**Official Title:** A Randomized, Investigator- /Subject- Blind, Single- and Multiple-Ascending Dose, Placebo-Controlled Study to Investigate Safety, Tolerability, Pharmacokinetics, Pharmacodynamics and Food Effect of RO6953958 (Including RO6953958 Effect on Midazolam) Following Oral Administration in Healthy Male Participants

**NCT Number:** NCT04475848

**Document Date:** Protocol Version 7: 05-November-2021

# PROTOCOL

| | |
|---|---|
| **TITLE:** | **A RANDOMIZED, INVESTIGATOR- /SUBJECT-BLIND, SINGLE- AND MULTIPLE-ASCENDING DOSE, PLACEBO-CONTROLLED STUDY TO INVESTIGATE SAFETY, TOLERABILITY, PHARMACOKINETICS, PHARMACODYNAMICS AND FOOD EFFECT OF RO6953958** ***(INCLUDING RO6953958 EFFECT ON MIDAZOLAM)*** **FOLLOWING ORAL ADMINISTRATION IN HEALTHY MALE PARTICIPANTS** |
| **PROTOCOL NUMBER:** | BP41695 |
| **VERSION:** | 7 |
| **EUDRACT NUMBER:** | 2019-004486-41 |
| **IND NUMBER:** | Not applicable |
| **TEST PRODUCT:** | RO6953958 |
| **SPONSOR:** | F. Hoffmann-La Roche Ltd |
| **DATE FINAL:** | Version 1: 13 December 2019 |
| **DATE AMENDED:** | Version 2: 28 February 2020<br>Version 3: 26 May 2020<br>Version 4: 06 Nov 2020<br>Version 5: 02 February 2021<br>Version 6: 19 April 2021<br>Version 7: See electronic date stamp below |

## FINAL PROTOCOL APPROVAL

| Date and Time (UTC) | Title | Approver's Name |
|---|---|---|
| 05-Nov-2021 10:46:36 | Company Signatory | [redacted] |

## PROTOCOL ACCEPTANCE FORM

| | |
|---|---|
| **TITLE:** | **A RANDOMIZED, INVESTIGATOR- /SUBJECT-BLIND, SINGLE- AND MULTIPLE-ASCENDING DOSE, PLACEBO-CONTROLLED STUDY TO INVESTIGATE SAFETY, TOLERABILITY, PHARMACOKINETICS, PHARMACODYNAMICS AND FOOD EFFECT OF RO6953958** ***(INCLUDING RO6953958 EFFECT ON MIDAZOLAM)*** **FOLLOWING ORAL ADMINISTRATION IN HEALTHY MALE PARTICIPANTS** |
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| **IND NUMBER:** | Not applicable |
| **TEST PRODUCT:** | RO6953958 |
| **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 Site Monitor.

## PROTOCOL AMENDMENT, VERSION 7: RATIONALE

Protocol BP41695 has been amended to include an additional study part (Part 3 Drug-drug interaction [DDI]) to assess the effect of RO6953958 on the pharmacokinetics of the cytochrome P450 (CYP) 3A substrate midazolam. Changes to the protocol, along with a rationale for each change, are summarized below:

- Addition of Study Part 3 (DDI)
  - The study title has been updated.
  - Figure 2 has been added to Section 1.2 (Schematic of Study Design).
  - Tables have been added to Section 1.3 (Schedule of Activities) (Table 5 overall schedule, Tables 6 and 7, detailed schedules).
  - Section 2.1 (Study Rationale) has been updated to include Part 3 DDI
  - Section 3 (Objectives) has been updated to include Part 3 objectives.
  - Section 4.1 (Study Design) has been updated to include Part 3 overall design.
  - Section 4.1.3 (Part 3 DDI) has been added to include Part 3 study design.
  - Section 4.1.4 (Length of study) has been updated to include the duration and activities of Part 3.
  - Section 4.2.7 (Rationale for Drug-Drug Interaction Evaluation and the Use of Midazolam) has been added to provide background for microdosing with midazolam.
  - Section 4.2.8 (Rationale for Measuring 4β-Hydroxycholesterol/Cholesterol Ratio) has been added to provide background for using the 4β-hydroxycholesterol/cholesterol ratio in plasma as an endogenous marker for hepatic CYP3A4 inhibition. Section 8.7.14 was added to cover the blood samples required for this analysis.
  - Section 4.3.2 (Part 3 Dose Justification) has been updated to include dose justifications for RO6953958 and midazolam.
  - Section 5.2 (Exclusion criteria), Criterion #20 was added to account for use of midazolam.
  - Section 6 (Treatment) and 6.1 (Treatment administered) were updated to include midazolam as non-investigational medicinal product (NIMP).
  - Section 6.3.1 (Method of Treatment Assignment) was updated to include enrolment details for Part 3.
  - Section 8.5 (PK) was updated to include plasma concentration determination of midazolam.
  - Section 9.2.2 (Part 3 Drug-Drug Interaction) was added to include sample size determination in Part 3.

  - Section 9.4.3.1 (Pharmacokinetic Parameters) has been updated to include Part 3 PK parameters.
  - Section 9.4.3.2 (Statistical Analysis Methods) has been updated to include Part 3.
- Further changes:
  - Dose escalation: Figure 1, Table 11, and Table 12 have been updated to reflect the actual doses given in the ongoing study (Part 1 and Part 2).
  - Section 2.1 (Study Rationale), Section 2.2.1 (background on indication), and Section 2.2.2 (Background on RO6953958) have been updated to align with the most recent Investigator's Brochure, now referring to "neurodevelopmental disorders" (NDDs) as an indication instead of "autism spectrum disorder (ASD) and autism-related sleep disorders". Section 4.2.2 (Rationale for Study Population) has been updated accordingly. The requirement to exclude participants with sleep disorder from study Part 2 has been removed from exclusion criterion #31.
  - Section 2.2.2.2 (Preliminary Blinded Safety and Pharmacokinetic and Safety Data Observed in Part 1 and Part 2) has been updated to include new participant data.
  - Because the theoretical risk of blood pressure decrease has not been fully evaluated, Section 2.3 (Benefit/Risk Assessment) has been updated to indicate that care should be taken by participants not to stand up abruptly or without using the support of a stable object following dosing with RO6953958.
  - Section 8.7.1.1 (Clinical genotyping) was updated to include additional genotyping assessments.

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

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## LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| Abbreviation | Definition |
|---|---|
| *4βHC* | *4β-hydroxycholesterol* |
| **AE** | Adverse event |
| **ALT** | Alanine aminotransferase |
| *AR* | *Accumulation ratio* |
| **ASD** | Autism Spectrum Disorder |
| **AST** | Aspartate aminotransferase |
| **AUC** | Area under the curve |
| **$AUC_{inf}$** | Area under the concentration–time curve from Time 0 to infinity |
| **$AUC_{0-24}$** | Area under the plasma concentration–time curve from 0 to 24 hours |
| *BCRP* | *Breast cancer resistance protein* |
| **BID** | Twice daily |
| **C-SSRS** | Columbia Suicide Severity Rating Scale |
| **CI** | Confidence interval |
| **$C_{max}$** | Maximum observed concentration |
| **CNS** | Central nervous system |
| **COVID-19** | Corona virus pandemic: the name designation refers to COVI for the acronym of coronavirus, D for the word disease, and 19 for the year of the outbreak. |
| **CSF** | Cerebrospinal Fluid |
| **CSR** | Clinical study report |
| **CTCAE** | Common terminology criteria for adverse events |
| *CV* | *Coefficient of variation* |
| **CYP** | Cytochrome P450 |
| *DDI* | *Drug-drug interaction* |
| *DRF* | *Dose-range finding* |
| **EC** | Ethics Committee |
| **ECG** | Electrocardiogram |
| **eCRF** | Electronic case report form |
| **EDC** | Electronic data capture |
| *EFD* | *Embryo-fetal development* |
| **ESS** | Epworth Sleepiness Scale |
| **EU** | European Union |
| **FDA** | Food and Drug Administration |
| **FE** | Food effect |
| **FERT** | Facial Emotion Recognition Task |

| Abbreviation | Definition |
|---|---|
| **GCP** | Good Clinical Practice |
| **GLP** | Good Laboratory Practice |
| **HBsAg** | Hepatitis B surface antigen |
| **HCV** | Hepatitis C virus |
| **HED** | human equivalent dose |
| *hERG* | *Human ether à go-go* |
| **HIV** | Human immunodeficiency virus |
| **HR** | Heart rate |
| **ICF** | Informed Consent Form |
| **ICH** | International Council on Harmonisation |
| **IEC** | Independent Ethics Committee |
| **IMP** | Investigational medicinal product |
| **INR** | International normalized ratio |
| **IRB** | Institutional Review Board |
| **IRC** | Independent Review Committee |
| **IV** | Intravenous |
| $K_b$ | *Dissociation constant* |
| **KSS** | Karolinska Sleepiness Scale |
| **LPLO** | Last participant, last observation |
| *M1* | *RO7045755* |
| *M3* | *RO7021594* |
| **MAD** | Multiple-ascending dose |
| **MEQ** | Morningness–eveningness Questionnaire |
| **MFD** | Maximum feasible dose |
| **MHRA** | Medicines and Healthcare products Regulatory Agency |
| **NCI** | National Cancer Institute |
| *NDD* | *Neurodevelopmental disorder* |
| *NIMP* | *Non-investigational product* |
| **NOAEL** | No-observed-adverse-effect level |
| **NSAESI** | Non-serious adverse event of special interest |
| **OATP** | Organic anion-transporting polypeptide |
| **OTC** | Over-the-counter |
| **PAA** | Platelet aggregation assay |
| **PAD** | Pharmacologically active dose |
| **PD** | Pharmacodynamic(s) |
| **P-gp** | P-glycoprotein |
| **PK** | Pharmacokinetic(s) |

| Abbreviation | Definition |
|---|---|
| **PR** | PR interval of the ECG |
| **PROMIS** | Patient-Reported Outcomes Measurement Information System |
| **PSG** | Polysomnography |
| **PSQI** | Pittsburgh Sleep Quality Index |
| **QD** | Once daily |
| **QRS** | QRS interval of the ECG |
| **QT** | QT interval of the ECG |
| **QTc** | Corrected QT interval |
| **QTcF** | Corrected QT interval using the Fridericia's formula |
| **RBR** | Research biosample repository |
| **REC** | Research Ethics Committee |
| **RR** | RR interval of the ECG |
| **SAD** | Single-ascending dose |
| **SAE** | Serious adverse event |
| **SARS-CoV-2** | Severe acute respiratory syndrome coronavirus 2 |
| **SE** | Sleep efficiency |
| *SMS* | *Smith-Magenis syndrome* |
| **SoA** | Schedule of activities |
| **SUSAR** | Suspected unexpected serious adverse reactions |
| *TEAE* | *Treatment-emergent adverse event* |
| **$t_{max}$** | Time of maximum concentration observed |
| **ULN** | Upper limit of normal |
| **US** | United States |
| **V** | Volume |
| **V/F** | Apparent volume of distribution |
| **$V_{ss}$** | Volume of distribution under steady-state conditions |
| **WOCBP** | Women of childbearing potential |

# 1. PROTOCOL SUMMARY

## 1.1 SYNOPSIS

| | |
|---|---|
| **PROTOCOL TITLE:** | **A RANDOMIZED, INVESTIGATOR- /SUBJECT-BLIND, SINGLE- AND MULTIPLE-ASCENDING DOSE, PLACEBO-CONTROLLED STUDY TO INVESTIGATE SAFETY, TOLERABILITY, PHARMACOKINETICS, PHARMACODYNAMICS AND FOOD EFFECT OF RO6953958** *(INCLUDING RO6953958 EFFECT ON MIDAZOLAM)* **FOLLOWING ORAL ADMINISTRATION IN HEALTHY MALE PARTICIPANTS** |
| **SHORT TITLE** | A Randomized, SAD/MAD/*DDI*, Placebo-Controlled Study for Safety, Tolerability, PK, PD, and Food Effect following RO6953958 Administration in Healthy Participants |
| **PROTOCOL NUMBER:** | BP41695 |
| **VERSION:** | 7 |
| **TEST PRODUCT:** | RO6953958 |
| **PHASE:** | I |

**RATIONALE**

RO6953958 is a potent and selective human vasopressin V1a receptor antagonist that blocks the activation of the V1a G protein-coupled receptor. *Evidence from mouse studies implicates V1a receptor antagonism in the accelerated resynchronization of circadian rhythm.* RO6953958 is highly potent on the human V1a receptor (*dissociation constant* [Kb] = 0.22 nM) and may provide a novel and first approach to treat *circadian disturbances and social* deficits *in neurodevelopmental* disorders *(NDDs).* This is the first study with RO6953958 in humans, designed to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of single- and multiple-ascending doses (MAD)*,* food effect (FE)*, and drug-drug interaction (DDI) potential* following oral administration to healthy male participants. The study results will support further clinical development of RO6953958.

**OBJECTIVES AND ENDPOINTS**

| | Objectives | Endpoints |
|---|---|---|
| **Primary** | | |
| **Parts 1*, 2*, and *3*** | • To assess the safety and tolerability of single- and multiple-ascending doses of RO6953958 *alone and in combination with midazolam (Part 3 only)* in healthy participants | • Incidence and severity of AEs. Changes in vital signs, physical findings, ECG parameters (12-lead safety *in all parts* and 24-hour Holter *in Part 1 and Part 2 only*), and clinical laboratory results during and following RO6953958 administration<br>• Change in suicide risk (using Columbia Suicide Severity Rating Scale) in Part 2 *and 3* only |
| **Secondary** | | |
| **Parts 1*, 2*, and *3*** | • To investigate the PK of RO6953958 and its metabolites RO7021594 and RO7045755 following single and multiple doses *of RO6953958 alone and in combination with midazolam (Part 3 only)*<br>• To investigate the dose proportionality of RO6953958 | • PK concentrations and PK parameters of RO6953958 and its metabolites RO7021594 and RO7045755 |
| **Part 1** | • To assess the effect of food on the PK of a single dose of RO6953958 | • PK concentrations and PK parameters of RO6953958 and its metabolites RO7021594 and RO7045755 in fasted and fed state |
| ***Part 3*** | • *To investigate the effect of RO6953958 on the PK of the cytochrome P450 (CYP) 3A substrate midazolam* | • *PK concentrations and parameters of midazolam* |

**OVERALL DESIGN**

The study is a *three*-part design. Part 1 (single-ascending dose [SAD]) of this study will examine safety and tolerability, and characterize PK and PD effect by evaluating the Karolinska sleepiness scale (KSS). Part 1 will also evaluate FE after RO6953958 administration at the dose(s) proposed for clinical use. In Part 2 (multiple-ascending dose [MAD]) the safety, tolerability, PK and PD effects by evaluating cortisol, melatonin, and sleep parameters of multiple doses of RO6953958 will be evaluated in fed conditions, based on preliminary PK data in Part 1. In addition, the concentration of RO6953958 and its metabolites will be measured in CSF.

The doses in Part 2 will be determined based on review of the Part 1 data. Ascending doses are planned in order to establish the safety and tolerability at low dose levels before proceeding to higher dose levels. Dose levels may be adjusted (increased, decreased, or repeated), or intermediate doses may be used depending on the emerging safety and PK data at each dose level.

*In Part 3, the safety, tolerability, and effect of RO6953958 on the pharmacokinetics of the cytochrome P450 (CYP) 3A substrate midazolam will be assessed by evaluating PK parameters of midazolam. In addition, concentrations of 4β-hydroxycholesterol and total cholesterol will be measured and the 4β-hydroxycholesterol/cholesterol ratio will be calculated as an endogenous marker of CYP3A activity.*

**Study Design**

**Part 1 (SAD/FE)**

Part 1 will be a randomized, investigator-/subject-blind, placebo-controlled SAD study with a parallel design and sentinel dosing, to explore the safety, tolerability, PK, and PD of single doses of orally administered RO6953958 in the fasted state and using one cohort to administer RO6953958 in the fed state.

Participants will be recruited in seven sequential cohorts. In each cohort, participants will receive either a single oral dose of RO6953958 or placebo while fasted. Participants in the FE cohort (90 mg) will return to receive the same dose repeated in the fed state. Each participant in the selected cohort will receive a single dose of RO6953958 30 minutes after starting a standardized high fat, high calorie breakfast. If participants are not willing to complete the fed part, additional participants will be recruited to ensure that a minimum number of participants complete food effect investigations (6 on active treatment and 2 on placebo in each period).

The starting dose of 5 mg is anticipated to be safe and have minimal pharmacological effects. Subsequent doses will be selected during study conduct based on emerging safety and PK data. In all cohorts, sentinel dosing will be employed to allow for an evaluation of safety data by the Investigator up to 23 hours after the start of treatment before subsequent participants are dosed. There will be at least 1 week between each dose level in order to permit adequate time for collation and review of emerging data before the next dose is administered. After review of the PK exposures in Cohort 1 and in the case that the observed exposure (maximum observed concentration [$C_{max}$] and area under the concentration–time curve from Time 0 to infinity [$AUC_{inf}$]) of the first dose in humans is significantly below the predicted concentrations, the dose of Cohort 2 may be adjusted by a greater than 3.3-fold increase in order to reach the expected exposure level for the original starting dose (with the same maximum increase of 3.3-fold multiples thereafter for further dose-escalation steps).

**Part 2 (MAD)**

Part 2 will be a randomized, Investigator- /subject-blind, placebo-controlled MAD study with the purpose of evaluating the safety, tolerability, PK, and PD of multiple doses of orally administered RO6953958. Participants from Part 1 cannot participate in Part 2.

Part 2 will explore the safety, tolerability, PK, and PD of multiple doses of RO6953958. The starting dose for Part 2 is 45 mg RO6953958 once daily (QD), established on the basis of safety and PK data in Part 1. The first dosing in Part 2 will only commence once safety is confirmed on the basis of dosing in Part 1 that allows at least 4-times' coverage (e.g., start 40 mg MAD once safety confirmed at a dose of 160 mg or higher in SAD). A maximum of five dose levels are anticipated. For each dose level, a minimum of 8 and a maximum of 16 participants will receive either a multiple oral dose of RO6953958 or placebo once daily for 10 days (active to placebo ratio 3:1). The treatment period may be extended to 14 days in subsequent cohorts depending on emerging data.

In addition, a lumbar puncture for CSF sampling will be performed in all participants in each cohort on Day 7 or alternatively Day 6 or 8. In a cohort, CSF sampling will be done predose in 4 participants and approximately 5 to 7 h after dosing of RO6953958 (around $T_{max}$ of the M3 metabolite) in the other 4 participants for determination of RO6953958, M3 (RO7021594) and M1 (RO7045755) concentrations in CSF. A corresponding blood sample will be taken at the same time point. The time point of CSF sampling in a participant may be adjusted with emerging

PK or CSF samples from preceding cohorts. In addition, these results might also lead to the conclusion to stop CSF sampling in subsequent cohorts.

The dose may be adjusted during the treatment period depending on the safety and/or PK observations. In addition, a different dosing regimen may be explored if warranted based on the safety and/or PK profile of RO6953958 (e.g., twice daily or exploration of titration). The starting dose in Part 2 Cohort 1 selected based on the safety and PK from Part 1 is 45 mg RO6953958 QD. Based on the outcome of Part 1, RO6953958 will be administered under fed conditions (a standard breakfast). Subsequent doses in Part 2 will be based on emerging safety and PK data. Additionally, the timing of dosing may be changed for additional participants in a cohort at a specific dose level, in order to explore the effect of time of dosing on the pharmacokinetics. A decision to include additional participants dosed in the evening at a specific dose level would only be made following review of the safety and PK following morning administration at the chosen dose level. CSF sampling would not be done in participants who are administered the dose in the evening.

***Part 3 (DDI)***

*Part 3 will be a single-center, non-randomized, open-label, five-treatment, fixed sequence crossover study with the purpose of evaluating the safety and tolerability of RO6953958 alone and in combination with midazolam and to assess the effect of RO6953958 on the PK of the CYP3A substrate midazolam. In addition, the effect of RO6953958 treatment on an endogenous marker of CYP3A activity will be assessed.*

*RO6953958 will be administered QD following a standardized breakfast on Day 3 to Day 14 at the maximum dose QD that was tested in the ongoing Part 2 (MAD). Midazolam will be administered as single IV bolus injection of 100 µg on Day 1 and Day 13, and as single oral dose of 300 µg on Day 2 and Day 14.*

**Treatment Groups and Duration**

The following confirmed and tentative dose levels are planned for Part 1 and 2:

- SAD Cohort 1: 5 mg RO6953958 or matching placebo
- SAD Cohort 2: 15 mg RO6953958 or matching placebo
- SAD Cohort 3: 45 mg RO6953958 or matching placebo
- SAD Cohort 4: 90 mg RO6953958 or matching placebo and FE*
- SAD Cohort 5: 180 mg RO6953958 or matching placebo
- SAD Cohort 6: 360 mg RO6953958 or matching placebo
- SAD Cohort 7: RO6953958 or matching placebo** – *dose to be determined*
- MAD Cohort 1: 45 mg RO6953958 or matching placebo
- MAD Cohort 2: *140* mg RO6953958 or matching placebo
- MAD Cohort 3: *210 mg* RO6953958 or matching placebo
- MAD Cohort 4: RO6953958 or matching placebo – dose to be determined
- MAD Cohort 5: RO6953958 or matching placebo – dose to be determined

* Cohort 4 will return to receive the same dose in the fed state (a single dose of RO6953958 or placebo 30 minutes after starting a standardized high fat, high calorie breakfast).

** Cohort 7 may run pending emerging data review from the MAD. Timing of dosing of RO6953958 may be in the evening instead of the morning, and dosing may be done under different food condition (fed vs fasted).

