Official Title: **A RANDOMIZED, SPONSOR-OPEN, INVESTIGATOR-BLINDED, SUBJECT-BLINDED, PLACEBO-CONTROLLED, SINGLE AND MULTIPLE ASCENDING DOSE STUDY TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF RO7020531 AND METABOLITES FOLLOWING ORAL ADMINISTRATION TO CHINESE HEALTHY VOLUNTEERS**

NCT Number: NCT03530917

Document Date: Protocol Version 2: 18 November 2017

PROTOCOL

TITLE:	**A RANDOMIZED, SPONSOR-OPEN, INVESTIGATOR-BLINDED, SUBJECT-BLINDED, PLACEBO-CONTROLLED, SINGLE AND MULTIPLE ASCENDING DOSE STUDY TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF RO7020531 AND METABOLITES FOLLOWING ORAL ADMINISTRATION TO CHINESE HEALTHY VOLUNTEERS**
PROTOCOL NUMBER:	YP39553
VERSION:	2
TEST PRODUCT:	RO7020531
SPONSOR:	F. Hoffmann-La Roche Ltd
DATE FINAL:	Version 1: 07 April 2017
DATE AMENDED:	Version 2: See electronic date stamp below

FINAL PROTOCOL APPROVAL

Approver's Name	Title	Date and Time (UTC)
[redacted]	Company Signatory	18-Nov-2017 06:26:39

PROTOCOL ACCEPTANCE FORM

TITLE:	**A RANDOMIZED, SPONSOR-OPEN, INVESTIGATOR-BLINDED, SUBJECT-BLINDED, PLACEBO-CONTROLLED, SINGLE AND MULTIPLE ASCENDING DOSE STUDY TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF RO7020531 AND METABOLITES FOLLOWING ORAL ADMINISTRATION TO CHINESE HEALTHY VOLUNTEERS**
PROTOCOL NUMBER:	YP39553
VERSION NUMBER:	2
TEST PRODUCT:	RO7020531
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 2
RATIONALE

Protocol YP39553 has been amended to incorporate the following changes:

- **The dose sequence in the single ascending dose (SAD) portion of the study has been modified based on availability of new safety, pharmacokinetic, and pharmacodynamic (PD) data in the ongoing entry-in-human (EIH) study NP39305 (Sections 3.1.1, 3.1.1.1, 3.1.2, 3.2.1, 4.4.1.1, and 4.4.2.1).**

  The newly available data in NP39305 indicated very good safety and tolerability with single RO7020531 doses up to 170 mg and PD effects documented only from doses $\geq$ 100 mg. The starting dose in this study was modified from 10 mg to 40 mg. This is expected to avoid very low doses/exposures that may not produce PD effects, while ensuring appropriate safety margins expected with the first dose. A planned dose escalation sequence of the SAD cohorts is anticipated to be 40 mg, 100 mg, and 140 mg and 170 mg. This sequence is based on the global EIH study results and ranges from one inactive dose through safe doses found to have PD activity.

- **The protocol text and reference to the Investigator's Brochure have been updated to incorporate new clinical safety, pharmacokinetic, and pharmacodynamic information from the ongoing study NP39305 (Sections 1.2.1, 1.2.2, and 10).**

- **Pharmacodynamic outcome measures (e.g., ISG15, OAS-1, MX1 and TLR7) have been added to evaluate markers of transcriptional responses to improve dose selection for the MAD part of the study (Section 1.3.1, 3.1.1.2, 3.2.4, 3.3.3, and 4.6.1.7, and 6.7 and Appendices 1-4).**

  The determination of TLR7 response by measuring various markers including ISG15, OAS-1, MX1 and TLR7 at the transcriptional level (mRNA determinations) has been shown to be a sensitive method to demonstrate TLR7- dependent response in the NP39305 study. In that study, higher level of response was noted for changes in the transcriptional markers than for the cytokines or neopterin at the 100 mg dose. This enabled clear determination of suitable doses for evaluation in the MAD portion of that study and it is anticipated will better enable dose selection in YP39553.

  The PD activity in the SAD part, including changes in the expression of the interferon response genes, will be used to help choose the starting dose in the MAD part of the study.

Additional minor changes have been made to correct typographical errors and to improve clarity and consistency. Substantial new information appears in italics. This amendment represents cumulative changes to the original protocol.

PROTOCOL AMENDMENT, VERSION 2: SUMMARY OF CHANGES

PROTOCOL SYNOPSIS

The protocol synopsis has been updated to reflect the changes to the protocol, where applicable.

1.2.1 Previous Non-Clinical Studies

[…]

No genotoxic potential was observed for RO7020531 in the Ames test, nor in the in vitro and in vivo micronucleus test. No evidence of teratogenicity or embryo-fetal toxicity was observed in definitive (GLP) embryo-fetal toxicity studies in rats (up to 150 mg/kg/day) or rabbits (up to 100 mg/kg/day), other than increased incidences of non-adverse fetal skeletal variations. In a definitive (GLP) fertility study in rats, no adverse effects of RO7011785 on mating performance, fertility, or early embryonic development were noted (up to 300 mg/kg, every other day [QOD]), despite slightly reduced body weight gain. See the RO7020531 *Investigator's Brochure* ~~IB and RO7020531 IB Addendum~~ for details on the properties of RO7020531 and RO7011785.

1.2.2 Previous Clinical Studies

A global entry-into-human (EIH) study (NP39305) is ongoing, where RO7020531 is being evaluated in healthy volunteers in single ascending doses (SAD) and multiple ascending doses (MAD) and subsequently will be evaluated in virologically suppressed CHB patients. ~~The first two cohorts (3 and 10 mg) have been completed and these doses were considered safe and well tolerated. The dose escalation is ongoing.~~

As of 1 October, 2017, 80 healthy volunteers (HVs; 64 males and 16 females) have been dosed with RO7020531/placebo in eight SAD cohorts. All dose levels (3 mg, 10 mg, 20 mg, 40 mg, 60 mg, 100 mg, 140 mg and 170 mg) were considered safe and well tolerated. There had been few adverse events (AEs) per cohort, all were reported as of mild intensity, and only three subjects (two in the second cohort [10mg] and one in the 170 mg cohort) had AEs that were considered "related" to study drug. No serious adverse events (SAEs) or discontinuations due to AEs had been reported; and there were no dose related trends in nature, incidence or severity of AEs. In addition, there have been no clinically significant changes (or trends) in ECG parameters, vital signs, or laboratory safety test results in any subjects.

The main active metabolite, RO7011785, exposure ($AUC_{0\text{-}inf}$) values increased proportionally with dose and was consistent with values predicted for humans from preclinical pharmacokinetics (PK) models. PD biomarkers did not have significant changes after single doses up to 60 mg in SAD Cohorts 1-5. Three out of eight subjects

in SAD Cohort 6 who received a single RO7020531 dose of 100 mg exhibited changes in interferon alpha indicative of TLR7 activation. Two of these subjects exhibited increased activity for IP10 and neopterin. The fraction of subjects responding as well as the amplitude of the response increased at the 140 mg dose. Changes in mRNA levels for ISG15, OAS-1, MX1 and TLR7 initially found at the 100 mg dose were amplified at the 140 mg dose.

1.3.1 Study Rationale

[…]

Evidence for TLR activation in the SAD and MAD studies will be provided by measuring post-dose changes in ~~select~~*selected* biomarkers including *the protein and metabolite markers* IFN-α, IP-10, TNF-α, IL-6, IL-10, IL-12p40 and neopterin~~,~~ *and markers of transcriptional responses (ISG15, OAS-1, MX1 and TLR7),* known to be stimulated by activation of TLR7 and/or TLR8.

3.1.1 Overview of Study Design

For both SAD and MAD parts of the study, each cohort will enroll ten subjects, with eight subjects randomly assigned to RO7020531 and two subjects randomly assigned to placebo (Table 1). Each cohort will include a minimum of two females, with at least one female receiving active drug. All single and multiple doses will be administered in the fasted state.

Table 1 Study Cohorts

Cohort	Stage	Dose (mg)[a]	Total Doses Administered	Route	Planned Number of Healthy Volunteers	
					Active Drug	Placebo
1	SAD	~~10~~*40*	1	PO	8	2
2	SAD	~~30~~*100*	1	PO	8	2
3	SAD	~~90~~*140*	1	PO	8	2
4	SAD	~~TBD~~[b]*170*[b]	1	PO	8	2
1	MAD	TBD	7 QOD	PO	8	2
2	MAD	TBD	7 QOD	PO	8	2

SAD = single ascending doses; MAD = multiple ascending doses; TBD = to be determined; QOD= every other day.

a. Proposed doses, which will be refined based on PK, safety and tolerability found in the global EIH study NP39305 *and/or in the previous cohorts in the current study YP39553*;

b. Optional cohort.

Additional cohorts may be explored in the SAD and MAD part as required.

3.1.1.1 Single Ascending Dose Study

For dose escalation, subjects will be sequentially enrolled into approximately four to six dose cohorts that may range between ~~10~~*40* mg and ~~180~~*170* mg, the maximum dose proposed in the global EIH study, NP39305. *Based on the newly available data in NP39305 indicating very good safety and tolerability with single RO7020531 doses up to 170 mg and PD effects documented only with doses ≥ 100 mg, the starting dose in this study was selected to be 40 mg, which is expected to avoid very low doses/exposures that may not produce pharmacodynamic effects, while ensuring appropriate safety margins expected with the first dose (See Section 3.2.1).*

The anticipated ascending dose scheme in the ~~first three~~ *SAD* cohorts is ~~10~~*40* mg, ~~30~~*100* mg *140 mg* and ~~90~~*170* mg. Additional cohorts may be added within a dose range between 10 mg and ~~180~~*170* mg (Figure 1). As an additional safety precaution in this study, the subjects participating in each single dose cohort will be dosed according to a sentinel dosing design. Initially, two subjects will be dosed: one subject with RO7020531 and one subject with placebo. If the safety and tolerability results from the first 24 hours following dosing for the initial subjects are acceptable to the Investigator, the remaining subjects of each cohort may be dosed soon after.

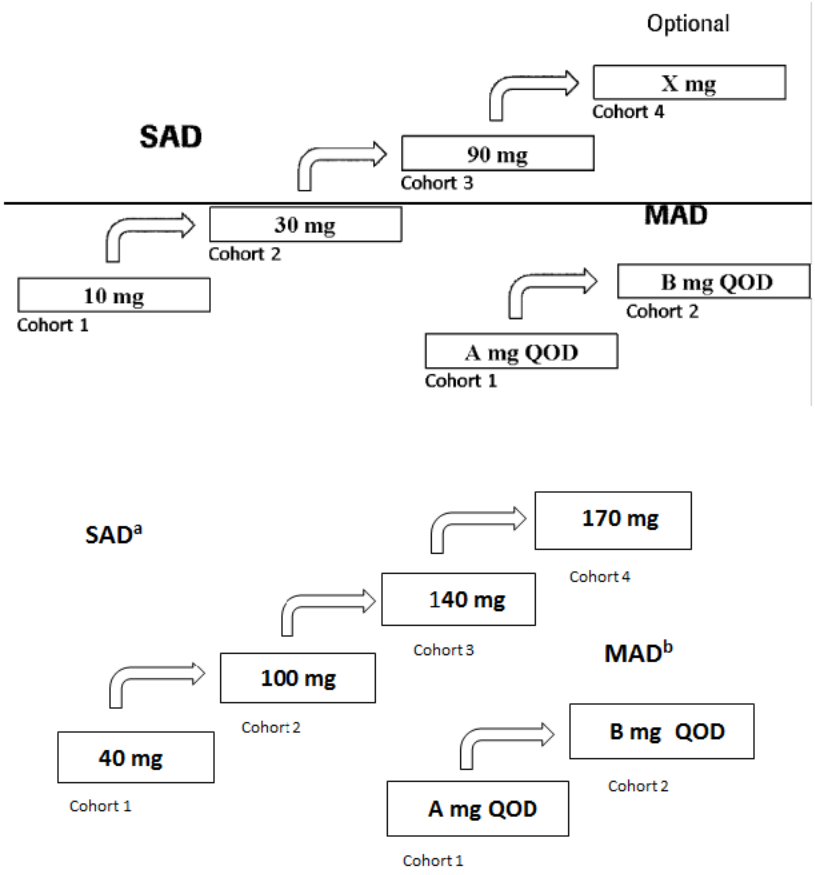
[…]

3.1.1.2 Multiple Ascending Dose Study

The trigger to start the first cohort in the MAD includes documentation of adequate safety and tolerability in the SAD cohorts and evidence of PD effects. For a given dose evaluated in the SAD, evidence in at least two subjects exhibiting responses above placebo-defined baseline for select PD biomarkers, including *the protein and metabolite markers* IFN-α, IP-10, TNF-α, IL-6, IL-10, IL-12p40 ~~or~~*and* neopterin~~,~~ *and markers of transcriptional responses (ISG15, OAS-1, MX1 and TLR7),* should be demonstrated. If the next dose in the SAD has been considered safe, then the previous dose demonstrating TLR-associated PD activity will be considered as the starting dose for the MAD. In addition, the starting dose/exposure in the MAD part of this study should have been considered safe in the global EIH study (NP39305). Only doses covered by the dose range evaluated in the SAD part will be used in the MAD, and two to three dose levels will be evaluated (Figure 1).

[…]

Figure 1Study Design



a. Doses shown in this figure may be modified based on emerging data. ~~Approximately four~~*Four* to six cohorts ~~may~~*will* be evaluated in the SAD part *of the study with a dose range between 40 mg and* ~~two~~*170 mg.*

b. Two to three cohorts will be evaluated in the MAD part of the study. *Doses in the MAD part will not exceed the dose considered to be safe and well-tolerated in the SAD part.*

3.1.2 Dose Escalation Decision Criteria

A Safety Review Meeting will be conducted by the Principal Investigator and the Sponsor Clinical Team prior to each dose escalation.

The decision to escalate to the next dose level in the SAD cohorts will be based primarily on the available safety and tolerability information through Day 4 (including adverse events [AEs], electrocardiograms [ECGs], vital signs, clinical laboratory test results) and, secondarily, on available PK and PD data through 24 hours post-dose at the previous dosage level. In addition, all available safety and PK data from the previous dose level(s) will be reviewed. The anticipated dose escalation sequence for the SAD is ~~10~~*40* mg, ~~30~~*100 mg, 140 mg* and ~~90 mg, with a maximum dose of 180~~*170* mg ~~if considered necessary.~~*(See Table 1).*

[…]

3.2.1 Rationale for Dosage Selection and Dosage Regimen

The dose selection for this study is supported by the non-clinical safety data and may be modified based on the clinical data collected from the global EIH study, NP39305. The current study in Chinese healthy volunteers (YP39553) is planned to consist of four to six dose levels in SAD and two to three dose levels in MAD, which will be within the dose-range considered to be safe and with acceptable tolerability in the global EIH study, NP39305.

Based on the non-clinical data with RO7020531 (see Section 1.2.1), the half-life of the active TLR7 agonist RO7011785 is relatively short. However, previous experience in monkeys and humans with another TLR7 agonist (RO6864018) indicate that a short exposure of the TLR7 agonist initiates a series of pharmacodynamic events with biomarker activity that peaks as late as 48 hours post dose. Similar results are anticipated following dosing with RO7020531 and are supported by activity measured in non-clinical models.

In the ongoing global EIH study (NP39305), all dose levels tested in SAD (3 mg – 170 mg) have been observed to be safe and well tolerated. In SAD Cohort 6 (100 mg), 3/8 healthy volunteers demonstrated increases in interferon alpha and which met the predefined criteria for TLR7 marker activity.

The starting dose of ~~10~~ *40* mg was selected for the SAD portion of this study in Chinese patients based on the safety and tolerability documented in the study NP39305, avoiding very low doses/exposures that may not produce pharmacodynamic effects, while ensuring appropriate safety margins expected with the first dose.

[…]

3.2.4 Rationale for Pharmacodynamic Assessments

TLR7 is expressed on human pDC and B-cells, and its activation induces both humoral and cellular changes (Iwasaki and Medzhitov 2004, Lester and Li 2014). These changes include the production of cytokines and chemokines such as IFN-α, IP-10, TNF-α, IL-6, IL-10, IL-12p40 *and changes in the expression of ISGs, e.g., ISG15, OAS-1 and myxovirus resistance 1 gene (MX1) and of the TLR7 gene itself (Fidock et al 2011),* as well as changes in markers of immune stimulation such as neopterin.

To capture the immunomodulatory effects of TLR7 agonism, cytokines*,* ~~and~~ chemokines *and peripheral blood gene expression* will be measured at time-points outlined in the SoA (Appendix 1, Appendix 2, Appendix 3 and Appendix 4). These markers showed a dose-dependent PD profile with other TLR7 agonists when assessed in patients and

healthy volunteers. The PD *activity* ~~signature~~ in the SAD part of the study will be used to help choose the starting dose in the MAD part of the study.

In previous healthy volunteer clinical studies, IFN-α, neopterin, IL10 and IP-10 were activated with another TLR7-selective agonist, RO6864018 and no activation of the TLR8 markers, IL-6 and TNF-α was seen. Thus, in the current study, any activation of IL-6 and TNF-α may be indicative of activation of TLR8.

