition of a serious adverse event (see Section 5.1.2).

For Part 3, DAIDS toxicity grading scales (Appendix 15) will be used to assess adverse event severity.

5.3.4 Assessment of Causality of Adverse Events

Investigators should use their knowledge of the study subject, the circumstances surrounding the event, and an evaluation of any potential alternative causes to determine whether or not an adverse event is considered to be related to the study drug, indicating "yes" or "no" accordingly. The following guidance should be taken into consideration:

- Temporal relationship of event onset to the initiation of study drug.
- Course of the event, considering especially the effects of dose reduction, discontinuation of study drug, or reintroduction of study drug.
- Known association of the event with the study drug or with similar treatments.
- Known association of the event with the disease under study.
- Presence of risk factors in the study subject or use of concomitant medications known to increase the occurrence of the event.
- Presence of non-treatment-related factors that are known to be associated with the occurrence of the event.

For healthy volunteers receiving combination therapy, causality will be assessed individually for each protocol-mandated therapy.

5.3.5 Procedures for Recording Adverse Events

Investigators should use correct medical terminology/concepts when recording adverse events on the Adverse Event eCRF. Avoid colloquialisms and abbreviations.

Only one adverse event term should be recorded in the event field on the Adverse Event eCRF.

5.3.5.1 Diagnosis versus Signs and Symptoms

A diagnosis (if known) should be recorded on the Adverse Event eCRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded on the Adverse Event eCRF. If a diagnosis is subsequently established, all previously reported adverse events based on signs and symptoms should be nullified and replaced by one adverse event report based on the single diagnosis, with a starting date that corresponds to the starting date of the first symptom of the eventual diagnosis.

5.3.5.2 Adverse Events Occurring Secondary to Other Events

In general, adverse events occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause, with the exception of severe or serious secondary events. However, medically significant adverse events occurring secondary to an initiating event that are separated in time should be recorded as independent events on the Adverse Event eCRF. For example:

- If vomiting results in mild dehydration with no additional treatment in a healthy adult, only vomiting should be reported on the eCRF.
- If vomiting results in severe dehydration, both events should be reported separately on the eCRF.
- If a severe gastrointestinal hemorrhage leads to renal failure, both events should be reported separately on the eCRF.
- If dizziness leads to a fall and subsequent fracture, all three events should be reported separately on the eCRF.

All adverse events should be recorded separately on the Adverse Event eCRF if it is unclear as to whether the events are associated.

5.3.5.3 Persistent or Recurrent Adverse Events

A persistent adverse event is one that extends continuously, without resolution, between study subject evaluation time-points. Such events should only be recorded once on the

Adverse Event eCRF. The initial severity of the event should be recorded, and the severity should be updated to reflect the most extreme severity any time the event worsens. If the event becomes serious, the Adverse Event eCRF should be updated to reflect this.

A recurrent adverse event is one that resolves between patient evaluation time-points and subsequently recurs. Each recurrence of an adverse event should be recorded separately on the Adverse Event eCRF.

5.3.5.4 Abnormal Laboratory Values

Not every laboratory abnormality qualifies as an adverse event. A laboratory test result should be reported as an adverse event if it meets any of the following criteria:

- Accompanied by clinical symptoms.
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation).
- Results in a medical intervention (e.g., potassium supplementation for hypokalemia) or a change in concomitant therapy.
- Clinically significant in the Investigator's judgment.

It is the Investigator's responsibility to review all laboratory findings. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an adverse event.

If a clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 times the ULN associated with cholecystitis), only the diagnosis (i.e., cholecystitis) should be recorded on the Adverse Event eCRF.

If a clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded on the Adverse Event eCRF, along with a descriptor indicating if the test result is above or below the normal range (e.g., "elevated potassium", as opposed to "abnormal potassium"). If the laboratory abnormality can be characterized by a precise clinical term per standard definitions, the clinical term should be recorded as the adverse event. For example, an elevated serum potassium level of 7.0 mEq/L should be recorded as "hyperkalemia".

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded on the Adverse Event eCRF, unless the etiology changes. The initial severity of the event should be recorded, and the severity or seriousness should be updated any time the event worsens.

5.3.5.5 Abnormal Vital Sign Values

Not every vital sign abnormality qualifies as an adverse event. A vital sign result should be reported as an adverse event if it meets any of the following criteria:

- Accompanied by clinical symptoms.
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation).
- Results in a medical intervention or a change in concomitant therapy.
- Clinically significant in the Investigator's judgment.

It is the Investigator's responsibility to review all vital sign findings. Medical and scientific judgment should be exercised in deciding whether an isolated vital sign abnormality should be classified as an adverse event.

If a clinically significant vital sign abnormality is a sign of a disease or syndrome (e.g., high blood pressure), only the diagnosis (i.e., hypertension) should be recorded on the Adverse Event eCRF.

Observations of the same clinically significant vital sign abnormality from visit to visit should not be repeatedly recorded on the Adverse Event eCRF, unless the etiology changes. The initial severity of the event should be recorded, and the severity or seriousness should be updated any time the event worsens.

5.3.5.6 Abnormal Liver Tests

For Healthy Volunteers:

The finding of an elevated ALT or AST ($>3\times$ULN) in combination with either an elevated total bilirubin ($>2\times$ULN) or clinical jaundice in the absence of cholestasis or other causes of hyperbilirubinemia is considered to be an indicator of severe liver injury (Hy's law). Therefore, investigators must report as an adverse event the occurrence of either of the following:

- Treatment-emergent ALT or AST $>$ 3 $\times$ ULN in combination with total bilirubin $>$ 2 $\times$ ULN.
- Treatment-emergent ALT or AST $>$ 3 $\times$ ULN in combination with clinical jaundice.

For patients with chronic HBV infection:

CHB patients may have elevated baseline ALT levels without an increase in bilirubin. However, the finding of an elevated ALT in combination with either an elevated total bilirubin or clinical jaundice in the absence of cholestasis or other causes of hyperbilirubinemia is considered to be an indicator of potential severe liver injury, or worsening of disease. Therefore, investigators must report as an adverse event the occurrence of either of the following:

- Treatment-emergent ALT >3 × baseline* AND >3 × ULN in combination with total bilirubin >2 × ULN.
- Treatment-emergent ALT >3 × baseline* AND >3 × ULN in combination with clinical jaundice.

* The threshold of ALT >3 × baseline will be capped at 10 × ULN.

The most appropriate diagnosis or (if a diagnosis cannot be established) the abnormal laboratory values should be recorded on the Adverse Event eCRF (see Section 5.3.5.1) and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event), either as a serious adverse event or a non-serious adverse event of special interest (see Section 5.4.2).

5.3.5.7 Deaths

All deaths that occur during the protocol-specified adverse event reporting period (see Section 5.3.1), regardless of relationship to study drug, must be recorded on the Adverse Event eCRF and immediately reported to the Sponsor (see Section 5.4.2).

Death should be considered an outcome and not a distinct event. The event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the Adverse Event eCRF. Generally, only one such event should be reported. The term “sudden death” should only be used for the occurrence of an abrupt and unexpected death due to presumed cardiac causes in a patient with or without preexisting heart disease, within one hour of the onset of acute symptoms or, in the case of an unwitnessed death, within 24 hours after the patient was last seen alive and stable. If the cause of death is unknown and cannot be ascertained at the time of reporting, “unexplained death” should be recorded on the Adverse Event eCRF. If the cause of death later becomes available (e.g., after autopsy), “unexplained death” should be replaced by the established cause of death.

5.3.5.8 Preexisting Medical Conditions

A preexisting medical condition is one that is present at the screening visit for this study. Such conditions should be recorded on the General Medical History and Baseline Conditions eCRF.

A preexisting medical condition should be recorded as an adverse event only if the frequency, severity, or character of the condition worsens during the study. When recording such events on the Adverse Event eCRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

5.3.5.9 Lack of Efficacy or Worsening of Chronic HBV Infection

As this is the first study of RO7049389 in patients with chronic HBV infection, the effects on viral parameters are being assessed as part of study endpoints in this study. Lack of RO7049389 efficacy in terms of changes in HBeAg and HBsAg do not qualify for adverse events in this study.

Medical occurrences or symptoms of deterioration in a course of chronic HBV infection should be recorded as an adverse event if judged by the Investigator to have unexpectedly worsened in severity or frequency or changed in nature at any time during the study.

For virological breakthrough, see Section 4.6.1.1.2 and for hepatitis flares, see Section 5.3.5.6. Each of these should be reported as adverse events.

5.3.5.10 Hospitalization or Prolonged Hospitalization

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event (per the definition of serious adverse event in Section 5.1.2), except as outlined below.

The following hospitalization scenarios are not considered to be serious adverse events:

- Hospitalization for respite care.
- Planned hospitalization required by the protocol (e.g., for study drug administration and intensive PK sampling).
- Hospitalization for a preexisting condition, provided that all of the following criteria are met:
    - The hospitalization was planned prior to the study or was scheduled during the study when elective surgery became necessary because of the expected normal progression of the disease.
    - The study subject has not suffered an adverse event.

The following hospitalization scenarios are not considered to be serious adverse events, but should be reported as adverse events instead:

- Hospitalization for an adverse event that would ordinarily have been treated in an outpatient setting had an outpatient clinic been available.

5.3.5.11 Overdoses

Study drug (including IMP and Non-IMP) overdose is the accidental or intentional use of the drug in an amount higher than the dose being studied. An overdose or incorrect administration of study drug is not an adverse event unless it results in untoward medical effects.

Any study drug overdose or incorrect administration of study drug should be noted on the Study Drug Administration eCRF.

All adverse events associated with an overdose or incorrect administration of study drug should be recorded on the Adverse Event eCRF. If the associated adverse event fulfills serious criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2).

5.4 IMMEDIATE REPORTING REQUIREMENTS FROM INVESTIGATOR TO SPONSOR

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The Investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the Investigator learns of the event. The following is a list of events that the Investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- Serious adverse events
- Non-serious adverse events of special interest
- Pregnancies

The Investigator must report new significant follow-up information for these events to the Sponsor immediately (i.e., no more than 24 hours after becoming aware of the information). New significant information includes the following:

- New signs or symptoms or a change in the diagnosis
- Significant new diagnostic test results
- Change in causality based on new information
- Change in the event's outcome, including recovery
- Additional narrative information on the clinical course of the event.

Investigators must also comply with local requirements for reporting serious adverse events to the local health authority and IRB/EC.

5.4.1 Emergency Medical Contacts

To ensure the safety of study patients, access to the Medical monitors is available 24 hours a day 7 days a week. Details will be available separately.

5.4.2 Reporting Requirements for Serious Adverse Events and Non-Serious Adverse Events of Special Interest

For reports of serious adverse events and non-serious adverse events of special interest (see Sections 5.1.2 and 5.1.3), investigators should record all case details that can be gathered on the Serious Adverse Reporting Form and forward this form to the SAE Responsible within 24 hours

5.4.3 Reporting Requirements for Pregnancies

5.4.3.1 Pregnancies in Female Patients

Female healthy subjects of childbearing potential will not be allowed to participate in this study. Female patients of childbearing potential will be allowed to participate in this study, but they should avoid becoming pregnant during the study treatment and for at least 6 months post the last dose of study treatment as described in Inclusion Criteria. All female subjects will be instructed to immediately inform the Investigator if they become pregnant during the study, or within 6 months after the last dose of study drug.

A Clinical Trial Pregnancy Reporting Form should be completed by the Investigator and submitted to the Sponsor within 24 hours after learning of the pregnancy. Pregnancy should not be recorded on the Adverse Event eCRF. The Investigator should discontinue study drug and counsel the subject, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy.

5.4.3.2 Pregnancies in Female Partners of Male Subjects

Male study subjects will be instructed through the Informed Consent Form to immediately inform the Investigator if their partner becomes pregnant during the study and during the follow-up period, until 21 days in Part 1, or 28 days in Part 2, or 6 months in Part 3 after the last dose of study drug.