*Part 3 (DDI):*

- *RO6953958 - maximum dose QD tested in Part 2 – to be given QD for 12 days (Day 3 through Day 14)*
- *midazolam - 100 µg – two single IV doses on Day 1 and Day 13*
- *midazolam - 300 µg – two single oral doses on Day 2 and Day 14*

The investigational medicinal products (IMPs) for this study are RO6953958 capsules 2.5 mg, 10 mg, and 80 mg; and matching placebo. *Midazolam is a non-investigational product.*

**Dose-Escalation Decision Criteria**

The dose-escalation decision will be made by the Roche Clinical Pharmacologist, the Principal Investigator, and appropriate personnel as required. The dose will not be escalated further if the tolerability, safety, or PK at the preceding dose level is not acceptable as judged by the Principal Investigator and the Clinical Pharmacologist. After review of the safety and PK data and discussion between the Roche Clinical Pharmacologist and the Principal Investigator, the same dose or regimen or a lower dose within the tolerated dose range may be given again in subsequent treatments to increase data within the tolerated dose range.

Dose escalation in Part 1 and Part 2 for all participants will be stopped if any of the predefined stopping rules are met in participants receiving RO6953958. However, the same or a lower dose may be administered after the discussion between the Sponsor's Clinical Pharmacologist and the Investigator. Additionally, dosing will be stopped if any of the stopping rules applies during the conduct of the study and applies to an individual participant during the study.

Participants who prematurely discontinued from the study may be replaced to ensure adequate numbers (at least 6 participants; 4 on RO6953958) of evaluable participants for each dose escalation step.

Dose escalation is dependent on the safety and PK data at each dose level. Dose-level increments will be no more than 3.3-fold and may be adjusted downwards, or doses may be repeated if safety or PK concerns emerge from ongoing results. In the case that the observed exposure at the first SAD dose level (Cohort 1) is significantly below the predicted human exposure, a dose increment higher than 3.3-fold may be given subsequently at the second dose level (Cohort 2) to achieve the predicted exposure for the starting dose (with the same dose-escalation multiples thereafter).

Due to the exploratory nature of this clinical study, its conduct can be discontinued before the last foreseen dose level has been investigated. This will not constitute a premature termination of the study.

**Stopping Rules**

**Stopping Rules for the Study**

The trial will be stopped if the following criteria are met:

- A serious adverse reaction (i.e., a serious adverse event [SAE] considered as, at least, possibly related to the IMP administration) in one participant or
- Severe adverse reactions (i.e., severe non-serious adverse events considered as, at least, possibly related to the IMP administration) in two participants in the same cohort.

Following an internal safety review, if it is appropriate to restart the trial, a substantial amendment will be submitted to the Medicines and Healthcare products Regulatory Agency (MHRA) and Research Ethics Committee (REC). The trial will not restart until the amendment has been approved by the MHRA and REC.

**Stopping Rules for Individuals**

Dosing will be stopped at any time during the study in a given participant receiving RO6953958 if one of the following circumstances occurs:

- SAE considered related to study treatment.
- Alanine aminotransferase (ALT) $\geq 5 \times$ upper limit of normal (ULN).
- ALT $\geq 3 \times$ ULN and total bilirubin $\geq 2 \times$ ULN or international normalized ratio (INR) $> 1.5$ (if a participant meets this withdrawal criterion, serum bilirubin fractionation should be performed).
- ALT $\geq 3 \times$ ULN if associated with the appearance or worsening of rash or hepatitis symptoms (fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia). Participants who have ALT $\geq 3 \times$ ULN and $< 5 \times$ ULN, total bilirubin $< 2 \times$ ULN or

INR < 1.5, and who do not exhibit hepatitis symptoms or rash can continue in the study (and continue receiving trial medication) as long as they can be monitored at least weekly until abnormal results are within the reference range or close to pretreatment values.

If a dose administration is suspended to allow for additional safety assessments, but no safety concerns are raised to prevent continued dosing in the judgment of the Investigator, dosing may resume at the next planned dosing date. A missed dose will not be made up (applicable to Part 2 [MAD] only).

**Stopping Rules after Dosing of the Sentinel Group in Part 1 (all Dose Levels)**

At all dose levels in Part 1, in order to avoid simultaneous exposure of all the participants, only 2 participants (sentinel group; composed of 1 participant on active treatment and 1 on placebo) will be dosed first prior to dosing further participants at the same dose level. If the safety and tolerability data (AEs, electrocardiograms [ECG], vital signs, and clinical laboratory test results) of the first 23 hours following dosing for the initial 2 participants are acceptable in the judgment of the Investigator, 6 additional participants (5 on active treatment and 1 on placebo) will be dosed at the same dose level at least 24 hours after the sentinel group. Dosing will be stopped after the sentinel group if one of the following circumstances occurs in the participant receiving RO6953958, unless it is determined by the Investigator that the occurrence is not related to the administration of the study drug:

- One SAE
- Any other findings that, at the joint discretion of the Sponsor's Clinical Pharmacologist, the Medical and Safety monitor and the Investigator, indicate that the dosing should be stopped

**Dose-Escalation and Study Cohort Stopping Rules in Part 1 and Part 2**

Dose-escalation will be stopped and treatment within an ongoing cohort will be stopped in Part 1 and/or Part 2 if one of the following circumstances occurs in participants receiving RO6953958 within the same dose level, unless it is determined by the Investigator that the occurrence is not related to the administration of the study drug:

- SAE considered to be related to study drug in 1 participant receiving RO6953958
- Severe non-serious AEs considered to be related to study drug in 2 or more participants receiving RO6953958 in the same cohort
- Clinical significant RO6953958-related laboratory abnormalities of the same type in 2 or more participants
- Clinical significant RO6953958-related changes in vital signs or ECGs of the same type in 2 or more participants
- If maximum individual exposure is anticipated to exceed the predicted $C_{max}$ (approximately 3500 ng/mL) and AUC (35,000 ng·h/mL) at the next planned dose level based on PK from previous dose cohorts or maximum feasible dose level of 800 mg once daily (QD) is achieved
- Any other findings that, at the joint discretion of the Sponsor's study team and the Investigator, indicate that the dosing should be stopped

**Length of Study**

Part 1 (SAD/FE)

The total duration of the study for each participant in each cohort will be up to 7 weeks (14 weeks if the participant is part of the FE cohort) divided as follows:

- Screening: Up to 4 weeks

- In-clinic period: Days -2 to 5.
- Treatment period: Day 1
- Washout period between each treatment (FE cohort only): Approximately 21 days. This might be adjusted/extended based on PK data from Part 1 or to await safety information from the subsequent dose level to provide adequate safety coverage for the fed part.
- Safety follow-up: 7 to 14 days after the last dose.

Part 2 (MAD)

The total duration of the study for each participant will be up to 8 weeks divided as follows:

- Screening: Up to 4 weeks.
- In-clinic period: 16 days (Days -2[1] to 14; 2 baseline days, 10 dosing days, 6 follow-up days).
- Treatment period: Days 1 to 10 (possibly to Day 14).
- Safety follow-up: 7 to 14 days after last dose.

*Part 3 (DDI)*

*The total duration of the study for each participant will be up to 8 weeks approximately divided as follows:*

- *Screening: Up to 4 weeks.*
- *In-clinic period: 19 days (Days -1 to 18; 1 baseline day, 14 dosing days, 4 follow-up days).*
- *Five treatment periods: Days 1 to 14 (Days 1 and 2 midazolam, Days 3 through 14 RO6953958, Days 13 and 14 midazolam).*
- *Safety follow-up: 7 to 14 days after the final dose of RO6953958.*

**End of Study**

A participant is considered to have completed the study if the participant has completed all phases of the study including the last scheduled procedure.

The end of the study is defined as the date when the last participant, last observation (LPLO) occurs. LPLO is expected to occur approximately 2 weeks after the last participant last dose in Part 3.

## PARTICIPANT POPULATION

The participants in this study will be healthy male volunteers between 18 to 55 years of age, inclusive, who fulfill all of the given inclusion criteria.

**Inclusion/Exclusion Criteria**

**Inclusion Criteria**

Participants are eligible to be included in the study only if all of the following criteria apply:

**Informed Consent**

1. Able and willing to provide written informed consent and to comply with the study protocol according to International Council on Harmonisation (ICH) and local regulations.

[1] Participants may be admitted on Day-3 to ensure severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing can be reported prior to dosing.

**Age**

2. Participants must be 18 to 55 years of age, inclusive, at the time of signing the informed consent form.

**Type of Participants and Disease Characteristics**

3. Healthy, as judged by the Investigator. Healthy status will be defined as the absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination, vital signs, 12-lead ECG, hematology, blood chemistry, serology, and urinalysis.

**Weight**

4. Body mass index (BMI) within the range 18 to 31 kg/m2 (inclusive).

**Sleep (Part 2 MAD Only)**

**Habitual sleep pattern**

5. Participants must be prepared to collect a sleep log and wear an actigraphy device the week before participation in the study. Participants must also have scored 5 or less on the Pittsburgh Sleep Quality Index (PSQI), less than 13 on the Epworth sleepiness scale (ESS), and not be considered an extreme morning or evening type according to the morningness–eveningness questionnaire (MEQ) at screening to be eligible.

**Sex**

6. Male participants

   The contraception and abstinence requirements are intended to prevent exposure of an embryo to the study treatment. The reliability of sexual abstinence for male enrollment eligibility needs to be evaluated in relation to the duration of the clinical study and the preferred and usual lifestyle of the participant. Periodic abstinence and withdrawal are not acceptable methods of contraception.

During the treatment period and for at least 14 days after the last dose of study drug, agreement to:

- Remain abstinent (refrain from heterosexual intercourse) or use a condom with a female partner of childbearing potential, or pregnant female partner, to avoid exposing the embryo.
- Refrain from donating sperm for at least 14 days after last dose.

**<u>Exclusion Criteria</u>**

Participants are excluded from the study if any of the following criteria apply:

**Medical Conditions**

1. Any condition or disease detected during the medical interview/physical examination that would render the participant unsuitable for the study, place the participant at undue risk, or interfere with the ability of the participant to complete the study, as determined by the Investigator.

2. History or evidence of any medical condition potentially altering the absorption, metabolism, or elimination of drugs. This includes a surgical history of the gastrointestinal tract affecting gastric motility or altering the gastrointestinal tract.
3. History of any clinically significant gastrointestinal, renal, hepatic, bronchopulmonary, neurological, psychiatric, cardiovascular, endocrinological, hematological, or allergic disease, sleep disorders (Part 2 [MAD] only), unexplained syncope (within 12 months prior to screening), metabolic disorder, cancer, or cirrhosis.
4. Use of any psychoactive medication, or medications known to have effects on central nervous system (CNS), or blood flow taken within 4 weeks prior to the first dosing (or within 5 times the elimination half-life of the medication) prior to the first dosing (whichever is longer).
5. History of convulsions (other than benign febrile convulsions of childhood), including epilepsy, or personal history of significant cerebral trauma or CNS infections (e.g., meningitis).
6. History of clinically significant hypersensitivity (e.g., drugs, excipients) or allergic reactions.
7. Any major illness within 1 month before the screening examination or any febrile illness within 1 week prior to screening and up to the first study drug administration.
8. Abnormal blood pressure, defined as confirmed (based on the average of $\geq$3 consecutive measurements) systolic blood pressure (SBP) greater than 140 or less than 90 mmHg, and diastolic blood pressure (DBP) greater than 90 or less than 50 mmHg.
9. Presence of orthostatic hypotension at screening. Orthostatic hypotension is defined as decrease by 20 mmHg in SBP and/or 10 mmHg in DBP after erection from a supine position and/or clinical presyncopal/syncopal AE during test.
10. Abnormal pulse rate, defined as confirmed (based on the average of $\geq$ 3 consecutive measurements) resting pulse rate greater than 100 or less than 40 bpm.
11. History or presence of clinically significant ECG abnormalities before study treatment administration (e.g., PQ/PR interval $>$ 220 ms, QT interval corrected using Fridericia's formula [QTcF] $>$ 450 ms) or cardiovascular disease (e.g., cardiac insufficiency, coronary artery disease, cardiomyopathy, congestive heart failure, family history of congenital long QT syndrome, family history of sudden death).
12. Clinically significant abnormalities in laboratory test results (including hepatic and renal panels, complete blood count, chemistry panel, and urinalysis). In the case of uncertain or questionable results, tests performed during screening may be repeated before randomization to confirm eligibility.
13. ALT and/or bilirubin $>$ 1.5 $\times$ ULN (isolated bilirubin $>$ 1.5 $\times$ ULN is acceptable if bilirubin is fractionated and direct bilirubin $<$ 35%).
14. Current or chronic history of liver disease or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
15. Participants who, in the Investigator's judgment, pose a suicidal or homicidal risk, or any participant with a history of suicidal or homicidal attempts.

16. Known active or any major episode of infection within 4 weeks prior to the start of drug administration.
17. Participants who test positive for acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on admission to the study site should not be enrolled.

**Prior/Concomitant Therapy**

18. Have used or intend to use over-the-counter or prescription medication including herbal medications within 30 days prior to dosing
19. Participants likely to need concomitant medication during the study period (including for dental conditions).
20. *Part 3 (DDI) only - History of hypersensitivity to benzodiazepines (including midazolam) or its formulation ingredients.*

**Prior/Concurrent Clinical Study Experience**

21. Participation in an investigational drug or device study within 3 months before admission to this study or more than 4 times a year.

**Diagnostic Assessments**

22. Positive test for drugs of abuse or alcohol.
23. Positive test for human immunodeficiency virus (HIV) antibody at screening.
24. Positive result on hepatitis B virus or hepatitis C virus (HCV), presence of hepatitis B surface antigen (HBsAg), or positive hepatitis C antibody test result at screening or within 3 months prior to starting study treatment.

**Other Exclusions**

25. Dietary restrictions that would prohibit the consumption of standardized meals.
26. Inability or unwillingness to fully consume standardized breakfast at Day 1 (for Part 1, FE cohort).
27. Consumption of any prohibited medications and food/beverages before study start and during the study.
28. Any suspicion or history of alcohol abuse and/or suspicion of regular consumption of drug of abuse or previous history of or treatment for a dependence disorder.
29. Sensitivity to any of the study treatments, or components thereof, or drug or other allergy that, in the opinion of the Investigator, contraindicates the participation in the study.
30. Participants who are smokers for at least 90 days prior to screening.
31. Part 2 (MAD) only – Participants who have issues sleeping or who have travelled across 2 or more time zones in the month prior to screening.
32. Part 2 (MAD) only – Participants who cannot produce sufficient saliva for study assessments.

33. Participants who have donated more than 500 mL of blood or blood products or had significant blood loss within 3 months prior to screening.
34. Participants under judicial supervision, guardianship, or curatorship.

**For participants with planned lumbar punctures, the following additional exclusion criteria apply:**

35. Have a history of clinically significant back pain, back pathology, and/or back injury (e.g. degenerative disease, spinal deformity, or spinal surgery) that may predispose to complications from, or technical difficulty with, lumbar puncture,
36. Have criteria that would preclude a lumbar puncture, such as local infection at the site of the lumbar puncture, clinically significant coagulation parameter abnormalities, or treatment with an anticoagulant or with antiplatelet agents within 6 weeks prior to the day of lumbar puncture,
37. Have a clinically significant hypersensitivity to local anesthetics that may be used for lumbar puncture (e.g. lidocaine).

**NUMBER OF PARTICIPANTS**

Part 1 (SAD)

Fifty-six participants will be randomized, 8 per cohort. For all dose levels, a sentinel group of 2 participants will be randomized as 1 on active treatment and 1 on placebo and the remaining participants will be randomized as 5 on active treatment and 1 on placebo at the same dose level at least 24 hours later. If participants in the FE cohort do not agree to perform the fed part, additional participants will be recruited.

Part 2 (MAD)

Up to 80 participants will be randomized with a minimum of 6 active treatment and 2 placebo participants in each dose level with the option to expand up to a maximum of 12 active treatment and 4 placebo participants per dose level. All dose levels will be assigned to active treatment or placebo using a 3:1 ratio.

*Part 3 (DDI)*

*Sixteen participants will be enrolled to have at least 12 participants complete study Part 3. All participants will receive active treatment.*

**CONCOMITANT MEDICATIONS**

As a general rule, no concomitant medication or vaccine will be permitted, with the exception of COVID-19 vaccines and medications to treat AEs, unless the rationale for exception is discussed and clearly documented between the Investigator and the Sponsor. Participants are allowed to receive a dose of COVID-19 vaccine at least 21 days prior to the first dose of study treatment or a dose of COVID-19 vaccine at least 10 days after last dose of study treatment.

Use of the following therapies is prohibited during the study and for at least 30 days, or at least 5 half-lives of the medication, prior to initiation of study treatment (whichever is longer), unless otherwise specified below:

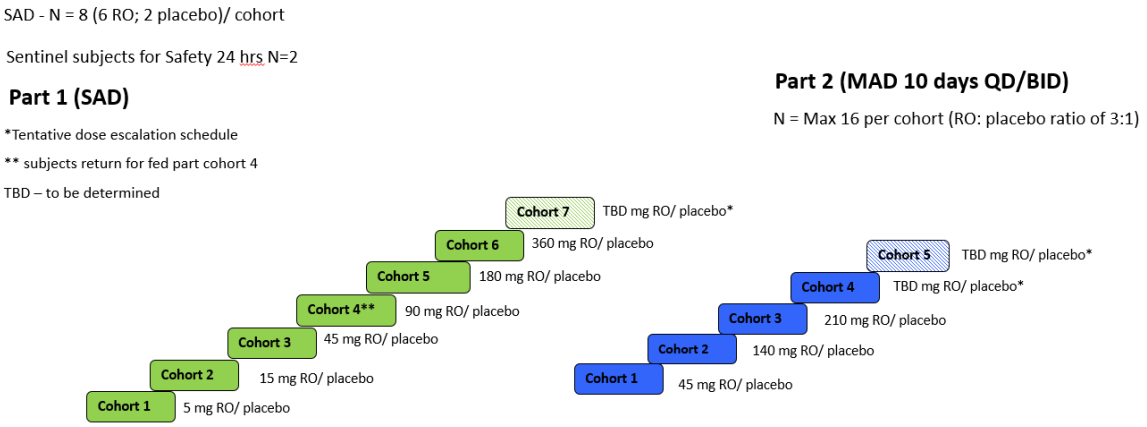
- Any prescribed or OTC medication (including herbal products, vitamin, mineral, dietary supplements, melatonin, and melatonin agonists).

- Any known inhibitor of cytochrome P450 (CYP) 3A4 or CYP2C19 taken within 4 weeks prior to start of administration of study drug (Day 1) or within 5 times the elimination half-life of the medication prior to start of study drug intake (whichever is longer), including but not limited to the following drugs: ritonavir, troleandomycin, telaprevir, danoprevir, elvitegravir, saquinavir, lopinavir, indinavir, nelfinavir, boceprevir, voriconazole, mifepristone, posaconazole, telithromycin, ketoconazole, itraconazole, fluconazole, erythromycin, clarithromycin, mibefradil, ceritinib, ribociclib, idelalisib, conivaptan, nefazodone, diltiazem, verapamil, cimetidine, fluvoxamine, fluoxetine, ASP8477, and ticlopidine.
- Any known inducer of CYP3A4 or CYP2C19 taken within 4 weeks prior to start of administration of study treatment (Day 1), including but not limited to the following drugs: rifampicin, rifabutin, glucocorticoids, carbamazepine, oxcarbazepine, phenytoin, phenobarbital, St. John's Wort extract, mitotane, avasimibe, rifapentine, ivosidenib, carbamazepine, lumacaftor, apalutamide, enzalutamide, and ritonavir.
- Use of drugs acting on platelets such as aspirin, clopidogrel, dipyridamole, ticlopidine, or with anticoagulant effect like heparin or warfarin.

## 1.2 SCHEMATIC OF STUDY DESIGN

An overview of the study design is provided in Figure 1.

**Figure 1 Overview of Study Design *for Part 1 and Part 2***




*BID = twice daily; MAD = multiple-ascending dose; QD = once daily; RO = RO6953958; SAD = single-ascending dose.*

***Figure 2 Overview of Study Design for Part 3***

| *Screening Visit* | *In-Clinic Period* | *Ambulatory Visits* | *Final Follow-up Visit* |
|---|---|---|---|
| *Day -28 to Day -2* | *Day -1 to Day 18;*<br>*1 baseline day (Day -1), 14 dosing days (see below),*<br>*4 follow-up days (Day 15 to Day 18)* | *Day 19 to Day 20* | *Day 21 to Day 28* |
| | *5 Treatment periods:*<br>• *Day 1 – IV midazolam microdosing (100 µg)*<br>• *Day 2 – oral midazolam microdosing (300 µg)*<br>• *Days 3 to 14 – RO6953958 QD (maximum dose QD tested in Part 2)*<br>• *Day 13 – RO6953958 in combination with a single 100 µg IV dose of midazolam (RO6953958 plus IV midazolam)*<br>• *Day 14 – RO6953958 in combination with a single 300 µg oral dose of midazolam (RO6953958 plus oral midazolam)* | | *Within 7 to 14 days after the final dose of RO6953958* |

*IV = intravenous; QD = once daily.*

## 1.3 SCHEDULE OF ACTIVITIES

The schedule of the activities is provided in Table 1 and Table 2 for Part 1, in Table 3 and Table 4 for Part 2, *and in Table 5, Table 6, and Table 7 for Part 3*.