3.3.3 Pharmacodynamic Outcome Measures

The PD outcome measures for this study are as follows:

- Blood samples will be collected to evaluate a number of PD outcome measures including the protein and metabolite markers of humoral response~~:~~(~~:~~IFN-α, IP-10, TNF-α, IL-6, IL-10, IL-12p40 and neopterin*) and markers of transcriptional responses (ISG15, OAS-1, MX1 and TLR7)*.

4.4.1 Formulation, Packaging, and Handling

4.4.1.1 RO7020531 and Placebo

RO7020531 and placebo will be supplied by Roche.

Investigational Medicinal Product (IMP): Hard gelatin capsule for oral administration containing ~~1 mg,~~ 10 mg, or 100 mg of RO7020531 drug substance.

[…]

4.4.2 Dosage, Administration and Compliance

4.4.2.1 RO7020531 and Placebo

Both RO7020531 and placebo will be administered orally in capsules.

In the SAD cohorts, RO7020531 or matching-placebo will be administered orally to the subjects by investigational staff on the morning of Day 1 after an overnight fast of at least 10 hours. Subjects in the SAD cohorts will not eat for 4 hours after the dose is administered. Water will be allowed ad libitum until one hour prior to dosing and after one hour post-dosing. Approximately 4 hours after dosing, subjects will be given lunch. The SAD part of the study will include an adaptive number of cohorts (approximately four). Each dose cohort will include 10 subjects (eight active and two placebo). ~~A~~*The* planned dose-escalation sequence for SAD is ~~10 mg, 30 mg and 90 mg. The actual dose~~*shown in* ~~Cohort 4 will be determined based on data collected in the global EIH study and/or from the previous cohorts in the current study, but will be maximally 180 mg.~~*Table 1.*

~~The starting dose~~*Doses of* ~~10 mg~~ *RO7020531* ~~will be administered as one 10 mg capsule. All other doses~~ in SAD and MAD cohorts will be administered as a combination of ~~1 mg,~~ 10 mg or 100 mg capsules with the requisite number of capsules being administered per specific dose cohort. Should an intermediate dose be required due to a change in the anticipated dose-escalation, the dose will be composed of the appropriate combination of ~~1 mg,~~ 10 mg and 100 mg capsules.

In the MAD cohorts, RO7020531 or matching-placebo will be administered orally to the subjects by investigational staff QOD from Day 1 through to Day 13. In total, seven doses will be given (for time-points see Appendix 3 and Appendix 4). Each dose will be given in a fasted state (after an overnight fast of at least 10 hours). Subjects will not eat for 4 hours after each dose is administered. Water will be allowed ad libitum until one hour prior to dosing and after one hour post-dosing. The MAD part of the study will include an adaptive number of cohorts (two to three). Each dose cohort will include ten subjects (eight active and two placebo). Dose levels for MAD will be defined during the study conduct based on emerging data~~.~~ *(See Table 1).*

Doses will be given orally with 240 mL of water. Additional amounts of water up to 100 mL could be given to assist dose administration only if needed.

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 subject 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 subject during the study.

Guidelines for dosage modification and treatment interruption or discontinuation are provided in Section 4.7.

4.5.3 Dietary and Special Requirements

There are data suggesting that concentrated green tea (including bottled green tea beverages) may inhibit aldehyde oxidase (Tayama et al 2011). Therefore, subjects should minimize the amount of bottled green tea beverages and other green tea preparations they drink from Day −7 until the follow-up visit at Day 8.

Subjects must fast overnight (at least 10 hours) before the dosing and must not eat for 4 hours after the dose is administered. Approximately 4 hours after dosing, subjects will be administered lunch. ~~Meals will be similar in composition and time of administration across all SAD/MAD cohorts.~~

[…]

4.6.1.7 Pharmacodynamic Assessments

Blood samples will be collected to evaluate a number of PD parameters including the protein and metabolite markers of humoral response~~:~~(~~:~~ IFN-α, IP-10, TNF-α, IL-6, IL-10, IL-12p40, and neopterin~~.~~)~~.~~ *and markers of transcriptional responses (ISG15, OAS-1, MX1 and TLR7)*.

6.1 Determination of Sample Size

~~Approximately four~~*Four* to six cohorts may be evaluated in the SAD part of the study (approximately 40-60 subjects in total), and ~~approximately~~*two to* three cohorts are anticipated for the MAD part of the study with approximately *20-*30 subjects in total.

Ten healthy volunteers are planned to be enrolled at each dose level. They will be randomized to either active treatment (eight healthy volunteers per dose level) or placebo (two healthy volunteers per dose level). With eight healthy volunteers per dose group or cohorts, there is a 90% chance to observe at least one AE that has an incidence rate of 25% in the population.

6.7 Pharmacodynamic Analyses

Summary descriptive statistics will be presented for the induction of cytokines, chemokines, and neopterin *and of interferon-response genes* separately by treatment arm. Exploratory analysis will be performed to assess the TLR7 agonist induced response under different dosing conditions. Graphical and statistical techniques including linear, non-linear, and logistic regression will be used to explore potential relationships between dosing regimen, PK and PD.

10. References

[…]

Fattovich G, Giustina G, Sanchez-Tapias J. Delayed clearance of serum HBsAg in compensated cirrhosis B: relation to interferon alpha therapy and disease prognosis. European Concerted Action on Viral Hepatitis (EUROHEP). Am J Gastroenterol. 1998;93:896-900.

Fidock MD, Souberbielle BE, Laxton C. The innate immune response, clinical outcomes, and ex vivo HCV antiviral efficacy of a TLR7 agonist (PF-4878691). Clin Pharmacol Ther. 2011;89:821-829.

Funk E, Kottilil S, Gilliam B. Tickling the TLR7 to cure viral hepatitis. J Transl Med. 2014;12:129.

Gane EJ, Lim YS, Gordon SC, et al. The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection. J of Hepatology. 2015; 63: 320–328.

Grippo J, Folitar I, van de Wetering de Rooij J, et al. Optimization of a Phase 2 Study Design in Chronic Hepatitis B (CHB) Patients Based on Safety, Pharmacokinetics (PK) and Pharmacodynamics (PD) from Phase 1 Studies Using Two Prodrugs of a Toll-like Receptor 7 (TLR7) Agonist. 2016; AASLD Abstract nr. 1869.

Horscroft NJ, Pryde DC, Bright H. Antiviral applications of toll-like receptor agonists. J Antimicrob Chemother. 2012;67:789-801.

Investigator's Brochure, RO7020531. ~~First version, September 2016.~~

~~Investigator's Brochure, RO7020531. Addendum 1, March 2017 (First version, September 2016).~~

[…]

Appendices 1 – 4

Appendices and footnotes have been updated to reflect changes to the protocol.

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PROTOCOL SYNOPSIS

TITLE:	**A RANDOMIZED, SPONSOR-OPEN, INVESTIGATOR-BLINDED, SUBJECT-BLINDED, PLACEBO-CONTROLLED, SINGLE AND MULTIPLE ASCENDING DOSE STUDY TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF RO7020531 AND METABOLITES FOLLOWING ORAL ADMINISTRATION TO CHINESE HEALTHY VOLUNTEERS**
PROTOCOL NUMBER:	YP39553
VERSION:	2
TEST PRODUCT:	RO7020531
PHASE:	I
SPONSOR:	F. Hoffmann-La Roche Ltd

OBJECTIVES

Primary Objectives

The primary objective of this study is:

- To assess the safety and tolerability of single and multiple ascending doses of oral RO7020531 in Chinese healthy subjects.

Secondary Objectives

The secondary objectives for this study are as follows:

- To investigate the plasma pharmacokinetics (PK) of RO7020531 and the main active metabolite, RO7011785, following single and multiple ascending doses of RO7020531 in Chinese healthy subjects. Additional metabolites may be measured, including RO7018822 and RO7033805.
- To investigate the urine PK of RO7020531 (if detectable) and the main active metabolite, RO7011785, in urine samples of Chinese healthy subjects after single ascending oral doses of RO7020531. Additional metabolites may be measured in urine, including RO7018822 and RO7033805.
- To investigate the effect of RO7020531 on pharmacodynamic (PD) parameters following single or multiple oral doses administered every other day (QOD) in Chinese healthy subjects.

STUDY DESIGN

Description of Study

This study will be a randomized, Sponsor-open, Investigator/subject-blinded, placebo controlled, single ascending dose (SAD) and multiple ascending dose (MAD) study to evaluate the safety, tolerability, PK and PD of RO7020531 and metabolites following oral administration to Chinese healthy subjects. PK, PD, safety and tolerability data collected in the SAD part of this study will be used to determine doses at which to initiate the MAD part of the study. The subjects participating in each single dose cohort will be dosed according to a sentinel dosing design. Initially, two subjects will be dosed: one subject with RO7020531 and one subject with placebo. If the safety and tolerability results from the first 24 hours following dosing for the initial subjects are acceptable to the Investigator, the remaining subjects of each cohort will be dosed.

NUMBER OF HEALTHY VOLUNTEERS

SAD

Approximately 40-60 subjects with an adaptive number of cohorts. There will be eight active and two placebo subjects *planned for* each cohort.

MAD

Approximately *20*-30 subjects with an adaptive number of cohorts. There will be eight active and two placebo subjects *planned for* each cohort.

TARGET POPULATION

Healthy male and female subjects between 18 to 60 years of age, inclusive.

INCLUSION/EXCLUSION CRITERIA

Inclusion criteria:

Healthy subjects must meet the following criteria for study entry:

1. Chinese healthy male and female subjects. 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, and urinalysis.
2. 18 to 60 years of age, inclusive.
3. A Body Mass Index (BMI) of 19 to less than 28 kg/m^2 and a body weight of at least 45 kg.
4. Informed of, and 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.

5. For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use two approved contraceptive methods, of which one must be a barrier method and the other should be an established non-barrier form of contraception with a failure rate of < 1% per year, during the treatment period and for at least one month after the last dose of study drug.
    - A woman is considered to be of childbearing potential if she 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 contraceptive methods with a failure rate of < 1% per year include bilateral tubal occlusion, male sterilization, established proper use of hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine device (IUDs), and copper IUDs.

    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.
6. For men: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive measures, and agreement to refrain from donating sperm, as defined below:
    - With female partners of childbearing potential or pregnant female partners, men must remain abstinent or be willing to use two methods of contraception with their partners, one of which must be a condom and the other should be an established form of contraception, during the treatment period and for at least one month after the last dose of study drug to avoid exposing the embryo. Other acceptable forms of contraception include vasectomy, bilateral tubal occlusion, IUD or proper use of hormonal contraceptives (e.g. contraceptive pills). 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 and withdrawal are not acceptable methods of contraception.
7. Negative pregnancy test on Day − 1 for female subjects.
8. Non-smokers, or use of < 10 cigarettes (or equivalent nicotine-containing product) per day.
9. Negative anti-nuclear antibody (ANA) test; or positive with dilutions not greater than 1:40 and with no associated history or symptoms of potential connective tissue disease or other immune mediated diseases.

Exclusion criteria:

Healthy subjects who meet any of the following criteria will be excluded from study entry:

1. Pregnant (positive pregnancy test) or lactating women, and male partners of women who are pregnant or lactating.
2. History of immunologically mediated disease (e.g., inflammatory bowel disease, idiopathic thrombocytopenic purpura, lupus erythematosus, autoimmune hemolytic anemia, scleroderma, severe psoriasis, rheumatoid arthritis, multiple sclerosis, or any other autoimmune disease).
3. History or symptoms of any clinically significant disease including (but not limited to), neurological, cardiovascular, endocrine, respiratory, hepatic, ocular, or renal disorder (as per Investigator's judgment).
4. Personal or family history of congenital long QT syndrome or sudden cardiac death.
5. Evidence of an active or suspected cancer or a history of malignancy, where in the

Investigator's opinion, there is a risk of recurrence.

6. History of having received or currently receiving any systemic anti-neoplastic (including radiation) or immune-modulatory treatment (including systemic oral or inhaled corticosteroids, IFN or PEG-IFN) within 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. Eye drop-containing and infrequent inhaled corticosteroids are permissible up to 4 weeks prior to the first dose of study drug.
7. History of clinically significant thyroid disease; also, subjects with clinically significant elevated thyroid stimulating hormone (TSH) concentrations at Screening.
8. Any confirmed clinically significant allergic reactions (anaphylaxis) against any drug, or multiple drug allergies (non-active hay fever is acceptable).
9. History of clinically significant psychiatric disease, especially major depression (significant psychiatric disease is defined as treatment with an antidepressant medication or a major tranquilizer at therapeutic doses for major depression or psychosis, respectively, or any history of the following: a suicide attempt, hospitalization for psychiatric disease, or a period of disability due to a psychiatric disease).
10. Clinically significant acute infection, e.g., influenza, local infection or any other clinically significant illness within two weeks of randomization.
11. History of clinically significant gastrointestinal (GI) disease including inflammatory bowel disease, peptic ulcer disease, GI hemorrhage.
12. Confirmed systolic blood pressure (BP) greater than 140 or less than 90 mmHg, and diastolic BP greater than 90 or less than 50 mmHg at Screening (based on the average of three separate resting BP measurements, properly measured with well-maintained equipment, after at least 5 minutes rest).
13. Clinically relevant electrocardiogram (ECG) abnormalities on Screening ECG: e.g.,
    - QTc interval (QTcF $>$ 450 msec or $<$ 300 msec)
    - Notable resting bradycardia (heart rate [HR] $<$ 45 bpm), or HR $>$ 90 bpm
    - Difference between highest and lowest of any Screening QTc $>$ 30 msec
    - ECGs with documented machine errors in the interval duration assessments
    - 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).
    - Evidence of atrial fibrillation, atrial flutter, complete bundle branch block, Wolff-Parkinson-White Syndrome, or cardiac pacemaker.
14. Any of the following laboratory parameters prior to dosing:
    - White blood cells (WBC) $<$ 3000 cells/mm^3
    - Neutrophil count $<$ 1500 cells/mm^3
    - Platelet count $<$ 140,000 cells/mm^3
    - Activated partial thromboplastin time (aPTT) $>$ 40 seconds, international normalized ratio (INR) $>$ 1.2
    - Hemoglobin (Hb) $<$ 12 g/dL in females or 13 g/dL in males
15. Abnormal renal function including serum creatinine $>$ upper limit of normal (ULN) or calculated CrCl $<$ 70 mL/min (using the Cockcroft Gault formula).
16. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) values at screening above ULN and judged clinically significant by the Investigator.
17. Positive results for anti-mitochondrial antibody (AMA), anti-smooth muscle antibody (ASMA) or thyroid peroxidase antibody.
18. Positive hepatitis A IgM antibody (HAV Ab IgM), hepatitis B surface antigen (HBsAg),

hepatitis C antibody (HCV Ab), or positive for human immunodeficiency virus (HIV) at screening.

19. 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 or judged to be clinically irrelevant for healthy subject*s*
20. History of alcohol abuse (consumption of more than two standard drinks per day on average; one standard drink = 10 grams of alcohol) and/or drug abuse within one year of randomization. Alcohol consumption will be prohibited at least 48 hours before screening, 48 hours before admission until discharge from the clinic, and 48 hours before each scheduled visit.
21. Positive test for drugs of abuse or positive alcohol test at screening or Day – 1.
22. Any clinically significant concomitant disease or condition that could interfere with, or for which the treatment of, 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.
23. Use of any medication (prescription or over the counter [OTC], including health supplements, vitamins or herbal remedies) within two weeks prior to the first dosing or within five half-lives of the medication prior to first dosing (whichever is longer). Exceptions may be made on a case-by-case basis following discussion and agreement between the Investigator and the Sponsor.
24. Participation in an investigational drug or device study within 90 days prior to randomization.
25. Donation or loss of blood over 500 mL, or administration of any blood product, within 90 days prior to starting study medication.
26. Subjects under judicial supervision, guardianship or curatorship.
27. Any medical or social condition which may interfere with the subject's ability to comply with the study visit schedule or the study assessments.

LENGTH OF STUDY

SAD: Up to 9 weeks (from screening through study completion) for each randomized subject as follows:

- Screening: Up to 28 days;
- Dosing period: 1 day;
- Follow up: 28 days after dosing.

MAD: Up to 10 weeks (from Screening through study completion) for each randomized subject as follows:

- Screening: Up to 28 days;
- Dosing period: 14 days;
- Follow-up: 28 days after dosing.

END OF STUDY

The end of the study is defined as the date when the last subject last observation (LSLO) occurs. LSLO is expected to occur 6 weeks after the last subject is randomized.

OUTCOME MEASURES

SAFETY OUTCOME MEASURES

The safety outcome measures for this study are as follows:

- Incidence and severity of adverse events (AE).
- Incidence of laboratory abnormalities based on hematology, clinical chemistry, coagulation and urinalysis test results.
- Incidence of vital signs or ECG abnormalities.