A Clinical Trial Pregnancy Reporting Form should be completed by the Investigator and submitted to the Sponsor within 24 hours after learning of the pregnancy. Attempts should be made to collect and report details of the course and outcome of any pregnancy in the partner of a male study subject exposed to study drug. The pregnant partner will need to sign an Authorization for Use and Disclosure of Pregnancy Health Information to allow for follow-up on her pregnancy. Once the authorization has been signed, the Investigator will update the Clinical Trial Pregnancy Reporting Form with additional information on the course and outcome of the pregnancy. An Investigator who is contacted by the male study subject or his pregnant partner may provide information

on the risks of the pregnancy and the possible effects on the fetus, to support an informed decision in cooperation with the treating physician and/or obstetrician.

5.4.3.3 Abortions

Any spontaneous abortion should be classified as a serious adverse event (as the Sponsor considers spontaneous abortions to be medically significant events), recorded on the Adverse Event eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2).

Any induced abortion due to maternal toxicity and/or embryo-fetal toxicity should also be classified as serious adverse event, recorded on the Adverse Event eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2).

Elective abortion not associated with toxicity (e.g., induced abortion for personal reasons) does not require expedited reporting but should be reported as outcome of pregnancy on the Clinical Trial Pregnancy Reporting Form.

5.4.3.4 Congenital Anomalies/Birth Defects

Any congenital anomaly/birth defect in a child born to a female study subject should be classified as a serious adverse event, recorded on the Adverse Event eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2).

5.5 FOLLOW-UP OF PATIENTS AFTER ADVERSE EVENTS

5.5.1 Investigator Follow-Up

The Investigator should follow each adverse event until the event has resolved to baseline grade or better, the event is assessed as stable by the Investigator, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all serious adverse events considered to be related to study drug or trial-related procedures until a final outcome can be reported.

During the study period, resolution of adverse events (with dates) should be documented on the Adverse Event eCRF and in the patient's medical record to facilitate source data verification. If, after follow-up, return to baseline status or stabilization cannot be established, an explanation should be recorded on the Adverse Event eCRF.

All pregnancies reported during the study should be followed until pregnancy outcome and reported according to the instructions provided in Section 5.4.3.

5.5.2 Sponsor Follow-Up

For serious adverse events, non-serious adverse events of special interest, and pregnancies, the Sponsor or a designee may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details and outcome information

(e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

5.6 POST-STUDY ADVERSE EVENTS

The Investigator is not required to actively monitor patients for adverse events after the end of the adverse event reporting period (Section 5.3.1).

If the Investigator becomes aware of any other serious adverse event occurring after the end of the adverse event reporting period, if the event is believed to be related to prior study drug treatment the event should be reported directly to the Sponsor or its designee, either by faxing or by scanning and emailing the Serious Adverse Event Reporting Form using the fax number or email address provided to investigators.

5.7 EXPEDITED REPORTING TO HEALTH AUTHORITIES, INVESTIGATORS, INSTITUTIONAL REVIEW BOARDS, AND ETHICS COMMITTEES

The Sponsor will promptly evaluate all serious adverse events and non-serious adverse events of special interest against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators, IRBs, ECs, and applicable Health Authorities based on applicable legislation.

To determine reporting requirements for single adverse event cases, the Sponsor will assess the expectedness of these events *through use of the reference safety information in the document listed below:*

Drug	*Document*
RO7049389	*RO7049389 Investigator's Brochure*

The Sponsor will compare the severity of each event and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document.

Reporting requirements will also be based on the Investigator's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed.

6. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

The primary objective of Part 1 of this study is to characterize safety and tolerability, and PK profile, associated with a single-dose and multiple-doses of RO7049389 in healthy subjects. The primary objective of Part 2 and Part 3 of this study is to characterize safety, tolerability, and antiviral effect of multiple-doses of RO7049389 in patients with chronic HBV infection. Statistical summaries will be descriptive in nature and will be reported separately for each part of the study. All study subjects who are randomized to receive

placebo will be pooled as respective placebo control groups, according to each part of the study.

6.1 DETERMINATION OF SAMPLE SIZE

For Part 1 of the study, up to 93 healthy volunteers will be enrolled. This comprises up to 53 for Parts 1a and 1b, and up to 40 for Part 1c. For Part 2 of the study, up to 35 patients with chronic HBV infection will be enrolled.

The number of study subjects to be randomized in Parts 1 and 2 was chosen based on practical considerations and complies with standard safety review rules. With six study subjects receiving active drug at a dose-level, there is an 82% chance to observe at least one adverse event that has an incidence rate of 25% in the population. The total sample size may increase adaptively based on the study findings for safety, PK or viral dynamic outcomes. Good operating characteristics for the model-based predictions of MTD with control for overdosing are shown in Appendix 12 for a variety of dose-response profiles.

This sample size for POM (Part 2) is to support the assessment of viral dynamic response to the study drug treatment. When the sample size is 6, a one-sided 90% confidence interval for the mean change from baseline of HBV DNA (Log_{10} IU/mL) will have an interval that extends no more than 0.904 from the observed mean assuming that the standard deviation is 1.5 and that the confidence interval is based on the t-statistic.

*Initially, a*pproximately 55 patients with chronic hepatitis B will be enrolled in the *first* three cohorts of Part 3 *with approximately 30 patients in* POM Cohort A, *10 patients in* POM Cohort B, and *15 patients in* POM Cohort C. *Based on emerging efficacy/safety data, the sample size in Cohort C can be increased to approximately 30 patients. In addition, additional treatment cohorts may be opened to explore different treatment durations, dose regimens, or different combinations.*

The sample size for Part 3 of the study is to support the assessment of response rate of HBV DNA <LLOQ with HBsAg loss at 24-weeks post-treatment. A sample size of 10, 15, or 30 would ensure that the lower 95% CI is above 5%, 9%, or 14%, respectively, if the observed response rate is 30% assuming binomial distribution.

6.2 SUMMARIES OF CONDUCT OF STUDY

The number of study subjects who enroll, discontinue, or complete the study will be summarized by treatment and dose level. Reasons for premature study withdrawal will be listed and summarized by treatment and dose-level. Protocol deviations will be listed and evaluated for their potential impact on interpretation of study results. Study drug administration will be summarized by treatment. Descriptive statistics will be used in evaluating the conduct of the study.

6.3 ANALYSIS POPULATIONS

6.3.1 Safety Analysis Population

All healthy volunteers (Part 1) and patients (Parts 2 and Part 3) who have received at least one dose of the study drug, whether prematurely withdrawn from the study or not, will be included in the safety analysis.

6.3.2 Pharmacokinetic Analysis Population

Study subjects will be excluded from the pharmacokinetic analysis population if they significantly violate the inclusion or exclusion criteria, deviate significantly from the protocol or if data are unavailable or incomplete which may influence the pharmacokinetic analysis. Excluded cases will be documented together with the reason for exclusion. All decisions on exclusions from the analysis will be made prior to database closure.

6.3.3 Efficacy Analysis Population

The analyses of viral dynamic response in Part 2 of the study will include all patients who were randomized and received at least one dose of study drug (RO7049389 or placebo). Patients will be analyzed according to the treatment group to which they were randomized.

The analyses of viral dynamic response in Part 3 of the study will include all patients who received at least one dose of RO7049389.

6.4 SUMMARIES OF TREATMENT GROUP COMPARABILITY

Descriptive statistics will be used for demographic and baseline disease characteristics as applicable for each part of the study and will include sex, race, ethnicity, origin (Asian and non-Asian, Chinese among Asian), age, weight, height, body mass index, HBV DNA and HBsAg levels, HBV history (duration of HBV disease, previous HBV treatments, length of time on nucleos(t)ides), and viral genotype.

For continuous variables, mean, standard deviation, median, and minimum and maximum values will be presented. For categorical data, the proportion of study subjects in each category will be summarized.

6.5 SAFETY ANALYSES

All safety analyses will be based on the safety analysis population.

6.5.1 Adverse Events

The original terms recorded on the eCRF by the Investigator for adverse events will be standardized by the Sponsor. Adverse events will be summarized by MedDRA mapped term and appropriate thesaurus level.

6.5.2 Clinical Laboratory Test Results

All clinical laboratory data will be stored on the database in the units in which they were reported. Study subjects' listings and summary statistics at each assessment time will be presented using the International System of Units (SI units; Système International d'Unités). Laboratory data not reported in SI units will be converted to SI units before processing.

Laboratory test values will be presented by individual listings with flagging of values outside the normal ranges.

The proportion of study subjects with laboratory abnormalities will be summarized, by treatment. Additionally, the value and change from baseline for each laboratory test will be summarized by treatment and visit using descriptive statistics.

6.5.2.1 Standard Reference Ranges and Transformation of Data

Roche standard reference ranges, rather than the reference ranges of the Investigator, will be used for all parameters. For most parameters, the measured laboratory test result will be assessed directly using the Roche standard reference range. Certain laboratory parameters will be transformed to Roche's standard reference ranges.

A transformation will be performed on certain laboratory tests that lack sufficiently common procedures and have a wide range of investigator ranges, e.g., enzyme tests that include AST, ALT, and alkaline phosphatase and total bilirubin. Since the standard reference ranges for these parameters have a lower limit of zero, only the upper limits of the ranges will be used in transforming the data.

6.5.2.2 Definition of Laboratory Abnormalities

For all laboratory parameters included, there exists a Roche predefined standard reference range. Laboratory values falling outside this standard reference range will be labeled "H" for high or "L" for low in study subject listings of laboratory data.

In addition to the standard reference range, a marked reference range has been predefined by Roche for each laboratory parameter. The marked reference range is broader than the standard reference range. Values falling outside the marked reference range that also represent a defined change from baseline will be considered marked laboratory abnormalities (i.e., potentially clinically relevant). If a baseline value is not available for a study subject, the midpoint of the standard reference range will be used as the study subject's baseline value for the purposes of determining marked laboratory abnormalities. Marked laboratory abnormalities will be labeled in the study subject listings as "HH" for very high or "LL" for very low.

6.5.3 Vital Signs

Vital signs data will be presented by individual listings with flagging of values outside the normal ranges and flagging of marked abnormalities. In addition, tabular summaries will be used, as appropriate.

6.5.4 ECG Data Analysis

ECG data will be presented by individual listings with flagging of values outside the normal ranges and flagging of marked abnormalities. Summary descriptive statistics for the actual values and changes from baseline will be tabulated by nominal time for HR, QRS duration, PR and QTcF (as calculated per Appendix 15). For multiple measurements taken at a nominal time-point, the average of these measurements will be used as the value at that nominal time-point in all summaries. In addition, QTcF will be categorized at each time-point as ≤ 450 msec, > 450-480 msec, > 480-500 msec and > 500 msec and summarized. Similarly, a summary will be provided of the QTcF changes from baseline at each time-point categorized as < 30 msec, 30-60 msec, and > 60 msec. Changes of the overall ECG interpretation, T-wave and U-wave morphology will be summarized.

In addition, an exposure-response relationship *may* be developed using the ECG data extracted from the Holter monitoring in both of Parts 1a (SAD) and 1c (MAD). The primary ECG analysis will be based on a mixed effects repeated measures model with change-from-time-matched baseline (i.e., ΔQTcF) as the dependent variable, drug plasma concentration as a continuous independent variable, time-point as a fixed effect, and an unstructured variance-covariance matrix. The absence of hysteresis will be checked graphically. If there is a delay in maximal change in placebo and baseline corrected QTcF compared to the peak plasma concentrations of the study drug, a PK model with an effect compartment will be explored to replace the direct effect model described above. The appropriateness of a linear model will be assessed by inspection of relevant goodness of fit plots. If there is an indication that a linear model is inappropriate, the non-linearity detected will be taken into account by an appropriate transformation of the concentration values.

From the model, the slope of the exposure versus response curve will be estimated along with the corresponding two-sided 90% confidence intervals (CIs). The model will be used to evaluate the average difference from the RO7049389 treatment at pre-determined plasma concentrations, with bootstrapping methods used to estimate the two-sided 90% CIs of the average difference. The upper bound of these two-sided 90% CIs will be compared to pre-specified thresholds (i.e., 10 msec).

*The exposure-response (*ΔQTcF) analysis results will be reported in a separated document.

6.5.5 Concomitant Medications

The original terms recorded on the study subjects' eCRF by the Investigator for concomitant medications will be standardized by the Sponsor by assigning preferred terms. Concomitant medications will be presented in summary tables and listings.

6.6 EFFICACY ANALYSES

Efficacy analyses are only applicable to Parts 2 and 3 of the study.