## Table 1 Overall Schedule of Activities – Part 1

| Day | Screening up to Day -28 | Day -2[o] | Day -1 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 8 | Follow Up Visit (7-14 days) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Assessments** | | | | | | | | | | | |
| Informed Consent | x | | | | | | | | | | |
| Demography | x | | | | | | | | | | |
| Medical History | x | | | | | | | | | | |
| Physical Examination | x[a] | | x[i] | | | | | x[i] | | | x[a] |
| In-house Period | | x | x | x | x | x | x | x | | | |
| Vital Signs [b] | x[j] | | x | 3[j] | 1 | | | x | | | x |
| ECG-12 lead (triplicate) [c] | x | | x | 3 | 1 | | | x | | | x |
| Holter ECG monitoring [d e] | | | | x | x | | | | | | |
| Serology | x | | | | | | | | | | |
| Urine Alcohol Test | x | | x | | | | | | | | x |
| Urine Drugs of Abuse | x | | x | | | | | | | | x |
| Urinalysis | x | | x | x | | x | | x | | | x |
| Blood Chemistry | x | | x | x | | x | | x | | | x |
| Hematology | x | | x | x | | x | | x | | | x |
| Coagulation | x | | | | | | | | | | x |
| Randomization [e] | | | | x | | | | | | | |
| Administration of Study Medication [f] | | | | x | | | | | | | |
| Standard Meal [g] | | x | x | x | x | x | x | x | | | |
| PK Sample plasma [k] | | | | 10 | x | x | x | x | x | x | x |
| Urine PK Sample [h,k] | | | | x | x | x | | | | | |
| PAA blood sampling [l,m] | | | x | x | | | | | | | |
| Karolinska Sleepiness Scale (KSS) [m] | | | | 6 | x | x | | | | | |
| SARS-CoV-2 testing | | x | | | | | | | | | |
| Sleep mat [n] | | | | | | | x | | | | |
| Clinical Genotyping | | | | x[e] | | | | | | | |
| Adverse Events | 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 |

## Table 1 Overall Schedule of Activities – Part 1 (cont.)

a. Full physical examination including body weight and height at screening when body mass index (BMI) will be derived.

b. Vital signs will include blood pressure, pulse rate, respiratory rate, and body temperature. Temperature will be recorded at screening, Day 1 predose, and follow-up.

c. Triplicate 12-lead ECGs will be measured after the participant has rested in a supine position for at least 10 minutes (see detailed Table 2).

d. Holter ECG data will be extracted at timepoints specified in Table 2. The participants should be at rest and in a supine position for at least 10 minutes prior to and remain in a supine position for at least 5 more minutes after the specified ECG extraction timepoints. When ECG extractions coincide with safety ECGs, vital signs assessment, and blood draws, procedures should be carried out in said order.

e. In first period only of FE cohort.

f. RO6953958 will be administered orally with approximately 240 mL water in the morning of Day 1. Water will be allowed ad libitum until 1 hour prior to dosing and from 1 hour postdosing. At higher dose levels, twice-daily dosing may be required.

g. Participants will stay fasted overnight (at least 10 hours) before dosing and remain fasted until lunch 4 hours postdose. Dinner will be provided approximately 8 hours postdose. Participants in the food-effect cohort will receive (in addition to lunch and dinner) a high fat, high calorie breakfast 30 minutes before drug intake. Three standard meals (breakfast, lunch, and dinner) will be served on Days 2, 3, and 4.

h. Bladder must be completely emptied at the time of study drug administration. Urine sample collection intervals are detailed in Table 2.

i. Brief, targeted physical exam.

j. Orthostatic challenge: Participants will rest in the supine position for 10 minutes and then quickly stand up and remain standing for 3 minutes. Pulse rate and blood pressure will be measured 3 times after the participant has rested in the supine position for 10 minutes (i.e., at 8, 9, and 10 minutes) and again after around 3 minutes in standing position to compare this with the latest supine measurements (Section 8.2.2.1).

k. Schedule of blood and urine samples may be modified to better characterize PK profile after analysis of emerging PK data from previous doses.

l. PAA – platelet aggregation assay.

m. The timing of PD measurements may be adapted based on emerging PK data.

n. Four participants will have sleep mat data collected overnight for testing purposes only.

o. Participants will be admitted on Day-2 to ensure severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing can be reported prior to dosing.

**Table 2 Detailed Schedule of Activities – Part 1**

| Day | Scheduled Time (h) | Vital Signs [a] | ECG-12 lead [b] | Holter ECG time points for extraction [c i] | Standard Meal [f] | Karolinska Sleepiness Scale (KSS) [h] | PK Sample | Urine PK Sample [d] | PAA blood sampling [g,h] | Clinical Genotyping |
|---|---|---|---|---|---|---|---|---|---|---|
| Day -1 | | x | x | | x | | | | x | |
| Day 1 | Predose | x [e] | x | 3 | x [f] | x | x | | | x [i] |
| | 0h | | | | | | | 0 to 4h | | |
| | 30 min | | | x | | | x | | | |
| | 1h | | | x | | x | x | | x | |
| | 2 h | x [j] | x | x | | | x | | | |
| | 3h | | | x | | x | x | | | |
| | 4h | | | | x | | | 4 to 12h | | |
| | 5h | | | x | | x | x | | | |
| | 6h | | | x | | | x | | | |
| | 7h | x | x | x | | x | x | | x | |
| | 8h | | | | x | | | | | |
| | 10h | | | x | | | x | | | |
| | 12h | | | x | | x | x | 12 to 24h | | |
| Day 2 | 24h | x | x | x | x | x | x | 24 to 48h | | |
| Day 3 | 48h | | | | x | x | x | | | |
| Day 4 | 72h | | | | x | | x | | | |
| Day 5 | 96h | x | x | | x | | x | | | |
| Day 6 | 120 h | | | | | | x | | | |
| Day 8 | 168 h | | | | | | x | | | |
| Day 10 | 216 h | | | | | | x | | | |

## Table 2 Detailed Schedule of Activities – Part 1 (cont.)

a. Vital signs will include blood pressure, pulse rate, respiratory rate and (at selected timepoints) body temperature. Vital sign measurements will be taken after the participant has rested in a supine position for at least 5 minutes.

b. Triplicate 12-lead ECG will be collected after the participant has rested in a supine position for at least 10 minutes.

c. Participants should be at rest and in a supine position for at least 10 minutes prior to and remain in a supine position for at least 5 more minutes after the specified ECG extraction timepoints. Three readings will be taken as a baseline at predose on Day 1.

d. Participants will be asked to empty their bladder before dosing. Then, all urine voided up to 48 hours postdose will be collected in four lots.

e. Body temperature to be recorded.

f. Participants will stay fasted overnight (at least 10 hours) before dosing and remain fasted until lunch 4 hours postdose. Dinner will be provided approximately 8 hours postdose. Participants in the food-effect cohort will receive (in addition to lunch and dinner) a high fat, high calorie breakfast 30 minutes before drug intake. Three standard meals (breakfast, lunch, and dinner) will be served on Days 2, 3, and 4.

g. PAA – platelet aggregation assay.

h. The timing of PD measurements may be adapted based on emerging PK data.

i. In first period only for FE cohort.

j. Orthostatic challenge: Participants will rest in the supine position for 10 minutes and then quickly stand up and remain standing for 3 minutes. Pulse rate and blood pressure will be measured 3 times after the participant has rested in the supine position for 10 minutes (i.e., at 8, 9, and 10 minutes) and again after around 3 minutes in standing position to compare this with the latest supine measurements.

## Table 3 Overall Schedule of Activities – Part 2

| Day | Screening up to Day -28 | Day -2[q] | Day -1 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 12 | Day 13 | Day 14 | Day 15 | Day 17 | Follow Up Visit (7-14 days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Assessments | | | | | | | | | | | | | | | | | | | | |
| Informed Consent | x | | | | | | | | | | | | | | | | | | | |
| Demography | x | | | | | | | | | | | | | | | | | | | |
| Medical History | x | | | | | | | | | | | | | | | | | | | |
| Physical Examination | x[a] | x[f] | | | x[f] | | | | | | | | | | | | x[f] | | | x[a] |
| In-house Period [e] | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | | | |
| Discharge from Unit | | | | | | | | | | | | | | | | | x | | | |
| Vital Signs [b] | x[j] | x | x | 3[j] | x | x | x | x | x | x[j] | x | x | 3 | x | x | x | x | | | x |
| ECG-12 lead [c] | x | x | x | 3 | | x | | | x | | | | 3 | | x | | x | | | x |
| Holter ECG monitoring [d] | | | | x | x | | | | | | | | x | x | | | | | | |
| Serology | x | x | | | | | | | | | | | | | | | | | | |
| Urine Alcohol Test | x | x | | | | | | | | | | | | | | | | | | x |
| Urine Drugs of Abuse | x | x | | | | | | | | | | | | | | | | | | x |
| Urinalysis | x | x | | | x | | | x | | | | x | | | x | | x | | | x |
| Blood Chemistry | x | x | | | x | | | x | | | | x | | | x | | x | | | x |
| Hematology | x | x | | | x | | | x | | | | x | | | x | | x | | | x |
| Coagulation [r] | x | | | | | | | x [r] | x [r] | x [r] | | | | | | | x | | | x |
| Randomisation | | | | x | | | | | | | | | | | | | | | | |

## Table 3 Overall Schedule of Activities – Part 2 (cont.)

| Day | Screening up to Day -28 | Day -2[q] | Day -1 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 12 | Day 13 | Day 14 | Day 15 | Day 17 | Follow Up Visit (7-14 days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Assessments | | | | | | | | | | | | | | | | | | | | |
| Administration of Study Medication[l] | | | | x | x | x | x | x | x | x | x | x | x | | | | | | | |
| Standard Meals | | x | x | x[m] | x | x | x | x | x | x | x | x | x[m] | x | x | x | x | | | |
| PK Sample[g] | | | | 10[u] | x | x | x | x | x | x | x | x | 10[u] | x | x | x | x | x | x | x |
| Urine PK Sample[g, h] | | | | x | x | | | | | | | | x | x | x | | | | | |
| CSF Sampling[r] | | | | | | | | | x[r] | x[r] | x[r] | | | | | | | | | |
| Cortisol[o] | | | 10 | | 10 | | | | | | | | 10 | | | 10 | | | | |
| DLMO[n] | | 11 | | 11 | | | | | | | | 11 | | | 11 | | | | | |
| Epworth Sleepiness Scale | x | | | | | | | | | | | | | | | | | | | |
| Morningness-Eveningness Questionnaire (MEQ) | x | | | | | | | | | | | | | | | | | | | |
| Actigraphy and Sleep log | x[i] | | | | | | | | | | | | | | | | | | | |
| Pittsburgh Sleep Quality Index | x | | | | | | | | | | | | | | | | | | | |
| Promis Sleep Questionnaires[k] | | | x | | | | | | | x | | | | | | | x | | | |
| Karolinska Sleepiness Scale (KSS) | | | 6 | 6 | 6 | | | | | | | | 6 | | | 6 | | | | |
| Facial Expression Recognition Task (FERT) | | | x | x | | | | | | | | | x | | | | | | | |
| Polysomnography and sleep mat[p] | | x | x | x | | | | | | | | x | | | x | | | | | |
| Core body temperature assessment[s] | | x[s] | x[s] | x[s] | | | | | | | | x[s] | | | x[s] | | | | | |
| Wake EEG[t] | | | x[t] | | | | | x[t] | x[t] | | | | | | | | | | | |
| C-SSRS | x | | x | | | | | | | | | | | | x | x | x | | | x |
| Clinical Genotyping | | | | x | | | | | | | | | | | | | | | | |
| Adverse Events | x | x | x | x | 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 | x | x |

## Table 3 Overall Schedule of Activities – Part 2 (cont.)

a. Full physical examination including body weight and height at screening when body mass index (BMI) will be derived.

b. Vital signs will include blood pressure, pulse rate, respiratory rate, and at selected timepoints on Day 1 and Day 10 for body temperature.

c. Triplicate 12-lead ECGs will be collected after the participant has rested in a supine position for at least 10 minutes (see Detailed Table 4).

d. Holter monitoring will be measured from predose on Day 1 to 24 hours postdosing on the morning of Day 2. Holter ECG data will be extracted at timepoints specified in Table 4. The participants should be at rest and in a supine position for at least 10 minutes prior to and remain in a supine position for at least 5 more minutes after the specified ECG extraction timepoints.

e. Dim lighting protocol to be applied before bedtime (lights out at 11:00 pm) starting 1 hour before melatonin sampling begins; participants should be recumbent (not supine), with no napping during days where this protocol is applied.

f. Brief, targeted physical exam.

g. Schedule of blood and urine samples may be modified to better characterize the PK profile. Timing of blood draw will be determined after analysis of the SAD and the PK data review from the previous cohort in MAD as appropriate.

h. Urine sample collection interval are detailed in Table 4.

i. Sleep log will be collected and actigraphy watch will be worn during the week prior to admission to unit.

j. Orthostatic challenge: Participants will rest in the supine position for 10 minutes and then quickly stand up and remain standing for 3 minutes. Pulse rate and blood pressure will be measured 3 times after the participant has rested in the supine position for 10 minutes (i.e., at 8, 9, and 10 minutes) and again after around 3 minutes in standing position to compare this with the latest supine measurements.

k. PROMIS Sleep Disturbance and PROMIS Sleep-Related Impairment short forms (8 items).

l. RO6953958 administered under fasted conditions depending on data from Part 1. In case fed conditions are applied, the dose will be administered with breakfast. A dose level may have a different timing of daily drug administration (evening vs morning) in additional participants.

m. The same food conditions (in terms of meal constitution and time of administration) will apply on Day 1 and on Day 10.

n. DLMO –dim light melatonin onset. Melatonin in saliva and serum to be collected every 30 mins between 6 p.m. and 11 p.m under dim light conditions. Timings and number of samples (to a maximum of 12 samples per matrix) may be adjusted based on DLMO protocol.

o. Cortisol in serum to be collected every 15 minutes for an hour after waking up, then every 30 minutes up until 3.5 hours after waking. Leftover samples will be used for additional melatonin testing.

p. Polysomnography and sleep mat overnight on Day -2 (habituation), Day -1 (baseline), Day 1 and Day 9 (on drug), and Day 12 (off drug).

q. Participants may be admitted on Day-3 to ensure severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing can be reported prior to dosing.

r. An additional PK blood sample is also taken on either Day 6, 7 or 8 to coincide with CSF sampling, as well as a coagulation sample which should be measured on the day prior to CSF sampling e.g., Day 5, 6 or 7. Timing of CSF sampling may be adapted based on emerging PK data.

## Table 3 Overall Schedule of Activities – Part 2 (cont.)

s. Apply vest and swallow the equivital pill for measuring the core body temperature prior to the DLMO or equivalent time if no assessment. Remove vest after last cortisol sampling the day after or equivalent time if no assessment.

t. Wake EEG is performed on Day-1 (baseline) and Day 5 (or alternatively on Day 6) at approximately 2 pm for 30 min.

u. Leftover PK samples collected on Day 1 and Day 10 will be used for additional cortisol testing.

## Table 4 Detailed Schedule of Activities – Part 2

| Day | Scheduled Time (h) | Vital Signs [a] | ECG-12 lead [b] | Holter ECG time points for extraction [e,o] | Urinalysis | Blood Chemistry | Hematology | Coagulation | Administration of Study Medication [c] | Standard Meals [h,i] | PK Sample [d] | Urine PK Sample [d, n] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day -2[q] | | x | x | | x | x | x | | | x | | |
| Day -1 | Predose -24 | | x | | | | | | | | | |
| | 1h | | | | | | | | | | | |
| | 2h | x | | | | | | | | | | |
| | 3h | | | | | | | | | | | |
| | 4h | | | | | | | | | x | | |
| | 5h | | | | | | | | | | | |
| | 6h | | | | | | | | | | | |
| | 7h | | | | | | | | | | | |
| | 8h | | | | | | | | | x | | |
| | 10h | | | | | | | | | | | |
| | 12h | | | | | | | | | | | |
| Day 1 | Predose | x [l] | x | 3 | | | | | | | x[u] | |
| | 0h | | | | | | | | x | | | 0 to 4h |
| | 30 min | | | x | | | | | | | x[u] | |
| | 1h | | | x | | | | | | | x[u] | |
| | 2h | x [m] | x | x | | | | | | | x[u] | |
| | 3h | | | x | | | | | | | x[u] | |
| | 4h | | | | | | | | | x | | 4 to 12h |
| | 5h | | | x | | | | | | | x[u] | |
| | 6h | | | x | | | | | | | x[u] | |
| | 7h | x | x | x | | | | | | | x[u] | |
| | 8h | | | | | | | | | x | | |
| | 10h | | | x | | | | | | | x[u] | |
| | 12h | | | x | | | | | | | x[u] | 12 to 24h |
| Day 2 | Predose | x | | x | x | x | x | | | x | x | |
| | 0h | | | | | | | | x | | | |
| | 1h | | | | | | | | | | | |
| | 3h | | | | | | | | | | | |
| | 5h | | | | | | | | | | | |
| | 6h | | | | | | | | | | | |
| | 7h | | | | | | | | | | | |
| | 12h | | | | | | | | | | | |
| Day 3 | Predose | x | | | | | | | | | x | |
| | 0h | | | | | | | | x | | | |
| | 2h | | x | | | | | | | | | |

## Table 4 Detailed Schedule of Activities – Part 2 (cont.)

| Day | Scheduled Time (h) | Vital Signs [a] | ECG-12 lead [b] | Holter ECG time points for extraction [e,o] | Urinalysis | Blood Chemistry | Hematology | Coagulation | Administration of Study Medication [c] | Standard Meals [h,i] | PK Sample [d] | Urine PK Sample [d, n] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day 4 | Predose | x | | | | | | | | x | x | |
| | 0h | | | | | | | | x | | | |
| Day 5 | Predose | x | | | x | x | x | x [r] | | | x | |
| | 0h | | | | | | | | x | | | |
| | 2h | | | | | | | | | | | |
| | 5h | | | | | | | | | | | |
| | 6 h | | | | | | | | | | | |
| Day 6 | Predose | x | | | | | | x [r] | | | x | |
| | 0h | | | | | | | | x | | | |
| | 2h | | x | | | | | | | | | |
| | 5h | | | | | | | | | | x [r] | |
| | 6h | | | | | | | | | | | |
| | 7h | | | | | | | | | | | |
| Day 7 | Predose | | | | | | | x [r] | | | x | |
| | 0h | | | | | | | | x | | | |
| | 2h | x [m] | | | | | | | | | | |
| | 5 h | | | | | | | | | | x [r] | |
| | 7 h | | | | | | | | | | | |
| Day 8 | Predose | x | | | | | | | | | x | |
| | 0h | | | | | | | | x | | | |
| | 5 h | | | | | | | | | | x [r] | |
| | 7 h | | | | | | | | | | | |
| Day 9 | Predose | x | | | x | x | x | | | | x | |
| | 0h | | | | | | | | x | | | |
| | 6h | | | | | | | | | | | |
| | 12h | | | | | | | | | | | |

## Table 4 Detailed Schedule of Activities – Part 2 (cont.)

| Day | Scheduled Time (h) | Vital Signs [a] | ECG-12 lead [b] | Holter ECG time points for extraction [e,o] | Urinalysis | Blood Chemistry | Hematology | Coagulation | Administration of Study Medication [c] | Standard Meals [h,i] | PK Sample [d] | Urine PK Sample [d, n] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day 10 | Predose | x [l] | x | x | | | | | | | x[u] | 0 to 4h |
| | 0h | | | | | | | | x | | | |
| | 30 min | | | x | | | | | | | x[u] | |
| | 1 h | | | x | | | | | | | x[u] | |
| | 2h | x | x | x | | | | | | | x[u] | |
| | 3h | | | x | | | | | | | x[u] | 4 to 12h |
| | 4h | | | | | | | | | x | | |
| | 5h | | | x | | | | | | | x[u] | |
| | 6h | | | x | | | | | | | x[u] | |
| | 7h | x | x | x | | | | | | | x[u] | |
| | 8h | | | | | | | | | x | | |
| | 10h | | | x | | | | | | | x[u] | |
| | 12h | | | x | | | | | | | x[u] | 12 to 24h |
| Day 11 | 24h | x | | x | | | | | | x | x | 24 to 48h |
| Day 12 | 48h | x | x | | x | x | x | | | x | x | |
| Day 13 | 72h | x | | | | | | | | x | x | |
| Day 14 | 96h | x | x | | x | x | x [p] | | | x | x | |
| Day 15 | 120 h | | | | | | | | | | x | |
| Day 17 | 168 h | | | | | | | | | | x | |
| Day 19 | 216 h | | | | | | | | | | x | |

## Table 4 Detailed Schedule of Activities – Part 2 (cont.)

| Day | Scheduled Time (h) | CSF Sample | Clinical Genotyping | Cortisol [k] | DLMO [j] | Promis Sleep Questionnaires [f] | Karolinska Sleepiness Scale (KSS) | Polysomnography and sleep mat [g] | Core Body Temperature [s] | Wake EEG [t] | Facial Expression Recognition Task (FERT) | CSSRS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day -2 [q] | | | | | 11 | | | x | x | | | |
| Day -1 | Predose -24 | | | | | x | x | | | | | x |
| | 0h | | | 10 | | | | | | | | |
| | 1h | | | | | | x | | | | x | |
| | 2h | | | | | | | | | | | |
| | 3h | | | | | | x | | | | | |
| | 4h | | | | | | | | | | | |
| | 5h | | | | | | x | | | x | | |
| | 6h | | | | | | | | | | | |
| | 7h | | | | | | x | | | | | |
| | 8h | | | | | | | | | | | |
| | 10h | | | | | | | | | | | |
| | 12h | | | | | | x | x | x | | | |
| Day 1 | Predose | | x | | | | x | | | | | |
| | 0h | | | | | | | | | | | |
| | 30 min | | | | | | | | | | | |
| | 1h | | | | | | x | | | | x | |
| | 2h | | | | | | | | | | | |
| | 3h | | | | | | x | | | | | |
| | 4h | | | | | | | | | | | |
| | 5h | | | | | | x | | | | | |
| | 6h | | | | | | | | | | | |
| | 7h | | | | | | x | | | | | |
| | 8h | | | | | | | | | | | |
| | 10h | | | | 11 | | | | | | | |
| | 12h | | | | | | x | x | x | | | |
| Day 2 | Predose | | | | | | x | | | | | |
| | 0h | | | 10 | | | | | | | | |
| | 1h | | | | | | x | | | | | |
| | 3h | | | | | | x | | | | | |
| | 5h | | | | | | x | | | | | |
| | 6h | | | | | | | | | | | |
| | 7h | | | | | | x | | | | | |
| | 12h | | | | | | x | | | | | |
| Day 3 | Predose | | | | | | | | | | | |
| | 0h | | | | | | | | | | | |
| | 2h | | | | | | | | | | | |