PHARMACOKINETIC OUTCOME MEASURES

The PK evaluations for this study are as follows:

- Summary descriptive statistics of plasma PK parameters for RO7020531 and the main active metabolite, RO7011785, and additional metabolites, including RO7018822 and RO7033805 will be computed. These parameters include C_{max}, T_{max}, AUC_{inf}, AUC_{last}, and $t_{1/2}$ and will be presented by dose cohorts including mean, standard deviation (SD), co-efficient of variation (CV), medians and ranges.
- Where available, descriptive statistics of urine PK parameters (total amount excreted, fraction excreted of total administered dose, renal clearance), if available, for RO7020531, RO7011785 and other metabolites will be presented.

PHARMACODYNAMIC OUTCOME MEASURES

The PD outcome measures for this study are as follows:

Blood samples will be collected to evaluate a number of PD outcome measures including the protein and metabolite markers of humoral response *(*IFN-α, IP-10, TNF-α, IL-6, IL-10, IL-12p40 and neopterin*) and markers of transcriptional responses (ISG15, OAS-1, MX1 and TLR7).*

CLINICAL GENOTYPING OUTCOME MEASURES

The clinical genotyping outcome measures for this study are as follows:

- *Correlation of single nucleotide polymorphisms (SNP) status with the observed exposure.*
- *Correlation of SNP status with the observed cytokine/chemokine levels.*

BIOMARKER/GENOTYPING SAMPLE COLLECTION

Clinical Genotyping (CG) Samples

On Day 1 (or any time during the conduct of the clinical study), a mandatory whole blood sample will be taken for DNA extraction from every subject. The DNA will be used to determine if alleles of drug metabolizing enzymes (such as aldehyde oxidases) and *TLR7* affect the PK/PD of RO7020531. Data arising from this study will be subject to the same confidentiality as the rest of the study.

INVESTIGATIONAL MEDICINAL PRODUCT(S)

Test Product: RO7020531 will be supplied by Roche as hard gelatin capsules containing 10 mg, or 100 mg of RO7020531 drug substance.

Placebo: Placebo will be supplied by Roche as hard gelatin capsules identical in size and appearance to the corresponding active capsules, containing microcrystalline cellulose of compendial grade but no active substance.

Both RO7020531 and placebo will be administered orally in capsules. Doses will be given with 240 mL of water. Additional amounts of water up to 100 mL could be given to assist dose administration, if needed.

Doses of RO7020531 in SAD and MAD cohorts will be administered as a combination of 10 mg or 100 mg capsules with the requisite number of capsules being administered per specific dose cohort.

SAD

In the SAD cohorts, RO7020531 or matching placebo will be administered orally to the subjects by investigational staff on the morning of Day 1 after an overnight fast of at least 10 hours. Subjects in the SAD cohorts will not eat for 4 hours after the dose is administered. Water will be allowed ad libitum until one hour prior to dosing and after one hour post dosing. Approximately 4 hours after dosing, subjects will be given lunch. A planned dose escalation sequence of the SAD *cohorts* is anticipated to be *40* mg, *100* mg*, 140* mg *and 170mg. Additional cohorts may*

be added within a dose range between 10 mg and 170 mg.

MAD

In the MAD cohorts, RO7020531 or matching placebo will be administered orally to the subjects by investigational staff QOD from Day 1 through to Day 13. In total, seven doses will be given. Each dose will be given in a fasted state (after an overnight fast of at least 10 hours). Subjects will not eat for 4 hours after each dose is administered. Water will be allowed ad libitum until one hour prior to dosing and after one hour post-dosing. Dose levels for MAD will be defined during the study conduct based on the emerging study data.

PROCEDURES

Detailed Schedule of Assessments and procedures are tabulated in the appendices of the protocol.

BLINDING OR UNBLINDING:

This study is observer-blinded and Sponsor open.

In exceptional cases, (e.g., if deemed important for dose decisions or for the more thorough evaluation of safety-related concerns that may impact dosing of future subjects on this or other currently conducted or shortly to start studies involving administration of RO7020531) and in the interest of the subjects' safety, the Investigator may be unblinded after approval by the Clinical Pharmacologist.

STATISTICAL METHODS

SAFETY ANALYSES

All healthy volunteers who have received at least one dose of the study medication, whether prematurely withdrawn from the study or not, will be included in the safety analysis. The safety data, including AEs, reasons for withdrawal from study, laboratory data, ECG, concomitant medications, vital signs, and physical examination results will be listed and summarized descriptively.

As appropriate, listings, summary tables and graphs (subject plot and/or mean plots) will be provided for safety and tolerability assessments.

Adverse events will be listed and summarized by body system and preferred term.

For laboratory data, subject listings will be presented with abnormalities flagged.

PHARMACOKINETIC ANALYSES

Non-compartmental analysis using WinNonlin software will be used to calculate PK parameters where appropriate. Summary descriptive statistics of plasma PK parameters including C_{max}, T_{max}, AUC_{inf}, AUC_{last} and $t_{1/2}$ for RO7020531 and RO7011785 and additional metabolites, including RO7018822 and RO7033805, will be presented by treatment arm including means, SD, CV, geometric means, medians and ranges. Where appropriate, data may be pooled and analyzed. Listings, summary tables and graphs (individual plots and/or mean plots) by treatment group will be provided. Descriptive statistics of urine PK parameters including total amount excreted, fraction excreted of total administered dose; renal clearance for RO7020531 and RO7011875 and additional metabolites including RO7018822 and RO7033805 will be presented, where available.

Additional PK analyses, including population PK and/or PD analyses, will be conducted as appropriate.

PHARMACODYNAMIC ANALYSES

Summary descriptive statistics will be presented for the induction of cytokines, chemokines, and neopterin *and of interferon-response genes* separately by treatment arm. Exploratory analysis will be performed to assess the TLR7 agonist induced response under different dosing conditions. Graphical and statistical techniques including linear, non-linear, and logistic regression will be used to explore potential relationships between dosing regimen, PK and PD.

CLINICAL GENOTYPING ANALYSES:

Potential PD and/or PK differences will be studied by clinical genotyping, exploring whether genetic polymorphisms of drug metabolizing enzymes (such as aldehyde oxidases) and *TLR7* can be correlated to PK/PD profile of RO7020531.

SAMPLE SIZE JUSTIFICATION

Ten healthy volunteers are planned to be enrolled at each dose level. They will be randomized to either active treatment (eight healthy volunteers per dose level) or placebo (two healthy volunteers per dose level). The current planned study design and sample size complies with standard safety review rules applied in single and multiple ascending dose studies.

With eight healthy volunteers per dose group or cohorts, there is a 90% chance to observe at least one AE that has an incidence rate of 25% in the population.

LIST OF PROHIBITED MEDICATIONS

As a general rule, no concomitant medication (including health supplements, vitamins or herbal remedies) will be permitted, unless the rationale for use is discussed between the Investigator and Sponsor and is clearly documented (with the exception of medications to treat AEs [after discussion with Sponsor, unless the subject is at risk] and use of hormone replacement therapy can continue if initiated 2 months prior to study start. Use of acetaminophen/paracetamol is allowed up to a maximum dose of 2 g per day (with restricted use in relation to study drug administration).

Use of the following therapies is prohibited:

- Any prescribed or OTC medications (except for the cases given in Section 4.5.1), including health supplements, vitamins or herbal remedies within 5 half-lives or 2 weeks, whichever is longer, prior to the first dosing until the follow up visit.
- Any systemic anti-neoplastic (including radiation) or immune-modulatory treatment (including systemic oral or inhaled corticosteroids, IFN or PEG-IFN; see exclusion criteria). Eye drop-containing and infrequent inhaled corticosteroids are prohibited from 4 weeks prior to the first dose of study drug until the follow up visit.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
Ab	Antibody
AE	Adverse events
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMA	Anti-mitochondrial antibody
ANA	Anti-nuclear antibody
aPTT	Activated partial thromboplastin time
ASMA	Anti-smooth muscle antibody
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
AUC_{inf}	Area under the plasma concentration versus time curve extrapolated to infinity
AUC_{last}	Area under the plasma concentration versus time curve up to the last measurable concentration
BMI	Body mass index
BP	Blood pressure
cccDNA	Covalently closed circular DNA
CHB	Chronic hepatitis B
CL	Clearance
C_{max}	Maximum observed plasma concentration
CRO	Contract research organization
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EEA	European economic area
EIH	Entry into human
ESF	Eligibility Screening Form
EU	European Union
FSH	Follicle-stimulating hormone
GGT	Gamma glutamyl transpeptidase
GI	Gastrointestinal
GLP	Good Laboratory Practice
HAV	Hepatitis A virus
Hb	Hemoglobin

HbA1c	Glycated hemoglobin
HBeAg	Hepatitis B envelope antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCG	Human chorionic gonadotropin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Heart rate
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IFN	Interferon
IgM	Immunoglobulin M
IL	Interleukin
IMP	Investigational medicinal product
IND	Investigational New Drug
INR	International normalized ratio
IRB	Institutional Review Board
IUD	Intrauterine device
MAD	Multiple ascending dose
NOAEL	No-observed-adverse-effect level
NUC	Nucleoside/nucleotide analogue
OATP	Organic anion transporting polypeptide
PD	Pharmacodynamic
pDC	Plasmacytoid dendritic cell
PEG-IFN	Pegylated interferon
pH	Measure of acidity or alkalinity
PK	Pharmacokinetic
PQ	PQ interval
PR	PR interval
QOD	Every other day
QRS	QRS complex
QT	QT interval
QTc	Corrected QT interval
QTcF	Fridericia's correction of QT interval
QW	Once a week
SAD	Single ascending dose
SAE	Serious adverse event

SD	Standard deviation
SI	Système International d'Unités
SNP	Single nucleotide polymorphism
SoA	Schedule of assessments
$t_{1/2}$	Half-life
TLR	Toll-like receptor
T_{max}	Time to maximum observed plasma concentration
TNF	Tumor necrosis factor
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
V_{ss}	Volume of distribution at steady-state
WBC	White blood cell
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND ON DISEASE

Chronic hepatitis B (CHB) and its sequelae are major global healthcare problems. Despite the implementation of effective vaccination in many countries, hepatitis B is one of the most common infectious diseases in the world. It is estimated that more than 2 billion people or one third of the world's population have been infected with the hepatitis B virus (HBV) at some time in their lives, and an estimated 240 million are now chronically infected, with approximately one-third of those subjects in China (WHO 2002, WHO 2016). Nearly 25% of all chronic HBV carriers develop serious liver diseases such as chronic hepatitis, cirrhosis, and primary hepatocellular carcinoma. More than 686 000 people die every year due to the consequences of hepatitis B (WHO 2016).

The endemicity of HBV varies substantially by region, with East Asia and sub-Saharan Africa having prevalence rates of CHB above 8% (Ott et al 2012). In these highly endemic areas, the most common means of transmission is by perinatal infection, and up to 90% of the population has serological evidence of prior infection (Alter et al 2003). Although prevalence levels in developed countries are relatively low, immigration from highly endemic regions has had a significant influence on the local need for therapy, and even countries with low endemicity are currently experiencing the burden of CHB (Wasley et al 2010).

HBV belongs to the Hepadnaviridae family. It is a partly double-stranded DNA virus with approximately 3200 base pairs. The transcriptional template of HBV is the covalently closed circular DNA (cccDNA), which resides inside the hepatocyte nucleus as a minichromosome (Locarnini et al 2010). Several HBV subtypes have been identified. Most CHB patients are infected with the wild-type strain of HBV, which produces large amounts of the hepatitis B envelope antigen (HBeAg) resulting in the HBeAg-positive form of CHB. However, in a significant proportion of patients, variant forms of the virus predominate later in the course of the disease, which have diminished ability to produce HBeAg. Another serological marker, hepatitis B surface antigen (HBsAg), is a hallmark of the infection and remains persistently positive in CHB patients. There is a correlation between the presence of HBsAg and patients' outcome with HBsAg level being predictive of fibrosis severity, development of hepatocellular carcinoma and survival rates (Fattovich et al 1998, Tseng et al 2012, Martinot-Peignoux et al 2013).

HBV is not cytopathic: both liver damage and viral control are immunomediated (Trepo et al 2014). The clinical outcome of infection is dependent on the complex interplay between HBV replication and both the innate and adaptive immune responses. Patients with CHB have defects in innate and adaptive immune responses, characterized by exhaustion of antigen specific T cells, suboptimal antigen presentation, and insufficient antibody production (Bertoletti et al 2012). The dominant cause of the long-term viral persistence and pathogenesis of HBV liver disease is the development of an inefficient antiviral response to the viral antigens (Bertoletti et al 2012).

Currently available treatments for CHB include interferon (IFN), pegylated interferon (PEG-IFN), and nucleos(t)ide analogues (NUC): lamivudine, adefovir, entecavir, tenofovir and telbivudine (Papatheodoridis et al 2012; Sarin et al 2016; Terrault et al 2016). Although these therapies achieve long-term effects in lowering HBV DNA levels, chronic HBV infection cannot be completely eradicated with currently approved therapeutics due to the persistence of cccDNA in the nucleus of infected hepatocytes (Lucifora et al 2014). With these treatments, rates of HBsAg clearance and seroconversion, which are associated with reduced or reversed cirrhosis and prevention of HCC development, are low (<15% HBsAg seroconversion after 1 to 5 years follow-up) (Chang et al 2010, Marcellin et al 2013). In addition, the notable deficiencies of current HBV treatments include indefinite duration of NUCs and risk of viral resistance with some NUC treatments, while PEG-IFN therapy is poorly tolerated and a significant portion of patients do not have a virological response (Papatheodoridis et al 2008).

Due to the therapeutic limitations of the currently available agents for the management of HBV infection, there is a need for new treatments of CHB that can provide clinical cure (HBsAg loss) and sustained suppression of HBV replication (Wang and Chen 2014).

One promising strategy to stimulate the immune system in CHB is by the use of toll-like receptors (TLRs). TLRs are a family of pathogen-recognition receptors that activate the innate immune response. Stimulation of TLRs leads to the release of multiple cytokines, including type I and type II IFNs, to the induction of pathways and enzymes that destroy intracellular pathogens, and to the maturation of professional antigen-presenting cells, resulting in the activation of the adaptive immune response (Iwasaki and Medzhitov 2004). To date, 11 functional TLRs have been identified in humans. Most TLRs are located in the plasma membrane, except TLR3, TLR7, TLR8 and TLR9, which are intracellularly expressed, particularly in endosomes. TLR7 receptors are able to recognize viral components and induce IFN production and downstream responses (Lester and Li 2014).

A number of small molecule agonists for TLR7 have been identified (Horscroft et al 2012). The stimulation of TLR7 mediates an endogenous type I IFN response, which is critical in development of a broad, effective and protective immunity against hepatitis viruses (Horscroft et al 2012, Funk et al 2014). Compared to PEG-IFN therapy, treatment with a TLR7 agonist induces broader immuno-modulatory effects that are likely to lead to more effective control and functional cure of chronic HBV infection (Strader et al 2004, Isogawa et al 2005). TLR7 agonists induce the production of multiple isotypes of IFN from plasmacytoid dendritic cells (pDCs) which have been shown in vitro to possess additive or synergistic antiviral effects compared to exogenous PEG-IFN.

1.2 BACKGROUND ON RO7020531

RO7020531, an oral double prodrug of the TLR7-specific agonist, RO7011785, is being developed for the treatment of CHB patients. A prodrug approach was chosen for oral delivery of the TLR7 agonist RO7011785 in order to improve bioavailability and

potentially limit TLR7 activation in the gastrointestinal (GI) tract, which may be associated with GI intolerability. Non-clinical studies with RO7020531 suggest that it is rapidly converted to the active metabolite RO7011785. Data from in vivo studies with RO7020531 and in vitro studies with RO7011785 support immune activation as the mechanism of action. A clinical study (NP39305) of RO7020531 exploring safety, PK and PD effects is ongoing (see Section 1.2.2).

See the RO7020531 Investigator's Brochure (RO7020531 IB) for details on non-clinical studies.

1.2.1 Previous Non-Clinical Studies

RO7011785 showed in vitro potency to activate TLR7 and triggered downstream TLR7-mediated nuclear factor-kappa B (NF-κB) signaling in an engineered HEK293 reporter cell line expressing human TLR7. RO7011785 was shown to be a selective hTLR7 agonist with less potency to activate hTLR8 in vitro. Ex vivo stimulation of human peripheral blood mononuclear cells (PBMCs) from healthy donors by RO7011785 resulted in dose-dependent induction of various cytokines and chemokines, including IFN-α, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IFN gamma-inducible protein 10 (IP-10).

In an HBV mouse model, C57BL/6 mice were injected with recombinant adeno-associated virus harboring 1.3 copies of the HBV genome, and demonstrated high levels of HBV viral makers including HBV DNA, HBsAg, and HBeAg. Oral administration of RO7020531, once a week (QW) or every other day (QOD) for 6 weeks, proved to have in vivo anti-HBV activity in this mouse model, i.e., clear reduction in HBV DNA and HBsAg (by more than 1 log after the 42-day treatment period). With the same administration frequency, higher doses resulted in faster and greater reduction in HBV DNA and HBsAg. With the same dose of 100 mg/kg, QOD administration resulted in greater viral reduction on Day 42 than QW administration. In addition, RO7020531 induced interferon-stimulated gene expression in peripheral whole blood in this mouse model.