Plasma*/serum* samples for viral dynamic data (HBV DNA, HBsAg, HBeAg, anti-HB*s,* anti-HB*e*, *HBV RNA, HBcrAg, anti*-HBc, and viral genotypes) will be collected as detailed in the SoA.

6.6.1 Primary Efficacy Analyses

The primary efficacy endpoint in Part 2 will be mean change from baseline in quantitative HBV DNA levels.

Summary descriptive statistics will be used to summarize HBV DNA levels, including actual and change from baseline (absolute and percentage) and maximum change from baseline (absolute and percentage) at each time-point.

The primary efficacy endpoint in Part 3 will be proportion of patients achieving functional cure (HBV DNA <LLOQ *(20 IU/mL)* with HBsAg loss at 24 weeks post-treatment). Response rate and 95% CI will be calculated using Clopper-Pearson method at week 12, 24, 36, 48 (EOT), and 24 weeks after EOT. Patients who cannot stop NUC therapy after week 48 because their HBV DNA have never been below LLOQ during the whole study period will be treated as not achieving functional cure. Patients with missing HBsAg or HBV DNA measures at 24 weeks visit after EOT and thereafter will be treated as not achieving functional cure.

6.6.2 Secondary Efficacy Analyses

Summary descriptive statistics will be used to summarize secondary efficacy outcome measures (HBV DNA, HBsAg, HBeAg, HBV RNA, *and* HBcrAg), including actual and change from baseline and maximum change from baseline at each time point. In addition, the rate of loss of HBeAg, HBeAg seroconversion (loss of HBeAg and presence of anti-HBe), loss of HBsAg, HBsAg seroconversion (loss of HBsAg and presence of anti-HBs)*,* HBV DNA levels less than 20 IU/mL, HBV RNA levels less than LLOQ/LOD*,* and HBcrAg levels below LLOQ/LOD will also be summarized at each time-point.

6.6.3 Exploratory Efficacy Analyses

Dynamics of HBV viral markers as described below:

The relationship between PK parameters and the viral response measures will be explored by graphical analysis. Further analysis of this relationship may be performed depending upon the findings in the graphical analysis.

Summary descriptive statistics will be used to summarize the exploratory outcome measures, including actual and change from baseline and maximum change from baseline at each time-point. Exploratory efficacy analyses may be performed including, but not limited to total HBsAg and liver stiffness measurement by Fibroscan (see section 3.3.4).

The findings from the resistance analyses will be listed for each patient selected for analysis*, and report separately*. Exploratory analysis may also be performed to identify markers and/or marker panels correlating with and/or potentially predictive of an in vivo PD response, the occurrence of certain AEs (to be defined) and/or changes in viral parameters.

6.7 PHARMACOKINETIC ANALYSES

Non-compartmental analysis will be employed for estimation of PK parameters. Individual plasma concentrations at each sampling time-point for RO7049389 of each cohort will be presented by listings and appropriate descriptive summary statistics, including means, medians, geometric means, ranges, standard deviations and coefficients of variation. Individual and mean plasma concentration of each cohort versus time data will be plotted on semi-logarithmic scales.

All PK parameters will be presented by individual listings and summary statistics for each cohort including means, geometric means, medians, ranges, standard deviations and coefficients of variation.

The relationship of selected PK parameters from Day 1 with dose will be evaluated graphically to establish dose-linearity. Dose-proportionality may be assessed by estimating the slope of the linear regression of logarithmically transformed variables C_{max}, AUC_{last} and $AUC_{0-\infty}$ versus the logarithmically transformed dose. A 95% confidence interval for the slope which includes the value 1 would be consistent with dose-proportionality.

To assess the effect of food, the logarithmically transformed variables C_{max}, AUC_{last} and $AUC_{0-\infty}$ will be subjected to an ANOVA (factors fasted/fed state and subject) for which measurements are available for healthy subjects in both the fasted and fed state. Ninety percent confidence intervals for the ratio of geometric means will be calculated using the fasting state as the reference.

A population PK model development and analysis may be performed. The Population PK results will be reported in a separated document.

6.8 ANALYSIS TO SUPPORT DOSE-ESCALATION DECISION AND DOSE SELECTION

A continual re-assessment method (CRM), with control for the probability of over-dosing, based on occurrence of dose-limiting event (DLE) will be used to inform decision about the dose of RO7049389 to be given to the next cohort during dose-escalation. A DLE will be defined as any treatment-related adverse reaction per investigator assessment (e.g., adverse event, laboratory abnormality, change in vital signs, ECG) that would prevent another drug administration at the same dose-level in a given study subject.

Details on the model/algorithm are given below. The relationship between the dose and the probability of observing a DLE will be described by the following two-parameter logistic regression model:

$$\text{logit}(p) = \log\left(\frac{p}{1-p}\right) = \alpha + \beta \log\left(\frac{dose}{ref}\right)$$

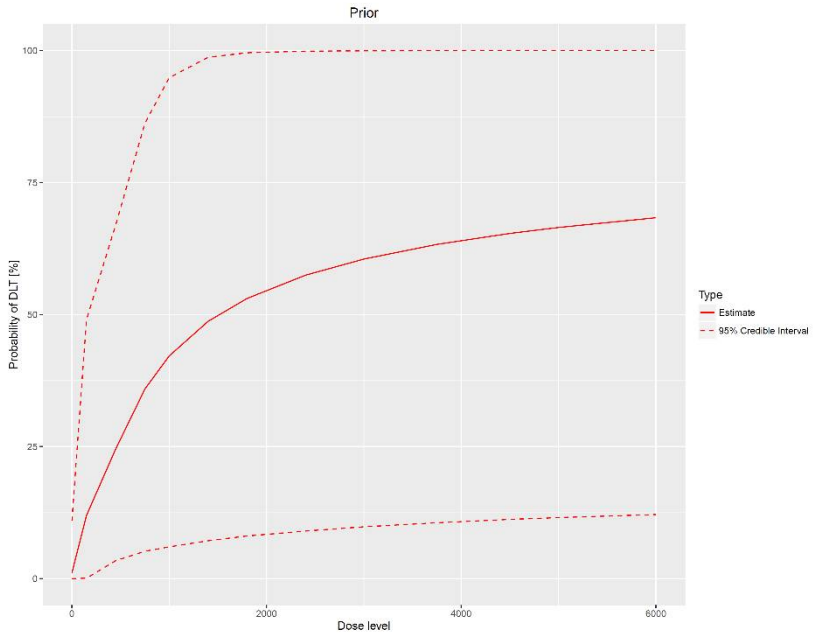
where ρ is the probability of observing a DLE at a given dose, α is the log of the odds for ρ at the reference dose (ref, here equal to 450 mg) and β is the change in the log odds for an e^1-fold increase in dose (detailed discussion about this model parameterization can be found in Neuenschwander et al 2008).

Since the model will be estimated using a Bayesian framework, priors need to be specified for α and β. The following bivariate normal distribution will be used for α and β for the SAD Part 1a. For Part 1c (MAD) and Part 2 (POM), data from the already completed cohorts of the previous study parts may be used as prior information on the model.

$$\begin{pmatrix} \alpha \\ \log(\beta) \end{pmatrix} = N\left(\mu = \begin{pmatrix} -1.355 \\ -0.159 \end{pmatrix}, \Sigma = \begin{pmatrix} 1.063 & -0.018 \\ -0.018 & 0.751 \end{pmatrix}\right)$$

A visual representation of this prior (translated into the prior for the probability of DLE) is provided in Figure 3, showing the mean and 95% credible interval for the probability of DLE (y-axis) across different doses of RO7049389 (x-axis). This prior was built on the assumption that the starting-dose (150 mg) is safe with a 5% probability of observing a proportion of DLEs above 35% and that there is a 5% probability of observing a proportion of DLEs below 20% at the 6000 mg level. This information is conveyed into a 'minimally informative prior', as described in Neuenschwander et al 2008, which would allow to model different possible dose-toxicity profiles.

Figure 3 Probability of DLE versus Dose of RO7049389



After each cohort of study subjects completes dosing, the model will be updated with the DLE–observed occurrence and a new MTD will be defined as the dose such that:

- The probability of being within the target safety interval (of 20% to 35% DLE rate) is maximized and
- The probability of being within the excessive toxicity interval (above 35% DLE rate) is below 30%.
- However, clinical judgment will always override model estimates in the dose-selection process.

Details about the operating characteristics of this Bayesian model-based approach for the planned study design for Part 1a are presented in Appendix 11.

It is expected that for the Part 1c (MAD) and Part 2 (POM) designs, good operating characteristics would be obtained for the dose-levels to be examined. For Part 2 (POM), a Bayesian dose-escalation approach may be applied to identify the dose giving an optimal trade-off between safety and viral response. Viral response (e.g., Week 4 HBV DNA change from baseline) would be modeled as a linear log-log function of dose and a gain function applied to select the next best dose (Yeung et al 2015).

6.9 EXPLORATORY ANALYSES

Urine and plasma samples collected in the study will be qualitatively analyzed for the presence of metabolites of RO7049389 with the use of non-validated methods.

Endogenous substances levels will be investigated to assess the effect of multiple oral dosing of RO7049389 on CYP3A4 activity. Individual ratio (before and after 2 weeks of RO7049389 treatment in MAD cohorts) of plasma 4β-hydroxycholesterol/cholesterol will be listed and summarized using descriptive statistics. Geometric mean ratio of after RO7049389 treatment relative to before RO7049389 treatment and the 90% confidence intervals will be computed.

The relationship between dose or exposure and efficacy response (e.g., HBV DNA, HBsAg) in patients will be assessed by modelling approach.

For exploratory analyses for Part 2 and Part 3 of the study, please see Section 6.6.3.

6.10 INTERIM ANALYSES

No interim analysis is planned for Parts 1 and 2 of this study.

Given the hypothesis-generating nature of this study, the Sponsor may choose to conduct interim efficacy analyses in Part 3. The decision to conduct an optional interim analysis and the timing of the analysis will be documented in the Sponsor's trial master file prior to the conduct of the interim analysis. The interim analysis will be performed and interpreted by Sponsor study team personnel.

7. DATA COLLECTION AND MANAGEMENT

7.1 DATA QUALITY ASSURANCE

The Sponsor will be responsible for data management of this study, including quality checking of the data. Sites will be responsible for data entry into the Electronic Data Capture system.

A comprehensive validation check program will verify the data. Discrepancies will be generated automatically in the system at the point of entry or added manually for resolution by the Investigator.

The Sponsor will produce a Data Handling Manual and a Data Management Plan that describes the quality checking to be performed on the data. Laboratory electronic data will be sent directly to the Sponsor, using the Sponsor's standard procedures to handle and process the electronic transfer of these data.

System backups for data stored by the Sponsor and records retention for the study data will be consistent with the Sponsor's standard procedures.

7.2 ELECTRONIC CASE REPORT FORMS

Data for this study will be captured via an on line EDC system. The data collected in the source documents is entered onto the study eCRF. An audit trail will maintain a record of initial entries and changes made; reasons for change; time and date of entry; and user

name of person authorizing entry or change. For each healthy volunteer/patient enrolled, an eCRF must be completed and electronically signed by the Principal Investigator or authorized delegate from the study staff. If a healthy volunteer/patient withdraws from the study, the reason must be noted on the eCRF. If a healthy volunteer/patient is withdrawn from the study because of a treatment-limiting adverse event, thorough efforts should be made to clearly document the outcome.

The Investigator should ensure the accuracy, completeness and timeliness of the data reported to the Sponsor in the eCRFs and in all required reports.

eCRFs will be submitted electronically to the Sponsor and should be handled in accordance with instructions from the Sponsor.

At the end of the study, the investigator will receive patient data for his or her site in a readable format on a compact disc that must be kept with the study records. Acknowledgement of receipt of the compact disc is required.

7.3 SOURCE DATA DOCUMENTATION

Study monitors will perform ongoing source data verification to confirm that critical protocol data (i.e., source data) entered into the eCRFs by authorized site personnel are accurate, complete, and verifiable from source documents.

Source documents (paper or electronic) are those in which patient data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, patient-reported outcomes, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, patient files, and records kept at pharmacies, laboratories, and medico-technical departments involved in a clinical trial.

Before study initiation, data to be entered directly into the eCRFs (i.e., no prior written or electronic record of the data) and considered source data must be defined in the Trial Monitoring Plan.