## Table 4 Detailed Schedule of Activities – Part 2 (cont.)

| Day | Scheduled Time (h) | CSF Sample | Clinical Genotyping | Cortisol [k] | DLMO [j] | Promis Sleep Questionnaires [f] | Karolinska Sleepiness Scale (KSS) | Polysomnography and sleep mat [g] | Core Body Temperature [s] | Wake EEG [t] | Facial Expression Recognition Task (FERT) | CSSRS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day 4 | Predose | | | | | | | | | | | |
| | 0h | | | | | | | | | | | |
| Day 5 | Predose | | | | | | | | | | | |
| | 0h | | | | | | | | | | | |
| | 2h | | | | | | | | | | | |
| | 5h | | | | | | | | | x | | |
| | 6 h | | | | | | | | | | | |
| Day 6 | Predose | x [r] | | | | | | | | | | |
| | 0h | | | | | | | | | | | |
| | 2h | | | | | | | | | | | |
| | 5h | x [r] | | | | | | | | x | | |
| | 6h | | | | | | | | | | | |
| | 7h | | | | | | | | | | | |
| Day 7 | Predose | x [r] | | | | x | | | | | | |
| | 0h | | | | | | | | | | | |
| | 2h | | | | | | | | | | | |
| | 5 h | x [r] | | | | | | | | | | |
| | 7 h | | | | | | | | | | | |
| Day 8 | Predose | x [r] | | | | | | | | | | |
| | 0h | | | | | | | | | | | |
| | 5 h | x [r] | | | | | | | | | | |
| | 7 h | | | | | | | | | | | |
| Day 9 | Predose | | | | | | | | | | | |
| | 0h | | | | | | | | | | | |
| | 6h | | | | | | | | | | | |
| | 12h | | | | 11 | | | x | x | | | |

## Table 4 Detailed Schedule of Activities – Part 2 (cont.)

| Day | Scheduled Time (h) | CSF Sample | Clinical Genotyping | Cortisol [k] | DLMO [j] | Promis Sleep Questionnaires [f] | Karolinska Sleepiness Scale (KSS) | Polysomnography and sleep mat [g] | Core Body Temperature[s] | Wake EEG[t] | Facial Expression Recognition Task (FERT) | CSSRS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day 10 | Predose | | | | | | x | | | | | |
| | 0h | | | 10 | | | | | | | | |
| | 30 min | | | | | | | | | | | |
| | 1 h | | | | | | x | | | | x | |
| | 2h | | | | | | | | | | | |
| | 3h | | | | | | x | | | | | |
| | 4h | | | | | | | | | | | |
| | 5h | | | | | | x | | | | | |
| | 6h | | | | | | | | | | | |
| | 7h | | | | | | x | | | | | |
| | 8h | | | | | | | | | | | |
| | 10h | | | | | | | | | | | |
| | 12h | | | | | | x | | | | | |
| Day 11 | 24h | | | | | | | | | | | |
| Day 12 | 48h | | | | 11 | | | x | x | | | x |
| Day 13 | 72h | | | 10 | | | 6 as above | | | | | x |
| Day 14 | 96h | | | | | x | | | | | | x |
| Day 15 | 120 h | | | | | | | | | | | |
| Day 17 | 168 h | | | | | | | | | | | |
| Day 19 | 216 h | | | | | | | | | | | |

## Table 4 Detailed Schedule of Activities – Part 2 (cont.)

a. Vital signs will include blood pressure, pulse rate, respiratory rate, and (at selected timepoints) body temperature. Vital sign measurements will be taken after the participant has rested in a supine position for at least 5 minutes.

b. Triplicate 12‑lead ECG will be collected after the participant has rested in a supine position for at least 10 minutes.

c. RO6953958 will be given orally with water from Day 1 to Day 10, once daily in the morning. A dose level may have a different timing of daily drug administration (evening vs morning) in additional participants.

d. Schedule of blood and urine samples may be modified to better characterize the PK profile. Timing of blood draw will be determined after analysis of the SAD and the PK data review from the previous cohort in MAD as appropriate.

e. 3 timepoints at predose.

f. PROMIS Sleep Disturbance and PROMIS Sleep-Related Impairment short forms (8 items).

g. Polysomnography and sleep mat overnight on Day -2 (habituation), Day -1 (baseline), Day 1 and Day 9 (on drug), and Day 12 (off drug).

h. RO6953958 administered under fasted conditions depending on data from Part 1. In case fed conditions are applied, the dose will be administered with breakfast.

i. The same food conditions (in terms of meal constitution and time of administration) will apply on Day 1 and on Day 10.

j. DLMO – dim light melatonin onset. Melatonin in saliva and serum to be collected every 30 minutes between 6 p.m. and 11 p.m under dim light conditions. Timings and number of samples (to a maximum of 12 samples per matrix) may be adjusted based on DLMO protocol.

k. Cortisol in serum to be collected every 15 minutes for an hour after waking up, then every 30 minutes up until 3.5 hours after waking. Leftover samples will be used for additional melatonin testing.

l. Body temperature will be measured at predose on Day 1 and Day 10.

m. Orthostatic challenge: Participants will rest in the supine position for 10 minutes and then quickly stand up and remain standing for 3 minutes. Pulse rate and blood pressure will be measured 3 times after the participant has rested in the supine position for 10 minutes (i.e. at 8, 9, and 10 minutes) and again after around 3 minutes in standing position to compare this with the latest supine measurements.

n. Participants will be asked to empty their bladder before dosing on Day 1 and Day 10. Then, all urine voided during 24 hours postdose on Day 1 and up to 48 hours on Day 10 will be collected.

o. Participants should be at rest and in a supine position for at least 10 minutes prior to and remain in a supine position for at least 5 more minutes after the specified ECG extraction timepoints. When ECG extractions coincide with safety ECGs, vital signs assessment and blood draws, procedures should be carried out in said order.

p. Coagulation should also be measured.

q. Participants may be admitted on Day-3 to ensure severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing can be reported prior to dosing.

## Table 4 Detailed Schedule of Activities – Part 2 (cont.)

r. Sample will be taken pre-dose in 4 participants per cohort and at 6 hours after dosing (allowed time window was between 5 to 7 hours after dosing) in 4 participants in each cohort (i.e., one CSF sample per participant per cohort). At the same timepoint an additional PK sample is taken. As an alternative to Day 7, CSF sampling and PK sampling can also be performed on Day 6 or Day 8 at predose or 6 hours after dosing (allowed time window was between 5 to 7 hours after dosing). Coagulation sample to be taken a day prior to CSF sampling. Timing of CSF sampling may be adapted based on emerging PK data.

s. Apply vest and swallow the equivital pill for measuring the core body temperature prior to the DLMO. Remove vest after last cortisol sampling the day after.

t. Wake EEG is performed on Day-1 (baseline) and Day 5 (alternatively on Day 6) at 2 pm for 30 min.

u. Leftover PK samples collected on Day 1 and Day 10 will be used for additional cortisol testing.

## *Table 5 Overall Schedule of Activities – Part 3*

| Day relative to first Dose Midazolam | Screening up to Day -28 | Baseline Day -1 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 12 | Day 13 | Day 14 | Day 15 | Day 16 | Day 17 | Day 18 | Day 19 | Day 20 | Final Follow-up Visit [c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Informed Consent | x | | | | | | | | | | | | | | | | | | | | | | |
| Demography | x | | | | | | | | | | | | | | | | | | | | | | |
| Medical History | x | | | | | | | | | | | | | | | | | | | | | | |
| Inclusion/Exclusion Criteria Review | x | x | | | | | | | | | | | | | | | | | | | | | |
| Physical Examination [a] | x | x | | x | | | | | | | | | | | | x | | | | | | | x |
| Admission to Unit | | x | | | | | | | | | | | | | | | | | | | | | |
| In-house Period | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | | | | |
| Discharge from Unit | | | | | | | | | | | | | | | | | | | | x | | | |
| Ambulatory Visit | x | | | | | | | | | | | | | | | | | | | | x | x | x |
| Vital Signs [b] | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | | | x |
| ECG-12 Lead [d] | x | x | x | x | x | | x | | | x | | | x | | x | x | | x | | x | | | x |
| Serology | x | | | | | | | | | | | | | | | | | | | | | | |
| SARS-CoV-2 PCR Test [e] | | x | | | | | | | | | | | | | | | | | | | | | |
| Urine Alcohol Test [f] | x | x | | | | | | | | | | | | | | | | | | | | | x |
| Urine Drugs of Abuse [f] | x | x | | | | | | | | | | | | | | | | | | | | | x |
| Urinalysis | x | x | | | | x | | | x | | | | x | | | x | | x | | x | | | x |
| Blood Chemistry | x | x | | | | x | | | x | | | | x | | | x | | x | | x | | | x |
| Hematology | x | x | | | | x | | | x | | | | x | | | x | | x | | x | | | x |
| Coagulation | x | | | | | | | | | | | | | | | | | | | x | | | x |
| Administration of RO6953958 [g] | | | | | x | x | x | x | x | x | x | x | x | x | x | x | | | | | | | |
| Oral Administration of Midazolam [h] | | | | x | | | | | | | | | | | | x | | | | | | | |
| IV Administration of Midazolam [h] | | | x | | | | | | | | | | | | x | | | | | | | | |
| Standard Meal [i] | | | x | x | | | | | | | | | | | x | x | | | | | | | |
| Clinical Genotyping | | | x | | | | | | | | | | | | | | | | | | | | |
| PK Sample RO6953958, M1, and M3 | | | | | 10 | x[j] | | x[j] | | x[j] | | x[j] | | x[j] | x[j] | 10 | x | x | x | x | x | x | x |
| PK Sample Midazolam [k] | | | 14 | 13 | x | | | | | | | | | | 14 | 13 | x | | | | | | |
| 4β -hydroxycholesterol Sampling [l] | 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 | x | x | x | x | x |
| Adverse Events [m] | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| C-SSRS | x | x | | | | | | | | | | | | x | x | x | | | | | | | x |

## *Table 5 Overall Schedule of Activities – (cont.)*

*BMI = body mass index; C-SSRS = Columbia Suicide Severity Scale; ECG = electrocardiogram; IV = intravenous; M1 = RO7045755; M3 = RO7021594; PCR=polymerase chain reaction; PK=pharmacokinetic; QD = once daily.*

a. *Full physical examination including body weight at Screening and Follow-up; height at Screening when BMI will be derived. All other visits will include only a brief, targeted physical examination.*
b. *Vital signs will include blood pressure, pulse rate, respiratory rate, and body temperature. Temperature will be recorded at Screening, Day 1 (predose), and Follow-up. All measurements will be taken after the participant has rested in a supine position for at least 5 minutes.*
c. *The final follow-up visit is 7 to 14 days after the last dosing of RO6953958.*
d. *Triplicate 12 lead ECGs will be collected after the participant has rested in a supine position for at least 10 minutes.*
e. *Sampling of nasal and throat mucosal cells. If deemed necessary, additional tests may be conducted during the study.*
f. *At indicated visits and any other time at the discretion of the Investigator.*
g. *On Days 3 to 14, a single oral dose of RO6953958 (highest safe QD dose tested in Part 2) will be given in the morning under fed conditions with water.*
h. *Midazolam will be administered in the morning as a bolus injection on Days 1 and 13 or orally (liquid) on Days 2 and 14.*
i. *See details of mealtimes in Table 6 and Table 7.*
j. *Predose PK samples only to be taken on Days 4, 6, 8, 10, 12, and 13; full PK profile to be taken on Days 3 and 14 (see Table 7 for details) through to the follow-up visit.*
k. *See details for midazolam PK sampling in Table 6 and Table 7.*
l. *Sample taken predose relative to RO6953958 and midazolam dosing on dosing days.*
m. *Adverse events and use of concomitant medication during outpatient periods to be captured at the next study centre visit or from spontaneous reports.*

*Table 6 Detailed Schedule of Activities on Day 1 and Day 13 – Part 3*

| Procedure | Time prior to IV midazolam dosing (h) | | | | | Time post IV midazolam dosing (h) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ~3 | 2.5 | 2 | 0.5 | 0 | 5 min | 0.25 | 0.5 | 0.75 | 1 | 1.5 | 2 | 2.5 | 3 | 4 | 6 | 8 | 10 |
| Standard Meal [a] | | x | | | | | | | | | | x | | | | | x | |
| PK Sample RO6953958, M1 and M3 [b] | x | | | | | | | | | | | | | | | | | |
| ECG-12 lead | x | | | | | | | | | | | | | | | | | |
| Vital signs [c] | x | | | | | | | | | | | x | | | x | | | |
| Administration of RO6953958 [d] | | | x | | | | | | | | | | | | | | | |
| PK Sample midazolam | | | | x | | x | x | x | x | x | x | x | x | x | x | x | x | x |
| 4β -hydroxycholesterol sampling | x | | | | | | | | | | | | | | | | | |
| IV Administration of midazolam [e] | | | | | x | | | | | | | | | | | | | |

*ECG = electrocardiogram; h = hour; IV = intravenous, min = minute; M1 = RO7045755; M3 = RO7021594; PK = pharmacokinetic.*

a. *Standardized meals at -2.5 hours predose and at approximately 2 and 8 hours postdose relative to midazolam dosing. PK sampling should be performed before providing meals.*
b. *On Day 13 only.*
c. *Vital signs will include blood pressure, pulse rate, respiratory rate, and body temperature. Temperature will be recorded at Screening, Day 1 (predose), and Follow-up. All measurements will be taken after the participant has rested in a supine position for at least 5 minutes. Perform vital signs assessment prior to PK sampling*
d. *On Day 13 only, RO6953958 will be administered orally 30 minutes after starting a standardized breakfast (2 hours prior to midazolam dosing).*
e. *Midazolam will be administered in the morning as a bolus injection on Days 1 and 13 (2 hours after RO6953958 dosing).*

*Table 7 Detailed Schedule of Activities on Day 2 (Day 3 for RO6953958 PK Profile) and Day 14 – Part 3*

| Procedure | Time pre oral midazolam dosing (h) | | | | | Time post oral midazolam dosing (h) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 1.5 | 1 | 0.5 | 0 | 0.25 | 0.5 | 0.75 | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 | 4.5 | 5.5 | 6 | 8 | 8.5 | 10 | 10.5 |
| ECG-12 lead | x | | | | | | | | | | | | | | | | | | | | | |
| Vital signs [a] | x | | | | | | | | | | x | | | | x | | | | | | | |
| Standard Meal [b] | x | | | | | | | | | | x | | | | | | | | x | | | |
| PK Sample RO6953958, M1 and M3 [c] | x | | x | x | | | x | | | x | | | | x | | x | x | | | x | | x |
| PK Sample midazolam | | | | x | | x | x | x | x | x | x | x | x | | x | | | x | x | | x | |
| 4β -hydroxycholesterol sampling | x | | | | | | | | | | | | | | | | | | | | | |
| Administration of RO6953958 [d] | | x | | | | | | | | | | | | | | | | | | | | |
| Oral Administration of midazolam [e] | | | | | x | | | | | | | | | | | | | | | | | |

*ECG = electrocardiogram, min = minute; h = hour; M1 = RO7045755; M3 = RO7021594; PK = pharmacokinetics.*

a. *Vital signs will include blood pressure, pulse rate, respiratory rate, and body temperature. Temperature will be recorded at Screening, Day 1 (predose), and Follow-up. All measurements will be taken after the participant has rested in a supine position for at least 5 minutes. Perform vital signs prior to PK sampling.*
b. *Standardized meals at -2 hours predose and at approximately 2 and 8 hours postdose relative to midazolam dosing. PK sampling should be performed before providing meals.*
c. *Full PK profile for RO6953958 on Days 3 and 14 only. On Day 3, PK sample times will be relative to RO6953958 administration.*
d. *On Day 14 only, RO6953958 will be administered orally 30 minutes after starting a standardized breakfast (1.5 hours prior to midazolam dosing).*
e. *Midazolam will be administered orally on Days 2 and 14 (1.5 hours after RO6953958 dosing).*

# 2. INTRODUCTION

There have been no clinical studies with RO6953958 to date. The planned Phase I study BP41695 in healthy male volunteers will be the first study conducted in humans.

## 2.1 STUDY RATIONALE

RO6953958 is a potent and selective human vasopressin V1a receptor antagonist that blocks the activation of the V1a G protein-coupled receptor. *Evidence from mouse studies implicates a V1a receptor antagonism in the accelerated resynchronization of circadian rhythm.* RO6953958 is highly potent on the human V1a receptor (*dissociation constant* [Kb] = 0.22 nM) and may provide a novel and first approach to treat *circadian disturbances and social* deficits *in neurodevelopmental* disorders *(NDDs).* This is the first study with RO6953958 in humans, designed to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of single- and multiple-ascending doses (MAD), food effect (FE)*, and drug-drug interaction (DDI) potential* following oral administration to healthy male participants. The study results will support further clinical development of RO6953958.

The rationale for the study design is provided in Section 4.2.

## 2.2 BACKGROUND

### 2.2.1 Background on *Neurodevelopmental Disorders*

*NDDs are defined as a group of disorders caused by changes in early brain development, resulting in behavioral and cognitive alterations in sensory and motor systems, speech, and language (van Loo and Martens 2007). The prevalence of these disorders has been estimated to be approximately 1 in 6 children (approximately 17%) between the ages of 3 to 17 years in the United States (with the most common, attention-deficit/hyperactivity disorder [affecting between 8.5%–9.5% of children] and Autism Spectrum Disorder [ASD; between 1.1%–2.5% children, or 1%–2% of the world population]) (Elsabbagh et al 2012; Baio et al 2018; Xu et al 2019; Zablotsky et al 2019). Up to 80% of children with NDDs are reported to have disrupted sleep (Grigg–Damberger and Ralls 2013), with associated deleterious effects on daytime behaviors, cognition, physical and/or emotional outcomes and on their overall development and caregiver burden (Stores 2001; Schreck et al 2004; Jan et al 2008; Annaz et al 2011; Taylor et al 2012). Furthermore, greater sleep difficulties in children with ASD are associated with greater impairment in core deficits of the disorder compared to ASD in children with less sleep difficulty (Cohen 2017; MacDuffie et al 2020; Yavuz–Kodat 2020), suggesting that improving sleep is an important approach to treatment of NDDs.*

*Sleep disorders in children with NDDs are often multifactorial in etiology, including medical, neurologic, and psychiatric co-morbidities. However, some specific conditions such as Smith-Magenis syndrome (SMS), ASD, Prader-Willi, Angelman, and Rett syndromes are associated with specific dysfunctions in circadian rhythms (Potocki et al*

*2000; De Leersnyder 2001; Tordjman 2005; Melke et al 2008; Powell and LaSalle 2015).*

*Abnormalities in the vasopressin systems have also been investigated in psychiatric disorders (Iovino et al 2018) and NDDs (Francis et al 2014) that are characterized by the presence of social deficits. Moreover, disturbed sleep-wake patterns have also been described in a number of psychiatric disorders and NDDs including ASD (Pinato et al 2019), SMS, Prader-Willi, Angelman, and Rett syndromes (Powell and LaSalle 2015). Based on current literature and nonclinical observations, V1a receptor antagonism has the potential to offer therapeutic benefit for a wide range of NDDs and psychiatric disorders that involve circadian rhythm sleep-wake disturbances.*

There are three known receptors of the nine amino acid vasopressin peptide. These G protein-coupled receptors are called vasopressin 1a (V1a), vasopressin 1b (V1b), and vasopressin 2 (V2) receptors. V1a and V1b are coupled to *Gαq* protein and stimulate intracellular calcium release. V2 is coupled to Gs protein alpha-subunit (Gαs) and stimulates cyclic adenosine monophosphate formation. A very closely related peptide receptor is the oxytocin receptor, which is also coupled to Gαq. The V1a, V1b, and oxytocin receptors are expressed in limbic areas of the brain, including the hypothalamus, septum, ventral tegmental area, hippocampus, and amygdala (Loup et al 1991; Ostrowski et al 1994; Ostrowski 1998). These areas are known to be involved in emotional processes and play a critical role in affective disorders and the regulation of social behavior. *The V1a receptor is also expressed in a high number of neurons of the dorsal SCN, which plays an important role in keeping the endogenous circadian clock synchronized to the light/dark rhythm (Tsuji et al 2017).*

In the periphery, V1a receptors are expressed in platelets and vascular smooth muscles, mediating vasopressin-induced platelet aggregation and vasoconstriction, respectively. V1a receptors expressed in the medulla of adrenals may be involved in the release of catecholamines. *V1a receptors expressed in dorsal root ganglia and peripheral nerves have been claimed to mediate analgesic effects of oxytocin and vasopressin (Schorscher-Pectu et al 2010).* Renal expression of V1a receptors has been suggested to be involved in mediating the renal effects of aldosterone and their luminal expression in the renal tubules in sodium excretion. However, the quantitative relevance of such effects in *humans* is not known. *In clinical studies with another V1a receptor antagonist (balovaptan) developed by the Sponsor for social and communication deficits in ASD, no relevant changes in blood pressure, heart rate, blood sodium and potassium levels, or changes in bleeding risk were observed. V2 receptors are mainly expressed in the kidneys where they mediate the anti-diuretic effects of vasopressin.*

### 2.2.2 Background on RO6953958

The human vasopressin V1a receptor is a Gq protein coupled receptor and RO6953958 was found to be a potent and selective human V1a antagonist. In vitro, RO6953958 exhibited an inhibition constant of 0.5 nM for the human V1a receptor, with at least

6000-fold binding selectivity over 133 other receptors, enzymes, and ion channels including the related human vasopressin V2 and V1b receptors, as well as the human oxytocin receptor. In functional assays of calcium fluxes using fluorescent imaging (fluorometric imaging plate reader), RO6953958 showed antagonist activity at the human V1a with *a* dissociation constant (Kb) *of* 0.22 nM. The dissociation half-life of RO6953958 from the human V1a receptor is 102 minutes at room temperature. *Metabolites M1 (RO7045755) and M3 (RO7021594) were also shown to inhibit the human V1a receptor in a functional assay, with Kb values of 24 nM and 0.75 nM, respectively.*

In an ex vivo functional assay, RO6953958 was shown to inhibit V1a-mediated vasopressin-induced platelet aggregation in human whole blood, with a Kb of 12 nM. This value is consistent with the dissociation constant measured in vitro (Kb = 0.22 nM), taking into account the measured human plasma free fraction of 1.7%, and it demonstrates that *RO6953958* can inhibit the native V1a receptor.