RO7020531 showed high oral bioavailability and rapid conversion to the active moiety, RO7011785. RO7011785 exhibited a relatively short half-life ($t_{1/2}$) of 1.51 hours in rats and 2.12 hours in monkeys, moderate systemic clearance (CL) of ~50% liver blood flow in rats, and CL of approximately 30% liver blood flow in monkeys. RO7011785 had a moderate volume of distribution (V_{ss}) in the range of body water or slightly higher for both rats and monkeys. Differences in metabolite exposure were noted between species, although RO7011785 was the predominant metabolite in both rats and monkeys, confirming good conversion to active drug.

Conversion of RO7020531 to the active form RO7011785 was observed in animal and human hepatocytes, with both the ester hydrolysis and aldehyde oxidase-mediated pathways active. The relative strength of the oxidase versus esterase pathways varied

between species and influenced the concentrations of the different intermediate prodrugs: RO7033805 was the predominant intermediate for cynomolgus monkey, whereas RO7018822 was the predominant intermediate for mouse, rat, and human.

RO7020531 was found to be a good substrate of multidrug resistance protein 1 (MDR1; P-glycoprotein), with a high apparent membrane permeability. Therefore, in vivo drug-drug interactions due to co-administration with MDR1 inhibitors cannot be excluded. However, interactions of strong MDR1 inhibitors with the absorption of RO7020531 are considered unlikely due to the high permeability, high solubility, and high oral bioavailability of RO7020531, which makes it unlikely that MDR1 plays a major role in RO7020531 trans-intestinal absorption in vivo.

RO7011785 can be directly glucuronidated by UDP-glucuronosyltransferase 1A1 (UGT1A1); thus, drug-drug interactions with UGT1A1 inhibitors cannot be excluded. Drug-drug interaction potential was low, as determined in vitro with cytochrome P450 (CYP) enzymes and with transporters (organic anion transporting polypeptide 1B1 [OATP1B1], OATP1B3, organic anion transporter 1 [OAT1], OAT3, and breast cancer resistance protein [BCRP]). Therefore, the interactions with CYPs and substrates for hepatic uptake transporters and kidney transporters (for example NUCs) in vivo are unlikely.

Repeat-dose toxicity studies with treatment duration of up to 13 weeks have been performed with RO7020531 in rats and monkeys. The effects observed were considered to be consistent with exaggerated pharmacology, i.e., TLR7 agonism leading to the intended immune/cytokine stimulation and subsequent tolerability or pro-inflammatory findings. QOD and QW dosing regimens were investigated in Good Laboratory Practice (GLP) toxicology studies. The no-observed-adverse-effect level (NOAEL) for the 13-week rat toxicology study was 3 mg/kg QOD (area under the concentration–time curve for males and females [AUC M/F] = 186/503 h • ng/mL) and 30 mg/kg QW (AUC M/F = 2950/4660 h • ng/mL). The NOAEL for the 13-week monkey toxicology study was 3 mg/kg QOD (AUC M/F = 858/706 h • ng/mL) and 10 mg/kg QW (AUC M/F = 3340/2960 h • ng/mL).

Neither the double prodrug RO7020531, nor its metabolites RO7011785 and RO7018822, significantly affected potassium channels in the in vitro human ether-à-go-go-related gene (hERG) assay. Dosing at ≥ 3 mg/kg in monkeys caused reversible heart rate (HR) increase (with decreased RR interval and shortened QT and/or heart-rate corrected QT [QTc] interval), non-adverse increase in PR interval and increase in body temperature. There were no RO7020531-related effects on respiratory or central nervous system function in rats up to the highest dose tested (300 mg/kg).

No genotoxic potential was observed for RO7020531 in the Ames test, nor in the in vitro and in vivo micronucleus test. No evidence of teratogenicity or embryo-fetal toxicity was observed in definitive (GLP) embryo-fetal toxicity studies in rats (up to 150 mg/kg/day) or

rabbits (up to 100 mg/kg/day), other than increased incidences of non-adverse fetal skeletal variations. In a definitive (GLP) fertility study in rats, no adverse effects of RO7011785 on mating performance, fertility, or early embryonic development were noted (up to 300 mg/kg, every other day [QOD]), despite slightly reduced body weight gain. See the RO7020531 Investigator's Brochure for details on the properties of RO7020531 and RO7011785.

1.2.2 Previous Clinical Studies

A global entry-into-human (EIH) study (NP39305) is ongoing, where RO7020531 is being evaluated in healthy volunteers in single ascending doses (SAD) and multiple ascending doses (MAD) and subsequently will be evaluated in virologically suppressed CHB patients.

As of 1 October, 2017, 80 healthy volunteers (HVs; 64 males and 16 females) have been dosed with RO7020531/placebo in eight SAD cohorts. All dose levels (3 mg, 10 mg, 20 mg, 40 mg, 60 mg, 100 mg, 140 mg and 170 mg) were considered safe and well tolerated. There had been few adverse events (AEs) per cohort, all were reported as of mild intensity, and only three subjects (two in the second cohort [10mg] and one in the 170 mg cohort) had AEs that were considered "related" to study drug. No serious adverse events (SAEs) or discontinuations due to AEs had been reported; and there were no dose related trends in nature, incidence or severity of AEs. In addition, there have been no clinically significant changes (or trends) in ECG parameters, vital signs, or laboratory safety test results in any subjects.

Exposure ($AUC_{0\text{-}inf}$) of the main active metabolite, RO7011785, increased proportionally with dose and was consistent with values predicted for humans from preclinical pharmacokinetics (PK) models. PD biomarkers did not have significant changes after single doses up to 60 mg in SAD Cohorts 1-5. Three out of eight subjects in SAD Cohort 6 who received a single RO7020531 dose of 100 mg exhibited changes in interferon alpha indicative of TLR7 activation. Two of these subjects exhibited increased activity for IP10 and neopterin. The fraction of subjects responding as well as the amplitude of the response increased at the 140 mg dose. Changes in mRNA levels for ISG15, OAS-1, MX1 and TLR7 initially found at the 100 mg dose were amplified at the 140 mg dose.

1.3 STUDY RATIONALE AND BENEFIT–RISK ASSESSMENT

1.3.1 Study Rationale

Study YP39553, is a Phase I study to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of RO7020531 following oral administration of SAD and MAD in Chinese healthy subjects.

Safety and tolerability of ascending doses of RO7020531 will be the primary endpoint in this study. In addition, PK and PD measured via the appearance of select biomarkers

indicating activation of TLR7 and/or TLR8, will be evaluated. These biomarkers will be evaluated for each cohort in the SAD and will be used in a decision to initiate the MAD.

The MAD part is designed to dose subjects with RO7020531 using a QOD regimen that will proceed for two weeks. Previous clinical and non-clinical data with another TLR7 agonist, RO6864018, have shown that the QOD dose schedule provides additional priming of the TLR7 response and is thought to be a dose regimen that may optimize the benefit-risk ratio for HBV treatment.

Evidence for TLR activation in the SAD and MAD studies will be provided by measuring post-dose changes in select*ed* biomarkers including *the protein and metabolite markers* IFN-α, IP-10, TNF-α, IL-6, IL-10, IL-12p40 and neopterin *and markers of transcriptional responses (ISG15, OAS-1, MX1 and TLR7)*, known to be stimulated by activation of TLR7 and/or TLR8.

1.3.2 Benefit-Risk Assessment

The evaluation of potential risks of treatment and specific tests, observations, and precautions required for clinical studies with RO7020531 are based on information from non-clinical toxicology and safety pharmacology studies, and on information/in house experience from clinical studies with other TLR7 agonists (Gane 2015, Grippo, 2016). Furthermore, information from clinical data collected from the global EIH Study NP39305 will be available prior to starting to dose subjects in this study. The doses/exposures to be explored in this study should be considered safe and with acceptable tolerability based on study NP39305.

Safety and tolerability will be carefully assessed, and healthy volunteers will be closely monitored. The RO7020531 IB summarizes potential risks and key risk management activities to consider when dosing this compound.

As for all early clinical studies in healthy volunteers, there is no direct benefit to the subjects participating in this study. However, this study will be essential in the development of a new treatment for chronic HBV infection, and for selecting the most appropriate dose for a study in HBV-infected patients.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVES

The primary objective of this study is:

- To assess the safety and tolerability of single and multiple ascending doses of oral RO7020531 in Chinese healthy subjects.

2.2 SECONDARY OBJECTIVES

The secondary objectives for this study are as follows:

- To investigate the plasma PK of RO7020531 and the main active metabolite, RO7011785, following single and multiple ascending doses of RO7020531 in Chinese healthy subjects. Additional metabolites may be measured, including RO7018822 and RO7033805.
- To investigate the urine PK of RO7020531 (if detectable) and the main active metabolite, RO7011785, in urine samples of Chinese healthy subjects after single ascending oral doses of RO7020531. Additional metabolites may be measured in urine, including RO7018822 and RO7033805.
- To investigate the effect of RO7020531 on PD parameters following single or multiple oral doses administered QOD in Chinese healthy subjects.

3. STUDY DESIGN

This study will be a randomized, Sponsor-open, Investigator/subject-blinded, placebo-controlled, SAD and MAD study to evaluate the safety, tolerability, PK and PD of RO7020531 and metabolites following oral administration to Chinese healthy subjects. PK, PD, safety and tolerability data collected in the SAD part of this study will be used to determine doses at which to initiate the MAD part of the study. A QOD dosing regimen will be evaluated in the MAD portion of this study.

3.1 DESCRIPTION OF STUDY

3.1.1 Overview of Study Design

For both SAD and MAD parts of the study, each cohort will enroll ten subjects, with eight subjects randomly assigned to RO7020531 and two subjects randomly assigned to placebo (Table 1). Each cohort will include a minimum of two females, with at least one female receiving active drug. All single and multiple doses will be administered in the fasted state.

Table 1 Study Cohorts

Cohort	Stage	Dose (mg)[a]	Total Doses Administered	Route	Planned Number of Healthy Volunteers	
					Active Drug	Placebo
1	SAD	*40*	1	PO	8	2
2	SAD	*100*	1	PO	8	2
3	SAD	*140*	1	PO	8	2
4	SAD	*170*[b]	1	PO	8	2
1	MAD	TBD	7 QOD	PO	8	2
2	MAD	TBD	7 QOD	PO	8	2

SAD = single ascending doses; MAD = multiple ascending doses; TBD = to be determined; QOD = every other day.

a. Proposed doses, which will be refined based on PK, safety and tolerability found in the global EIH study NP39305 *and/or in the previous cohorts in the current study YP39553*;

b. Optional cohort.

Additional cohorts may be explored in the SAD and MAD part as required.

3.1.1.1 Single Ascending Dose Study

For dose escalation, subjects will be sequentially enrolled into approximately four to six dose cohorts that may range between *40* mg and 1*7*0 mg, the maximum dose proposed in the global EIH study, NP39305. *Based on the newly available data in NP39305 indicating very good safety and tolerability with single RO7020531 doses up to 170 mg and PD effects documented only with doses ≥ 100 mg, the starting dose in this study was selected to be 40 mg, which is expected to avoid very low doses/exposures that may not produce pharmacodynamic effects, while ensuring appropriate safety margins expected with the first dose (See Section 3.2.1).*

The anticipated ascending dose scheme in the *SAD* cohorts is *40* mg, *100* mg *140 mg* and *170* mg. Additional cohorts may be added within a dose range between 10 mg and *170* mg (Figure 1). As an additional safety precaution in this study, the subjects participating in each single dose cohort will be dosed according to a sentinel dosing design. Initially, two subjects will be dosed: one subject with RO7020531 and one subject with placebo. If the safety and tolerability results from the first 24 hours following dosing for the initial subjects are acceptable to the Investigator, the remaining subjects of each cohort may be dosed soon after.

As described below, PK/PD and safety data from the SAD cohorts will be utilized to select the starting dose for the MAD. If only three cohorts are required to choose a starting dose for the MAD, then further cohorts may not be evaluated. The final doses chosen for evaluation may be modified based on data collected in Study NP39305 and/or from the previous cohorts in the current study. If adequate safety and tolerability are documented in a dosing cohort, further dose progression may be explored but not

until a consensus agreement is reached between the Sponsor and the Investigator; the Ethics Committee will be informed. RO7020531 doses planned for this study in Chinese healthy volunteers will not be evaluated if they are expected to result in RO7011785 exposures which are not found to be safe and tolerable in the global EIH study. In addition, doses will not be escalated in both the SAD and MAD if stopping rules have been met (see Section 3.1.3). Safety and tolerability at each dose level will determine eligibility for dose escalation. Measurement of PK and select PD markers will be made with each cohort and, upon availability, will be utilized along with safety data to determine the first dose with which to initiate the MAD. The MAD part of this study may be initiated before the SAD part is completed. Therefore, both SAD and MAD parts of this study may run in parallel.

In the SAD cohorts, subjects will be screened up to 28 days before randomization and asked to remain in the unit from Day −2 until Day 3 with dosing occurring on Day 1. After being discharged on Day 3, subjects will return for an outpatient visit on Day 5 and a follow-up visit on Day 8. All subjects will have a follow-up phone call 28 days after the study drug administration. Subjects will be asked to report any AEs that occur during this period.

3.1.1.2 Multiple Ascending Dose Study

The trigger to start the first cohort in the MAD includes documentation of adequate safety and tolerability in the SAD cohorts and evidence of PD effects. For a given dose evaluated in the SAD, evidence in at least two subjects exhibiting responses above placebo-defined baseline for select PD biomarkers, including *the protein and metabolite markers* IFN-α, IP-10, TNF-α, IL-6, IL-10, IL-12p40 *and* neopterin *and markers of transcriptional responses (ISG15, OAS-1, MX1 and TLR7)*, should be demonstrated. If the next dose in the SAD has been considered safe, then the previous dose demonstrating TLR-associated PD activity will be considered as the starting dose for the MAD. In addition, the starting dose/exposure in the MAD part of this study should have been considered safe in the global EIH study (NP39305). Only doses covered by the dose range evaluated in the SAD part will be used in the MAD, and two to three dose levels will be evaluated (Figure 1).

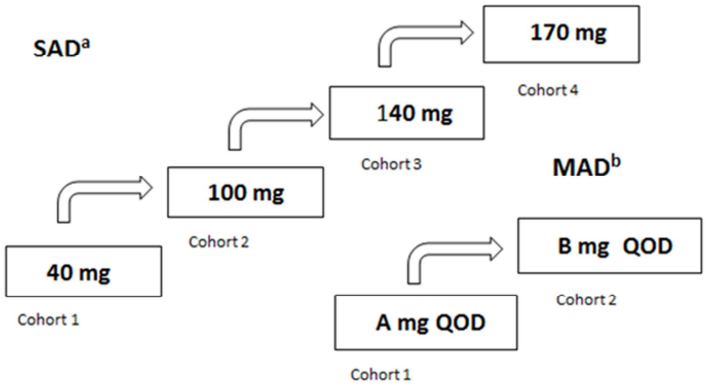
Once criteria have been reached to determine an initial dose for MAD, subjects will be screened up to 28 days before enrollment into the MAD part of this study. They will be asked to remain in the unit from Day -2 until Day 14 with QOD dosing initiated on Day 1. In total, 7 QOD doses will be administered with the last dose given on Day 13. Subjects will be discharged on Day 14. After completing 7 QOD doses, each subject in the MAD cohorts will return for an outpatient follow-up visit 7 days after the last dose. All subjects will have a follow-up phone call 28 days after their last dose. Subjects will be asked to report any AEs that occur during this period.

Upon safety review of the MAD cohort(s) data, dose escalation will proceed in the MAD part of this study. The maximum dose investigated in MAD will not exceed doses

evaluated in the SAD part of this study nor will it exceed doses and exposures considered to be safe and with acceptable tolerability in the global EIH study.

Based on the PK, PD and safety evaluation of the previous dose cohorts, additional cohorts of subjects may be enrolled at higher or lower dose levels, or at a dose level already tested.

Figure 1 Study Design



a. Doses shown in this figure may be modified based on emerging data. Four to six cohorts *will be evaluated in the SAD part of the study with a dose range between 40 mg and 170 mg.*

b. *Two* to three *cohorts will be evaluated* in the MAD part of the study. *Doses in the MAD part will not exceed the dose considered to be safe and well-tolerated in the SAD part.*

3.1.2 Dose Escalation Decision Criteria

A Safety Review Meeting will be conducted by the Principal Investigator and the Sponsor Clinical Team prior to each dose escalation.

The decision to escalate to the next dose level in the SAD cohorts will be based primarily on the available safety and tolerability information through Day 4 (including adverse events [AEs], electrocardiograms [ECGs], vital signs, clinical laboratory test results) and, secondarily, on available PK and PD data through 24 hours post-dose at the previous dosage level. In addition, all available safety and PK data from the previous dose level(s) will be reviewed. The anticipated dose escalation sequence for the SAD is *40* mg, *100* mg*, 140 mg* and *170* mg *(See Table 1).*

The decision to escalate to the next dose level in the MAD cohorts will be based primarily on the safety and tolerability information (including AEs, ECGs, vital signs, clinical laboratory test results) through Day 14 and secondarily on available PK data. In addition, all available safety and PK data from the previous dose level(s), including SAD cohorts, will be reviewed.