Source documents that are required to verify the validity and completeness of data entered into the eCRFs must not be obliterated or destroyed and must be retained per the policy for retention of records described in Section 7.5.

To facilitate source data verification, the investigators and institutions must provide the Sponsor direct access to applicable source documents and reports for trial-related monitoring, Sponsor audits, and IRB/EC review. The investigational site must also allow inspection by applicable Health Authorities.

7.4 USE OF COMPUTERIZED SYSTEMS

When clinical observations are entered directly into an investigational site's computerized medical record system (i.e., in lieu of original hardcopy records), the electronic record can serve as the source document if the system has been validated in accordance with Health Authority requirements pertaining to computerized systems used in clinical research. An acceptable computerized data collection system allows preservation of the original entry of data. If original data are modified, the system should maintain a viewable audit trail that shows the original data as well as the reason for the change, name of the person making the change, and date of the change.

7.5 RETENTION OF RECORDS

Records and documents pertaining to the conduct of this study and the distribution of IMP, including eCRFs, ePRO data (if applicable), Informed Consent Forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for at least 15 years after completion or discontinuation of the study, or for the length of time required by relevant national or local Health Authorities, whichever is longer. After that period of time, the documents may be destroyed, subject to local regulations. No records may be disposed of without the written approval of the Sponsor. Written notification should be provided to the Sponsor prior to transferring any records to another party or moving them to another location.

8. ETHICAL CONSIDERATIONS

8.1 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in full conformance with the ICH E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Studies conducted in the United States (U.S.) or under a U.S. Investigational New Drug (IND) application will comply with U.S. FDA regulations and applicable local, state, and federal laws. Studies conducted in the EU/EEA will comply with the EU Clinical Trial Directive (2001/20/EC).

8.2 INFORMED CONSENT

The Sponsor's sample Informed Consent Form (and ancillary sample Informed Consent Forms such as a Child's Assent or Caregiver's Informed Consent Form, if applicable) will be provided to each site. If applicable, it will be provided in a certified translation of the local language. The Sponsor or its designee must review and approve any proposed deviations from the Sponsor's sample Informed Consent Forms or any alternate consent forms proposed by the site (collectively, the "Consent Forms") before IRB/EC

submission. The final IRB/EC‑approved Consent Forms must be provided to the Sponsor for Health Authority submission purposes according to local requirements.

The Consent Forms must be signed and dated by the patient before his or her participation in the study. The case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained prior to participation in the study.

The Consent Forms should be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the patient to participate. The final revised IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes.

Patients must be re-consented to the most current version of the Consent Forms (or to a significant new information/findings addendum in accordance with applicable laws and IRB/EC policy) during their participation in the study. For any updated or revised Consent Forms, the case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained using the updated/revised Consent Forms for continued participation in the study.

A copy of each signed Consent Form must be provided to the patient. All signed and dated Consent Forms must remain in each patient's study file or in the site file and must be available for verification by study monitors at any time.

8.3 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE

This protocol, the Informed Consent Forms, any information to be given to the patient, and relevant supporting information must be submitted to the IRB/EC by the Principal Investigator and reviewed and approved by the IRB/EC before the study is initiated. In addition, any patient recruitment materials must be approved by the IRB/EC.

The Principal Investigator is responsible for providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. Investigators are also responsible for promptly informing the IRB/EC of any protocol amendments (see Section 9.5).

In addition to the requirements for reporting all adverse events to the Sponsor, investigators must comply with requirements for reporting serious adverse events to the local Health Authority and IRB/EC. Investigators may receive written IND safety reports or other safety‑related communications from the Sponsor. Investigators are responsible for ensuring that such reports are reviewed and processed in accordance with health authority requirements and the policies and procedures established by their IRB/EC, and archived in the site's study file.

8.4 CONFIDENTIALITY

The Sponsor maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient identification number. This means that patient names are not included in data sets that are transmitted to any Sponsor location.

Patient medical information obtained by this study is confidential and may only be disclosed to third parties as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law.

Medical information may be given to a patient's personal physician or other appropriate medical personnel responsible for the patient's welfare, for treatment purposes.

Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA and other national and local Health Authorities, Sponsor monitors, representatives, and collaborators, and the IRB/EC for each study site, as appropriate.

8.5 FINANCIAL DISCLOSURE

Investigators will provide the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate Health Authorities. Investigators are responsible for providing information on financial interests during the course of the study and for one year after completion of the study (i.e., LPLV).

9. STUDY DOCUMENTATION, MONITORING, AND ADMINISTRATION

9.1 STUDY DOCUMENTATION

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented, including but not limited to the protocol, protocol amendments, Informed Consent Forms, and documentation of IRB/EC and governmental approval. In addition, at the end of the study, the Investigator will receive the patient data, which includes an audit trail containing a complete record of all changes to data.

Roche shall also submit a Development Safety Update Report (DSUR) once a year to the IEC and CAs according to local regulatory requirements and timelines of each country participating in the study.

It is the understanding of the Sponsor that this protocol (and any modifications) as well as appropriate consent procedures and advertisements, will be reviewed and approved by an Institutional Review Board (IRB). This board must operate in accordance with the current Federal Regulations. The Sponsor will be sent a letter or certificate of approval

prior to initiation of the study, and also whenever subsequent amendments /modifications are made to the protocol. Roche shall also submit an IND Annual Report to FDA according to local regulatory requirements and timelines.

9.2 SITE INSPECTIONS

Site visits will be conducted by the Sponsor or an authorized representative for inspection of study data, patients' medical records, and eCRFs. The Investigator will permit national and local Health Authorities, Sponsor monitors, representatives, and collaborators, and the IRBs/ECs to inspect facilities and records relevant to this study.

9.3 ADMINISTRATIVE STRUCTURE

The Sponsor of the trial is F. Hoffmann-La Roche Ltd. The Sponsor is responsible for the study management, data management, statistical analysis and medical writing for the clinical study report.

9.4 PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor prior to submission. This allows the Sponsor to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the Investigator.

The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter trials only in their entirety and not as individual center data. In this case, a coordinating Investigator will be designated by mutual agreement.

Any formal publication of the study in which contribution of Sponsor personnel exceeded that of conventional monitoring will be considered as a joint publication by the Investigator and the appropriate Sponsor personnel.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Any inventions and resulting patents, improvements, and/or know-how originating from the use of data from this study will become and remain the exclusive and unburdened property of the Sponsor, except where agreed otherwise.

9.5 PROTOCOL AMENDMENTS

Any substantial protocol amendments will be prepared by the Sponsor. Substantial protocol amendments will be submitted to the IRB/EC and to regulatory authorities in accordance with local regulatory requirements.

Approval must be obtained from the IRB/EC and regulatory authorities (as locally required) before implementation of any changes, except for changes necessary to eliminate an immediate hazard to patients or any non-substantial changes, as defined by regulatory requirements.

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Appendix 1 Schedule of Assessments: Parts 1a (SAD) and 1b (FE) – Main Table

Protocol Activity	Screening	All SAD cohorts								Food Effect (FE) Cohort Only							Follow Up call[k]	Follow Up call (FE)[k]	Early Termination/ Study Drug Discontinuation[l]
Day	D-28 to D-3	Day -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Follow-up Day 8[j]	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Follow-up Day 23[j]	Day 29	Day 44	
Assessments																			
Informed Consent	X																		
Eligibility	X		X																
Demography	X																		
Medical History	X																		
Physical Examination[a]	X		X			X			X							X			X
Vital Signs[b]	X		4	8	X	X	X	X	X	X	8	X	X	X	X	X			X
ECG-12 lead[c]	X		X	3	X				X	X	3	X				X			X
ECG-Holter Start[d]			X	X															
ECG-Holter End[d]				X	X														
ECG-Holter Extraction[d]			6	7	X														
Hematology[e]	X		X		X				X	X		X				X			X
Blood Chemistry[e]	X		X		X				X	X		X				X			X
Coagulation[e]	X		X		X				X	X		X				X			X
Urinalysis[e]	X		X		X				X	X		X				X			X
Viral markers[f]	X																		
Substance Use	X		X							X									
Pregnancy Test[g]	X		X							X									X
Follicle Stimulating Hormone[h]	X																		
Randomisation				X															
High-fat, high calorie meal											X								
Administration of Study Medication				X							X								
Plasma PK				12	2	X	X	X			12	2	X	X	X				X
Urine PK Sample				5	X														
Clinical Genotyping[i]				X															
In-Clinic Stay		X	X	X	X					X	X	X							
Discharge						X							X						
Ambulatory Visit							X	X						X	X				
Adverse Events	<----------X---------->																		
Previous and Concomitant Treatments	<----------X---------->																		

Appendix 1 Schedule of Assessments: Parts 1a (SAD) and 1b (FE) – Main Table (cont.)

a. A full physical exam is required at screening and follow-up. At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations should be performed at the discretion of the Investigator. Height will only be recorded at screening. BMI will be calculated at screening.

b. Vital signs include triplicate measurements of blood pressure and heart rate and single assessment of body temperature.

c. 12-lead ECGs will be obtained in triplicate (three consecutive interpretable 12-lead ECGs within a 3 – 5 minute interval) after the subject has been in a supine position for at least 10 minutes. Automated ECG intervals (PR (PQ), QRS, QT, QTcF) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented.

d. Triplicate ECG measurements from the continuous recordings within ± 10 minutes of the time points will be extracted. Subject should be in a supine position for at least 10 minutes prior to and remain in a supine position for at least 10 minutes after the ECG extraction time-point. ECG intervals (PR (PQ), QRS, QT, QTcF) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented. The absence of any environmental distractions (e.g., television, radio, conversation, or phone calls) during the pre- and post-ECG rest period, and at every ECG time point specified in the SoA must be emphasized. In particular, activities known to cause changes in heart rate should be avoided.

e. Samples for clinical laboratory tests (hematology, chemistry, coagulation, urinalysis) should be collected in the morning of Days 2 and 17 approximately the same time as the 24 hour post-dose PK samples are collected. At all other time points these samples should be taken at the most appropriate time.

f. Hepatitis A (HAV IgM Ab), Hepatitis B (HBsAg), Hepatitis C (HCV RNA or HCV Ab), human immunodeficiency virus (HIV-1 and HIV-2 Ab).

g. Serum or plasma beta-human chronic gonadotropic (β-HCG) at screening, urine on all other occasions (females only).

h. Follicle stimulating hormone (females only to confirm post-menopausal status).

i. If the genetic blood sample is not collected at Day 1, it may be collected at any time during the conduct of the clinical study.

j. A follow up visit to be completed in 7 days (±1) after the last dose of study medication. Subjects who participate in Part 1b (FE) will not have follow-up on Day 8.

k. A follow up call to be completed in 28 days (±3) after the last dose of study medication. Subjects who participate in Part1a will have a follow up call on Day 29 only. Subjects who participate in both Part1a and Part 1b (FE) will have a follow-up call on Day 44 only.

l. In case of early termination or study drug discontinuation, a subject should be called for an unscheduled visit and assessments should be performed as listed in the table.