In a mouse circadian study, wild-type mice subjected to a 6–hour-phase advance of the light/dark cycle resynchronized significantly faster to the new cycle after *one intraperitoneal administration of 60 mg/kg RO6953958* than vehicle-treated mice. This indicates that RO6953958 has the potential to resynchronize circadian rhythms, which may improve sleep-wake patterns that are known to be *dysregulated* in patients with *NDDs*.

The PK properties of RO6953958 were assessed in rats, cynomolgus monkeys, dogs, and minipigs. RO6953958 was well absorbed in all of these species following oral administration. In rats and cynomolgus monkeys, RO6953958 displayed a low systemic clearance, whereas in dogs and minipigs the systemic clearance was medium to high. The volume of distribution under steady-state conditions (Vss) was low to moderate in all of the species tested. *RO6953958* has low intrinsic clearance in vitro and a moderate plasma protein binding across species, including humans. This results in a low to moderate predicted systemic clearance in humans. Cerebrospinal fluid *(CSF)* exposures in rats indicated RO6953958 has good brain penetration, consistent with weak P-glycoprotein (P-gp) substrate properties. RO6953958 is cleared in vitro and in animal species primarily by oxidative metabolism. After single dose intravenous (IV) administration in rats, less than 1% of the administered dose was found in urine and feces as parent compound, suggesting that the renal clearance plays a minor role in RO6953958 elimination in rats.

The primary steps in RO6953958 oxidation *by human liver microsomes or human hepatocytes* mainly result in the generation of metabolites M1 (N-oxide), M2, and M3 (phenol), with minor secondary metabolites also identified (secondary oxidation products and glucuronides). RO6953958 is a substrate for human cytochrome P450 (CYP) 3A4 and CYP2C19. RO6953958 is *an* inhibitor (CYP2C19, 2C8, 3A4) and inducer (CYP2B6, *2C8,* 3A4) of the major human CYP enzymes *in vitro*. *However, based on anticipated*

*human dose and target exposures,* it is unlikely that co-administration with RO6953958 will alter the *pharmacokinetics* of other drugs whose disposition is influenced by CYP metabolism*, with the possible exception of CYP3A4 depending on the clinical dose. Requirement for clinical DDI studies will be refined upon availability of data from this ongoing Phase I study (BP41695).* RO6953958 is highly permeable and displays no substrate interaction with human P-gp.

In vitro studies have shown that RO6953958 does not significantly inhibit P-gp at the digoxin binding site, nor does it significantly inhibit the *breast cancer resistance protein (BCRP) efflux transporter. In addition, RO6953958 does not significantly inhibit the* organic anion-transporting polypeptides (OATP) 1B1 or OATP1B3*, the organic anion transporter OAT1, or the multidrug and toxin extrusion transporter MATE2-K. However, it can inhibit OAT3, the organic cation transporter OCT2 and MATE1 at high-substrate concentrations. The relevance of this finding will be evaluated further once the clinical dose for Phase II studies has been defined.*

*The DDI potential of M1 and M3, the major circulating metabolites identified so far, was also assessed. No perpetrator DDI risk potential due to enzymes or transporters was observed for M1 in vitro. At high concentrations only (i.e., substrate concentrations not considered to be clinically relevant), M3 was found to be an in vitro reversible inhibitor of CYP2C8, CYP3A4, and UGT1A1 and also an inhibitor of OAT3. M3 did not significantly inhibit P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OCT2, MATE1, or MATE2-K.*

The nonclinical safety program was conducted in rats and cynomolgus monkeys, which are pharmacologically relevant species.

RO6953958 was administered in the 13-week Good Laboratory Practice (GLP) toxicity study in rats at doses up to 500 mg/kg/day in males and up to 250 mg/kg/day in females, the highest doses administered, with no adverse effects. The no-observed-adverse-effect level (NOAEL) for this study *was* 500 mg/kg/day in males and 250 mg/kg/day in females.

RO6953958 was administered in the 13-week GLP toxicity study in male and female cynomolgus monkeys at doses up to 600 mg/kg/day, which was the maximum feasible dose (MFD) with no adverse effects.

*Cardiovascular safety of RO6953958 and M3 was assessed in vitro in human ether à go-go (hERG) cardiac ion channel assays. Based on the results of these studies, RO6953958 and M3 are not expected to affect hERG cardiac ion channels at the intended clinical dose. No in vivo cardiovascular effects were observed in cynomolgus monkeys after single or repeat dosing up to 600 mg/kg/day, which was the highest dose evaluated.*

*GLP safety pharmacology studies were conducted in rats to assess central nervous system (CNS) and respiratory function. No effects on the CNS were observed at doses up to 500 mg/kg in males and 250 mg/kg in females using the functional observation battery and nerve conduction velocity evaluations included in the GLP 13-week oral gavage toxicity study in rats. These were the highest doses evaluated. No effects in respiratory function were observed after a single dose of 500 mg/kg, which was the highest dose administered.*

The in vitro and in vivo mutagenicity assays did not reveal any indication *of* genotoxic potential *for RO6953958 or M3. No teratogenic effects were observed at the highest doses tested in the rat embryo-fetal development (EFD), rabbit EFD, or rat dose-range finding (DRF) juvenile studies. The NOAEL was 250 mg/kg/day in the rat EFD study and 300 mg/kg/day in the rabbit EFD study. The NOAEL was 500 mg/kg/day for males and 250 mg/kg/day for females in the rat DRF juvenile study.*

A detailed description of the chemistry, pharmacology, and safety of RO6953958 is provided in the RO6953958 Investigator's Brochure.

#### 2.2.2.1 Safety Information from Drugs of the Same Class

Safety information from a selective V1a receptor antagonist is available for balovaptan (RO5285119) which was in Phase II/Phase III clinical development for treatment of core symptoms of ASD. However, further development of balovaptan has been stopped based on the planned futility analysis of one of the Phase III studies in adults with ASD and final results in a Phase II study in children and adolescents with ASD. Balovaptan was considered to be safe and well tolerated in both studies.

No adverse event (AE) pattern thought to be causally linked to the blockade of the V1a receptor has been identified as of now.

Animal toxicities noted with balovaptan emerged at plasma levels higher than required to block the V1a receptor and are considered to be *related to the chemical structure of balovaptan and its* metabolite*(s)* and not target-related *(RO5285119 [balovaptan] Investigator's Brochure)*. As RO6953958 is chemically distinct from balovaptan and from balovaptan's major metabolite in animals, the animal toxicities observed with balovaptan are considered irrelevant for RO6953958. Indeed, the available 13-week GLP toxicology results with RO6953958 in rats and monkeys did not reveal toxicities found in animals with balovaptan.

Additionally, the effects of a single, 20-mg IV dose of RO5028442, another V1a receptor antagonist, was assessed in a multicenter, randomized, double-blind, placebo-controlled, crossover study in 19 high functioning male participants with ASD Single doses of 20 mg, associated with a predicted 90% receptor occupancy at $C_{trough}$, were well tolerated (Umbricht et al 2017).

Therefore, no particular target-related safety concerns are expected for this novel target of V1a receptors RO6953958 in this proposed entry-into-human study.

Taken together, safety experience from other V1aR receptor antagonists does not point to a safety alert related to mode of action and no relevant toxicities were observed in 13-week GLP toxicology animal studies with RO6953958 indicating a particular non-target related safety risk.

#### 2.2.2.2 Preliminary Pharmacokinetic *and Safety* Data Observed in Part 1 *and Part 2*

At the time of writing version *7* of this protocol, *64 male* participants (active and placebo *3:1*) had been enrolled in *S*tudy BP41695. *In Part 1 (SAD) 48 participants* received single oral doses of 5 mg, 15 mg, 45 mg, 90 mg, 180 mg, and 360 mg respectively, with 6 participants on active, and 2 on placebo at each dose level. Subjects receiving 90 mg returned around 4 weeks later for another dose of 90 mg under fed conditions. *In the ongoing Part 2 (MAD) of the study, a further 16 male participants (active and placebo 3:1) have been dosed with 45 mg and 140 mg QD for 10 days, with 6 participants on active, and 2 on placebo at each dose level. On 12 Jul 2021, a new cohort was started within the MAD part with a dose of 210 mg QD for 10 days. This cohort is currently ongoing.*

***Clinical Pharmacology***

A full PK data set was available for *Part 1 (SAD) of this study.* A preliminary PK parameter summary based on the available PK data of RO6953958 and its metabolites (RO7045755 and RO7021594) is presented in *Table 8*.

*Following single oral doses ranging from 5 mg to 360 mg in the fasted state,* RO6953958 was rapidly absorbed with a median $T_{max}$ of between 1-2 h *postdose*. A later $T_{max}$ for the metabolites, RO7045755 and RO7021594, was observed with median values of approximately 2 h which was further delayed to between 4 - 7h for 45 to 360 mg, respectively. The $C_{max}$ of RO6953958 increased in a less than dose proportional manner, for *single doses of* 5, 15, 45, 90 180, and 360 mg. However*,* the $AUC_{inf}$ of RO6953958 appears to increase with dose for 5, 15, 45, 90, 180, and 360 mg, respectively in a relatively dose-proportional manner. The metabolites RO7045755 and RO7021594 show a similar trend with dose for $C_{max}$ and $AUC_{inf}$. In terms of overall exposure compared to parent (RO6953958), RO7021594 is approximately 7-fold higher than RO6953958 whilst RO7045755 is comparable to RO6953958 *after a single dose*. When 90 mg RO6953958 was administered with a high fat breakfast, the $C_{max}$ of RO6953958 increased 2-fold compared to fasted conditions, whereas $AUC_{inf}$ remained similar (*Table 8*). This trend was also observed for both metabolites (RO7045755 and RO7021594).

Urinary excretion of RO6953958 after single dosing was quantifiable in urine, however, the fraction excreted was very small and generally less than 1% of the dose administered (range: 0.27% to 0.64%). Excretion of M3 was comparable to parent, however, M1 urinary excretion was around 10-fold higher.

**Table 8 Summary of pharmacokinetic parameters for RO6953958 and its metabolites (RO7045755 *[M1]* and RO7021594 *[M3]*) following single doses of 5, 15, 45, 90, 180, and 360 mg under fasted and fed conditions**

| | | Analyte | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | RO6953958 | | | RO7021594 | | | RO7045755 | | |
| Dose and fed status | | Tmax* (hr) | Cmax (ng/ml) | AUCINF_obs (hr*ng/ml) | Tmax (hr) | Cmax (ng/ml) | AUCINF_obs (hr*ng/ml) | Tmax (hr) | Cmax (ng/ml) | AUCINF_obs (hr*ng/ml) |
| RO6953958 5MG | Geometric Mean | 1 | 70.3 | 206 | 2 | 61.3 | 1490 | 2 | 31.9 | 238 |
| Fasted | Geometric CV% | | 31.4 | 39.7 | | 45 | 37.7 | | 33 | 64 |
| | Min | 0.5 | 49.5 | 132 | 1 | 35 | 1030 | 1 | 21.2 | 105 |
| | Max | 2 | 99.7 | 401 | 3 | 127 | 2820 | 3 | 43.4 | 518 |
| RO6953958 15MG | Geometric Mean | 1 | 150 | 676 | 2 | 187 | 5130 | 2 | 77.3 | 740 |
| Fasted | Geometric CV% | | 34.2 | 52.2 | | 41 | 49.9 | | 25.2 | 49 |
| | Min | 0.5 | 106 | 317 | 2 | 122 | 3220 | 1 | 59.9 | 423 |
| | Max | 2.12 | 250 | 1180 | 6 | 375 | 11400 | 6 | 119 | 1400 |
| RO6953958 45MG | Geometric Mean | 2 | 299 | 1780 | 5.5 | 480 | 12600 | 4 | 169 | 1700 |
| Fasted | Geometric CV% | | 50 | 26.2 | | 76 | 90.5 | | 36.9 | 35.5 |
| | Min | 1 | 135 | 1180 | 5 | 224 | 4420 | 2.02 | 108 | 1010 |
| | Max | 3 | 525 | 2530 | 7 | 1180 | 33200 | 6 | 248 | 2500 |
| RO6953958 90MG | Geometric Mean | 2 | 451 | 5120 | 6.51 | 490 | 21100 | 5 | 272 | 5120 |
| Fasted | Geometric CV% | | 30.7 | 46.5 | | 18.8 | 42 | | 32.5 | 32.2 |
| | Min | 1 | 324 | 2720 | 5 | 388 | 12000 | 3 | 194 | 3190 |
| | Max | 2.02 | 704 | 7890 | 10 | 597 | 37100 | 6 | 473 | 7170 |
| RO6953958 90MG | Geometric Mean | 4 | 932 | 5410 | 5.5 | 819 | 21600 | 5 | 491 | 5770 |
| Fed | Geometric CV% | | 34.6 | 39.3 | | 21.2 | 40.1 | | 29.1 | 33.2 |
| | Min | 2 | 618 | 3020 | 3 | 606 | 12700 | 2 | 311 | 3730 |
| | Max | 5 | 1450 | 7780 | 10 | 1120 | 36500 | 7 | 662 | 8140 |
| RO6953958 180MG | Geometric Mean | 2 | 618 | 9710 | 6.5 | 1230 | 74600 | 4 | 305 | 6900 |
| Fasted | Geometric CV% | | 47.4 | 31.3 | | 67.2 | 79.6 | | 71.1 | 62.4 |
| | Min | 1 | 365 | 6760 | 2 | 625 | 35900 | 2 | 107 | 2940 |
| | Max | 5 | 1110 | 16400 | 48 | 2890 | 179000 | 7.05 | 630 | 14500 |
| RO6953958 360MG | Geometric Mean | 2 | 866 | 15200 | 7.01 | 1280 | 79100 | 5.01 | 481 | 11700 |
| Fasted | Geometric CV% | | 39.5 | 78.7 | | 28.3 | 46.9 | | 42.4 | 87.8 |
| | Min | 0.5 | 460 | 5930 | 5 | 856 | 43600 | 3 | 265 | 3040 |
| | Max | 3 | 1220 | 30300 | 24.1 | 1950 | 156000 | 10 | 817 | 25600 |

* For all $T_{max}$ values the median is reported, not the geometric mean.

In the ongoing Part 2 (MAD) of this study, oral doses of 45 mg and 140 mg were administered for 10 days QD under fed conditions. Table 9 provides a summary of the available results for PK parameters on Day 1 and 10 for RO6953958 and the metabolites RO7045755 (M1) and RO7021594 (M3)

Based on the half-lives of RO6953958, M1, and M3 - with M3 showing the longest geometric mean half-life ranging between 15.0 and 29.5 hours - steady state is expected to have been reached by Day 10. RO6953958 was rapidly absorbed with a $t_{max}$ of approximately 2 hours postdose. The total AUC from 0 to next dose ($AUC_{tau}$) for RO6953958 increased in a mostly dose-proportional fashion over the two doses. However, a less than dose-proportional increase in $C_{max}$ on both Day 1 and 10 was

evident, suggesting solubility-limited absorption. The accumulation ratios (AR) from Day 1 to Day 10 were ~1 for both $C_{max}$ and $AUC_{tau}$ for the parent compound (RO6953958) and M1, whereas they were ranging from 1.6-1.9 for M3.

For M3, $AUC_{tau}$ was approximately 4.6- and 8-fold higher than the parent compound, on Days 1 and 10, respectively; while exposure levels of M1 were 0.7-fold of the parent compound on both Days 1 and 10, for both doses administered.

***Table 9 Summary of Pharmacokinetic Parameters for RO6953958 and the Metabolites RO7045755 (M1) and RO7021594 (M3) on Day 1 and Day 10 Following Multiple Oral Doses of 45 mg and 140 mg for 10 Days Under Fed Conditions***

| | | | Analyte | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | RO6953958 | | | | | | RO7021594 (M3) | | | | | | | | RO7045755 (M1) | | | | | | | |
| Dose (mg) | Day | n=6 | $t_{max}$* (hr) | $C_{max}$ (ng/ml) | AR $C_{max}$ | $AUC_{tau}$ (hr*ng/ml) | AR $AUC_{tau}$ | $t_{1/2}$ (hr) | $t_{max}$* (hr) | $C_{max}$ (ng/ml) | AR $C_{max}$ | $AUC_{tau}$ (hr*ng/ml) | AR $AUC_{tau}$ | $t_{1/2}$ (hr) | MPR_Cmax | MPR_ $AUC_{tau}$ | $t_{max}$* (hr) | $C_{max}$ (ng/ml) | AR $C_{max}$ | $AUC_{tau}$ (hr*ng/ml) | AR $AUC_{tau}$ | $t_{1/2}$ (hr) | MPR_ $C_{max}$ | MPR_ $AUC_{tau}$ |
| 45 | 1 | Geomean | 2.53 | 469 | | 1830 | | 2.54 | 5.00 | 677 | | 9040 | | 15.0 | 1.39 | 4.75 | 4.00 | 171 | | 1450 | | 3.87 | 0.350 | 0.761 |
| | | GM CV% | 21.9 | 38.7 | | 44.7 | | 45.9 | 33.1 | 47.9 | | 43.8 | | 13.6 | 77.7 | 72.5 | 28.5 | 43.0 | | 74.1 | | 44.6 | 32.3 | 36.2 |
| | | Min | 2.00 | 268 | | 833 | | 1.52 | 3.00 | 455 | | 5910 | | 11.8 | 0.726 | 2.67 | 3.00 | 97.3 | | 485 | | 2.15 | 0.227 | 0.465 |
| | | Max | 3.00 | 798 | | 2810 | | 3.80 | 6.03 | 1330 | | 17700 | | 17.0 | 3.46 | 11.9 | 5.00 | 266 | | 2450 | | 6.01 | 0.542 | 1.12 |
| 45 | 10 | Geomean | 2.02 | 490 | 1.05 | 2010 | 1.10 | 3.32 | 5.02 | 1100 | 1.63 | 16400 | 1.81 | 29.5 | 2.16 | 7.85 | 3.01 | 194 | 1.13 | 1550 | 1.07 | 4.32 | 0.380 | 0.744 |
| | | GM CV% | 41.8 | 25.0 | 19.0 | 38.4 | 8.41 | 41.9 | 39.2 | 41.4 | 12.1 | 47.9 | 13.9 | 34.6 | 50.7 | 69.3 | 35.3 | 44.9 | 10.0 | 79.7 | 8.13 | 50.3 | 37.3 | 40.1 |
| | | Min | 1.00 | 384 | 0.876 | 1000 | 1.01 | 1.96 | 2.08 | 704 | 1.45 | 9440 | 1.60 | 18.4 | 1.09 | 4.19 | 2.08 | 98.8 | 1.01 | 500 | 0.989 | 2.13 | 0.248 | 0.436 |
| | | Max | 3.00 | 699 | 1.43 | 2840 | 1.24 | 4.72 | 5.05 | 1930 | 1.94 | 31000 | 2.28 | 44.8 | 3.91 | 17.0 | 5.02 | 306 | 1.31 | 2540 | 1.21 | 7.48 | 0.654 | 1.05 |
| 140 | 1 | Geomean | 2.01 | 1190 | | 5820 | | 5.81 | 3.02 | 1790 | | 27400 | | 19.9 | 1.45 | 4.53 | 3.02 | 452 | | 3980 | | 6.07 | 0.366 | 0.658 |
| | | GM CV% | 36.9 | 43.5 | | 64.0 | | 17.5 | 52.4 | 66.7 | | 69.6 | | 72.0 | 99.3 | 93.8 | 26.7 | 31.5 | | 70.8 | | 45.8 | 35.6 | 46.5 |
| | | Min | 1.00 | 720 | | 3330 | | 4.40 | 3.00 | 887 | | 13300 | | 11.6 | 0.444 | 1.68 | 3.00 | 332 | | 1760 | | 3.34 | 0.248 | 0.367 |
| | | Max | 3.02 | 1920 | | 12900 | | 7.16 | 10.0 | 3490 | | 67800 | | 50.0 | 3.34 | 11.0 | 5.00 | 779 | | 8500 | | 12.4 | 0.639 | 1.14 |
| 140 | 10 | Geomean | 2.04 | 1250 | 1.05 | 6060 | 1.04 | 7.28 | 4.00 | 3180 | 1.77 | 51500 | 1.88 | 26.4 | 2.46 | 8.17 | 3.04 | 456 | 1.01 | 4290 | 1.08 | 6.71 | 0.352 | 0.681 |
| | | GM CV% | 41.2 | 49.7 | 24.0 | 58.0 | 15.9 | 46.4 | 40.5 | 76.7 | 32.2 | 85.4 | 19.9 | 32.1 | 110 | 101 | 26.5 | 68.3 | 45.2 | 97.2 | 23.4 | 42.8 | 29.4 | 52.4 |
| | | Min | 1.02 | 616 | 0.706 | 2850 | 0.858 | 3.22 | 3.00 | 1360 | 1.21 | 22000 | 1.54 | 15.9 | 0.719 | 2.59 | 3.00 | 186 | 0.560 | 1390 | 0.791 | 3.56 | 0.239 | 0.359 |
| | | Max | 3.00 | 2310 | 1.40 | 11600 | 1.25 | 11.4 | 7.00 | 9710 | 2.78 | 181000 | 2.67 | 37.2 | 6.87 | 19.8 | 5.00 | 1020 | 2.09 | 11900 | 1.55 | 11.2 | 0.527 | 1.15 |

*AR = accumulation ratio; Geomean = Geometric Mean; GM CV% = geometric coefficient of variation; Max = maximum; Min = minimum; MPR = metabolite to parent ratio; * = Median for $t_{max}$.*

***Safety***

*A total of 48 healthy male participants enrolled in the SAD part received single, oral doses of 5, 15, 45, 90, 180, and 360 mg with 6 participants on RO6953958 and 2 on placebo at each dose level. There were no deaths or serious adverse events (SAEs) reported. The majority of the AEs were mild and self-limited (resolved within 24 hours), with no dose-limiting AEs or dose-limiting toxicities observed. There were no clinically significant changes in vital signs, laboratory tests, or ECGs including QTc and PR interval.* Oral administration of RO6953958 at single doses *has been generally safe and* well tolerated *and no dose relationship was observed for any of the reported AEs.*

*In the SAD part (Part 1)*, a total of *22 treatment-emergent AEs (TEAEs)* were *recorded for 17* participants*, of which 15 received RO6953958 and 2 received placebo. Of the 22 TEAEs, 15 were considered to be related to study drug administration by the Principal Investigator.* The majority of AEs involved the system organ class of nervous system disorders. The most commonly reported *AEs* were headache (n = 5) and somnolence (n = 3)*; these events were reported by participants who received 5, 15, 90, or 360 mg RO6953958. Sixteen TEAEs* were *reported with* mild intensity, including all of the somnolence events. Six of the AEs were reported with moderate intensity*:* ear infection, sore throat, headache, presyncope and syncope *in* participants *who received either*15 mg RO6953958 or placebo. Back pain *was reported by a participant who received* 180 mg RO6953958.