Depending on the evaluation of the safety and/or PK data: the same dose may be repeated; a lower dose may be administered; or intermediate dose escalation steps other than those anticipated above may be used at the discretion of the Study Investigator and the Sponsor.

The recommendation for the subsequent cohort dose level will be shared with the Ethics Committee.

3.1.3 Treatment Stopping Rules

Any serious adverse event (SAE) considered related to study drug will lead to an immediate halt of study drug dosing and enrollment of new subjects until a thorough investigation has been conducted by the Sponsor's Clinical Team and the Principal Investigator. The sponsor or designee will notify all investigators and IRBs/EC regarding the outcome of such investigation, and the study will only continue if agreed by the IRBs/EC.

Planned dose escalation will be stopped if one of the following circumstances occurs in subjects dosed with RO7020531, unless it is apparent that the occurrence is not related to the administration of study medication:

- Within a cohort, three or more subjects on active drug experience:
  - Severe and clinically significant (as defined by the Investigator) RO7020531-related AEs of the same character, or
  - Clinically significant RO7020531-related laboratory abnormalities of the same character, or
  - Clinically significant RO7020531-related changes in vital signs or ECGs of the same character (e.g., confirmation of mean corrected QT interval [QTc] 500 msec or 60 msec longer than the pre-dose baseline).
- Within two consecutive dose cohorts, four occurrences of any of the above conditions in subjects receiving active drug.
- Any other findings (regardless of the incidence rates) that, at the joint discretion of the Sponsor and the Investigator, indicate dose escalation should be halted.
- Dose escalation will not proceed if it is predicted that higher doses of RO7020531 will not result in a further increase in RO7011785 plasma exposure; or if the mean systemic exposure (AUC) reaches the RO7011785 plasma levels found to be associated with dose-limiting toxicities in the global EIH study.

If dose escalation is stopped, lower doses could be investigated by mutual agreement between the Sponsor and the Investigator.

For an individual subject in the MAD cohorts, dose continuation will not occur if the subject experiences any of the following:

- Clinically significant RO7020531-related changes in safety parameters that are considered not acceptable by the Investigator and/or the Sponsor; or
- Poor tolerability, which is considered to affect the subject's well-being and/or the PK evaluation.

Due to the exploratory nature of this clinical study, its conduct can be discontinued at any time at the discretion of the Sponsor.

3.1.4 Length of Study

SAD: The total duration of the study will be up to 9 weeks (from Screening through study completion) for each randomized subject as follows:

- Screening: Up to 28 days;
- Dosing period: 1 day;
- Follow up: 28 days after dosing.

The in-clinic period will be from Day −2 until Day 3.

MAD: The total duration of the study will be up to 10 weeks (from Screening through study completion) for each randomized subject as follows:

- Screening: Up to 28 days;
- Dosing period: 14 days;
- Follow-up: 28 days after last dosing.

3.1.5 End of Study

The end of the study is defined as the date when the last subject last observation (LSLO) occurs. LSLO is expected to occur 6 weeks after the last subject is randomized.

3.2 RATIONALE FOR STUDY DESIGN

3.2.1 Rationale for Dosage Selection and Dosage Regimen

The dose selection for this study is supported by the non-clinical safety data and may be modified based on the clinical data collected from the global EIH study, NP39305. The current study in Chinese healthy volunteers (YP39553) is planned to consist of four to six dose levels in SAD and two to three dose levels in MAD, which will be within the dose-range considered to be safe and with acceptable tolerability in the global EIH study, NP39305.

Based on the non-clinical data with RO7020531 (see Section 1.2.1), the half-life of the active TLR7 agonist RO7011785 is relatively short. However, previous experience in monkeys and humans with another TLR7 agonist (RO6864018) indicate that a short exposure of the TLR7 agonist initiates a series of pharmacodynamic events with biomarker activity that peaks as late as 48 hours post dose. Similar results are

anticipated following dosing with RO7020531 and are supported by activity measured in non-clinical models.

In the ongoing global EIH study (NP39305), all dose levels tested in SAD (3 mg – 170 mg) have been observed to be safe and well tolerated. In SAD Cohort 6 (100 mg), 3/8 healthy volunteers demonstrated increases in interferon alpha and which met the predefined criteria for TLR7 marker activity.

The starting dose of *40* mg was selected for the SAD portion of this study in Chinese patients based on the safety and tolerability documented in the study NP39305, avoiding very low doses/exposures that may not produce pharmacodynamic effects, while ensuring appropriate safety margins expected with the first dose.

The phenomena of response priming (increase in PD effects with the second dose) and tachyphylaxis (reduced response with chronic and frequent doses) are expected with RO7020531 based on clinical and non-clinical data observed with another TLR7 agonist, RO6864018. The phenomenon of TLR7 priming was frequently observed with RO6864018 in cynomolgus monkeys after the second dose administration and was followed by a subsequent response tachyphylaxis. Similarly, in healthy volunteers and HCV patients (Study ANA773-601), there is evidence of response priming with QOD schedule of RO6864018 showing an increase in PD response with the second dose, and evidence of tachyphylaxis to systemic IFN with repeated dosing on QOD schedule.

The QOD schedule has been selected for the multiple dosing part of this study based on information acquired from non-clinical studies with RO7020531 and clinical studies with RO6864018, and is aimed to optimize PD responses while minimizing safety risks, such as poor tolerability with every day dosing.

Based on clinical data with another TLR7 agonist (Study ANA773-601), it is expected that priming of PD effects and tachyphylaxis to systemic IFN release provided by the QOD schedule might be a desirable effect that can potentially limit AEs, and improve tolerability and efficacy.

3.2.2 Rationale for Study Population

Healthy subjects were chosen for this SAD and MAD study to allow (in a safe and well-controlled setting) better understanding of the tolerability, safety and PK of oral dosing with RO7020531 while avoiding the confounding variables of co-medications and co-morbidities found in HBV patients. Another prodrug, RO6864018, which is rapidly converted to the TLR7 agonist, RO6871765, has been tested in both healthy volunteers, hepatitis C virus (HCV) and is being tested in an ongoing study in HBV patients. Data from single and multiple dose administrations indicate that PK/PD relationships determined in healthy volunteers and HCV patients were comparable. These in house data and other literature data with TLR7 agonists comparing healthy volunteers and CHB patients indicate that PD/dose relationships determined in healthy volunteers, CHB

and HCV patients were comparable, suggesting that healthy volunteers represent a suitable population for the evaluation of safety, tolerability, PK and PD of TLR agonists that will be predictive of the dose-effect relationships anticipated in CHB patients.

Healthy status in study subjects will be confirmed during the screening period. Due to immunomodulatory mechanism of action of RO7020531, all subjects with a history of immunologically mediated disease will be excluded. All healthy volunteers will be tested for autoimmune markers to exclude participants with potential connective tissue disease or other immune-mediated diseases.

In this SAD and MAD study, each cohort will include ten subjects (eight active, two placebo). Sex differences in TLR7-mediated response have been reported, including higher immune activation and IFN-α production by pDCs in females (Berghofer et al 2006). Females appear to be more sensitive to TLR7 activation than males and exhibit greater PD responses in peripheral blood such as fold change from baseline for several genes and cyto/chemokines investigated in previous clinical studies of another TLR7 agonist, RO6864018. It was also shown that female healthy volunteers appeared to have higher exposure of the active TLR7 agonist, RO6871765. These facts warrant further investigation in this study, and so, to allow evaluation of potential sex differences in PK and PD response, a minimum number of two female subjects per cohort (with at least one female receiving active drug) is implemented in each one of the study cohorts.

3.2.3 Rationale for Pharmacokinetic Assessments

In general, PK data will be used to describe the concentration-time profile for RO7020531 and its metabolites, as well as key PK characteristics including elimination half-life, dose/exposure and PK/PD relationships for safety and specific TLR7-dependent biomarkers.

For the SAD part of the study, subjects will be asked to remain in the unit for up to 3 days post-dose for safety monitoring and to collect plasma samples for PK. With the proposed collection of up to 48 hours post-dose, it is assumed that the full concentration-time profile for RO7020531 and its metabolites will be determined. Urine samples will be collected in intervals to determine the fraction of RO7020531 and its metabolites that are eliminated in the urine. Previous work with the TLR7 agonist RO6864018 has shown extensive elimination of the active TLR7 agonist RO6871765 in the urine. As both RO7020531 and RO6864018 are from the same chemical class, it is expected that extensive renal elimination of the active TLR7 agonist RO7011785 will occur. It is anticipated that the 24-hour urine collection will allow characterization of this elimination pathway.

For the MAD part of the study, a QOD dosing schedule will be used, and extensive PK sampling will be made over a 24-hour period on Day 1 following the first dose and on Day 13 following multiple doses to determine if PK parameters change with multiple doses. With subsequent doses throughout the dosing period, sparse PK sampling will

also be used to characterize trough values and C_{max} values to determine that exposure of RO7011785 is maintained during this dose regimen. No urine samples will be collected in the MAD part of the study.

3.2.4 Rationale for Pharmacodynamic Assessments

TLR7 is expressed on human pDC and B-cells, and its activation induces both humoral and cellular changes (Iwasaki and Medzhitov 2004, Lester and Li 2014). These changes include the production of cytokines and chemokines such as IFN-α, IP-10, TNF-α, IL-6, IL-10, IL-12p40 *and changes in the expression of ISGs, e.g., ISG15, OAS-1 and myxovirus resistance 1 gene (MX1) and of the TLR7 gene itself (Fidock et al 2011),* as well as changes in markers of immune stimulation such as neopterin.

To capture the immunomodulatory effects of TLR7 agonism, cytokines,-chemokines *and peripheral blood gene expression* will be measured at time-points outlined in the SoA (Appendix 1, Appendix 2, Appendix 3 and Appendix 4). These markers showed a dose-dependent PD profile with other TLR7 agonists when assessed in patients and healthy volunteers. The PD *activity* in the SAD part of the study will be used to help choose the starting dose in the MAD part of the study.

In previous healthy volunteer clinical studies, IFN-α, neopterin, IL10 and IP-10 were activated with another TLR7-selective agonist, RO6864018 and no activation of the TLR8 markers, IL-6 and TNF-α was seen. Thus, in the current study, any activation of IL-6 and TNF-α may be indicative of activation of TLR8.

3.2.5 Rationale for Clinical Genotyping

Various single nucleotide polymorphisms (SNPs) have been described for the *TLR7* gene. These SNPs may be associated with differential expression of *IFN*, *TLR7* and certain cytokines, or carry an increased risk of developing autoimmune diseases such as lupus erythematosus (Askar et al 2010, Kawasaki et al 2011). Furthermore, there seems to be a pronounced difference in the frequency of these SNPs in Asians and Caucasians (IGSR: 1000 Genomes Project).

Potential PD and/or PK differences will be studied by clinical genotyping, exploring whether genetic polymorphisms of drug metabolizing enzymes (such as aldehyde oxidase) and *TLR7* can be related to the PK/PD profile of RO7020531.

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.

- Incidence of vital signs (blood pressure [BP], pulse rate, respiratory rate and body temperature) or ECG (PR [PQ], QRS, QT, QTcF) abnormalities.

3.3.2 Pharmacokinetic Outcome Measures

The PK evaluations for this study are as follows:

- Summary descriptive statistics of plasma PK parameters for RO7020531 and the main active metabolite, RO7011785, and additional metabolites, including RO7018822 and RO7033805, will be computed. These parameters include C_{max}, T_{max}, AUC_{inf}, AUC_{last}, and $t_{1/2}$ and will be presented by dose cohorts including means, standard deviation (SD), coefficient of variation (CV), geometric means, medians and ranges.
- Where available, descriptive statistics of urine PK parameters (total amount excreted, fraction excreted of total administered dose, renal clearance), if available, for RO7020531, RO7011785 and other metabolites will be presented.

3.3.3 Pharmacodynamic Outcome Measures

The PD outcome measures for this study are as follows:

- Blood samples will be collected to evaluate a number of PD outcome measures including the protein and metabolite markers of humoral response(IFN-α, IP-10, TNF-α, IL-6, IL-10, IL-12p40 and neopterin*) and markers of transcriptional responses (ISG15, OAS-1, MX1 and TLR7).* .

3.3.4 Clinical Genotyping Outcome Measures

The outcome measures for this study are as follows:

- Correlation of SNP status with the observed exposure.
- Correlation of SNP status with the observed cytokine/chemokine levels.

See rationale Section 3.2.5 for information on SNP analysis.

4. MATERIALS AND METHODS

4.1 CENTER

One site is currently planned; however, additional site(s) may be included for back-up purposes and may be activated if needed. Administrative and Contact Information, and List of Investigators are provided separately.

4.2 STUDY POPULATION

The target population consists of Chinese male and female healthy subjects between the ages of 18 and 60, inclusive. Healthy subjects must satisfy all inclusion and exclusion criteria to be enrolled into the study. Under no circumstances are subjects who are randomized in this study permitted to be re-randomized to another cohort of this study.

Study subjects who drop out of the study for non-safety reasons may be replaced to ensure sufficient data to characterize the safety, tolerability, and PK and/or to make

dose-escalation decisions. Study subjects who withdraw from the study due to poor tolerability or for study drug-related adverse events will not be replaced.

4.2.1 Recruitment Procedures

Healthy volunteers will be identified for potential recruitment using pre-screening enrollment logs, IEC/IRB approved newspaper, radio or other advertisements and mailing lists prior to consenting to take place on this study.

4.2.2 Inclusion Criteria

Healthy subjects must meet the following criteria for study entry:

1. Chinese healthy male and female subjects. 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, and urinalysis.
2. 18 to 60 years of age, inclusive.
3. A Body Mass Index (BMI) of 19 to less than 28 kg/m^2 and a body weight of at least 45 kg.
4. Informed of, and 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.
5. For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use two approved contraceptive methods, of which one must be a barrier method and the other should be an established non-barrier form of contraception with a failure rate of $< 1\%$ per year, during the treatment period and for at least one month after the last dose of study drug.
    - A woman is considered to be of childbearing potential if she 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 contraceptive methods with a failure rate of $< 1\%$ per year include bilateral tubal occlusion, male sterilization, established proper use of hormonal contraceptives that inhibit ovulation, hormone-releasing IUDs, and copper IUDs.

    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.
6. For men: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive measures, and agreement to refrain from donating sperm, as defined below:

- With female partners of childbearing potential or pregnant female partners, men must remain abstinent or be willing to use two methods of contraception with their partners, one of which must be a condom and the other should be an established form of contraception, during the treatment period and for at least one month after the last dose of study drug to avoid exposing the embryo. Other acceptable forms of contraception include vasectomy, bilateral tubal occlusion, IUD or proper use of hormonal contraceptives (e.g. contraceptive pills). 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 and withdrawal are not acceptable methods of contraception.

7. Negative pregnancy test on Day -1 for female subjects.
8. Non-smokers, or use of <10 cigarettes (or equivalent nicotine-containing product) per day.
9. Negative anti-nuclear antibody (ANA) test; or positive with dilutions not greater than 1:40 and with no associated history or symptoms of potential connective tissue disease or other immune-mediated diseases.

4.2.3 Exclusion Criteria

Healthy subjects who meet any of the following criteria will be excluded from study entry:

1. Pregnant (positive pregnancy test) or lactating women, and male partners of women who are pregnant or lactating.
2. History of immunologically mediated disease (e.g., inflammatory bowel disease, idiopathic thrombocytopenic purpura, lupus erythematosus, autoimmune hemolytic anemia, scleroderma, severe psoriasis, rheumatoid arthritis, multiple sclerosis, or any other autoimmune disease).
3. History or symptoms of any clinically significant disease including (but not limited to), neurological, cardiovascular, endocrine, respiratory, hepatic, ocular, or renal disorder (as per Investigator's judgment).
4. Personal or family history of congenital long QT syndrome or sudden cardiac death.
5. Evidence of an active or suspected cancer or a history of malignancy, where in the Investigator's opinion, there is a risk of recurrence.
6. History of having received or currently receiving any systemic anti-neoplastic (including radiation) or immune-modulatory treatment (including systemic oral or inhaled corticosteroids, IFN or PEG-IFN) within 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. Eye drop-containing and infrequent inhaled corticosteroids are permissible up to 4 weeks prior to the first dose of study drug.
7. History of clinically significant thyroid disease; also, subjects with clinically significant elevated thyroid-stimulating hormone (TSH) concentrations at Screening.