Appendix 2 Schedule of Assessments: Parts 1a (SAD) and 1b (FE) – Detailed Table

Protocol Activity	All SAD cohorts																													
Day	Day -1							Day 1																			Day 2			Day 3	Day 4	Day 5
Scheduled Time (h)	0	1	2	3	4	6	12	Predose	0	0.25	0.5	1	1.5	2	3	4	0-4	6	8	4-8	10	12	8-12	12-24	24	36	24-48	48	72	96
Vital Signs[a]	X	X			X		X	X			X	X		X	X	X		X				X			X			X	X	X
ECG-12 lead[b]	X							X						X				X							X					
ECG-Holter Start	X							X																						
ECG-Holter End								X																	X					
ECG-Holter Extraction[c]		X	X	X	X	X	X	X				X		X	X	X		X				X			X					
Randomisation								X																						
Administration of Study Medication									X																					
Plasma PK								X		X	X	X	X	X	X	X		X	X		X	X			X	X		X	X	X
Urine PK Sample[d]								X									X			X			X	X			X			

Protocol Activity	Food Effect (FE) Cohort Only																		
Day	Day 16													Day 17		Day 18	Day 19	Day 20	
Scheduled Time (h)	Predose	0	0.25	0.5	1	1.5	2	3	4	6	8	10	12	24	36	48	72	96	
Vital Signs[a]	X			X	X		X	X	X	X			X	X		X	X	X	
ECG-12 lead[b]	X						X			X				X					
ECG-Holter Start																			
ECG-Holter End																			
ECG-Holter Extraction[c]																			
Randomisation																			
Administration of Study Medication		X																	
Plasma PK	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine PK Sample[d]																			

Appendix 2 Schedule of Assessments: Parts 1a (SAD) and 1b (FE) – Detailed Table (cont.)

a. Vital signs include triplicate measurements of blood pressure and heart rate and single assessment of body temperature.

b. 12-lead ECGs will be obtained in triplicate (three consecutive interpretable 12-lead ECGs within a 3- 5 minute interval) after the subject has been in a supine position for a least 10 minutes. Automated ECG intervals (PR (PQ), QRS, QT, QTcF) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented.

c. Triplicate ECG measurements from the continuous recordings within ± 10 minutes of the time points will be extracted. Subject should be in a supine position for at least 10 minutes prior to and remain in a supine position for at least 10 minutes after the ECG extraction time-point. ECG intervals (PR (PQ), QRS, QT, QTcF) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented. The absence of any environmental distractions (e.g., television, radio, conversation, or phone calls) during the pre- and post-ECG rest period, and at every ECG extraction time point specified in the SoA must be emphasized. In particular, activities known to cause changes in heart rate should be avoided. Holter extractions will not be collected in period 2 of FE cohort (Day 16 and beyond).

d. Urine samples will be collected at pre-dose, 0-4, 4-8, 8-12, 12-24, 24-48 hours post-dose. Urine samples will not be collected in period 2 of FE cohort (Day 16 and beyond).

Appendix 3 Schedule of Assessments: Part 1c (MAD) – Main Table

Protocol Activity	Screening	Week 1									Week 2							Week 3		Follow Up Visit[j]	Follow Up call[k]	Early Termination/ Study Drug Discontinuation[l]
Day	D-28 to D-3	Day -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 21	Day 42	
Assessments																						
Informed Consent	X																					
Eligibility	X		X																			
Demography	X																					
Medical History	X																					
Physical Examination[a]	X		X								X									X		X
Vital Signs[b]	X		4	7	X	X	X	X	X	X	X	X	X	X	X	X	7	X	X	X		X
ECG-12 lead[c]	X		X	3	X	X				X							3	X		X		X
ECG-Holter Start[d]			X	X													X					
ECG-Holter End[d]				X	X													X				
ECG-Holter Extraction[d]			6	7	X												7	X				
Hematology[e]	X		X			X				X							X			X		X
Blood Chemistry[e]	X		X			X				X							X			X		X
Coagulation[e]	X		X			X				X							X			X		X
Urinalysis[e]	X		X			X				X							X			X		X
Exploratory liver biomarkers	X		X			X				X							X			X		
Viral markers[f]	X																					
Substance Use	X		X																			
Pregnancy Test[g]	X		X																	X		X
Follicle Stimulating Hormone[h]	X																					
Randomisation				X																		
Administration of Study Medication				2	2	2	2	2	2	2	2	2	2	2	2	2	X					
Plasma PK				12	X	X	X	X		X							12	2	X			X
Urine PK Sample				5													5					
Blood Sample for 4 betahydroxycholesterol/cholesterol				X													X					
Midazolam Microdosing			X														X					
Midazolam PK sample			10	X													10	X				
Clinical Genotyping[i]				X																		
In-Clinic Stay		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Discharge																			X			
Adverse Events	<----------x---------->																					
Previous and Concomitant Treatments	<----------x---------->																					

Appendix 3 Schedule of Assessments: Part 1c (MAD) – Main Table (cont.)

a. A full physical exam is required at screening and follow-up. At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations should be performed at the discretion of the Investigator. Height will only be recorded at screening. BMI will be calculated at screening.

b. Vital signs include triplicate measurements of blood pressure and heart rate and single assessment of body temperature.

c. 12-lead ECGs will be obtained in triplicate (three consecutive interpretable 12-lead ECGs within a 3 – 5 minute interval) after the subject has been in a supine position for at least 10 minutes. Automated ECG intervals (PR (PQ), QRS, QT, QTcF) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented.

d. Triplicate ECG measurements from the continuous recordings within ± 10 minutes of the time points will be extracted. Subject should be in a supine position for at least 10 minutes prior to and remain in a supine position for at least 10 minutes after the ECG extraction time-point. ECG intervals (PR (PQ), QRS, QT, QTcF) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented. The absence of any environmental distractions (e.g., television, radio, conversation, or phone calls) during the pre- and post-ECG rest period, and at every ECG time point specified in the SoA must be emphasized. In particular, activities known to cause changes in heart rate should be avoided.

e. Samples for clinical laboratory tests (hematology, chemistry, coagulation, urinalysis) should be collected in the morning of Days 3, 7, and 14 approximately the same time before the morning dose (i.e., pre-dose). At all other time points these samples should be taken at the most appropriate time.

f. Hepatitis A (HAV IgM Ab), Hepatitis B (HBsAg), Hepatitis C (HCV RNA or HCV Ab), human immunodeficiency virus (HIV-1 and HIV-2 Ab).

g. Serum or plasma beta-human chronic gonadotropic (β-HCG) at screening, urine on all other occasions (females only).

h. Follicle stimulating hormone (females only to confirm post-menopausal status, performed at screening only).

i. If the genetic blood sample is not collected at Day 1, it may be collected at any time during the conduct of the clinical study.

j. A follow up visit to be completed in 7 days (±1) after the last dose of study medication.

k. A follow up call to be completed in 28 days (±3) after the last dose of study medication.

l. In case of early termination or study drug discontinuation, a subject should be called for an unscheduled visit and assessments should be performed as listed in the table.

Appendix 4 Schedule of Assessments: Part 1c (MAD) – Detailed Table

Day	Day -1												Day 1																	
Scheduled Time (h)	Predose	0	0.25	0.5	1	2	3	4	6	8	10	12	Predose	0	0.25	0.5	1	1.5	2	3	4	0-4	6	8	4-8	10	12	8-12	12-24
Vital Signs[a]		x			x			x				x	x			x	x		x		x		x				x		
ECG-12 lead[b]		x											x						x				x						
ECG-Holter Start		x											x																
ECG-Holter End													x																
ECG-Holter Extraction					x	x	x	x	x			x	x				x		x	x	x		x				x		
Randomisation													x																
Administration of Study Medication[c]														x													x		
Plasma PK[d]													x		x	x	x	x	x	x	x		x	x		x	x		
Urine PK Sample[e]													x									x			x			x	x
Blood Sample for 4 betahydroxycholesterol /cholesterol													x																
Midazolam Microdosing		x																											
Midazolam PK sample	x		x	x	x	x		x	x	x	x	x	x																

Day	Day 2	Day 3	Day 4	Day 5	Day 7	Day 14																		Day 15		Day 16
Scheduled Time (h)	Predose	Predose	Predose	Predose	Predose	Predose	0	0.25	0.5	1	1.5	2	3	4	0-4	6	8	4-8	10	12	8-12	12-24	24	36	48
Vital Signs[a]	x	x	x	x	x	x			x	x		x		x		x				x			x		x
ECG-12 lead[b]	x	x			x	x						x				x							x		
ECG-Holter Start						x																			
ECG-Holter End	x																						x		
ECG-Holter Extraction	x					x				x		x	x	x		x				x			x		
Randomisation																									
Administration of Study Medication[c]							x																		
Plasma PK[d]	x	x	x	x	x	x		x	x	x	x	x	x	x		x	x		x	x			x	x	x
Urine PK Sample[e]						x									x			x			x	x			
Blood Sample for 4 betahydroxycholesterol /cholesterol						x																			
Midazolam Microdosing							x																		
Midazolam PK sample						x		x	x	x		x		x		x	x		x	x			x		

Appendix 4 Schedule of Assessments: Part 1c (MAD) – Detailed Table (cont.)

a. Vital signs include triplicate measurements of blood pressure and heart rate and single assessment of body temperature

b. 12-lead ECGs will be obtained in triplicate (three consecutive interpretable 12-lead ECGs within a 3 – 5 minute interval) after the subject has been in a supine position for at least 10 minutes. Automated ECG intervals (PR (PQ), QRS, QT, QTcF) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented.

c. BID dosing (12 hours apart) except for Day 14, where only one dose in the morning will be given. If PK data from SAD cohorts support QD regimen, subjects will be dosed for two weeks once daily.

d. Plasma samples will be collected at pre-dose, 0.25,0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours after the first dose (Day 1); and pre-dose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, and 48 hours after the last morning dose (Day 14). Trough samples will be collected on Days 2, 3, 4, 5 and 7 before the morning dose (i.e., pre-dose).

e. Urine samples will be collected at pre-dose, 0-4, 4-8, 8-12,12-24 hours post-morning dose on Days 1 and 14.

Appendix 5 Schedule of Assessments: Part 2 (POM) – Main Table

Protocol Activity	Screening		Treatment period													Week 5	Follow Up Visit[j]	Follow Up Visit[k]	Follow Up Visit[m]	Follow Up Visit[n]	Early Termination/ Study Drug Discontinuation[l]
			Week 1					Week 2		Week 3		Week 4									
Day	D-28 to D-2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5-7	Day 8	Day 9-14	Day 15	Day 16-21	Day 22	Day 23-26	Day 27	Day 28	Day 29	Day 35 (+/- 1 day)	Day 56 (+/- 3 days)	Day84 (+/- 2 weeks)	Day112 (+/- 2 weeks)	
Assessments																					
Informed Consent	x	x																			
Eligibility	x	x																			
Demography	x																				
Medical History	x																				
Physical Examination[a]	x	x						x									x				x
Vital Signs[b]	x	x	5	x	x	x		x		x		x			5	x	x	x	x	x	x
ECG-12 lead[c]	x	x	3					x		x					3	x	x				x
Hematology[d]	x	x		x		x		x		x		x			x		x	x	x	x	x
Blood Chemistry[d]	x	x		x		x		x		x		x			x		x	x	x	x	x
Exploratory liver biomarkers	x	x		x		x		x		x		x			x		x	x			x
Coagulation[d]	x	x		x		x		x		x		x			x		x	x			x
Urinalysis[d]	x	x		x		x		x		x		x			x		x	x			x
Liver Biopsy/Fibroscan[e]	x																				
Alpha-fetoprotein	x																				
Viral markers (HAV, HCV, HDV, HIV)[f]	x																				
RBR DNA			x					x		x		x			x		x				
RBR RNA			x					x		x		x			x		x				
RBR serum			x					x		x		x			x		x				
RBR plasma			x																		
HBV DNA HBV total nucleic acid, Viral resistance monitoring	x	x						x		x		x			x		x	x	x	x	x
HBsAg quantitative	x	x						x		x		x			x		x	x	x	x	x
HBcAg	x	x						x		x		x			x		x	x	x	x	x
HBsAg, HBeAg qualitative	x	x						x		x		x			x		x	x	x	x	x
HBsAg/anti-HBsAg complex	x	x						x		x		x			x		x	x	x	x	x
anti-HBs, anti-HBe, anti-HBc	x	x						x		x		x			x		x	x	x	x	x
HBV Viral genotypes		x																			
Substance Use	x	x																			
Pregnancy Test[g]	x	x															x				x
FSH[h]	x																				
Randomisation			x																		
Administration of Study Medication			2	2	2	2	2	2	2	2	2	2	2	2	x						
Plasma PK Sample[i]			7	x	x	x		x		x		x			7	x					x
Clinical Genotyping			x																		
Admission (optional)		x												x							
Discharge (optional)				x												x					
Ambulatory Visit	x	x	x	x	x	x		x		x		x			x	x	x	x	x	x	
Adverse Events	<----------x---------->																				
Previous and Concomitant Treatments	<----------x---------->																				

Appendix 5 Schedule of Assessments: Part 2 (POM) – Main Table (cont.)

a. A full physical exam is required at screening and follow-up. At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations should be performed at the discretion of the Investigator. Height will only be recorded at screening. BMI will be calculated at screening.

b. Vital signs include triplicate measurements of blood pressure and heart rate and single assessment of body temperature.

c. 12-lead ECGs will be obtained in triplicate (three consecutive interpretable 12-lead ECGs within a 3 – 5 minute interval) after the subject has been in a supine position for at least 10 minutes. Automated ECG intervals (PR (PQ), QRS, QT, QTcF) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented.

d. Samples for clinical laboratory tests (hematology, chemistry, coagulation, urinalysis) should be collected in the morning of Days 2, 4, 8, 15, 22 and 28 approximately the same time pre-dose (i.e., before the morning dose). At all other time points, samples should be taken at the most appropriate time.

e. Screening biopsy, Fibroscan or equivalent documented within 6 months of randomization.

f. Hepatitis A (HAV IgM Ab), Hepatitis C (HCV RNA or HCV Ab), Hepatitis D virus (total anti-HDV), human immunodeficiency virus (HIV-1 and HIV-2 Ab).

g. Plasma or Serum beta-human chronic gonadotropic (β-HCG) at screening, urine on all other occasions (females only).

h. Follicle stimulating hormone (females only to confirm post-menopausal status, performed at screening only).

i. Plasma samples on Days 2, 3, 4, 8, 15, and 22 will be collected before the morning dose (i.e., pre-dose).

j. A follow up visit to be completed in 7 days (±1) after the last dose of study medication.

k. A follow up visit to be completed in 28 days (±3) after the last dose of study medication.

l. In case of early termination or study drug discontinuation, a subject should be called for an unscheduled visit and assessments should be performed as listed in the table.

m. A follow up visit to be completed in 56 days (±2 weeks) after the last dose of study medication.

n. A follow up visit to be completed in 84 days (±2 weeks) after the last dose of study medication.