*In the ongoing, and still-blinded MAD part (Part 2), an additional 16 participants have been enrolled who received 45 mg or 140 mg for 10 days QD with 6 participants on active and 2 on placebo at each dose level. There have been no deaths or SAEs reported and no clinically significant changes in vital signs, laboratory tests, or ECG including QTc and PR interval. On 12 July 2021, a new cohort in MAD with the dose of 210 mg QD for 10 days started and is currently ongoing. So far, a similar AE profile has been observed in Part 2 as that in Part 1 of the study.*

*A detailed description of the chemistry, pharmacology, and safety of RO6953958 is provided in the Investigator's Brochure.*

## 2.3 BENEFIT/RISK ASSESSMENT

For this first study with RO6953958 in humans, healthy male participants aged 18 to 55 (inclusive) were chosen because of the absence of potentially confounding disease processes, which will lead to a clearer and more consistent assessment of drug disposition and biological activity. In addition, healthy participants are unlikely to require concomitant medication that could interfere with the study drug or its PD effects. Female participants have been excluded as nonclinical teratogenicity studies have not been conducted to date and not to expose female participants to undue risk.

In the single-ascending dose (SAD) part (Part 1), in order to avoid simultaneous exposure of all participants on the same day, a sentinel group at each dose level will be dosed first with 2 participants randomized; 1 on the active treatment and 1 on placebo. If the safety and tolerability from the first 2 participants are acceptable in the judgment of the Investigator 23 hours after dosing, the remaining participants (randomized as 5 on the active treatment and 1 on placebo) will be dosed at the same dose level at least 24 hours later. Ascending doses are planned in order to establish the safety and tolerability at low dose levels before proceeding to higher dose levels. Dose levels may be adjusted (increased, decreased, or repeated) or intermediate doses may be used depending on the emerging safety and PK data at each dose level. In order to ensure safety of the participants, the effects of each dose level including safety and PK will be reviewed carefully before escalating to the next dose level. Criteria and stopping rules for dose-escalation decisions are detailed in Section 4.1.5 and Section 4.1.7. No therapeutic benefit is anticipated for participants of this study, as is common for most Phase I studies involving healthy participants. The evaluation of potential risks of RO6953958 in humans is based primarily on available data from nonclinical toxicology, safety pharmacology studies documented class risks from other drugs developed in house that bind V1a receptors as antagonists (balovaptan and RO5028442; see Section 2.2.2.1) and preliminary blinded safety data from Part 1 of the study (Section 2.2.2.2).

To ensure the safety of participants, safety monitoring/assessments covering broadly the various organ systems have been implemented, while nonclinical studies do not mandate a particular dedicated safety measure. During the conduct of the study, participants will be hospitalized at the clinical research unit for the entire treatment duration and for at least 48 hours after the last dose. Safety and tolerability will be monitored closely throughout the study. Tolerability will be assessed by recorded AEs. In addition, any cardiovascular changes will be monitored with extensive and repeated measurements of vital signs (blood pressure [BP] and pulse rate), triplicate 12-lead and Holter electrocardiograms (ECGs) (in Part 1, no Holter ECG for the fed part in the FE cohort*; in Part 3 no Holter ECG*), and laboratory safety parameters, which will be recorded in *all* parts of the study. In addition, RO6953958 concentrations will be measured in plasma and urine on specific days, and plasma PK concentrations will be used to support dose escalation. Furthermore, CSF sampling by lumbar puncture will be performed in Part 2 of the study in all participants per cohort to support dose selection for future clinical studies.

To ensure the safety of study participants and staff and minimise their risk of COVID-19 infection, appropriate trial and site specific COVID-19 risk mitigation measures will be implemented as required per local and site regulations.

Additionally, a risk assessment was conducted to determine whether there is any impact of the COVID-19 vaccines on the benefit/risk assessment of this study protocol including but not limited to the healthy population under study and study treatment being

evaluated. Based on this assessment, in order not to delay study participant from receiving a COVID-19 vaccination when it is offered to them, participants are allowed to receive a dose of the vaccine 21 days prior to the first dose of study treatment or a dose of the vaccine at least 10 days after the last dose of study treatment. The existing safety monitoring and management guidelines, as well as the risk minimization measures outlined in the clinical protocol, are considered appropriate to support administration of COVID-19 vaccines.

Following the FDA guidance on estimating the maximum safe starting dose in adult healthy volunteers (U.S. FDA 2005), a maximum starting dose of 240 mg was calculated without taking into consideration the pharmacologically active dose (PAD). However, as the dose of 240 mg daily is anticipated to be within the estimated therapeutic dose range in humans (10 mg to 160 mg twice daily [BID]) which is to be explored during Part 1, a starting dose of 5 mg has been selected (Section 4.3). An alternative approach is a starting dose offering the possibility to assess safety of dose levels that are below the anticipated therapeutic PAD range in humans based on PK/PD (EMA 2017). A 5-mg starting dose is expected to be safe and associated with no or only minimal PD effect(s). The conservative and careful selection of the starting dose ensures safety margins of more than 565- and 975-fold on the basis of free concentrations for $C_{max}$ and area under the curve (AUC) against the NOAEL of cynomolgus monkey. Furthermore, subsequent dose-escalation-decision criteria include a maximum individual exposure that does not exceed a predicted exposure for $C_{max}$ of approximately 3500 ng/mL and/or $AUC_{0-24}$ of 35,000 ng·h/mL.

In Part 1, dose escalation will be stopped if the subsequent dose is predicted to result in maximum individual exposures that would exceed a $C_{max}$ of approximately 3500 ng/mL and/or $AUC_{0-24}$ of 35,000 ng·h/mL or the MFD of 10 dose units of 80 mg (800 mg) QD. A $C_{max}$ of approximately 3500 ng/mL $AUC_{0-24}$ of 35,000 ng·h/mL has a 9-fold safety margin below the NOAEL (based on free concentrations) reported for cynomolgus monkey. The maximum dose in Part 2 (MAD) will not exceed maximum individual total and peak exposures observed in Part 1 or doses tested in Part 1 (SAD). Part 1 of the study will also investigate the effect of food on the PK of RO6953958 to establish the appropriate timing of dosing relative to food intake for Part 2 and subsequent studies. The dose for the FE is to be performed at the anticipated therapeutic dose tested in Part 1 but may be adjusted based on the review of the PK data.

In addition to the potential risks associated with study drug administration, other potential risks include discomfort from planned study procedures such as CSF sampling. CSF sampling by lumbar puncture carries a risk of post-puncture headache, and, although rare, a potential risk of spinal infection or hemorrhage. These risks will be minimized by careful selection of the participants and by having an experienced anesthesiologist perform the lumbar punctures. The rationale for CSF sampling in this study can be found in Section 4.2.6.

*For Part 3, please refer to Section 4.2.7 for rationale of midazolam and its associated risks.*

Furthermore, additional safety measures are required during the assessment of the core body temperature. After the core body temperature pill (capsule sensor) is swallowed and until the device has left the body, magnetic resonance imaging (MRI) is strongly forbidden. In order to make sure that this is not overlooked it is recommended to affix an MRI warning wrist band to the participant with the instruction to wear it until the sensor has left the body. It is recommended to swallow the capsule sensor with water or any other suitable liquid to avoid choking. In rare instances, the capsule sensor may become lodged in the intestines. Participants are therefore asked to report any gastrointestinal discomfort following ingestion of the capsule, such as nausea, vomiting or pain immediately.

The administration of RO6953958 is anticipated to be safe and well-tolerated following single and multiple oral dose administration based on available nonclinical data and under the study conditions described above and outlined in Section 1.2 and Section 4. *However, because the theoretical risk of blood pressure decrease has not been fully evaluated, care should be taken by participants not to stand up abruptly, or without using the support of a stable object following dosing with RO6953958.*The potential risks for any healthy participants due to the treatment of RO6953958 or study-related procedures are considered minimal and outweighed by the potential to develop a new treatment for ASD and autism-related sleep disorders. More detailed information about the known and expected benefits in the context of potential risks and reasonably expected AEs of RO6953958 is provided in the RO6953958 Investigator's Brochure.

# 3. OBJECTIVES AND ENDPOINTS

The objectives and corresponding endpoints are provided in Table 10.

## Table 10 Objectives and Endpoints

| | Objectives | Endpoints |
|---|---|---|
| **Primary** | | |
| **Parts 1, 2, and** *3* | • To assess the safety and tolerability of single- and multiple-ascending doses of RO6953958 *alone and in combination with midazolam (Part 3 only)* in healthy participants | • Incidence and severity of AEs. Changes in vital signs, physical findings, ECG parameters (12-lead safety *in all parts* and 24-hour Holter *in Part 1 and Part 2 only*), and clinical laboratory results during and following RO6953958 administration<br>• Change in suicide risk (using Columbia Suicide Severity Rating Scale) in Part 2 only |
| **Secondary** | | |
| **Parts 1, 2, and** *3* | • To investigate the PK of RO6953958 and its metabolites RO7021594 and RO7045755 following single and multiple doses *of RO6953958 alone and in combination with midazolam (Part 3 only)*<br>• To investigate the dose proportionality of RO6953958 | • PK concentrations and PK parameters of RO6953958 and its metabolites RO7021594 and RO7045755 |
| **Part 1** | • To assess the effect of food on the PK of a single dose of RO6953958 | • PK concentrations and PK parameters of RO6953958 and its metabolites RO7021594 and RO7045755 in fasted and fed state |
| ***Part 3*** | • *To investigate the effect of RO6953958 on the PK of the cytochrome P450 (CYP) 3A substrate midazolam* | • *PK concentrations and parameters of midazolam* |

## Table 10 Objectives and Endpoints (cont.)

| | Objectives | Endpoints |
|---|---|---|
| **Exploratory** | | |
| **Parts 1, 2, and 3** | • To screen for the presence of RO6953958 derived metabolites<br>• To assess the relative abundance and PK parameters of any metabolite as appropriate | • PK concentrations of RO6953958 derived metabolites, if appropriate<br>• PK parameters of RO6953958 derived metabolites, if appropriate |
| **Parts 1, 2, and 3** | • To investigate whether genetic variants (e.g., at drug-metabolizing enzymes transporters, receptors) affect the PK, PD, and/or safety of RO6953958 | • Clinical genotyping data |
| **Part 1 and 2** | • Optional cardiodynamic ECG assessment:<br>To evaluate the effect of RO6953958 on ECG parameters, including Concentration-QTc (C-QT) analysis | • Change-from-baseline heart rate (HR), PR, QRS and QTcF (ΔHR, ΔPR, ΔQRS and ΔQTcF)<br>• Placebo-corrected ΔHR, ΔPR, ΔQRS and ΔQTcF(ΔΔHR, ΔΔPR, ΔΔQRS and ΔΔQTcF)<br>• Categorical outliers for HR, QTcF, PR, and QRS<br>• Frequency of treatment emergent changes of T-wave morphology and U-wave presence |
| **Part 2** | • To investigate the effect of RO6953958 on sleep and circadian rhythm | • PD parameters including sleep quality questionnaires (PROMIS Sleep Disturbance and Sleep-Related Impairment), sleepiness scale (KSS), melatonin *onset and offset*, cortisol, polysomnography (PSG) *and* sleep mat-*derived sleep parameters*, *nocturnal* core body temperature, wake EEG and FERT |

**Table 10 Objectives and Endpoints (cont.)**

| | Objectives | Endpoints |
|---|---|---|
| **Exploratory** | | |
| **Part 2** | • To determine cerebrospinal fluid (CSF) concentrations of RO6953958 and its metabolites RO7021594 and RO7045755 following multiple doses | • CSF concentrations of RO6953958 and its metabolites RO7021594 and RO7045755 |
| ***Part 3*** | • *To evaluate the endogenous biomarker 4β-hydroxycholesterol (4βHC)/cholesterol ratio for the assessment of CYP3A activity following oral administration of RO6953958* | • *Plasma concentrations and percent change from baseline of 4βHC and total cholesterol, and the 4βHC/cholesterol ratio* |

# 4. STUDY DESIGN

## 4.1 OVERALL DESIGN

An overview of the study design is provided in Section 1.2.

The study is a *three*-part design*:*

Part 1 (SAD) of this study will examine safety and tolerability, and characterize PK and PD effect by evaluating the Karolinska sleepiness scale (KSS). Part 1 will also evaluate FE after RO6953958 administration at the dose(s) proposed for clinical use.

In Part 2 (multiple-ascending dose [MAD]) the safety, tolerability, PK, and PD effects by evaluating cortisol, melatonin, and sleep parameters of multiple doses of RO6953958 will be evaluated in fed conditions, based on preliminary PK data in Part 1. In addition, concentrations of RO6953958 and its metabolites will be measured in CSF. The doses in Part 2 will be determined based on review of the Part 1 data (see Section *4.1.5.2*). Ascending doses are planned in order to establish the safety and tolerability at low dose levels before proceeding to higher dose levels. Dose levels may be adjusted (increased, decreased, or repeated), or intermediate doses may be used depending on the emerging safety and PK data at each dose level.

*In Part 3 (to be run after the highest dose in MAD QD dosing is completed) the safety, tolerability, and effect of RO6953958 on the PK of the CYP3A substrate midazolam will be assessed by evaluating PK parameters of midazolam. In addition, concentrations of*

*4β-hydroxycholesterol (4βHC) and total cholesterol will be measured, and the 4βHC/cholesterol ratio will be calculated as an endogenous marker of CYP3A activity.*

### 4.1.1 Part 1 (SAD/FE)

Part 1 will be a randomized, Investigator-/ subject-blind, placebo-controlled SAD study with a parallel design and sentinel dosing, to explore the safety, tolerability, PK, and PD of single doses of orally administered RO6953958 in the fasted state and using one cohort to administer RO6953958 in the fed state.

Participants will be recruited in seven sequential cohorts. In each cohort, participants will receive either a single oral dose of RO6953958 or placebo while fasted. Participants in the FE cohort (90 mg) will return to receive the same dose repeated in the fed state. Each participant in the selected cohort will receive a single dose of RO6953958 30 minutes after starting a standardized high fat, high calorie breakfast. If participants are not willing to complete the fed part, additional participants will be recruited to ensure that a minimum number of participants complete food effect investigations (6 on active treatment and 2 on placebo in each period).

The starting dose of 5 mg is anticipated to be safe and have minimal pharmacological effects. Subsequent doses will be selected during study conduct based on emerging safety and PK data. In all cohorts, sentinel dosing will be employed to allow for an evaluation of safety data by the Investigator up to 23 hours after the start of treatment before subsequent participants are dosed (see Section 4.1.6.3). There will be at least 1 week between each dose level in order to permit adequate time for collation and review of emerging data before the next dose is administered. A tentative dose escalation scheduled is provided in Table 11. After review of the PK exposures ($C_{max}$ and area under the concentration–time curve from Time 0 to infinity [$AUC_{inf}$]) in Cohort 1 and in the case that the observed exposure of the first dose in humans is significantly below the predicted concentrations, the dose of Cohort 2 may be adjusted by a greater than 3.3-fold increase in order to reach the expected exposure level for the original starting dose (with the same maximum increase of 3.3-fold multiples thereafter for further dose-escalation steps).

**Table 11 Confirmed and Tentative Dose Escalation in the SAD (Part 1)**

| | |
|---|---|
| Cohort 1 | 5 *mg* (2*2.5 *mg*) |
| Cohort 2 | 15 *mg* (1*10 *mg* and 2*2.5 *mg*) |
| Cohort 3 | 45 *mg* (4*10 *mg* and 2*2.5 *mg*) |
| Cohort 4 | 90 *mg* (1*80 *mg* and 1*10 *mg*) and FE* |
| Cohort 5 | 180 *mg* (2*80 *mg* and 2*10 *mg*) |
| Cohort 6 | 360 *mg* (4*80 *mg* and 4*10 *mg*) |
| Cohort 7** | *to be determined* |

*FE = food effect; SAD = single-ascending dose.*

* Cohort 4 will return to receive the same dose in the fed state.

** Cohort 7 may run pending emerging data review from the MAD. Timing of dosing of RO6953958 may be in the evening and dosing may be done under different food condition (fed vs fasted).

The dose for FE part of Part 1 is confirmed to be performed at the dose tested in Cohort 4 (90 mg dose). The fed part will not proceed until adequate safety coverage from a higher dose (Cohort 5) is confirmed.

### 4.1.2 Part 2 (MAD)

Part 2 will be a randomized, Investigator-/ subject-blind, placebo-controlled MAD study with the purpose of evaluating the safety, tolerability, PK, and PD of multiple doses of orally administered RO6953958. Participants from Part 1 cannot participate in Part 2. The criteria to move from Part 1 to Part 2 of the study are detailed in Section 4.1.5.2.

Part 2 will explore the safety, tolerability, PK, and PD of multiple doses of RO6953958. The starting dose for Part 2 is 45 mg RO6953958 QD, established on the basis of safety and PK data in Part 1. The first dosing in Part 2 will only commence once safety is confirmed on the basis of dosing in Part 1 that allows at least 4 times' coverage (e.g., start 40 mg MAD once safety confirmed at a dose of 160 mg or higher in SAD). A maximum of five dose levels are anticipated. For each dose level, a minimum of 8 and a maximum of 16 participants will receive either a multiple oral dose of RO6953958 or placebo QD for 10 days (active to placebo ratio 3:1). The treatment period may be extended to 14 days in subsequent cohorts depending on emerging data. In addition, a lumbar puncture for CSF sampling will be performed in all participants in each cohort on Day 7 or alternatively Day 6 or 8. In a cohort CSF sampling will be done predose in 4 participants and approx. 5 to 7 h after dosing of RO6953958 (around $T_{max}$ of the M3 metabolite) in the other 4 participants for determination of RO6953958, M3 (RO7021594) and M1 (RO7045755) concentrations in CSF. A corresponding blood sample will be taken at the same time point. As an alternative to CSF sampling on Day 7, it may also be performed on Day 6 or Day 8. The time points of CSF sampling in a participant may be adjusted with emerging PK or CSF samples from preceding

cohorts. In addition, these results might also lead to the conclusion to stop CSF sampling in subsequent cohorts.

The data requirements for dose escalations and stopping rules for each dose group are outlined in Sections 4.1.5.1, 4.1.5.3, and 4.1.6.4. The dose may be adjusted during the treatment period depending on the safety and/or PK observations. In addition, a different dosing regimen (Section 6.1) may be explored if warranted based on the safety and/or PK profile of RO6953958 (e.g., BID or exploration of titration). A dose escalation schedule is provided in Table 12. The starting dose in Part 2 Cohort 1 selected based on the safety and PK from Part 1 is 45 mg RO6953958 QD. Based on the outcome of Part 1, RO6953958 will be administered under fed conditions (a standard breakfast). Subsequent doses in Part 2 will be based on emerging safety and PK data. Additionally, the timing of dosing may be changed for additional participants in a cohort at a specific dose level, in order to explore the effect of time of dosing on the pharmacokinetics. A decision to include additional participants dosed in the evening at a specific dose level would only be made following review of the safety and PK following morning administration at the chosen dose level. CSF sampling would not be done in participants who are administered the dose in the evening.

**Table 12 Confirmed and Tentative Dose Escalation in the MAD (Part 2)**

| | |
|---|---|
| Cohort 1 | 45 *mg* (4*10 *mg* and 2*2.5 *mg*) QD |
| Cohort 2 | *140 mg (1*80 mg and 6*10 mg)* QD |
| Cohort 3 | *210 mg (2*80 mg and 5*10 mg) QD* |
| Cohort 4 | to be determined |
| Cohort 5 | to be determined |

*MAD = multiple-ascending dose; QD = once daily.*

### 4.1.3 *Part 3 (DDI)*

*Part 3 will be a single-center, non-randomized, open-label, five-treatment, fixed sequence crossover study with the purpose of evaluating the safety and tolerability of RO6953958 alone and in combination with midazolam and to assess the effect of RO6953958 on the PK of the CYP3A substrate midazolam. In addition, the effect of RO6953958 treatment on an endogenous marker of CYP3A activity will be assessed.*

*RO6953958 will be administered QD following a standardized breakfast on Day 3 to Day 14 at the maximum dose QD that was tested in the ongoing Part 2 (MAD). Midazolam will be administered as single IV bolus injection of 100 µg on Day 1 and Day 13, and as single oral dose of 300 µg on Day 2 and Day 14.*

### 4.1.4 Length of the Study

**Part 1 (SAD/FE)**

The total duration of the study for each participant in each cohort will be up to 7 weeks (14 weeks if the participant is part of the FE cohort) divided as follows:

- Screening: Up to 4 weeks
- In-clinic period: Days -2 to 5
- Treatment period: Day 1

  Washout period between each treatment (FE cohort only): Approximately 21 days. This might be adjusted/extended based on PK data from Part 1 or to await safety information from the subsequent dose level to provide adequate safety coverage for the fed part.
- Safety follow-up: 7 to 14 days after the last dose

**Part 2 (MAD)**

The total duration of the study for each participant will be up to 8 weeks approximately divided as follows:

- Screening: Up to 4 weeks
- In-clinic period: 16 days (Days -2[2] to 14; 2 baseline days, 10 dosing days, 6 follow-up days)
- Treatment period: Days 1 to 10 (possibly to Day 14)
- Safety follow-up: 7 to 14 days after last dose

***Part 3 (DDI)***

*The total duration of the study for each participant will be up to 8 weeks approximately divided as follows:*

- *Screening: Up to 4 weeks.*
- *In-clinic period: 19 days (Days -1 to 18; 1 baseline day, 14 dosing days, 4 follow-up days).*
- *Five treatment periods: Days 1 to 14 (Days 1 and 2 midazolam, Days 3 through 14 RO6953958, Days 13 and 14 midazolam).*
- *Safety follow-up: 7 to 14 days after the final dose of RO6953958.*

### 4.1.5 Dose-Escalation Decision Criteria

The dose-escalation decision will be made by the Roche Clinical Pharmacologist, the Principal Investigator, and appropriate personnel as required. The dose will not be escalated further if the tolerability, safety, or PK at the preceding dose level is not

[2] Participants may be admitted on Day-3 to ensure severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing can be reported prior to dosing.

acceptable as judged by the Principal Investigator and the Clinical Pharmacologist. After review of the safety and PK data and discussion between the Roche Clinical Pharmacologist and the Principal Investigator, the same dose or regimen or a lower dose within the tolerated dose range may be given again in subsequent treatments to increase data within the tolerated dose range (see Section 4.1.7).