8. Any confirmed clinically significant allergic reactions (anaphylaxis) against any drug, or multiple drug allergies (non-active hay fever is acceptable).
9. History of clinically significant psychiatric disease, especially major depression (significant psychiatric disease is defined as treatment with an antidepressant medication or a major tranquilizer at therapeutic doses for major depression or psychosis, respectively, or any history of the following: a suicide attempt, hospitalization for psychiatric disease, or a period of disability due to a psychiatric disease).
10. Clinically significant acute infection, e.g., influenza, local infection or any other clinically significant illness within two weeks of randomization.
11. History of clinically significant GI disease including inflammatory bowel disease, peptic ulcer disease, GI hemorrhage.
12. Confirmed systolic BP greater than 140 or less than 90 mmHg, and diastolic BP greater than 90 or less than 50 mmHg at Screening (based on the average of three separate resting BP measurements, properly measured with well-maintained equipment, after at least 5 minutes rest).
13. Clinically relevant ECG abnormalities on screening ECG, e.g.:
    - QTc interval (QTcF > 450 msec or < 300 msec)
    - Notable resting bradycardia (HR < 45 bpm), or HR > 90 bpm
    - Difference between highest and lowest of any screening QTc >30 msec
    - ECGs with documented machine errors in the interval duration assessments
    - 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).
    - Evidence of atrial fibrillation, atrial flutter, complete bundle branch block, Wolff-Parkinson-White Syndrome, or cardiac pacemaker.
14. Any of the following laboratory parameters prior to dosing:
    - White blood cells (WBC) < 3000 cells/mm^3
    - Neutrophil count < 1500 cells/mm^3
    - Platelet count < 140,000 cells/mm^3
    - Activated partial thromboplastin time (aPTT) > 40 seconds, international normalized ratio (INR) > 1.2
    - Hemoglobin (Hb) < 12 g/dL in females or 13 g/dL in males
15. Abnormal renal function including serum creatinine > upper limit of normal (ULN) or calculated CrCl < 70 mL/min (using the Cockcroft Gault formula; see Appendix 7).
16. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) values at Screening above ULN and judged clinically significant by the Investigator.

17. Positive results for anti-mitochondrial antibody (AMA), anti-smooth muscle antibody (ASMA) or thyroid peroxidase antibody.
18. Positive hepatitis A IgM antibody (HAV Ab IgM), hepatitis B surface antigen (HBsAg), hepatitis C antibody (HCV Ab), or positive for human immunodeficiency virus (HIV) at Screening.
19. 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 or judged to be clinically irrelevant for healthy subjects.
20. History of alcohol abuse (consumption of more than two standard drinks per day on average; one standard drink = 10 grams of alcohol) and/or drug abuse within one year of randomization. Alcohol consumption will be prohibited at least 48 hours before screening, 48 hours before admission until discharge from the clinic, and 48 hours before each scheduled visit.
21. Positive test for drugs of abuse or positive alcohol test at Screening or Day -1.
22. Any clinically significant concomitant disease or condition that could interfere with, or for which the treatment of, 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.
23. Use of any medication (prescription or over-the-counter [OTC], including health supplements, vitamins or herbal remedies) within two weeks prior to the first dosing or within five half-lives of the medication prior to first dosing (whichever is longer). Exceptions may be made on a case-by-case basis following discussion and agreement between the Investigator and the Sponsor.
24. Participation in an investigational drug or device study within 90 days prior to randomization.
25. Donation or loss of blood over 500 mL, or administration of any blood product, within 90 days prior to starting study medication.
26. Subjects under judicial supervision, guardianship or curatorship.
27. Any medical or social condition which may interfere with the subject's ability to comply with the study visit schedule or the study assessments.

4.3 METHOD OF TREATMENT ASSIGNMENT AND BLINDING

This study is observer-blinded. This means that the subjects, the Investigator, and all individuals in direct contact with the subjects at the investigative site will be blinded, except for the pharmacy staff handling the study drug distribution.

This study is Sponsor-open; members of the Sponsor's project and study teams who do not have direct contact with the subjects will be unblinded. This may exclude contract research organization (CRO) staff in direct contact with the site.

Healthy volunteers will be randomized 4:1 to RO7020531 or to placebo in each dose cohort. The randomization numbers will be generated by the Sponsor or its designee. The randomized treatment assignment will be allocated from the list sequentially to subjects in the order in which they are randomized. A minimum of two females per cohort will be randomized, with at least one female receiving active drug. The treatment allocation will be managed by the unblinded site pharmacist(s) and will be based on the randomization code list.

To allow informed recommendations or decisions regarding the dose decisions in this study, an integrated assessment of the available data on PK, select PD, safety and tolerability will be made prior to each dose decision.

In exceptional cases, (e.g., if deemed important for dose decisions or for the more thorough evaluation of safety-related concerns that may impact dosing of future subjects on this or other currently conducted or shortly to start studies involving administration of RO7020531) and in the interest of the subjects' safety, the Investigator may be unblinded after approval by the Clinical Pharmacologist.

The Principal Investigator(s) will receive a set of sealed treatment codes. These may 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 healthy volunteer's condition, the treatment code for that healthy volunteer may be broken.

As per Health Authority reporting requirements, the Sponsor will break the treatment code for all unexpected serious adverse events (see Section 5.1) that are considered by the Investigator to be related to study drug.

All treatment code breakages should be documented in the study file. Treatment codes should not be broken except in emergency situations and, if possible, the responsible clinical pharmacologist should be contacted before the code is opened. At the final monitoring visit, the unused treatment codes 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 destroyed by the investigator site after verification by the Study Monitor, and once permission is granted by Roche.

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, at the investigating site end, will be documented in the study report with date, reason for identifying the drug and the name of all the person(s) who had to be unblinded.

4.4 STUDY TREATMENT

4.4.1 Formulation, Packaging, and Handling

4.4.1.1 RO7020531 and Placebo

RO7020531 and placebo will be supplied by Roche.

Investigational Medicinal Product (IMP): Hard gelatin capsule for oral administration containing 10 mg, or 100 mg of RO7020531 drug substance.

Placebo: Hard gelatin capsule identical in size and appearance to the corresponding active capsules, containing microcrystalline cellulose of compendial grade but no active substance.

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 medication will be in accordance with Roche standard and local regulations.

The study drug must be stored according to the details on the product label: “Store at 2°C-8°C, protect from light and moisture”.

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, refer to the most updated RO7020531 IB.

4.4.2 Dosage, Administration and Compliance

4.4.2.1 RO7020531 and Placebo

Both RO7020531 and placebo will be administered orally in capsules.

In the SAD cohorts, RO7020531 or matching-placebo will be administered orally to the subjects by investigational staff on the morning of Day 1 after an overnight fast of at least 10 hours. Subjects in the SAD cohorts will not eat for 4 hours after the dose is administered. Water will be allowed ad libitum until one hour prior to dosing and after one hour post-dosing. Approximately 4 hours after dosing, subjects will be given lunch. The SAD part of the study will include an adaptive number of cohorts (approximately four). Each dose cohort will include 10 subjects (eight active and two placebo). *The* planned dose-escalation sequence for SAD is *shown in Table 1.*

*D*oses *of RO7020531* in SAD and MAD cohorts will be administered as a combination of 10 mg or 100 mg capsules with the requisite number of capsules being administered per specific dose cohort. Should an intermediate dose be required due to a change in the

anticipated dose-escalation, the dose will be composed of the appropriate combination of 10 mg and 100 mg capsules.

In the MAD cohorts, RO7020531 or matching-placebo will be administered orally to the subjects by investigational staff QOD from Day 1 through to Day 13. In total, seven doses will be given (for time-points see Appendix 3 and Appendix 4). Each dose will be given in a fasted state (after an overnight fast of at least 10 hours). Subjects will not eat for 4 hours after each dose is administered. Water will be allowed ad libitum until one hour prior to dosing and after one hour post-dosing. The MAD part of the study will include an adaptive number of cohorts (two to three). Each dose cohort will include ten subjects (eight active and two placebo). Dose levels for MAD will be defined during the study conduct based on emerging data *(See Table 1)*.

Doses will be given orally with 240 mL of water. Additional amounts of water up to 100 mL could be given to assist dose administration only if needed.

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 subject 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 subject during the study.

Guidelines for dosage modification and treatment interruption or discontinuation are provided in Section 4.7.

4.4.3 Investigational Medicinal Product Accountability

IMP (RO7020531) and placebo required for completion of this study will be provided by the Sponsor. The investigational site will acknowledge receipt of IMPs, to confirm the shipment condition and content. Any damaged shipments will be replaced.

The Investigator 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 healthy volunteer to whom the study drug was dispensed (for example healthy volunteer initials and date of birth).
- All records and drug supplies must be available for inspection by the Roche 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 destroyed.
- Quantity of investigational product destroyed.
- Date of destruction.
- Method of destruction.
- Name and signature of responsible person (or company) who destroyed investigational products.

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.

4.5 CONCOMITANT THERAPY, DIETARY AND SPECIAL REQUIREMENTS

4.5.1 Permitted Therapy

As a general rule, no concomitant medication (including health supplements, vitamins or herbal remedies) will be permitted, unless the rationale for use is discussed between the Investigator and Sponsor and is clearly documented. The following medications are exceptions:

- Medications used to treat an AE 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 AEs should be recorded on the AE electronic case report form (eCRF).
- Hormone replacement therapy (HRT), continue using if initiated two months prior to study start.
- Acetaminophen/paracetamol is allowed up to a maximum dose of 2 g per day. 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 AE by the Investigator.

All medications (prescription drugs, over-the-counter [OTC] drugs, approved dietary and herbal supplements, nutritional supplements) taken within 4 weeks of study screening will be recorded on the appropriate eCRF.

4.5.2 Prohibited Therapy

Use of the following therapies is prohibited:

- Any prescribed or OTC medications (except for the cases given in Section 4.5.1), including health supplements, vitamins or herbal remedies within 5 half-lives or 2 weeks, whichever is longer, prior to the first dosing until the follow-up visit.
- Any systemic anti-neoplastic (including radiation) or immune-modulatory treatment (including systemic oral or inhaled corticosteroids, IFN or PEG-IFN) within 6 months prior to the first dose of study drug until the follow-up visit (Day 8 for SAD and Day 20 for MAD). Eye drop-containing and infrequent inhaled corticosteroids are prohibited from 4 weeks prior to the first dose of study drug until the follow up visit.

4.5.3 Dietary and Special Requirements

There are data suggesting that concentrated green tea (including bottled green tea beverages) may inhibit aldehyde oxidase (Tayama et al 2011). Therefore, subjects should minimize the amount of bottled green tea beverages and other green tea preparations they drink from Day −7 until the follow-up visit at Day 8.

Subjects must fast overnight (at least 10 hours) before the dosing and must not eat for 4 hours after the dose is administered. Approximately 4 hours after dosing, subjects will be administered lunch.

Water will be allowed ad libitum until one hour prior to dosing and from one hour post-dosing. However, the excessive consumption of fluids (greater than 3 liters per day) should be avoided until completion of the follow-up visit.

Laboratory safety assessments should be conducted after subjects have been fasted for a minimum of 8 hours.

Alcohol must not be consumed from 48 hours before screening, 48 hours before admission until discharge from the clinic, and 48 hours before each scheduled visit. Please refer to Appendix 8 for the calculation of alcohol standard drinks as relates to eligibility criteria.

Caffeine (i.e., beverage, chocolate or supplements) must not be consumed from 48 hours prior to study drug administration until discharge from the clinic.

Strenuous exercise is not permitted during the study from 96 hours before admission until completion of the follow-up visit.

The use of tobacco is not permitted from Day -2 till Day 3 for the SAD part and from Day -2 till Day 14 for the MAD part of the study.

4.6 STUDY ASSESSMENTS

4.6.1 Description of Study Assessments

All examinations listed below will be performed according to the Schedule of Assessments (SoA) outlined in Appendix 1, Appendix 2, Appendix 3 and Appendix 4.

At time-points when several pre-dose assessments coincide, the following sequence should be followed:

- Urine collection (urinalysis, urine PK pre-dose sample).
- ECG recordings.
- Vital signs.
- Pre-dose blood sampling (pre-dose PK, pre-dose PD, exploratory samples, safety blood tests).
- RO7020531/placebo intake.

For the post-dose assessments, the following sequence should be followed with the PK blood sample (or PD blood sample if there is no PK sampling) to be taken at the nominal time-point:

- ECG recordings.
- Vital signs.
- Post-dose blood sampling (post-dose PK, post-dose PD).

4.6.1.1 Medical History and Demographic Data

Medical history includes clinically significant diseases, surgeries, 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 healthy volunteer within 4 weeks prior to the screening visit.

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

4.6.1.2 Physical Examinations

A complete physical examination should be performed at time-points indicated in the SoA (Appendix 1, Appendix 2, Appendix 3 and Appendix 4) and includes an evaluation of the head, eyes, ears, nose, throat, neck and lymph nodes, and the cardiovascular, dermatological, musculo-skeletal, respiratory, GI and neurological systems. A genitourinary examination may be performed in case of evocative symptoms at the Investigator's discretion. Any abnormality identified at Screening or at baseline should be recorded on the Medical History eCRF.

At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations should be performed. Changes from baseline abnormalities should be recorded in the healthy volunteer's notes. New or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF.

Height will only be recorded at Screening. Weight will be recorded at time-points for complete Physical Examination as specified in the SoA (Appendix 1, Appendix 2, Appendix 3 and Appendix 4). BMI will be calculated at Screening in accordance with the formula provided in Appendix 5.

4.6.1.3 Vital Signs

BP, pulse rate, respiratory rate and body temperature will be recorded at the time-points specified in the SoA (Appendix 1, Appendix 2, Appendix 3 and Appendix 4).

BP, pulse rate and respiratory rate should be obtained in a quiet room at a comfortable temperature, with the healthy volunteer's arm unconstrained by clothing or other material. BP, respiratory rate and pulse rate will be obtained after the subject has been in a supine or sitting position for at least 5 minutes. All BP and pulse rate measurements will be obtained from the same arm (where possible), in the same position and with the same cuff size, using a well-calibrated automatic instrument with a digital readout, throughout the study. BP measurement will be performed in triplicate (can be as short as 20 second to 1 minute interval between measurements). The mean of three consecutive replicates will be used as the value for the defined time-point.

Body temperature measurement can be either oral or tympanic; the method should be maintained throughout the study.

4.6.1.4 Electrocardiograms

Triplicate 12-lead ECG recordings (i.e., three useful ECGs without artifacts) will be obtained within approximately 2-5 minutes at each specified time‑point. The average of the three readings will be used to determine ECG intervals (PR [PQ], QRS, QT, QTcF). The intervals 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.

Whenever possible, the same brand/model of a standard digital high‑quality, high-fidelity electrocardiograph machine equipped with computer-based interval measurements should be used for each subject. The conditions should be as close as possible to pre-dose time-points; this includes but is not limited to food intake, activity level, stressors and room temperature.

To minimize variability, it is important that subjects be in a resting position for≥10 minutes prior to each ECG evaluation. Body position should be consistently

maintained for each ECG evaluation to prevent changes in heart rate. Environmental distractions (e.g., television, radio, conversation) should be avoided during the pre-ECG resting period and during ECG recording. ECGs should be performed prior to any scheduled vital sign measurements and blood draws. In some cases, it may be appropriate to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality.

For safety monitoring purposes, the Investigator must review, sign and date all ECG tracings. Paper copies will be kept at the study centers with the subject's clinical file as part of the permanent record. 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.

If any ECG recording documents QT/QTc values > 500 msec or increases from Day -1 QTc > 60 msec (as provided by the machine), the site should repeat the ECG triplicate 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.

4.6.1.5 Laboratory Assessments

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 (Appendix 1).

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 healthy volunteer'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 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 or central laboratory.

Samples for the following laboratory tests will be sent to one or several laboratories or to the Sponsor for analysis. Instruction manuals and supply kits will be provided for all laboratory assessments.

- Hematology: Hb, hematocrit, total WBC count, differential WBC count (including basophils, eosinophils, lymphocytes, monocytes and neutrophils), platelet count, red blood cells count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and reticulocyte (%) counts.
- Coagulation: INR and aPTT.
- Blood chemistry: ALT, AST, total and indirect bilirubin, alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT; only at Screening), total protein, albumin, urea, creatinine, uric acid, total cholesterol, triglycerides, glucose, sodium, chloride, potassium, calcium, phosphorus, bicarbonate, cystatin C, HbA1c (only at Screening).
- Urinalysis: A midstream, clean-catch urine specimen will be collected for dipstick analysis of protein, blood, glucose, leucocytes 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.
- Substance use: Drugs of abuse urine test and alcohol test (only at Screening and Day −1). A breath test may be used to test for alcohol.
- Viral serology (only at Screening):

  HIV

  HAV Ab IgM

  HBsAg

  HCV Ab
- Pregnancy test:

  Serum or plasma beta-human chorionic gonadotropin (β-HCG) at Screening, urine on all other occasions (females only).
- Hormones (only at screening): follicle-stimulating hormone (females only to confirm post-menopausal status).
- Thyroid function tests (only at screening): TSH, free T3 and free T4.
- Autoimmune panel (only at screening): ANA, AMA, ASMA and thyroid peroxidase antibody.

Based on continuous analysis of the data in this study and other studies, any sample type not considered to be critical for safety may be stopped at any time if the data from the samples collected does not produce useful information.

4.6.1.6 Pharmacokinetic Assessments

Blood and urine (SAD only) samples for determination of plasma and urine concentrations of RO7020531, the main active metabolite RO7011785, and additional

metabolites (as applicable) including RO7018822 and RO7033805, will be collected at time-points specified in the SoA (Appendix 1, Appendix 2, Appendix 3 and Appendix 4).