Appendix 6 Schedule of Assessments: Part 2 (POM) – Detailed Table

Day	Day 1									Day2	Day3	Day4	Day8	Day15	Day22	Day 28								Day 29
Scheduled Time (h)	Predose	0	1	2	3	4	6	8	12	Predose	Predose	Predose	Predose	Predose	Predose	Predose	0	1	2	3	4	6	8	24
Vital Signs[a]	x		x	x		x	x			x	x	x	x	x	x	x		x	x		x	x		x
ECG-12 lead[b]	x			x			x						x	x		x			x			x		x
Randomisation	x																							
Administration of Study Medication		x							x								x							
Plasma PK Sample[c]	x		x	x	x	x	x	x		x	x	x	x	x	x	x		x	x	x	x	x	x	x

a. Vital signs include triplicate measurements of blood pressure and heart rate and single assessment of body temperature.

b. 12-lead ECGs will be obtained in triplicate (three consecutive interpretable 12-lead ECGs within a 3 – 5 minute interval) after the subject has been in a supine position for at least 10 minutes. Automated ECG intervals (PR (PQ), QRS, QT, QTcF) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented.

c. Plasma samples will be collected at pre-dose, 1, 2, 3, 4, 6, and 8 hours after the first dose (Day 1); and pre-dose, 1, 2, 3, 4, 6, 8, 24 hours after the last morning dose (Day 28). The time points of these samples may be adjusted based on PK data in SAD and MAD cohorts. Trough samples will be collected on Days 2, 3, 4, 8, 15, and 22 before the morning dose (i.e., pre-dose).

Appendix 7 Schedule of Assessments: Part 3 (POM) – Main Table

	Screening		Treatment period														Follow-up period									Early Termination/ Study Drug Discontinuation [u]
Protocol Activity	**Day -28 to -2**	**Day -1**	**Day 1**	**2w ±3d**	**4w ±3d**	**8w ±6d**	**12w ±6d**	**16w ±6d**	**20w ±6d**	**24w ±6d**	**28w ±6d**	**32w ±6d**	**36w ±6d**	**40w ±6d**	**44w ±6d**	**48w ±6d**	**50w ±3d** [s]	**52w ±3d** [s]	**54w ±3d** [s]	**56w ±3d/ 6d** [t]	**58w ±3d** [s]	**60w ±3d** [s]	**64w ±6d**	**68w ±6d** [s]	**72w ±6d**	
Assessment																										
Informed consent	x																									
Eligibility	x	x																								
Demography	x																									
Medical history	x																									
Physical examination [a]	x	x					x			x			x			x						x			x	x
Vital signs [b]	x	x	x	x	x	x	x			x			x			x						x			x	x
ECG 12-lead [c]	x	x	x	x	x	x	x			x			x			x						x			x	x
Hematology [d]	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Blood chemistry [d]	x	2		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
GLDH		x		x	x	x	x	x	x	x			x			x		x		x		x	x	x	x	x
Coagulation [d]	x	x		x	x	x	x	x	x	x			x			x		x		x		x	x	x	x	x
Urinalysis [d]	x	x		x	x	x	x	x	x	x			x			x		x		x		x	x	x	x	x
α-fetoprotein	x	x					x			x			x			x						x			x	x
FT3,FT4, TSH	x	x					x			x			x			x						x			x	x
Liver biopsy/ Fibroscan [e]	x	x					x			x			x			x						x			x	x
B ultrasound	x						x			x			x			x						x			x	x
Viral markers (HAV, HCV, HIV, HDV, HEV) [f]	x																									
HBV Viral genotyping based on HBV RNA [g]		x																								
HBV Viral genotyping based on HBV DNA [h]		x																								
Viral dynamic response measures (HBV DNA, HBsAg and HBeAg, anti-HBs, anti-HBe)	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Total HBsAg [i, q]		x		x	x	x	x	x	x	x	x	x	x	x	x	x		x		x		x			x	x

Appendix 7 Schedule of Assessments: Part 3 (POM) – Main Table (cont.)

	Screening		Treatment period														Follow-up period									Early Termination/ Study Drug Discontinuation [u]
Protocol Activity	**Day -28 to -2**	**Day -1**	**Day 1**	**2w ±3d**	**4w ±3d**	**8w ±6d**	**12w ±6d**	**16w ±6d**	**20w ±6d**	**24w ±6d**	**28w ±6d**	**32w ±6d**	**36w ±6d**	**40w ±6d**	**44w ±6d**	**48w ±6d**	**50w ±3d [s]**	**52w ±3d [s]**	**54w ±3d [s]**	**56w ±3d/ 6d [t]**	**58w ±3d [s]**	**60w ±3d [s]**	**64w ±6d**	**68w ±6d [s]**	**72w ±6d**	
Viral resistance monitoring, HBV RNA, HBcrAg, anti-HBc [j]		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Substance use	x	x																								
Pregnancy test [k]	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
FSH [l]	x																									
RBR DNA [q]			x																							
RBR RNA [q]			x																							
RBR serum [q]			x																							
RBR plasma [q]			x																							
Clinical Genotyping [q]			x																							
Plasma PK for RO7049389 [m]			7	2	7	2	2	2	2	7	2	2	2	2	2	8										x
Plasma PK for NUC [m]				x	x	x	x	x	x	x	x	x	x	x	x	x										
Serum PK for Peg-IFN [m, q]				x	x	x	x	x	x	x	x	x	x	x	x	x										
Dispense RO7049389 (IMP)			x		x	x	x	x	x	x	x	x	x	x	x [r]											
Dispense NUC and Peg-IFN (non-IMP)			x		x	x	x	x	x	x	x	x	x	x	x											
Patient diary review and drug accountability (IMP and non-IMP) [n]			x	x	x	x	x	x	x	x	x	x	x	x	x	x										x
Adverse events & concomitant treatments [o]	←------------------------------------ x ------------------------------------→																									
Optional hospitalization [p]		x								x						x										

Appendix 7 Schedule of Assessments: Part 3 (POM) – Main Table (cont.)

a. A full physical exam is required at screening and follow-up. At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations should be performed at the discretion of the Investigator. Height will only be recorded at screening. BMI will be calculated at screening.

b. Vital signs include blood pressure, pulse rate, and body temperature. To be obtained at least 5 minutes after patient has been in *a* supine position.

c. *A single interpretable* 12-lead ECG will be obtained after the subject has been in a supine position for at least 10 minutes. Automated ECG intervals (PR (PQ), QRS, QT, QTcF) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented.

d. Samples for clinical laboratory tests (hematology, chemistry, coagulation, urinalysis) should be collected in the morning of the same time pre-dose (i.e., before the morning dose) under fasting status. At all other time points, samples should be taken at the most appropriate time. On Day -1 a duplicate sample will be taken for liver tests (including at least ALT, AST, total bilirubin) and sent to the local laboratory. The local laboratory result will be used to confirm eligibility for liver parameters before dosing on Day 1.

e. At screening biopsy, Fibroscan or equivalent *test* documented within 6 months of randomization will be used to assess the eligibility. During the study, only Fibroscan is used to monitor the change of liver stiffness. *If Fibroscan is obtained during Day* –28 to *–2 with the same device to be used throughout the study, then Fibroscan on Day –1 can be optional.*

f. Hepatitis A (HAV IgM Ab), Hepatitis C (HCV RNA *and* HCV Ab), Hepatitis D (HDV *Ab*), Hepatitis E (HEV IgM Ab and IgG Ab), human immunodeficiency virus (HIV-1 and HIV-2 Ab).

g. HBV viral genotyp*ing based on HBV RNA* will be *conducted* only for NUC-suppressed patients *(e.g., Cohort A)*.

h. HBV viral genotyp*ing based on HBV DNA* will be *conducted* only for *treatment* naïve patients *(e.g., Cohort B and Cohort C)*.

i. Total HBsAg = post-dissociation of HBsAg/*anti*-HBs complexes.

j. Serum will be collected as scheduled for potential resistance surveillance.

k. Plasma or *s*erum beta-human chronic gonadotropic (β-HCG) at screening, urine on all other occasions (females only). If urine pregnancy test is positive, it must be confirmed by a serum*/plasma* pregnancy test.

l. Follicle stimulating hormone (females only to confirm post-menopausal status, performed at screening only).

m. For RO7049389 and its three metabolites (RO7121986, RO7255420 and RO7255422), plasma PK samples will be collected at pre-dose, 1, 2, 3, 4, 6, and 8 hours post-dose (Day 1 and week 24); and pre-dose, 1, 2, 3, 4, 6, 8, 24 hours after the last dose (week 48). Additional plasma PK samples will be collected at weeks 2, 4, 8, 12, 16, 20, 28, 32, 36, 40 and 44 pre-dose and between 1-4 hours post-dose. But for POM Cohort B, at week 4, plasma PK samples will be collected at pre-dose, 1, 2, 3, 4, 6, and 8 hours post-dose. For SoC, pre-dose PK samples will be collected at weeks 2 (Cohort A and C only), 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 and 48. For Peg-IFN, when the pre-dose PK samples cannot be collected, the post dose PK samples will be collected. Peg-IFN PK samples are only for POM Cohort C.

n. The patient's diary will be dispensed at each visit (except for the week 2 visit) and reviewed at the following visit.

o. Pre-treatment, only serious adverse events should be reported.

p. Optional hospitalization from Study Day-1/Day 1, *from Day 168 to Day 169,* or from Day 336 to Day 337, to facilitate collection of the post dose PK sample.

q. Since these measures are set to be exploratory, in some circumstances, these measures will only be tested in a subset of the patients or part of the visits.

r. The patients should be continually dosed with RO7049389 until the actual end-of-treatment (EOT) visit at week 48, during which the pre- and post-dose PK samples will be collected for the last dose of RO7049389.

s. Only applicable to participants who meet NUC Stopping Criteria (HBsAg < 100 IU/mL and HBV DNA < 20 IU/mL) at EOT visit.

t. For week 56 follow-up visit, ±3 days window is allowed for participants who meet NUC Stopping Criteria at EOT visit, while ±6 day window is allowed for participants who do not meet NUC Stopping Criteria at EOT visit.

u. In case of early termination or study drug discontinuation, a subject should complete an "early termination" visit with the assessments listed in this column within 7 days after the last dose of study drug or at the earliest possible time after "early termination" occurs, and then enter into the follow-up period in 2 weeks (±3 days) (meeting NUC Stopping Criteria) or 8 weeks (±6 days) (not meeting NUC Stopping Criteria) after the last dose of study drug with the assessments listed in respective columns.