Dose escalation in Part 1 and Part 2 for all participants will be stopped if any of the changes detailed in Section 4.1.6.3 (Part 1 only) and/or Section 4.1.6.4 occur in participants receiving RO6953958. However, the same or a lower dose may be administered after the discussion between the Sponsor's Clinical Pharmacologist and the Investigator. Additionally, dosing will be stopped if any of the stopping rules in Section 4.1.6.1 applies during the conduct of the study and Section 4.1.6.2 applies to an individual participant during the study.

Participants who prematurely discontinued from the study may be replaced (see Section 7.2) to ensure adequate numbers (at least 6 participants; 4 on RO6953958) of evaluable participants (for the definition of evaluable participant, see Section 9.3) for each dose-escalation step.

Dose escalation is dependent on the safety and PK data at each dose level. Dose-level increments will be no more than 3.3-fold and may be adjusted downwards, or doses may be repeated if safety or PK concerns emerge from ongoing results. In the case that the observed exposure ($C_{max}$ and $AUC_{inf}$) at the first SAD dose level (Cohort 1) is significantly below the predicted human exposure, a dose increment higher than 3.3-fold may be given subsequently at the second dose level (Cohort 2) to achieve the predicted exposure for the starting dose (with the same dose-escalation multiples thereafter).

Due to the exploratory nature of this clinical study, its conduct can be discontinued before the last foreseen dose level has been investigated. This will not constitute a premature termination of the study.

#### 4.1.5.1 Data Requirements for Dose-Escalation Decision for Sentinel Group and Dose Groups in Part 1

In Part 1 for each sentinel group, if the safety and tolerability results of the first 23 hours following dosing for the initial 2 participants (sentinel group) are acceptable in the judgment of the Investigator and Sponsor (see Section 4.1.7), the remaining 6 participants within the cohort will be dosed at least 24 hours after the sentinel group. After a satisfactory review of the safety, tolerability (i.e., at minimum AEs, ECG, vital signs), and PK data, progression to the next dose level will occur if the last dose and regimen is well tolerated. There will be at least 7 days between dosing in each cohort in order to permit adequate time for collation and review of emerging data before the next dose is administered.

As a minimum, the following data will be required from at least 6 participants (4 on RO6953958) of the previous dose level for dose escalation in Part 1 (SAD/FE):

- PK data over 24 hours postdose (plasma only)
- Safety data from dosing to 48 hours postdose

#### 4.1.5.2 Decision Criteria to Progress from Part 1 to Part 2

Progression to Part 2 will be based on satisfactory review of the safety and PK data by the Investigator and Sponsor from Part 1 (SAD) data. The starting dose for Part 2 (MAD) will be determined by analysis of safety and tolerability data and using PK data from the SAD part of the study. The starting dose in Part 2 will be chosen so as to produce exposures at steady-state close to those predicted to target an average V1a receptor occupancy of approximately 90% over 24 hours.

The first dosing in Part 2 will commence once safety is confirmed on the basis of dosing in Part 1 that allows at least 4 times' coverage (i.e., start 40 mg MAD once safety has been confirmed in 160 mg SAD).

#### 4.1.5.3 Data Requirements for Dose-Escalation Decision Criteria for Part 2

Doses in Part 2 (MAD) will be selected based on satisfactory review of the safety and PK data by the Investigator and Sponsor from Part 1 (SAD) data and emerging data from subsequent cohorts in Part 2. Dose escalation for each cohort in Part 2 will be based on data collected in at least 6 participants (4 on RO6953958) and from previous dose levels. If a dose de-escalation is planned at later cohorts (e.g., cohort 4), dosing would proceed based on initial safety of ongoing cohorts up to Day 10 (at least 3 on RO6953958) and safety and PK data from a preceding cohort that ensured at least 3-fold coverage.

As a minimum, the following data will be required from at least 6 participants of the previous dose level for dose escalation in Part 2 (MAD):

- PK data over 24 hours post-last dose (plasma only)
- Safety data from first dosing to 24 hours post-last dose

#### 4.1.5.4 Decision to Expand Cohorts in Part 2 Based on Review of Sleep Biomarkers

Following review of Part 2 and establishing a recommended dose range that supports characterization of the PK/PD relationship of the sleep biomarkers, additional participants may be enrolled in order to increase the number of participants within Part 2 or explore alternative doses (on doses that do not exceed the maximum exposures observed in Part 1 [SAD]). Up to a maximum of 8 additional participants will be included per cohort to bring the maximum per cohort to 16 participants (active to placebo ratio 3:1) in order to better characterize the relationship (as described above). The selected

doses will be chosen in a dose range that optimally informs PK/PD modelling of sleep biomarkers in the Part 2.

### 4.1.6 Stopping Rules

#### 4.1.6.1 Stopping Rules for the Study

The trial will be stopped if the following criteria are met:

- A serious adverse reaction (i.e., a serious adverse event considered as, at least, possibly related to the investigational medicinal product (IMP) administration) in one participant or
- Severe adverse reactions (i.e., severe non-serious adverse events considered as, at least, possibly related to the IMP administration) in two participants in the same cohort.

Following an internal safety review, if it is appropriate to restart the trial, a substantial amendment will be submitted to the Medicines and Healthcare products Regulatory Agency (MHRA) and Research Ethics Committee (REC). The trial will not restart until the amendment has been approved by the MHRA and REC.

#### 4.1.6.2 Stopping Rules for Individuals

Dosing will be stopped at any time during the study in a given participant receiving RO6953958 if one of the following circumstances occurs:

- SAE considered related to study treatment.
- Alanine aminotransferase (ALT) $\geq 5 \times$ upper limit of normal (ULN).
- ALT $\geq 3 \times$ ULN and total bilirubin $\geq 2 \times$ ULN or international normalized ratio (INR) $> 1.5$ (if a participant meets this withdrawal criterion, serum bilirubin fractionation should be performed).
- ALT $\geq 3 \times$ ULN if associated with the appearance or worsening of rash or hepatitis symptoms (fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia). Participants who have ALT $\geq 3 \times$ ULN and $< 5 \times$ ULN, total bilirubin $< 2 \times$ ULN or INR $< 1.5$, and who do not exhibit hepatitis symptoms or rash can continue in the study (and continue receiving trial medication) as long as they can be monitored at least weekly until abnormal results are within the reference range or close to pretreatment values.

If any of the above stopping rules are met, see Section 8.3.3 for the follow-up of AEs and SAEs.

If a dose administration is suspended to allow for additional safety assessments, but no safety concerns are raised to prevent continued dosing in the judgment of the Investigator, dosing may resume at the next planned dosing date. A missed dose will not be made up (applicable to Part 2 [MAD] only).

#### 4.1.6.3 Stopping Rules after Dosing of the Sentinel Group in Part 1 (all Dose Levels)

At all dose levels in Part 1, in order to avoid simultaneous exposure of all the participants, only 2 participants (sentinel group; composed of 1 participant on active treatment and 1 on placebo) will be dosed first prior to dosing further participants at the same dose level. If the safety and tolerability data (AEs, ECG, vital signs, and clinical laboratory test results) of the first 23 hours following dosing for the initial 2 participants are acceptable in the judgment of the Investigator, 6 additional participants (5 on active treatment and 1 on placebo) will be dosed at the same dose level at least 24 hours after the sentinel group. Dosing will be stopped after the sentinel group if one of the following circumstances occurs in the participant receiving RO6953958, unless it is determined by the Investigator that the occurrence is not related to the administration of the study drug:

- One SAE
- Any other findings that, at the joint discretion of the Sponsor's Clinical Pharmacologist, the Medical and Safety monitor and the Investigator, indicate that the dosing should be stopped

#### 4.1.6.4 Dose-Escalation and Study Cohort Stopping Rules in Part 1 and Part 2

Dose-escalation will be stopped and treatment within an ongoing cohort will be stopped in Part 1 and/or Part 2, if one of the following circumstances occurs in participants receiving RO6953958 within the same dose level, unless it is determined by the Investigator that the occurrence is not related to the administration of the study drug:

- SAE considered to be related to study drug in 1 participant receiving RO6953958
- Severe non-serious AEs considered to be related to study drug in 2 or more participants receiving RO6953958 in the same cohort
- Clinical significant RO6953958-related laboratory abnormalities of the same type in 2 or more participants
- Clinical significant RO6953958-related changes in vital signs or ECGs of the same type in 2 or more participants
- If maximum individual exposure is anticipated to exceed the predicted $C_{max}$ (approximately 3500 ng/mL) and AUC (35,000 ng·h/mL) at the next planned dose level based on PK from previous dose cohorts or MFD level of 800 mg QD is achieved
- Any other findings that, at the joint discretion of the Sponsor's study team and the Investigator, indicate that the dosing should be stopped

### 4.1.7 Communication Strategy for Preceding from the Sentinel Group and each Dose Groups in Parts 1 and 2

In Parts 1 and 2, there will be ongoing reviews of available data (based on datum requirements outlined in Section 4.1.5) prior to initiation of the next dose. For the

sentinel cohort, the Sponsor must confirm via email to Investigator that the Sponsor agrees to proceed with dosing the subsequent 6 participants based on Investigator's safety review and interpretation and ensuring none of the stopping rules as outlined in Sections 4.1.6.1, 4.1.6.2, or 4.1.6.3 have been met.

For each cohort (at least 6 participants; 4 on RO6953958), a dose-escalation meeting will be conducted between the Sponsor study team and the Investigator prior to dosing of the next cohort. In addition to review of the safety and PK data, in order to proceed, none of the stopping rules as outlined in Sections 4.1.6.1, 4.1.6.2, and 4.1.6.4 are allowed to have been met.

In Parts 1 and 2, after each participant receives RO6953958 or placebo and following completion of dosing (Day 1 [Part 1 only] and Day 10), the Investigator must confirm to the Sponsor that all participants have been dosed and provide a brief summary of the status of the participant in terms of safety and tolerability to RO6953958. Additional details for communication strategy and dose-escalation procedure are found in the separate Medical Data Review Plan.

## 4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The study rationale is provided in Section 2.1.

In order to ensure maximum safety, the effects of each dose level will be reviewed carefully before escalating to the next dose level. Dose escalation will take place after the review of safety, tolerability, and PK as described in Section 4.1.5. Dose-escalation stopping rules are detailed in Sections 4.1.6.2, 4.1.6.3, and 4.1.6.4.

### 4.2.1 Rationale for Number of Dosing Days and Regimen in Part 2

The treatment period in Part 2 (MAD) will be 10 days in duration, anticipated to be sufficient to reach steady-state PK conditions and support the first planned patient trials. A once-daily (QD) dosing regimen has been chosen for Part 2 on the basis of the predicted PK properties of RO6953958 to provide exposures for the efficacious dose anticipated to target an average V1a receptor occupancy of $>$90% over a 24-hour period. The treatment period may be extended to 14 days to better characterize the safety profile of RO6953958 over a longer period after achievement of the steady-state, if warranted. The timing of dosing may be changed for additional participants in a cohort at a specific dose level, in order to explore the effect of time of dosing on the pharmacokinetics (evening vs morning).

### 4.2.2 Rationale for Study Population

Prior to initiating studies in individuals diagnosed with $NDDs\ and\ sleep\ disorders$, it is considered important to assess RO6953958 in this entry-into-human study in healthy, adult, neurotypical participants. Females have been excluded as nonclinical teratogenicity studies have not been conducted to date. Since individuals with $NDDs$ are a particularly vulnerable population, it is prudent to assess safety and tolerability, PK and PD effects, and food effect of this novel compound first in neurotypical adult participants

prior to any dosing in the $NDD$ population. This population are further unlikely to require concomitant medication, which could interfere with the study drug or its PD effects. The absence of confounding disease factors will lead to a clearer and more consistent assessment of drug disposition and biological activity.

### 4.2.3 Rationale for Control Group

This study is designed to be adequate and well controlled. Participants will be randomized to RO6953958- or placebo-treatment groups. The randomization scheme to active and placebo groups is considered necessary to generate an adequate within study comparator dataset to allow proper evaluation of the magnitude of any treatment effects. The study is Investigator-/subject-blind to eliminate potential bias.

### 4.2.4 Rationale for Orthostatic Challenge

Whilst there is no indication for relevant blood pressure changes from nonclinical profiling because normal vasopressin levels are too low to activate V1a receptors in the periphery, orthostatic challenge testing is included to document absence of orthostatic dysregulation when participants are dosed with RO6953958.

### 4.2.5 Rationale for PD/Biomarker Assessments

The PD/biomarker assessments will be timed to coincide with the anticipated time of maximum pharmacological effect of RO6953958 at single dose and steady-state. The timing of PD assessments is based on experience from the nonclinical pharmacology studies, model-based predictions of RO6953958 plasma concentrations, and anticipated effects of RO6953958. The number and timing of the samples are considered adequate to allow characterization of key elements of the response versus time profile.

Exploratory PD assessments will include cortisol, melatonin, Patient-Reported Outcomes Measurement Information System (PROMIS) Sleep Disturbance and Sleep-Related Impairment questionnaires, KSS, polysomnography (PSG), sleep mat, Facial Emotion Recognition Task (FERT), core body temperature, wake EEGs and platelet aggregation assay (PAA). These exploratory assessments are included on the basis of the theoretical relationship of the vasopressin system to circadian rhythmicity and corresponding effects on sleep and sleepiness or related functions.

### 4.2.6 Rationale for CSF sampling

Cerebrospinal fluid sampling is planned to be performed in each cohort of Part 2. In order to confirm that RO6953958 and its metabolite M3 (RO7021594), which is a potent antagonist on the V1a receptor, enter the CSF/brain, CSF sampling and determination of RO6953958, M3 (RO7021594) and M1 (RO7045755) concentrations in CSF is included in the study.

### 4.2.7 *Rationale for Drug-Drug Interaction Study Design and the Use of Midazolam*

*An important aspect of drug development is to predict clinically relevant DDI. In vitro data have demonstrated that RO6953958 is metabolized by CYP3A and may be an inhibitor (time-dependent and direct) and/or an inducer of CYP3A. The DDI part (Part 3) of this study will assess the effect of RO6953958 on the PK of a sensitive CYP3A substrate (midazolam) as measured by midazolam systemic exposure. The potential induction/inhibitory potential of multiple oral doses of RO6953958 on CYP3A will be assessed, using both oral and IV microdose of midazolam. The extent of change in exposure (if any) of the CYP3A substrate midazolam will be assessed when dosed both in absence and in the presence of steady-state exposures of RO6953958. Dosing with food significantly increases RO6953958 bioavailability and, therefore, RO6953958 dosing will be with food to maximize drug exposure. Administration of RO6953958 and midazolam will be staggered for midazolam dosing to coincide with anticipated peak concentrations of RO6953958 in gut wall enterocytes and liver hepatocytes. On Day 13, RO6953958 dosing will be 2 hours prior to midazolam dosing, and on Day 14, 1.5 hours prior to midazolam dosing. In addition, plasma concentrations of 4βHC and total cholesterol will be measured according to the SoA (Section 1.3). 4βHC is a metabolite formed by CYP3A4 and CYP3A5-catalyzed metabolism of cholesterol and rises with increasing CYP3A activity. Recent studies have shown that 4βHC and the 4βHC/total cholesterol ratio is a convenient biomarker for preliminary assessment of CYP3A induction of drugs in the liver (Björkhem-Bergman et al 2013; Kasichayanula et al 2015).*

*The DDI part (Part 3) of the study will allow for an initial assessment of the effect of RO6953958 as a perpetrator on midazolam. Furthermore, this assessment may help design future clinical trials with treatment combinations that may have potential CYP3A interaction and inform concomitant drug inclusion and exclusion criteria with respect to CYP3A substrates. Midazolam is a short-acting benzodiazepine. It is selected because it is exclusively metabolized by CYP3A, is not a substrate of P-glycoprotein, and has been validated in a number of studies. It is recommended as a sensitive model substrate for CYP3A by regulatory agencies (e.g., the FDA and EMA) and is the most widely used CYP3A probe substrate in the literature (Hohmann et al 2014). Midazolam hydrochloride, given orally, is subject to extensive first-pass metabolism by CYP3A located in the intestine and the liver (Thummel et al 1996; Gorski et al 1998). Therefore, the induction or inhibition of CYP3A in the small intestine and/or liver will affect the oral bioavailability of midazolam. The 100 μg IV dose and 300 μg oral dose have been selected to adequately characterize the pharmacokinetics of midazolam and distinguish between CYP3A4 inhibition in the small intestine vs. the liver.*

*Midazolam was selected as a probe for CYP3A activity in this study also on the basis of its safety profile, rapid elimination half-life, simple metabolic scheme, and availability of a validated bioanalytical assay. Midazolam may transiently cause sedation*

*(including respiratory depression) and short-term memory loss, however with the use of a microdose, concentrations within the body will be far below the pharmacologically active levels of midazolam; thus no adverse events are anticipated (Hohmann et al 2014; midazolam Summary of product Characteristics [SmPC]). Nevertheless, participants will be monitored for safety and tolerability in the clinic after receiving midazolam.*

### 4.2.8 *Rationale for Measuring 4β-Hydroxycholesterol/Cholesterol Ratio*

*In Part 3 of the study, the 4βHC/cholesterol ratio in plasma will be measured as an endogenous marker for hepatic CYP3A4 inhibition. 4βHC is a metabolite formed by CYP3A4 and CYP3A5-catalyzed metabolism of cholesterol (Björkhem-Bergman et al 2013). The ratio of metabolite to parent will be calculated to adjust for between-subject differences in cholesterol levels. This ratio has been used to assess CYP3A4 activity in humans and has been shown to have concordance with the midazolam method. Unlike midazolam, which is extensively metabolized by the intestinal and liver CYP3A4, the metabolism of cholesterol in the intestine is not expected to be significant. Hence, the magnitude of the induction determined by oral midazolam can be greater than by 4βHC/cholesterol as it mostly reflects liver CYP3A4 only. Correlation between midazolam clearance and endogenous substances ratios will be explored in this study. If CYP3A4 activity needs be monitored for future RO6953958 clinical studies, the 4βHC/cholesterol ratio, instead of midazolam, can be conveniently employed in such studies.*

## 4.3 JUSTIFICATION FOR DOSE

### 4.3.1 Starting Dose

In Part 1, the starting dose will be a single dose of 5 mg. Further dose levels will be dependent on the safety and PK data of the preceding dose levels.

A starting dose of 5 mg RO6953958 has been selected for this first study in humans. The starting dose is predicted to be below the anticipated therapeutic dose of 80 mg QD in humans, whilst maintaining relevant safety margins of approximately 128-fold the average exposure at the NOAEL in cynomolgus monkeys (>900-fold based on unbound exposure). The NOAEL in cynomolgus monkeys was the highest feasible dose tested with no safety findings identified (Table 13). Furthermore, based on the NOAEL in rats, the 5-mg starting dose represents a margin of approximately 650-fold (>1,400 unbound in males) to >2,500-fold (>6,000-fold based on unbound in females; Table 13).

Two approaches were taken into consideration for the selection of starting dose of 5 mg single dose administration of RO6953958. One approach uses the U.S. Food and Drug Administration's Guidance estimating the maximum recommended starting-dose (U.S. FDA 2005). The NOAEL of cynomolgus monkey is converted to the human equivalent dose (HED) by using an appropriate scaling factor, followed by an application of a safety factor of 10. The NOAEL in the 13-week study in rats is 250 mg/kg in females. This

equates to an HED of 40 mg/kg, which corresponds to a flat dose of 2,400 mg/day in a 60-kg participant. After applying a safety factor of 10, a dose of 240 mg/day is derived. A dose of 240 mg would be within the anticipated therapeutic dose range (10 mg to 160 mg BID) but is considered too high for a starting dose.

Therefore, an alternative approach is a starting dose offering the possibility to assess safety of dose levels that are below the anticipated therapeutic PAD range in humans based on PK/PD (EMA 2017). The current hypothesis is that V1a receptor occupancy of > 90% over the dosing interval (at $C_{trough}$) may provide therapeutic benefit in the human ASD population. Therefore, a dose providing 20% of this range (i.e., V1a receptor occupancy at $C_{trough}$) is considered to have no pharmacological effect. Considering the range of projected human clearance (1 to 8 ml/hr/kg) of RO6953958, a single dose of 5 mg was selected. This dose is expected to translate into an average receptor occupancy of 20% at $C_{trough}$ and 89% at $C_{max}$. This dose is not expected to lead to a PD effect after a single dose based on the clinical data with balovaptan, a similar V1a antagonist currently in development (see Section 2.2.2.1), receptor occupancy of 88% at $C_{max}$ (77% at $C_{trough}$) showed no or minimal signs of efficacy under repeated dosing conditions but required receptor occupancies > 90% over 24 hours.