RO7020531, RO7011785, RO7018822 and RO7033805 plasma and urine concentrations will be measured by a specific and validated method. The plasma PK parameter will be read directly from the plasma concentration versus time profiles, or calculated using standard non-compartmental methods. Total drug concentrations of RO7020531, the main active metabolite RO7011785, and additional metabolites (as applicable) including RO7018822 and RO7033805, will be calculated over a 24-hour interval.

The volume and pH of urine voided at each interval will be recorded and aliquots will be collected from the pooled samples at each time interval for analysis.

Details on sampling procedures, sample storage, and shipment are given in the Laboratory Manual.

4.6.1.7 Pharmacodynamic Assessments

Blood samples will be collected to evaluate a number of PD parameters including the protein and metabolite markers of humoral response (IFN-α, IP-10, TNF-α, IL-6, IL-10, IL-12p40, and neopterin) *and markers of transcriptional responses (ISG15, OAS-1, MX1 and TLR7).*

Instruction manuals and supply kits will be provided for the collection of all PD samples.

Time-points of PD blood sample collection are provided in the SoA (Appendix 1, Appendix 2, Appendix 3 and Appendix 4).

These samples will be stored for up to 5 years for the protocol assessments defined above, unless otherwise indicated. 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.

4.6.1.8 Clinical Genotyping

Instruction manuals and supply kits will be provided for the collection of all biomarker samples. One blood sample will be collected before first intake of drug. If sample collection was missed, it can be collected at any time thereafter. Collection time is provided in the SoA (Appendix 1, Appendix 2, Appendix 3 and Appendix 4). Data arising from all biosamples will be subject to the confidentiality standards described in Section 8.4.

These samples will be stored for up to 5 years for the protocol assessments defined above, unless otherwise indicated. 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.

4.6.2 Timing of Study Assessments

4.6.2.1 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 healthy volunteer and for healthy volunteers 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 healthy volunteers meet all eligibility criteria. The Investigator will maintain a screening log to record details of all healthy volunteers screened and to confirm eligibility or record reasons for screening failure.

An Eligibility Screening Form (ESF) documenting the Investigator's assessment of each screened healthy volunteer with regard to the protocol's inclusion and exclusion criteria is to be completed by the Investigator and kept at the investigational site.

A screening examination should be performed from Day -28 to Day -2 for SAD and MAD parts of the study. Subjects must fulfill all entry criteria to be accepted into the study. Assessments as detailed in the SoA (Appendix 1, Appendix 2, Appendix 3 and Appendix 4) will be conducted.

4.6.2.2 Assessments during Treatment

Under no circumstances will healthy volunteers 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 study.

All assessments must be performed as per SoA (Appendix 1, Appendix 2, Appendix 3 and Appendix 4).

4.6.2.3 Follow Up Assessments

Healthy volunteers who complete the study (SAD and MAD parts) or discontinue from the dosing early (MAD part only) will be asked to return to the clinic approximately 7 days (±1 day) after the last dose of study drug for a follow-up visit and will be contacted for a follow-up call approximately 21 days (±3 days) after the follow-up visit to complete assessments as specified in the SoA (Appendix 1, Appendix 2, Appendix 3 and Appendix 4). After the follow-up call, adverse events should be followed as outlined in Section 5.5, Section 5.6.

4.6.2.4 Assessments at Unscheduled Visits

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

In case of early termination of a subject in MAD part, a blood sample for PK assessment may be collected at the time of discontinuation.

4.7 HEALTHY VOLUNTEER, STUDY, AND SITE DISCONTINUATION

4.7.1 Healthy Volunteer Discontinuation

The Investigator has the right to discontinue a healthy volunteer from RO7020531 or withdraw a healthy volunteer from the study at any time. In addition, healthy volunteers have the right to voluntarily discontinue study drug (MAD part only) 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*:*

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

Healthy volunteers in the MAD part of the study who discontinue study treatment due to poor tolerability to study drug, or other safety-related reasons will not be replaced. Healthy volunteers who discontinue study treatment early for non-safety reasons may be replaced to ensure sufficient data to make dose-escalation and drug development decisions.

The decision to replace a subject will be made by a mutual agreement between the Sponsor and Investigator.

4.7.1.1 Discontinuation from Study Drug during MAD

For an individual subject in the MAD part of the study, dose continuation will not occur if the subject experiences any of the following:

- Clinically significant RO7020531-related changes in safety parameters that are considered not acceptable by the Investigator and/or the Sponsor; or
- Poor tolerability, which is considered to affect the healthy volunteer's well-being and/or the PK evaluation.
- Pregnancy.

Healthy volunteers who discontinue study drug prematurely will be asked to return to the clinic within 7 days for a follow-up visit to complete follow‑up assessments (Section 4.6.2.3). The primary reason for premature study drug discontinuation should be documented on the appropriate eCRF. Healthy volunteers who discontinue study drug prematurely for safety reasons will not be replaced.

4.7.1.2 Withdrawal from Study

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

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

When a subject voluntarily withdraws from the study, or is withdrawn by the Investigator, samples collected until the date of withdrawal will be analyzed, unless the subject specifically requests for these to be discarded or local laws require their immediate destruction.

Healthy volunteers who withdraw from the study for safety reasons will not be replaced. Healthy volunteers who withdraw from the study for other reasons may be replaced.

4.7.2 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 healthy volunteers.
- Healthy volunteer enrollment is unsatisfactory.
- Previously unknown data become available which raise significant concerns about the potential risk to participants from continuation of the study.

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).
- 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 subject 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 5.3.5.9).
- Results in persistent or significant disability/incapacity (i.e., the adverse event results in substantial disruption of the healthy volunteer'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 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 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 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 for reporting instructions). Adverse events of special interest for this study include the following:

- Cases of an elevated ALT or AST in combination with either an 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 subject 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

Flu-like symptoms: TLR7 agonists can be associated with dose-dependent flu-like symptoms. A percentage of healthy volunteers may develop fever, chills, fatigue, myalgia, malaise, and headache within several hours after the dosing with RO7020531. Symptomatic treatment is recommended in case these events occur.

No dose modification of RO7020531/placebo for safety reasons will be implemented in the MAD part of this study. At the discretion of the Investigator, study treatment can be discontinued. For the treatment stopping rules in individual healthy volunteers and dose escalation stopping rules, please see Section 3.1.3 and Section 4.7.1.1.

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 Section 5.5 to Section 5.7.

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 healthy volunteer contact. All adverse events, whether reported by the healthy volunteer or noted by study personnel, will be recorded in the healthy volunteer'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 28 days after the last dose of study drug.

After a period of 28 days from the last dose of study drug, 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 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 2 provides guidance for assessing adverse event severity.

Table 2 Adverse Event Severity Grading Scale

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).

5.3.4 Assessment of Causality of Adverse Events

Investigators should use their knowledge of the healthy volunteer, 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.
- 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 healthy volunteer 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.

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 GI 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 healthy volunteer 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 subject 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 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 BP), 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 Function Tests

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. 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.

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).

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 subject with or without preexisting heart disease, within 1 hour of the onset of acute symptoms or, in the case of an unwitnessed death, within 24 hours after the subject 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 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:

- Planned in-clinic stay required by the protocol for the SAD and MAD parts of the study.

5.3.5.10 Overdoses

Study drug 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).

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 subjects, access to the Medical monitors is available 24 hours a day 7 days a week. Medical monitors' contact details are listed in the "Protocol Administrative and Contact Information & List of Investigators".

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

5.4.2.1 Events That Occur prior to Study Drug Initiation

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. The Serious Adverse Event/Adverse Event of Special Interest Reporting Form provided to investigators should be completed and submitted to the SAE Responsible immediately (i.e., no more than 24 hours after learning of the event).

5.4.2.2 Events That Occur after Study Drug Initiation

For reports of serious adverse events and non-serious adverse events of special interest (see Section 5.1.2 and Section 5.1.3) that occur after initiation of study drug, investigators should record all case details that can be gathered immediately (i.e., within 24 hours after learning of the event) on the appropriate Adverse Event of Special Interest/ Serious Adverse Event eCRF form and submit the report via the electronic data capture (EDC) system. A report will be generated and sent to the Sponsor's Safety Risk Management department.

In the case of electronic reporting, investigators should record all case details that can be gathered immediately (i.e., within 24 hours after learning of the event) on the appropriate Serious Adverse Event / Adverse Event of Special Interest eCRF form and submit the report via the electronic data capture (EDC) system. A report will be generated and sent to the Sponsor's Safety Risk Management department.

In the event that the EDC system is unavailable, the Serious Adverse Event/Adverse Event of Special Interest Reporting Form provided to investigators should be completed and submitted to the SAE Responsible immediately (i.e., no more than 24 hours after learning of the event).

Once the EDC system is available, all information will need to be entered and submitted via the EDC system.

5.4.3 Reporting Requirements for Pregnancies

5.4.3.1 Pregnancies in Female Healthy Volunteers

A female healthy volunteer of childbearing potential will be instructed to immediately inform the Investigator if she becomes pregnant during the study, and until the end of the follow-up period of 28 days.

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 subject should continue until conclusion of the pregnancy.

5.4.3.2 Pregnancies in Female Partners of Male Healthy Volunteer

Male healthy volunteers will be instructed through the Informed Consent Form to immediately inform the Investigator if their partner becomes pregnant during the study or within 28 days 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 healthy volunteer 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 healthy volunteer 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).

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)

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 subject or female partner of a male subject exposed to study drug 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).

5.5 FOLLOW-UP OF SUBJECTS 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 subject is lost to follow-up, or the subject 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 subject'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.

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 subjects for adverse events after the end of the adverse event reporting period (defined as 28 days after the last dose of study drug).

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 using the following reference document:

- RO7020531 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 analysis of complete data for the study will be performed when all healthy volunteers have either completed the follow-up period or have discontinued early from the study, all data from the study are in the database and have been cleaned and verified, and the database is locked.

6.1 DETERMINATION OF SAMPLE SIZE

*F*our to six cohorts may be evaluated in the SAD part of the study (approximately 40-60 subjects in total), and *two to* three cohorts are anticipated for the MAD part of the study with approximately *20*-30 subjects in total.

Ten healthy volunteers are planned to be enrolled at each dose level. They will be randomized to either active treatment (eight healthy volunteers per dose level) or placebo (two healthy volunteers per dose level). With eight healthy volunteers per dose group or cohorts, there is a 90% chance to observe at least one AE that has an incidence rate of 25% in the population.

6.2 SUMMARIES OF CONDUCT OF STUDY

The number of healthy volunteers who are randomized, discontinue, or complete the study treatment and/or the entire study (including follow-up period) will be summarized by treatment. Reasons for premature study withdrawal during MAD treatment and follow-up phase will be listed and summarized by treatment. Enrollment and protocol deviations will be listed and evaluated for their potential impact on interpretation of study results. Descriptive statistics will be used to evaluate the conduct of the study.

6.3 ANALYSIS POPULATIONS

6.3.1 Safety Analysis Population

All healthy volunteers who have received at least one dose of the study medication, whether prematurely withdrawn from the study or not, will be included in the safety analysis.

6.3.2 Pharmacokinetic Analysis Population

A per protocol analysis including all healthy volunteers randomized and adherent to the protocol will be performed. Healthy volunteers 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 Pharmacodynamic Analysis Population

PD analysis population will include all subjects who were randomized, received at least one dose of study medication (RO7020531 or placebo), and have PD data available. Subjects will be analyzed according to the treatment group to which they are randomized.

6.3.4 Clinical Genotyping Analysis Population

All healthy volunteers who have provided a sample will be included in the analysis.

6.4 SUMMARIES OF TREATMENT GROUP COMPARABILITY

Descriptive statistics will be generated for demographic and baseline disease characteristics including sex, age, weight, height, body mass index.

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 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. Healthy volunteers 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.

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 healthy volunteer 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 healthy volunteer, the midpoint of the standard reference range will be used as the healthy volunteer's baseline value for the purposes of determining marked laboratory abnormalities. Marked laboratory abnormalities will be labeled in the healthy volunteer 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. In addition, tabular summaries will be used, as appropriate.

Summary descriptive statistics for the actual values and changes from baseline will be tabulated by nominal time for HR, QRS duration, PR and QTcF (see Appendix 6). 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.

6.5.5 Concomitant Medications

The original terms recorded on the healthy volunteers' 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 PHARMACOKINETIC ANALYSES

Non-compartmental analysis using WinNonlin software will be used to calculate PK parameters where appropriate. Summary descriptive statistics of plasma PK parameters including C_{max}, T_{max}, AUC_{inf}, AUC_{last} and $t_{1/2}$ for RO7020531 and RO7011785 and additional metabolites including RO7018822 and RO7033805, will be presented by treatment arm including means, SD, CV, geometric means, medians and ranges. Where appropriate, data may be pooled and analyzed. Listings, summary tables and graphs (individual plots and/or mean plots) by treatment group will be provided. Descriptive statistics of urine PK parameters including total amount excreted; fraction excreted of total administered dose; renal clearance for RO7020531 and RO7011875 and additional metabolites including RO7018822 and RO7033805 will be presented, where available.

Additional PK analyses, including population PK and/or PD analyses will be conducted as appropriate.

6.7 PHARMACODYNAMIC ANALYSES

Summary descriptive statistics will be presented for the induction of cytokines, chemokines, and neopterin *and of interferon-response genes* separately by treatment arm. Exploratory analysis will be performed to assess the TLR7 agonist induced response under different dosing conditions. Graphical and statistical techniques including linear, non-linear, and logistic regression will be used to explore potential relationships between dosing regimen, PK and PD.

6.8 CLINICAL GENOTYPING ANALYSES

Potential PD and/or PK differences will be studied by clinical genotyping, exploring whether genetic polymorphisms of drug metabolizing enzymes (such as aldehyde oxidases) and *TLR7* can be correlated to PK/PD profile of RO7020531.

6.9 INTERIM ANALYSES

There will be no Interim Analysis for this study.

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 (EDC) 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/or a Data Management Plan that describes the quality checking to be performed on the 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 Electronic Data Capture (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 enrolled, an eCRF must be completed and electronically signed by the Principal Investigator or authorized delegate from the study staff. If a healthy volunteer withdraws from the study, the reason must be noted on the eCRF. If a healthy volunteer 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/CRO in the eCRFs and in all required reports.

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

At the end of the study, the Investigator will receive subject 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 subject 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, subject-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, subject 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).

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 healthy volunteer or the subject's legally authorized representative before his or her participation in the study. The case history or clinical records for each subject 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 subject to participate. The final revised IRB/EC-approved Consent Forms must be provided to the Sponsor for Health Authority submission purposes.

Subjects 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 subject 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 subject or the subject's legally authorized representative. All signed and dated Consent Forms must remain in each subject'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 subject, 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 subject 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 subject enrolled in the study through assignment of a unique subject identification number. This means that subject names are not included in data sets that are transmitted to any Sponsor location.

Subject 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 subject, unless permitted or required by law.

Medical information may be given to a subject's personal physician or other appropriate medical personnel responsible for the subject'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., after last subject last observation).

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 subject data, which includes an audit trail containing a complete record of all changes to data.

9.2 SITE INSPECTIONS

Site visits will be conducted by the Sponsor or an authorized representative for inspection of study data, subjects' 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.

The Sponsor is also responsible for managing CROs and central laboratories used in the study.

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 subjects or any non-substantial changes, as defined by regulatory requirements.

10. REFERENCES

Alter MJ. Epidemiology of hepatitis B in Europe and worldwide. J Hepatol. 2003;39:S54-S69.

Askar E, Ramadori G, Mihm S. Toll-like receptor 7 rs179008/Gln11Leu gene variants in chronic hepatitis C virus infection. J Med Virol. 2010;82:1859-1868.

Berghofer B, Frommer T, Haley G, et al. TLR7 ligands induce higher IFN-α production in females. J Immunol. 2006;177:2088–2096.

Bertoletti A, Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. Gut. 2012;61:1754–1764.

Boonstra A, Liu BS, Groothuismink ZM. Potent immune activation in chronic hepatitis C patients upon administration of an oral inducer of endogenous interferons that acts via Toll-like receptor 7. Antivir Ther. 2012;17:657-667.

Chang TT, Lai CL, Kew Yoon S, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. Hepatol. 2010;51:422–430.

Fattovich G, Giustina G, Sanchez-Tapias J. Delayed clearance of serum HBsAg in compensated cirrhosis B: relation to interferon alpha therapy and disease prognosis. European Concerted Action on Viral Hepatitis (EUROHEP). Am J Gastroenterol. 1998;93:896-900.

Fidock MD, Souberbielle BE, Laxton C. The innate immune response, clinical outcomes, and ex vivo HCV antiviral efficacy of a TLR7 agonist (PF-4878691). Clin Pharmacol Ther. 2011;89:821-829.

Funk E, Kottilil S, Gilliam B. Tickling the TLR7 to cure viral hepatitis. J Transl Med. 2014;12:129.

Gane EJ, Lim YS, Gordon SC, et al. The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection. J of Hepatology. 2015; 63: 320–328.