Appendix 8 Schedule of Assessments: Part 3 (POM) – Detailed Table

Week	0							2		4							8		12		16		20	
Day	Day 1							14		28							56		84		112		140	
Scheduled Time (h)	Predose	0	1	2	3	4	6	8	Predose	1-4 h	Predose	1	2	3	4	6	8	1-4 h	Predose	1-4 h	Predose	1-4 h	Predose	1-4 h	Predose	1-4 h
Vital Signs[a]	x								x		x								x		x					
ECG-12 lead[b]	x								x		x								x		x					
Administration of Study Medication at Clinical Site		x							x		x								x		x		x		*x*	
Plasma PK Sample (RO7049389/metabolites)[c]	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
Plasma PK for NUC[c]									x		x								x		x		x		x	
Serum PK for Peg-IFN[c]									x		x								x		x		x		x	

Week	24								28		32		36		40		44		48								
Day	168								196		224		252		280		308		336								337
Scheduled Time (h)	Predose	1	2	3	4	6	8		Pre dose	1-4 h	Pre dose	1-4 h	Pre dose	1-4 h	Pre dose	1-4 h	Pre dose	1-4 h	Pre dose	1	2	3	4	6	8		24
Vital Signs[a]	x												x						x								
ECG-12 lead[b]	x												x						x								
Administration of Study Medication at Clinical Site	x								x		x		x		x		x		x								
Plasma PK Sample (RO7049389/metabolites)[c]	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
Plasma PK for NUC[c]	x								x		x		x		x		x		x								
Serum PK for Peg-IFN)[c]	x								x		x		x		x		x		x								

a. Vital signs include blood pressure, pulse rate, and body temperature. To be obtained at least 5 minutes after patient has been in supine position.

b. *A single interpretable* 12-lead ECG will be obtained after the subject has been in a supine position for at least 10 minutes. Automated ECG intervals (PR (PQ), QRS, QT, QTcF) and HR will be captured or calculated, and the changes in T-wave and U-wave morphology will be documented.

Appendix 8 Schedule of Assessments: Part 3 (POM) – Detailed Table (cont.)

c. For RO7049389 and its three metabolites (RO7121986, RO7255420 and RO7255422), plasma PK samples will be collected at pre-dose, 1, 2, 3, 4, 6, and 8 hours post-dose (Day 1 and week 24); and pre-dose, 1, 2, 3, 4, 6, 8, 24 hours after the last dose (week 48). Additional plasma PK samples will be collected at weeks 2, 4, 8, 12, 16, 20, 28, 32, 36, 40 and 44 pre-dose and between 1-4 hours post-dose. But for POM Cohort B, at week 4, plasma PK samples will be collected at pre-dose, 1, 2, 3, 4, 6, and 8 hours post-dose. For SoC, pre-dose PK samples will be collected at weeks 2 (cohort A and C only), 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 and 48. For Peg-IFN, when the pre-dose PK samples cannot be collected, the post dose PK samples will be collected (i.e., if the day of weekly Peg-IFN administration does not coincide with the visit day). Peg-IFN PK samples are only for POM Cohort C.

Appendix 9 High-Fat Breakfast for the Food Effect Cohort

Subjects participating in the food effect cohort will fast overnight for at least 8 hours prior to Day 16. Subjects will then be fed a high fat breakfast 30 minutes before dosing. The meal should be completed within 30 minutes. All subjects will be administered lunch approximately 5 hours post-dose.

The high-fat meal will be as recommended by the FDA for food effect bioavailability and bioequivalence studies. The fat content of the breakfast will be approximately 50 percent of the total caloric content of the meal. The meal will be high-calorie (approximately 800 to 1000 calories). This test meal will derive approximately 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively.

The meal will consist of:

- Two eggs in butter
- Two strips of bacon
- Two slices of toast with butter
- Four ounces of hash brown potatoes
- Eight ounces of whole milk

Substitutions in this meal can be made (for example for vegetarians) as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity.

Appendix 10 Cockcroft Gault Equation for Calculation CrCl and eGFR Equation for eGFR

The Cockcroft-Gault equation:

Used to calculate creatinine clearance (CrCl) (Conventional units = mL/min or SI units = mL/sec). Baseline body weight (ABW) will be used for calculation of CrCl.

Conventional Units:

$$\text{Males (mL/min)} = \frac{(140 - \text{Age}) * \text{ABW (kg)}}{72 * \text{Serum Creatinine (mg/dL)}}$$

Females (mL/min) = Male value × 0.85

Conversion Factor for Creatinine Clearance:

- SI Units (mL/sec) = Conventional units (mL/min) × 0.0167
- Conventional Units (mL/min) = SI Units (mL/sec) / 0.0167

Conversion Factor for Serum Creatinine:

- Conventional units (mg/dL) = SI units (µmol/L) / 88.4
- SI Units (µmol/L) = Conventional Units × 88.4

The Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) is used to estimate eGFR:

$$eGFR = 141 \times \min\left(\frac{S_{cr}}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{S_{cr}}{\kappa}, 1\right)^{-1.209} \times 0.993^{age} \times [1.018\ if\ female] \times [1.159\ if\ black]$$

where:
S_{cr} is serum creatinine in mg/dL,
κ is 0.7 for females and 0.9 for males,
α is -0.329 for females and -0.411 for males,
min indicates the minimum of S_{cr}/κ or 1, and
max indicates the maximum of S_{cr}/κ or 1.

Appendix 11 Child-Pugh Classification of Severity of Liver Disease

Clinical and Biochemical Measurements		Points Scored for Increasing Abnormality		
		1	**2**	**3**
Encephalopathy (grade)*		None	1 and 2	3 and 4
Ascites		Absent	Slight	Moderate
Bilirubin	mg per 100 mL	1 - 2	2 - 3	> 3
	µmol/L	< 34	34 – 51	> 51
Albumin	g per 100 mL	> 3.5	2.8 – 3.5	< 2.8
	g/L	> 35	28 – 35	< 28
Prothrombin time	Second prolonged	1 - 4	4 - 6	> 6

* According to grading of Trey, Burns and Saunders (1966)

1, 2 or 3 points are scored for increasing abnormality of each of the 5 parameters measured.

- Grade A: 5 or 6
- Grade B: 7 to 9
- Grade C: 10 to 15

Appendix 12 Simulation of the Operating Characteristics of the Planned SAD Design (Part 1a)

This section documents the simulation of the Part1a (SAD) and the operating characteristics for the model-based predictions of MTD with control for overdosing for a variety of dose-response profiles.

The following possible dose-levels were used in the simulation for RO7049389:

0.1 (as placebo), 150, 450, 750, 1000, 1400, 1800, 2400, 3000, 3750, 4500, 5000 and 6000 mg.

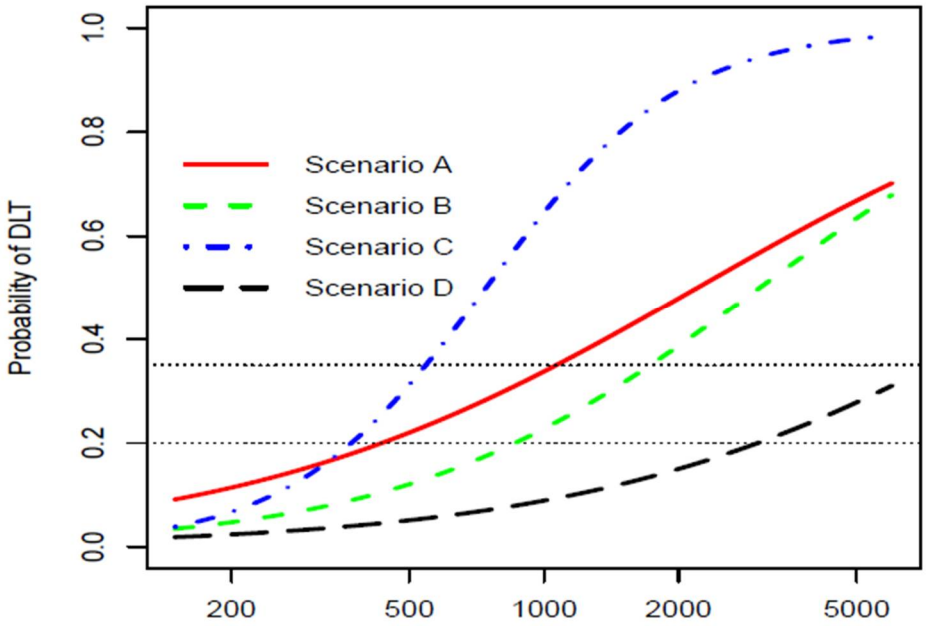
Four different scenarios for the true dose-toxicity profile were considered for the simulation:

- Scenario A: the doses of RO7049389 with true DLE rate in the target interval (between 20% and 35% DLE rate) are 150, 450, 750 and 1000 mg, i.e., similar to the prior
- Scenario B: the doses of RO7049389 with true DLE rate in the target interval (between 20% and 35% DLE rate) are 750, 1000 and 1400 mg.
- Scenario C: the doses of RO7049389 with true DLE rate in the target interval (between 20% and 35% DLE rate) are 150 and 450 mg.
- Scenario D: the doses of RO7049389 with true DLE rate in the target interval (between 20% and 35% DLE rate) are 3750, 4500, 5000 and 6000 mg.

For each scenario, 1000 simulations were run. The true relationship between the probability of DLE and dose of RO7049389 is shown in Figure A1 for each scenario.

Appendix 12 Simulation of the Operating Characteristics of the Planned SAD Design (Part 1a) (cont.)

Figure A1 Considered Scenarios for the True Dose-Toxicity Relationship



Each simulation mimicked a complete dose-escalation study outcome, starting with the first cohort receiving a dose of RO7049389 of 150 mg. After a new cohort was completed, the dose for the next cohort (current estimate of MTD) was predicted based on the observed pattern of DLEs, as the dose for which:

- The probability of being within the target safety interval (of 20% to 35% DLE rate) was maximized
- The probability of being within the excessive toxicity interval (above 35% DLE rate) was below 30%

In all trials, the dose for the next cohort did not exceed the fold increase of the dose from the previous cohort shown in the Table A1 below for the doses considered:

Table A1 Maximum Fold Increases in Dose from Previous Cohort

Dose of Previous Cohort (mg)	150 - 450	450 - 1000	1000 - 1800	1800 - 3000	3000 - 6000
Maximum Fold Increase	2	1.22	0.8	0.67	0.25

Appendix 12 Simulation of the Operating Characteristics of the Planned SAD Design (Part 1a) (cont.)

If no DLEs were observed, the dose-escalation would proceed as follows: 150 to 450 to 1000 to 1800 to 3000 to 6000 mg.

The initial cohort size was set to 3 (active) + 1 (placebo). Once a DLE occurred, the size of the next cohort was set to 6 (active) + 2 (placebo).

The following stopping rules were used in the simulation to stop the study:

- If 51 active subjects (maximum sample size in Part 1) were already dosed, then the study was stopped.
- If the predicted MTD had a probability of being within the target interval greater than or equal to 60%, and there were 6 subjects already dosed at that predicted MTD, then the study was stopped.
- If the probability of under-dosing (DLE rate below 20%) for the maximum dose was above 60% and there were 6 subjects already dosed at the maximum dose, then the study was stopped.

Note that if all doses were already too toxic, i.e., the probability of being within the excessive toxicity interval (above 35% DLE rate) was greater than 30%, the study was stopped.

The operating characteristics of the proposed design under the four alternative true scenarios, based on 1000 simulations, are presented in Table A2.

For each scenario, the following results are presented:

- True MTD: the RO7049389 doses with a true DLE rate in the target interval of 20%-35%.
- Predicted MTD: the predicted MTD (median, 10% and 90% quantiles) across all simulations (this includes no dose found).
- % Trials selecting MTD: the percentage of total times the MTD was correctly predicted across all simulations.
- True toxicity at doses selected: the true percentage of toxicity at the selected MTD across all simulations.
- n treated above true MTD: the number of subjects (median, 10% and 90% quantiles) receiving a RO7049389 dose above the true MTD across all simulations.
- n: number of subjects enrolled (median, 10% and 90% quantiles) across all simulations.
- n of cohorts: number of cohorts of size 3 or 6 (median, 10% and 90% quantiles) across all simulations.

Appendix 12 Simulation of the Operating Characteristics of the Planned SAD Design (Part 1a) (cont.)

Table A2 Operating Characteristics

Scenario	True MTD (mg)	Predicted MTD (mg)	% Trials' Selecting MTD	True Toxicity at Doses Selected (%)	n Treated above True MTD	n
A (red)	433.8 -1066.4	688 (150, 1000)	82	24 (9, 34)	2 (0, 6)	45 (33, 56)
B (green)	861- 1729.5	1148 (450, 1800)	55	25 (11, 36)	5 (0, 18)	47 (35, 56)
C (blue)	371-544.4	369.5 (150, 450)	67	21 (4, 27)	4 (0, 12)	46 (28, 56)
D (black)	3004.1- NA	4017.7 (2400, 6000)	64	24 (17, 31)	0 (0, 0)	53 (49, 56)

NA=Not applicable, Tox.= toxicity, MTD = Maximum Tolerated Dose.

Note: Median (10% and 90% quantiles) of 1000 simulations per scenario are shown. Simulations are based upon the low clearance dose levels and do not allow for an increase to 6 subjects for food effect dose level unless recommended by the algorithm.

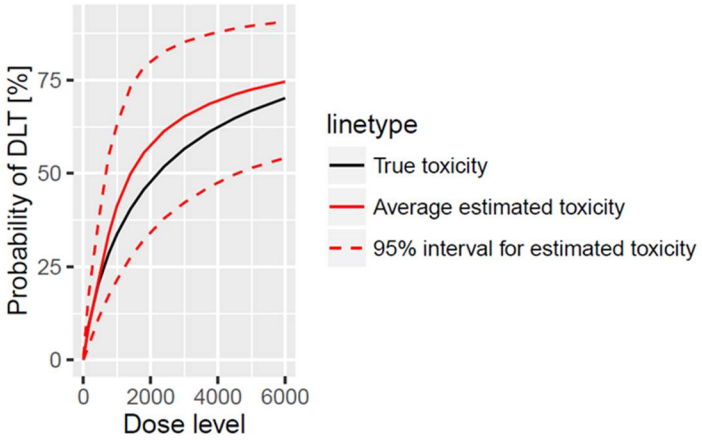
As shown in Table A2, for all scenarios, in median, the MTD was correctly estimated with true toxicity percentage in the target toxicity interval, and a low number of 6 subjects who received doses with toxicity greater than 35%.

In addition, Figure A2 shows for each scenario the true dose-toxicity profile and the model-based estimate of the probability of DLE (mean and 95% credible intervals) across different doses of RO7049389.

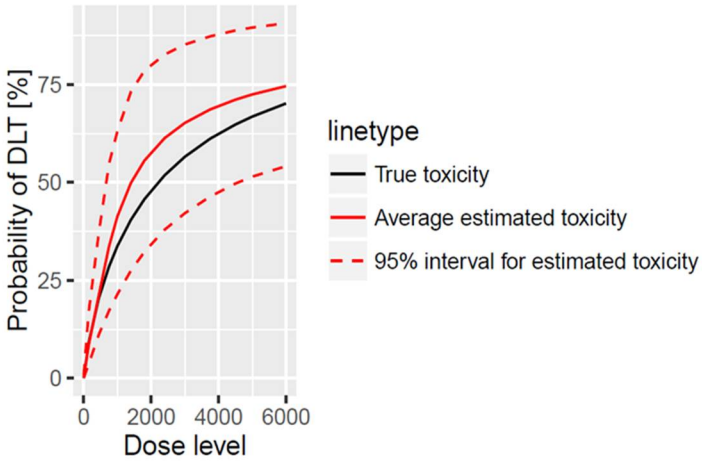
Appendix 12 Simulation of the Operating Characteristics of the Planned SAD Design (Part 1a) (cont.)

Figure A2 True Dose-Toxicity Profile and Model Based Estimate of DLE Rate across Different Doses of RO7049389

Scenario A



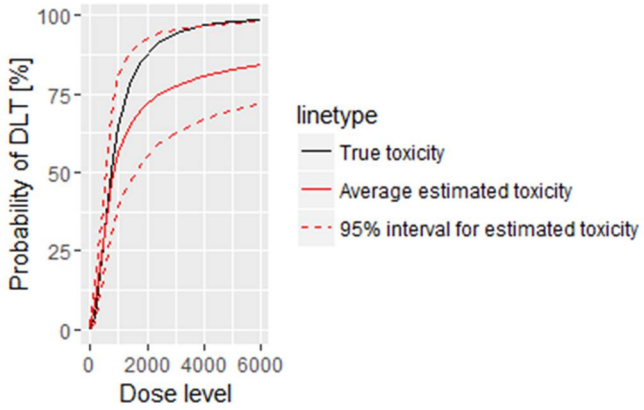
Scenario B



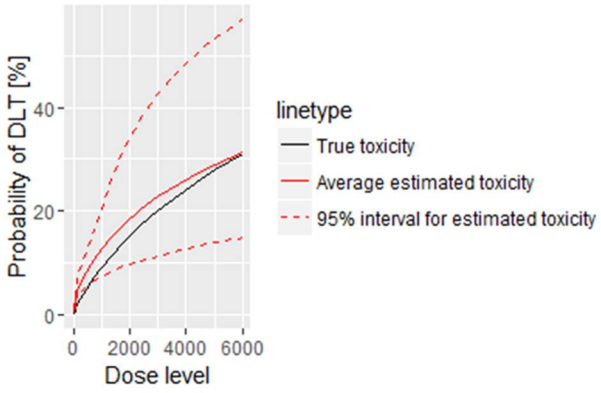
Appendix 12 Simulation of the Operating Characteristics of the Planned SAD Design (Part 1a) (cont.)

Figure A2 True Dose-Toxicity Profile and Model Based Estimate of DLE Rate across Different Doses of RO7049389 (cont.)

Scenario C



Scenario D



Appendix 13 Correction Formulas for QTc Intervals

Fridericia's correction for QTc Measurement - QTcF

$$QTcF\ (msec) = \frac{QT\ (ms)}{\sqrt[3]{RR(ms)/1000}}$$

Example: QTcF of a subject with a QT of 386 msec and a RR of 848 msec

QT (msec) = 386

RR(msec) = 848

$$\frac{QT\ (msec)}{\sqrt[3]{RR(msec)/1000}} = 408\ msec$$

Bazett's correction for QTc Measurement - QTcB

$$QTcB\ (msec) = \frac{QT\ (msec)}{\sqrt{RR(msec)/1000}}$$

Example: QTcB of a subject with a QT of 386 msec and a RR of 848 msec

QT (msec) = 386

RR (msec) = 848

$$\frac{QT\ (msec)}{\sqrt{RR(msec)/1000}} = 419\ msec$$

Appendix 14 Alcohol Volume Calculation

A standard drink is any drink containing 10 grams of alcohol. One standard drink contains the same amount of alcohol regardless of container size or alcohol type (i.e., beer, wine, or spirit).

Calculation for the standard number of drinks per day:

Drink volume (mL) x Percent alcohol by volume x Density of ethyl alcohol (g/mL)

Example: $375\text{mL} \times 0.05 \times 0.789\text{g/mL} = 15\text{g} = 1.5$ standard drinks

Examples from Australian Department of Health (Population Health Division)
http://www.alcohol.gov.au/internet/alcohol/publishing.nsf/Content/standard

Appendix 14 Alcohol Volume Calculation (cont.)

Appendix 15 Division of AIDS (DAIDS) Table for Grading the Severity of Adverse Events (For Part 3 of the study)

Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events Corrected Version 2.1 July 2017 will be used for assessing adverse event severity (see below table). For more detailed information, please use the following link to access the full DAIDS table： https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

Adverse Event Grading (Severity) Scale (For Part 3)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Clinical adverse event **NOT** identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

Appendix 16 *List of Inducers, Inhibitors and Substrates of Drug-Metabolizing Enzymes and Transporters*

This representative list is not intended to be exhaustive. Inducers and inhibitors should be prohibited in this study.

CYP3A Inducers	***CYP3A Inhibitors***
Aspirin, Carbamazepine, Efavirenz, Nevirapine, Nicotine, Phenobarbital, Phenytoin, Pioglitazone, Rifampin, Rifabutin, St. John's wort, Troglitazone.	*Amiodarone, Betamethaone, Cimetidine, Ciprofloxacin, Clarithromycin, Cyclosporine, Diltiazem, Erythromycin, Ergotamine, Fentanyl, Fluvoxamine, Hydroxyzine, Indinavir, Itraconazole, Ketoconazole, Nefazodone, Nelfinavir, Oxethazaine, Promethazine, Quinidine, St. John's wort, Suboxone, Troleandomycin, Verapamil, Voriconazole*
UGT Inducers	***UGT Inhibitors***
Carbamazepine, Nicotine	*Atazanavir, Gemfibrozil, Indinavir, Ketoconazole*
OATP1B Inhibitors	***BCRP Inhibitors***
Amoxicillin, Cloxacillin, Betamethaone, Cetirizine, Cyclosporine, Diclofenac, Domperidone, Eltrombopag, Gemfibrozil, Hydroxyzine, Isotretinoin, Lapatinib, Levofloxacin, Lopinavir, Loratadine, Metronidazole, Montelukast, Rifampicin, Ritonavir, Sirolimus, Tacrolimus	*Cyclosporine, Elacridar, Eltrombopag, Gefitinib, Loperamide, Prochlorperazine, Rabeprazole*
CYP3A4 Substrates With Narrow Therapeutic Range	***Substrates of OATP1B***
Alfentanil, Cyclosporine, Diegotamine, Ergotamine, Fentanyl, Pimozide, Quinidine, Sirolimus, Tacrolimus	*Atrasentan, Bosentan, Ezetimibe, Irinotecan, Statins (e.g., Atorvastatin, Rosuvastatin, Simvastatin, Pitavastatin, Pravastatin), Repaglinide, Rifampin, Valsartan, Olmesartan*

Appendix 17 *Alternative Study Visits and Study Drug (IMP and non-IMP) Dispensing during COVID-19 or Similar Epidemic*

This appendix describes the guidance on how the study visits may be conducted to ensure subjects' safety while continuing to participate in Study YP39364 during COVID-19 or similar epidemic. Everything in the protocol still applies unless otherwise noted in this appendix.

1. Telemedicine Visit

In case a study participant is not able to attend visits at the study site, it is recommended that a Telemedicine visit be scheduled by the Investigator/Delegate to conduct for some study assessments by telephone or video call.

The assessments shall include the review of intercurrent illness, adverse events, concomitant medications, drug compliance, etc., and the results shall be documented on eCRF with specific annotation that the assessments are done remotely due to COVID-19 or similar epidemic. Once the telemedicine visit confirms that there is no apparent safety concern leading to a change in study treatment, the investigator may continue study treatment to the subject.

This recommendation is based on the nature of the Chronic Hepatitis B disease and experience with standard of care i.e. nucleos(t)ide analogs in which premature discontinuation of anti-HBV treatment often leads to viral relapse and, in rare cases, fatal liver failure.

To further ensure patient's safety during administration of study medication, it is highly recommended for the patient to take safety laboratory tests (liver function test and hematology as a minimum) at other hospital, clinics or community lab services per standard of care for which the cost will be covered by Sponsor.

2. Study Drug (IMP and non-IMP) Dispensing

In addition, to ensure that all the subjects participating in the study can continue receiving their protocol defined treatment, in compliance with applicable global and local regulations and ICH GCP. Based on the self-administrable nature of the study drug, a courier service may be used to deliver study drugs from the site directly to a subject's home. This activity must ensure that the storage requirements according to the product label are maintained. Transporting without temperature monitoring is possible only in very specific circumstances and should only happen in very exceptional cases, and need to be assessed on a site by site basis. In all cases, decisions need to be made by the Investigator in consultation with the Sponsor and IRB/EC/HA as required.

Alternatively, study drugs can be dispensed to a relative or a person attending on behalf of the study subject. This scenario is possible only if the relative or person receiving the study drug demonstrates that it has been legally delegated by the subject according to local applicable regulatory requirements.

In both circumstances, the study participant will be asked to acknowledge the receipt of the study drug, and the Investigator/Delegate is requested to document this study drug transfer process appropriately.

3. Discontinuation of Study Treatment

Study participants will not be discontinued from the study purely due to missed visits or deviations due to COVID-19 or similar epidemic. However, if study drug cannot be dispensed to the subjects or subjects' safety cannot be assured by Telemedicine visit and minimum safety assessments, or lack of either of them, investigators can consider discontinuing the subject after assessing the risk/benefit. The Medical Monitors should be informed when such decision is made. An "early termination" visit should be arranged as soon as on-site visit becomes possible.