Grippo J, Folitar I, van de Wetering de Rooij J, et al. Optimization of a Phase 2 Study Design in Chronic Hepatitis B (CHB) Patients Based on Safety, Pharmacokinetics (PK) and Pharmacodynamics (PD) from Phase 1 Studies Using Two Prodrugs of a Toll-like Receptor 7 (TLR7) Agonist. 2016; AASLD Abstract nr. 1869.

Horscroft NJ, Pryde DC, Bright H. Antiviral applications of toll-like receptor agonists. J Antimicrob Chemother. 2012;67:789-801.

Investigator's Brochure, RO7020531.

Isogawa M, Robek MD, Furuichi Y, et al. Toll-like receptor signaling inhibits hepatitis B virus replication in vivo. J Virol. 2005;79:7269–7272.

Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol. 2004;5:987-995.

Kawasaki A, Furukawa H, Kondoet Y, et al. *TLR7* single-nucleotide polymorphisms in the 3'untranslated region and intron 2 independently contribute to systemic lupus erythematosus in Japanese women: a case-control association study. Arthritis Research & Therapy. 2011;13:R41.

Lester SN, Li K. Toll-like receptors in antiviral innate immunity. J Mol Biol. 2014;426:1246-1264.

Locarnini S, Zoulim F. Molecular genetics of HBV infection. Antivir Ther. 2010;15:3–14.

Lucifora J, Xia Y, Reisinger F. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science. 2014;343:1221-1228.

Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet. 2013;381:468–475.

Martinot-Peignoux M, Carvalho-Filho R, Lapalus M. Hepatitis B surface antigen serum level is associated with fibrosis severity in treatment-naïve, e antigen-positive patients. J Hepatol. 2013;58:1089-1095.

Ott JJ, Stevens GA, Groeger J. Global epidemiology of hepatitis B virus infection: New estimates of age-specific HBsAg seroprevalence and endemicity. J Vaccine. 2012;30:2212–2219.

Papatheodoridis G, Buti M, Cornberg M, et al. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol. 2012;57:167-185.

Papatheodoridis GV, Manolakopoulos S, Dusheiko G. Therapeutic strategies in the management of patients with chronic hepatitis B virus infection. Lancet Infect Dis. 2008;8:167-178.

Sarin SK, Kumar M, Lau GK, at al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016;10:1-98.

Strader DB, Wright T, Thomas L, et al. Diagnosis, management and treatment of hepatitis C. Hepatol. 2004;39:1147-1171.

Tayama Y, Sugihara K, Sanoh S. Effect of tea beverages on aldehyde oxidase activity. Drug Metab Pharmacokinet. 2011;26:94-101.

Terrault NA, Bzowej NH, Chang KM, et al. AASLD guidelines for treatment of chronic hepatitis B. Hepatol. 2016;63:261-283.

The International Genome Sample Resource (IGSR): 1000 Genomes Project. http://www.internationalgenome.org/home

Trepo C., Chan HL, Lok A. Hepatitis B virus infection. Lancet. 2014;384:2053-2063.

Tseng TC, Liu CJ, Yang HC. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. Gastroenterol. 2012;142:1140-1149.

Wang XY, Chen HS. Emerging antivirals for the treatment of hepatitis B. World J Gastroenterol. 2014;20:7707-7717.

Wasley A., Kruszon-Moran D, Kuhnert W. The Prevalence of Hepatitis B Virus Infection in the United States in the Era of Vaccination. J Infect Dis. 2010;202:192-201.

World Health Organization. Hepatitis B. Global Alert and Response (GAR) 2002.

World Health Organization. Hepatitis B. World Health Organization Fact Sheet. Updated July 2016.

Appendix 1 Schedule of Assessments: SAD

Visit	Screening	Treatment Period					Follow Up Visit	Follow Up Call
Day	D-28 to D-2	Day -1	Day 1	Day 2	Day 3	Day 5	Day 8[a]	Day 29[b]
Informed Consent	x							
Eligibility	x	x						
Demography	x							
Medical History	x							
Physical Examination[c]	x	x					x	
Vital Signs[d]	x	x	7	2	x		x	
ECG-12 lead[e]	x	x	7	x	x		x	
Plasma PK Sample			12	2	x			
Urine PK Sample			5	x				
PD for Protein Biomarkers		x	4	x	x[f]	x[f]	x	
Whole Blood for RNA		x	4	x			x	
Hematology, Blood Chemistry, Coagulation, Urinalysis	x	x		x			x	
Pregnancy Test[g]	x	x					x	
Administration of Study Medication[h]			x					
Randomisation			x					
Autoimmune panel, Thyroid Function	x							
Viral Serology (HAV, HBV, HCV, HIV)	x							
Follicle Stimulating Hormone[i]	x							
Substance Use[j]	x	x						
In-Clinic Stay[k]		x	x	x	x			
Ambulatory Visit	x					x	x	
Clinical Genotyping[l]			x					
Adverse Events[m]	x[m]	x[m]	x	x	x	x	x	x
Previous and Concomitant Treatments	x	x	x	x	x	x	x	x

Appendix 1 Schedule of Assessments: SAD (cont.)

a) A follow-up visit to be completed 7 days (±1) after the last dose of study medication.
b) A follow-up call to be completed 28 days (±3) after the last dose of study medication.
c) Full physical examination, including recording of weight, is required at Screening, Day −1 and Follow-Up visit. At all other 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.
d) Vital signs include blood pressure, pulse rate, respiratory rate and body temperature. Blood pressure, respiratory rate and pulse rate will be obtained after the subject has been in a supine *or sitting* position for at least 5 minutes. Blood pressure measurement will be performed in triplicate (can be as short as 20-second to 1-minute interval between measurements). Pulse rate and body temperature measurement will be performed as single assessments.
e) 12-lead ECGs will be obtained in triplicate (three consecutive interpretable 12-lead ECGs within a 2-5 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.
f) PD samples at 48 and 96 hours for neopterin only (no other PD markers).
g) Serum or plasma beta-human chorionic gonadotropin (β-HCG) at Screening, urine on all other occasions (females only).
h) Study drug will be administered in fasted state (after an overnight fast of at least 10 hours).
i) Follicle-stimulating hormone (females only to confirm post-menopausal status, performed at screening only).
j) At Screening and Day − 1 only (drugs of abuse and alcohol test).
k) Subjects will be admitted to the unit on Day − 1 to start time-matching ECG assessments. The discharge from the unit will be on Day 3 after 48-hour assessments.
l) If the clinical genotyping blood sample is not collected at Day 1, it may be collected at any time during the conduct of the clinical study.
m) At Screening, Day − 2 and Day − 1, only serious adverse events caused by a protocol-mandated intervention should be reported.

Appendix 2 Schedule of Assessments: SAD Detailed

Visit	Day	Scheduled Time (h)	Vital Signs[a]	ECG-12 lead[b]	Hematology, Blood Chemistry, Coagulation, Urinalysis	Plasma PK Sample[c]	Urine PK Sample[d]	PD for Protein Biomarkers[e]	Whole Blood for RNA[e]	Clinical Genotyping[f]	Administration of Study Medication[g]
	Day -1		x	x	x			x	x		
	Day 1	Predose	x	x		x	x	x	x	x	
		0					0-4				x
		0.25				x					
		0.5	x	x		x					
		1	x	x		x					
		1.5				x					
		2	x	x		x		x	x		
		3				x					
		4	x	x		x					
		6	x	x		x	4-8	x	x		
		8				x					
		12	x	x		x	8-12	x	x		
		18				x	12-24				
	Day 2	24	x	x	x	x		x	x		
		36	x			x					
	Day 3	48	x	x		x		x			
	Day 5	96						x			
Follow Up Visit	Day 8[h]		x	x	x			x	x		

Appendix 2 Schedule of Assessments: SAD Detailed (cont.)

a) Vital signs include blood pressure, pulse rate, respiratory rate and body temperature. Blood pressure, respiratory rate and pulse rate will be obtained after the subject has been in a supine *or sitting* position for at least 5 minutes. Blood pressure measurement will be performed in triplicate (can be as short as 20-second to 1-minute interval between measurements). Pulse rate and body temperature measurement will be performed as single assessments.

b) 12-lead ECGs will be obtained in triplicate (three consecutive interpretable 12-lead ECGs within a 2-5 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 PK samples will be collected pre-dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, and 48 hours post-dose.

d) A single void urine sample will be collected pre-dose on Day 1. Pooled urine PK samples will be collected 0 to 4 hours, 4 to 8 hours, 8 to 12 hours and 12 to 24 hours post-dose.

e) PD samples will be collected at pre-dose, 2, 6, 12, 24, 48 and 96 hours post-dose and follow up visit. Samples at 48 and 96 hours post-dose will be collected for neopterin only (no other PD markers).

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

g) Study drug will be administered in fasted state (after an overnight fast of at least 10 hours).

h) A follow up visit to be completed 7 days ($\pm$1) after the last dose of study medication.

Appendix 3 Schedule of Assessments: MAD

Visit	Screening	Treatment Period															Follow Up Visit	Follow Up Call
Day	D-28 to D-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 20[a]	Day 41[b]
Informed Consent	x																	
Eligibility	x	x																
Demography	x																	
Medical History	x																	
Physical Examination[c]	x	x							x							x	x	
Vital Signs[d]	x	x	6	x	4	x	2	x	2	x	2	x	2	x	6	x	x	
ECG-12 lead[e]	x	x	6		3				x						6		x	
Plasma PK Sample			12	x	3	x	3	x	3	x	3	x	3	x	12	x		
PD for Protein Biomarkers		x	4	x	3	x	3	x	3	x					3	x	x	
Whole Blood for RNA		x	4	x	3	x	3	x	3	x					3	x	x	
Hematology, Blood Chemistry, Coagulation, Urinalysis	x	x				x				x			x		x	x	x	
Pregnancy Test[f]	x	x															x	
Administration of Study Medication[g]			x		x		x		x		x		x		x			
In-Clinic Stay[h]		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Ambulatory Vist	x																x	
Randomisation			x															
Autoimmune panel, Thyroid function	x																	
Viral Serology (HAV, HBV, HCV, HIV)	x																x	
Follocle Stimulating Hormone[i]	x																	
Substance Use[j]	x	x																
Clinical Genotyping[k]			x															
Adverse Events	x[l]	x[l]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Previous and Concomitant Treatments	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Appendix 3 Schedule of Assessments: MAD (cont.)

a) A follow-up visit to be completed 7 days (±1) after the last dose of study medication.

b) A follow-up call to be completed 28 days (±3) after the last dose of study medication.

c) Full physical examination, including recording of weight, is required at Screening, Day −1 and Follow-Up visit. At all other 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.

d) Vital signs include blood pressure, pulse rate, respiratory rate and body temperature. Blood pressure, respiratory rate and pulse rate will be obtained after the subject has been in a supine *or sitting* position for at least 5 minutes. Blood pressure measurement will be performed in triplicate (can be as short as 20-second to 1-minute interval between measurements). Pulse rate and body temperature measurement will be performed as single assessments.

e) 12-lead ECGs will be obtained in triplicate (three consecutive interpretable 12-lead ECGs within a 2-5 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.

f) Serum or plasma beta-human chorionic gonadotropin (β-HCG) at Screening, urine on all other occasions (females only).

g) Study drug will be administered in fasted state (after an overnight fast of at least 10 hours).

h) Subjects will be admitted to the unit on Day −2 to start time-matching ECG assessments in the morning of Day −1. Subjects will stay in the unit till Day 14, with the first dose given on Day 1 and last dose given on Day 13.

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

j) At Screening and Day −1 only (drugs of abuse urine test and alcohol test).

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

l) At Screening, Day −2 and Day −1, only serious adverse events caused by a protocol-mandated intervention should be reported.

Appendix 4 Schedule of Assessments: MAD Detailed

Period	Day	Scheduled Time (h)	Vital Signs[a]	ECG-12 lead[b]	Hematology, Blood Chemistry, Coagulation, Urinalysis	Plasma PK Sample[c]	PD for Protein Biomarkers[d]	Whole Blood for RNA[d]	Clinical Genotyping[e]	Administration of Study Medication[f]
	Day -1		x	x	x		x	x		
Treatment Period	Day 1	Predose	x	x		x	x	x	x	
		0								x
		0.25				x				
		0.5	x	x		x				
		1	x	x		x				
		1.5				x				
		2	x	x		x	x	x		
		3				x				
		4	x	x		x				
		6				x	x	x		
		8				x				
		12	x	x		x	x	x		
		18				x				
	Day 2	24	x			x	x	x		
	Day 3	Predose	x	x		x	x	x		
		0								x
		2	x	x		x	x	x		
		4	x	x						
		6	x			x	x	x		
	Day 4	24	x		x	x	x	x		
	Day 5	Predose	x			x	x	x		
		0								x
		2				x	x	x		
		6	x			x	x	x		
	Day 6	24	x			x	x	x		
	Day 7	Predose	x	x		x	x	x		
		0								x
		2				x	x	x		
		6	x			x	x	x		
	Day 8	24	x		x	x	x	x		
	Day 9	Predose	x			x				
		0								x
		2				x				
		6	x			x				
	Day 10	24	x			x				
	Day 11	Predose	x		x	x				
		0								x
		2				x				
		6	x			x				
	Day 12	24	x			x				
	Day 13	Predose	x	x	x	x	x	x		
		0								x
		0.25				x				
		0.5	x	x		x				
		1	x	x		x				
		1.5				x				
		2	x	x		x	x	x		
		3				x				
		4	x	x		x				
		6				x	x	x		
		8				x				
		12	x	x		x				
		18				x				
	Day 14	24	x		x	x	x	x		
Follow Up Visit	Day 20[g]		x	x	x		x	x		

Appendix 4 Schedule of Assessments: MAD Detailed (cont.)

a) Vital signs include blood pressure, pulse rate, respiratory rate and body temperature. Blood pressure, respiratory rate and pulse rate will be obtained after the subject has been in a supine *or sitting* position for at least 5 minutes. Blood pressure measurement will be performed in triplicate (can be as short as 20-second to 1-minute interval between measurements). Pulse rate and body temperature measurement will be performed as single assessments.

b) 12-lead ECGs will be obtained in triplicate (three consecutive interpretable 12-lead ECGs within a 2-5 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 PK samples will be collected: pre-dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18 and 24 post-dose from Day 1 to Day 2 and from Day 13 to Day 14. On Days 3, 5, 7, 9, and 11, samples for PK will be taken at pre‑dose and 2, 6 and 24 hours post-dose.

d) PD samples will be collected: pre-dose and 2, 6, 12 and 24 post-dose from Day 1 to Day 2. On Days 3, 5, 7 and 13 samples for PD will be taken at pre‑dose and 2, 6 and 24 hours post-dose. PD samples will be collected on follow-up visit.

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

f) Study drug will be administered in fasted state (after an overnight fast of at least 10 hours).

g) A follow-up visit to be completed 7 days (±1) after the last dose of study medication.

Appendix 5 Formula for Calculation of BMI

$$BMI = \frac{\text{Weight (kg)}}{\text{Height (m)}^2}$$

Unit Conversion: 1 kg = 2.2 lbs

1 inch = 2.54 cm

Example: BMI of a subject being 1.70 m tall and weighing 80 kg:

$$\frac{80\text{kg}}{(1.70\text{m})^2} = 27.7\ \text{kg/m}^2$$

The subject's standing height will be measured in bare feet standing with his/her heels and back in contact with the vertical bar of a wall mounted measuring device. The head is held so the subject looks straight forward. A level will be placed on the subject's head to ensure that the subject is looking straight forward. The point at which the lower surface of the level intersects with the vertical measuring device will be the standing height.

Appendix 6 Correction Formulas for QTc Intervals

Fridericia's correction for QTc Measurement - QTcF

$$\text{QTcF (msec)} = \frac{\text{QT (msec)}}{\sqrt[3]{\text{RR(msec)}/1000}}$$

Example: QTcF of a subject with a QT of 386 msec and a RR of 848 msec

QT (msec) = 386

RR (msec) = 848

$$\frac{\text{QT (msec)}}{\sqrt[3]{\text{RR(msec)}/1000}} = \quad 408 \text{ msec}$$

Appendix 7 Cockcroft-Gault Equation for Calculation CrCl

The Cockcroft-Gault equation will be 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:

$$\textit{Males (ml/min)} = \frac{\textit{(140 – Age) * ABW (kg)}}{\textit{72 * Serum Creatinine (mg/dL)}}$$

Females (ml/min) = Male value x 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

Appendix 8 Alcohol Volume Calculation

This appendix is to facilitate the assessment of exclusion criterion in Section 4.2.3. It shows a calculation for the study entry assessment on whether a subject drinks more than two standard drinks per day.

A standard drink is any drink containing 10 grams of alcohol. One standard drink always contains the same amount of alcohol regardless of container size or alcohol type, that is beer, wine, or spirit.

The formula for calculating standard drinks:

Number of standard drinks = Volume (liter) x Vol% of alcoholic beverage x 0.789

For example, one bottle of 375 mL of full strength beer 5% alcohol by volume equals to 1.5 standard drinks:

$0.375 \times 5 \times 0.789 = 1.5$ standard drinks