**Table 13 Estimated Margins of Safety for Entry-Into-Human Starting and Therapeutic Dose**

| **Species**<br>**NOAEL (mg/kg/day)** | **$C_{max}$ / $AUC_{0-24}$***<br>**(ng/mL / ng·h/mL)** | **Exposure Margins to Cynomolgus Monkey (total [free])** |
|---|---|---|
| **Rat 13-week NOAEL:**<br>**500 mg/kg/day (M) and**<br>**250 mg/kg/day (F)** | 18,900 / 217,000 (M)<br>52,100 / 930,000 (F) | |
| **Cynomolgus monkey 13-week NOAEL: 600 mg/kg/day** | 4,140 / 42,600 | |
| **Human 5 mg anticipated starting dose** | 56 / 334 | 74 (565) / 128 (975) |
| **80 mg therapeutic dose** | 583 / 5,286 | 7 (54) / 8 (62) |

Abbreviations: $AUC_{0-24}$=area under the concentration-time curve from 0 to 24 hours; $C_{max}$=maximum plasma concentration; F=female, M=male; NOAEL=no-observed-adverse-effect level.

* Refers to $AUC_{tau}$ and $AUC_{inf}$ for animals and human, respectively.

** Free fraction in humans 1.7%, rat 3.8%, and cynomolgus monkey 13%.

### 4.3.2 *Part 3 Dose Justification*

*In Part 3 (DDI), the maximum dose QD that was tested in the ongoing Part 2 (MAD) which allows exploration of the highest exposures of RO6953958 will be given QD from Day 3 through Day 14 to maximize potential effect on CYP3A. The treatment duration of RO6953958 alone (12 days) has been selected based on data from Part 2, which indicate that steady-state plasma concentrations are achieved within 10 days in all*

*individuals (see Section 2.2.2.2). Exposure to RO6953958 is not expected to be impacted by co-administration with midazolam. The dose level will not exceed the exposure limits described in Section 4.3.3.*

*Part 3 will not begin before review of sufficient safety and PK data of the highest safe dose QD dose escalation data at a minimum (see Section 4.1.5.3).The available data on the kinetics of enzyme inhibition/induction by RO6953958 (Report 1098857) and the turnover by CYP3A enzymes suggest that maximal inhibition/induction of CYP3A activity should be achieved soon after achievement of steady state (Xu et al 2011).*

*The midazolam microdoses (i.e., single IV dose of 100 μg and single oral dose of 300 μg) have been selected based on published information from previous clinical studies and from scientific literature involving microdoses of midazolam administered to healthy participants. The recommended oral dose of midazolam for preoperative sedation, anxiolysis, and amnesia in pediatric patients is 0.25 to 0.5 mg/kg with a maximum dose of 20 mg. The single doses of midazolam selected in Part 3 of this study are far below the pharmacological active concentrations of midazolam but are still high enough to allow characterization of midazolam pharmacokinetics (Hohmann et al 2014). Local inflammation and pain at the injection site is considered unlikely because intravenous injection of midazolam is usually well tolerated and a very low dose is used in this study.*

### 4.3.3 Maximum Exposure/Dose

An estimated efficacious dose of RO6953958 has been projected assuming that target inhibition profiles need to match those estimated for balovaptan, a similar V1a antagonist currently in development. Based on in vitro binding data and stimulated plasma-free concentration at steady-state, there is confidence that the desired target inhibition profile can be achieved. A likely scenario projects a daily dose of 80 mg to match the inhibition profile for 10 mg balovaptan. Assuming a high clearance, a dose of 160 mg BID would be needed, with the low clearance scenario the dose would be only 10 mg/day. Given that RO6953958 is mainly metabolized by CYP3A, current predictions using a static model and assuming both gut and liver inhibition estimate a 7- to 10-fold increase in exposure could not be excluded with a strong CYP3A inhibitor. Therefore, maximum individual exposure parameters have been selected to allow exploration of exposures in Part 1 to cover a drug–drug-interaction scenario in combination with a strong CYP3A inhibitor. In Part 1, maximum individual exposures would not exceed a $C_{max}$ of approximately 3500 ng/mL and/or $AUC_{0\text{-}24}$ 35,000 ng·h/mL or a MFD of 10 dose units of 80 mg (800 mg) QD. The maximum dose in Part 2 (MAD) will not exceed total exposures observed in Part 1 (SAD) or maximum dose tested in Part 1 (SAD).

## 4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if the participant has completed all phases of the study including the last scheduled procedure shown in the Schedule of Activities (see Section 1.3).

The end of the study is defined as the date when the last participant, last observation (LPLO) occurs. LPLO is expected to occur approximately 2 weeks after the last participant last dose in Part 3.

# 5. STUDY POPULATION

The study population rationale is provided in Section 4.2.2.

The participants in this study will be healthy male volunteers between 18 to 55 years of age, inclusive, who fulfill all of the given inclusion criteria.

Prospective approval of protocol deviations, also known as protocol waivers or exemptions, is not permitted.

## 5.1 INCLUSION CRITERIA

Participants are eligible to be included in the study only if all of the following criteria apply:

**Informed Consent**

1. Able and willing to provide written informed consent and to comply with the study protocol according to International Council on Harmonisation (ICH) and local regulations.

**Age**

2. Participants must be 18 to 55 years of age, inclusive, at the time of signing the informed consent form (ICF).

**Type of Participants and Disease Characteristics**

3. Healthy, as judged by the Investigator. Healthy status will be defined as the absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination, vital signs, 12-lead ECG, hematology, blood chemistry, serology, and urinalysis.

**Weight**

4. Body mass index (BMI) within the range 18 to 31 kg/m2 (inclusive).

**Sleep (Part 2 MAD only)**

**Habitual sleep pattern**

5. Participants must be prepared to collect a sleep log and wear an actigraphy device the week before participation in the study. Participants must also have scored 5 or less on the Pittsburgh Sleep Quality Index (PSQI), less than 13 on the Epworth sleepiness scale (ESS), and not be considered an extreme morning or evening type according to the morningness–eveningness questionnaire (MEQ) at screening to be eligible.

**Sex**

6. Male participants

   The contraception and abstinence requirements are intended to prevent exposure of an embryo to the study treatment. The reliability of sexual abstinence for male enrollment eligibility needs to be evaluated in relation to the duration of the clinical study and the preferred and usual lifestyle of the participant. Periodic abstinence and withdrawal are not acceptable methods of contraception.

During the treatment period and for at least 14 days after the last dose of study drug, agreement to:

- Remain abstinent (refrain from heterosexual intercourse) or use a condom with a female partner of childbearing potential (WOCBP, as defined in Section 1 of Appendix 5), or pregnant female partner, to avoid exposing the embryo.
- Refrain from donating sperm for at least 14 days after last dose.

## 5.2 EXCLUSION CRITERIA

Participants are excluded from the study if any of the following criteria apply:

**Medical Conditions**

1. Any condition or disease detected during the medical interview/physical examination that would render the participant unsuitable for the study, place the participant at undue risk, or interfere with the ability of the participant to complete the study, as determined by the Investigator.
2. History or evidence of any medical condition potentially altering the absorption, metabolism, or elimination of drugs. This includes a surgical history of the gastrointestinal tract affecting gastric motility or altering the gastrointestinal tract.
3. History of any clinically significant gastrointestinal, renal, hepatic, bronchopulmonary, neurological, psychiatric, cardiovascular, endocrinological, hematological, or allergic disease, sleep disorders (Part 2 [MAD] only), unexplained syncope (within 12 months prior to screening), metabolic disorder, cancer, or cirrhosis.

4. Use of any psychoactive medication, or medications known to have effects on CNS, or blood flow taken within 4 weeks prior to the first dosing (or within 5 times the elimination half-life of the medication) prior to the first dosing (whichever is longer).
5. History of convulsions (other than benign febrile convulsions of childhood), including epilepsy, or personal history of significant cerebral trauma or CNS infections (e.g., meningitis).
6. History of clinically significant hypersensitivity (e.g., drugs, excipients) or allergic reactions.
7. Any major illness within 1 month before the screening examination or any febrile illness within 1 week prior to screening and up to the first study drug administration.
8. Abnormal BP, defined as confirmed (based on the average of $\geq 3$ consecutive measurements) systolic BP (SBP) greater than 140 or less than 90 mmHg, and diastolic BP (DBP) greater than 90 or less than 50 mmHg.
9. Presence of orthostatic hypotension at screening. Orthostatic hypotension is defined as decrease by 20 mmHg in SBP and/or 10 mmHg in DBP after erection from a supine position and/or clinical presyncopal/syncopal AE during test.
10. Abnormal pulse rate, defined as confirmed (based on the average of $\geq 3$ consecutive measurements) resting pulse rate greater than 100 or less than 40 bpm.
11. History or presence of clinically significant ECG abnormalities before study treatment administration (e.g., PQ/PR interval $> 220$ ms, QT interval corrected using Fridericia's formula [QTcF] $> 450$ ms) or cardiovascular disease (e.g., cardiac insufficiency, coronary artery disease, cardiomyopathy, congestive heart failure, family history of congenital long QT syndrome, family history of sudden death).
12. Clinically significant abnormalities in laboratory test results (including hepatic and renal panels, complete blood count, chemistry panel, and urinalysis). In the case of uncertain or questionable results, tests performed during screening may be repeated before randomization to confirm eligibility.
13. ALT and/or bilirubin $> 1.5 \times$ ULN (isolated bilirubin $> 1.5 \times$ ULN is acceptable if bilirubin is fractionated and direct bilirubin $< 35\%$).
14. Current or chronic history of liver disease or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
15. Participants who, in the Investigator's judgment, pose a suicidal or homicidal risk, or any participant with a history of suicidal or homicidal attempts.
16. Known active or any major episode of infection within 4 weeks prior to the start of drug administration.
17. Participants who test positive for acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on admission to the study site should not be enrolled.

**Prior/Concomitant Therapy**

18. Have used or intend to use over-the-counter (OTC) or prescription medication including herbal medications within 30 days prior to dosing.
19. Participants likely to need concomitant medication during the study period (including for dental conditions).
20. *Part 3 (DDI) only – History of hypersensitivity to benzodiazepines (including midazolam) or its formulation ingredients.*

**Prior/Concurrent Clinical Study Experience**

21. Participation in an investigational drug or device study within 3 months before admission to this study or more than 4 times a year.

**Diagnostic Assessments**

22. Positive test for drugs of abuse or alcohol.
23. Positive test for human immunodeficiency virus (HIV) antibody at screening.
24. Positive result on hepatitis B or hepatitis C virus (HCV), presence of hepatitis B surface antigen (HBsAg), or positive hepatitis C antibody test result at screening or within 3 months prior to starting study treatment.

**Other Exclusions**

25. Dietary restrictions that would prohibit the consumption of standardized meals.
26. Inability or unwillingness to fully consume standardized breakfast at Day 1 (for Part 1, FE cohort).
27. Consumption of any prohibited medications and food/beverages before study start and during the study.
28. Any suspicion or history of alcohol abuse and/or suspicion of regular consumption of drug of abuse or previous history of or treatment for a dependence disorder.
29. Sensitivity to any of the study treatments, or components thereof, or drug or other allergy that, in the opinion of the Investigator, contraindicates the participation in the study.
30. Participants who are smokers for at least 90 days prior to screening.
31. Part 2 (MAD) only – Participants who have issues sleeping or who have travelled across 2 or more time zones in the month prior to screening.
32. Part 2 (MAD) only – Participants who cannot produce sufficient saliva for study assessments.
33. Participants who have donated more than 500 mL of blood or blood products or had significant blood loss within 3 months prior to screening.
34. Participants under judicial supervision, guardianship, or curatorship.

**For participants with planned lumbar punctures, the following additional exclusion criteria apply:**

35. Have a history of clinically significant back pain, back pathology, and/or back injury (e.g. degenerative disease, spinal deformity, or spinal surgery) that may predispose to complications from, or technical difficulty with, lumbar puncture
36. Have criteria that would preclude a lumbar puncture, such as local infection at the site of the lumbar puncture, clinically significant coagulation parameter abnormalities, or treatment with an anticoagulant or with antiplatelet agents within 6 weeks prior to the day of lumbar puncture
37. Have a clinically significant hypersensitivity to local anesthetics that may be used for lumbar puncture (e.g., lidocaine).

## 5.3 LIFESTYLE CONSIDERATIONS

### 5.3.1 Meals and Dietary Restrictions

In *all* parts of the study, participants will have to be fasted for at least 4 hours prior to laboratory safety tests at screening.

On Day 1 of each dose in Part 1, study treatment administration will be performed after an overnight fast of at least 10 hours. Water will be allowed ad libitum until 1 hour prior to dosing and from 1 hour postdosing. A standard lunch will be provided 4 hours after dosing. On all other days of the in-house period, standard breakfast, lunch, dinner, and snack will be provided at the times deemed convenient by the site.

In Part 1, for study drug administration under fed conditions, participants will be given the RO6953958 dose 30 minutes after starting a standardized high fat, high calorie breakfast. This breakfast should be consumed within 30 minutes or less. In Part 2, based on review of Part 1 data, study drug administration will be under fed conditions, i.e., participants will be given the RO6953958 dose 30 minutes after starting a standardized breakfast. The same food conditions (in term of meal constitution and time of administration) will apply on Day -1, Day 1, and Day 10 (as appropriate). In each part of the study, participants will be served standardized meals.

Meals will be similar in composition and time of administration across all cohorts. Consumption of nutrients known to modulate CYP3A activity (e.g., grapefruit or grapefruit juice, Seville orange) will not be permitted within 2 weeks prior to first dosing until discharge from the clinical unit.

Participants should not consume any foods containing poppy seeds within 48 hours (2 days) prior to each admission to the clinical research center as this could cause a false positive drug screen result.

### 5.3.2 Caffeine, Alcohol, and Tobacco

The consumption of caffeine-containing food and beverages or other methylxanthine-containing products (e.g., tea, coffee, caffeinated soft drinks, cola, chocolate) will not be permitted from 48 hours before dosing until the end of the residential period. During the period from Screening to the follow-up visit when participants are not resident in the unit, they will be asked to limit coffee or tea consumption to no more than 3 cups per day and to limit methylxanthine-containing products (e.g., cola and chocolate) to a maximum of 1 L per day (cola) and 60 g per day (chocolate).

Consumption of alcohol will not be allowed from 48 hours before dosing until the end of the residential period, and participants will be asked to limit alcohol to a maximum of 2 units per day (1 unit equates to approximately 330 mL beer, 125 mL of wine, or 25 mL of spirits) during the out-clinic phase until follow-up.

Smokers will be excluded as nicotine withdrawal can affect circadian rhythm and impact the sleep wakefulness cycle.

### 5.3.3 Activity

Light ambulatory activities will be permitted, with the level of activities kept as similar as possible on all days in the clinical research unit. After the participants leave the unit, they will be asked to refrain from strenuous physical activity until the end of their participation in the study.

## 5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomized to study treatment.

The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure.

Individuals who do not meet the criteria for participation in this study (screen failure) will not be rescreened unless there is an agreement with the Sponsor. A repeat of a screening laboratory test because of a borderline result is not considered a rescreening.

## 5.5 RECRUITMENT PROCEDURES

Participants will be identified for potential recruitment using prescreening enrollment logs, clinical database, and Independent Ethics Committee (IEC)/ Institutional Review Board (IRB)-approved newspaper/radio/social-media advertisements prior to consenting to take part in this study

# 6. TREATMENTS

Study intervention is defined as any investigational product (including placebo) or marketed product intended to be administered to a study participant according to the study protocol.

The IMPs for this study are RO6953958 capsules of 2.5 mg, 10 mg, and 80 mg; and matching placebo.

All IMPs required for completion of this study (RO6953958 and matching placebo) will be provided by the Sponsor. All study drug administration will be at the study center under supervision of site staff.

*The non-investigational product (NIMP) for this study is midazolam. Midazolam will be supplied by the clinical site. For information on the formulation, packaging, and handling of midazolam, see the clinical site drug-dispensing form and the local prescribing information for midazolam. Further information on dosing of midazolam may be provided in a separate pharmacy manual.*

Cases of RO6953958 accidental overdose or medication error, along with any associated AEs, should be reported as described in Appendix 2, Section 5.2.

## 6.1 TREATMENTS ADMINISTERED

Table 14 summarizes the treatments administered. Guidelines for dosage modification and discontinuation are provided in Section 6.6 and Section 7, respectively.

## Table 14 Summary of Treatments Administered

| **Study Treatment Name:** | RO6953958 | Placebo *(Part 1 and Part 2 only)* | *Midazolam (Part 3 only)* |
|---|---|---|---|
| **IMP and NIMP** | IMP | | *NIMP* |
| **Dose Formulation:** | Capsule | | *Solution* |
| **Unit Dose Strength(s)/Dosage Level(s):** | 2.5 mg, 10 mg, and 80 mg | NA | *1 mg/mL solution (oral)*<br>*1 mg/mL or 2 mg/mL (IV)* |
| **Dose:** | Part 1 Cohort 1 = 5 mg.<br>Parts *1 and 2*: See Section 4.1.5 for dose-escalation information.<br>*Part 3 = Maximum dose QD tested in Part 2.* | NA | *100 µg (IV) and 300 µg (oral)* |
| **Route of Administration:** | Oral | | *Oral solution/IV bolus injection* |

*Table 14 Summary of Treatments Administered (cont.)*

| **Study Treatment Name:** | RO6953958 | Placebo *(Part 1 and Part 2 only)* | *Midazolam (Part 3 only)* |
|---|---|---|---|
| **Dosing Instructions:** | **Part 1 (SAD/FE) – Fasted Condition:** RO6953958 or matching placebo capsules will be administered orally and swallowed whole with approximately 240 mL water at room temperature in the morning of Day 1 after an overnight fast of at least 10 hours. Water will be allowed ad libitum until 1 hour prior to dosing and from 1 hour postdosing. A standard lunch will be provided 4 hours after dosing. If solubility is limited at higher doses, the dose may be given BID. For BID dosing, details on timing of dosing will be provided separately following review of PK data. Cohort 7 may have a different timing of study drug administration (evening vs morning) and food condition (fed vs fasted).<br>**FE cohort – Fed Condition:** RO6953958 will be administered 30 minutes after starting a standardized high fat, high calorie breakfast. Breakfast should be consumed within 30 minutes or less.<br>**Part 2 (MAD):** RO6953958 or matching placebo capsules will be administered orally and swallowed whole with approximately 240 mL water at room temperature from Day 1 to Day 10 once daily (in the morning) or twice daily (BID) depending on study data. If BID dosing, dosing on Day 10 will only be in the morning. Fasted conditions for administration were determined from Part 1 data. A dose level may have a different timing of daily drug administration (evening vs morning) in additional participants.<br>***Part 3 (DDI):*** *RO6953958 will be administered orally 30 minutes after starting a standardized breakfast. Breakfast should be consumed within 30 minutes or less. Capsule should be swallowed whole with approximately 240 mL of water at room temperature from Day 3 to Day 14 once daily. On Day 13 dosing will be 2 hours prior to midazolam dosing, and on Day 14, 1.5 hours prior to midazolam dosing.* | | ***Part 3 (DDI):*** *Midazolam at a 100 µg dose will be administered via a single bolus injection on Day 1 and Day 13. Midazolam at a 300 µg dose will be administered via a single oral dose (solution) on Day 2 and Day 14. On Day 13 dosing of midazolam will be 2 hours after RO6953958 dosing and on Day 14 dosing of midazolam will be 1.5 hours after RO6953958 dosing.* |
| **Packaging and Labeling:** | Study treatment will be provided in bottles. Each bottle will be labeled as required per country requirement. | | *Information as per local drug suppliers.* |
| **Storage Conditions** | Store at 2°C–8°C. Protect from light. | | |
| **Manufacturer** | F. Hoffmann-La Roche Ltd Basel, Switzerland | | |

Please see RO6953958 Investigator's Brochure for more details.

## 6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

Study drug packaging will be overseen by the Sponsor's clinical study 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 the Sponsor's standard and local regulations.

The study site should follow all instructions included with each shipment of IMP. The investigational site will acknowledge receipt of IMPs and confirm the shipment condition and content. Any damaged shipments will be replaced. The Investigator or designee must confirm that appropriate temperature conditions have been maintained during transit for all IMPs received and that any discrepancies have been reported and resolved before use of the IMPs. All IMPs must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions, with access limited to the investigator and authorized staff.

Only participants enrolled in the study may receive IMPs, and only authorized staff may supply or administer IMPs.

The study site (i.e., Investigator or other authorized personnel [e.g., pharmacist]) is responsible for maintaining records of IMP delivery to the site, IMP inventory at the site, IMP use by each patient, and disposition or return of unused IMP, thus enabling reconciliation of all IMP received, and for ensuring that participants are provided with doses specified by the protocol.

Upon arrival of the IMPs at the site, site personnel will complete the following:

- Check the IMPs for damage.
- Verify proper identity, quantity, integrity of seals and temperature conditions.
- Report any deviations or product complaints to the Study Monitor upon discovery.

The qualified individual responsible for dispensing the study treatment will prepare the correct dose according to the randomization schedule.

The Investigator or delegate must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.

Only participants enrolled in the study may receive study treatment, and only authorized site staff may supply or administer study treatment. All study treatments must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

The Investigator is responsible for study treatment accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

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

Accurate records of all IMPs received at, dispensed from, returned to, and disposed of by the study site should be recorded on the drug accountability log.

Refer to the pharmacy manual and/or the RO6953958 Investigator's Brochure for information on IMP formulation, IMP handling, including preparation and storage, and accountability.

## 6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

### 6.3.1 Method of Treatment Assignment

The randomization numbers will be generated by the Sponsor or its designee. The randomized treatment assignment will be allocated from the list sequentially to participants in the order in which they are enrolled. The Investigator or designee will enter the corresponding participant number for allocation to the dosing groups/cohorts in the appropriate place on each participant's electronic case report form (eCRF).

Participants can be replaced (for details see Section 7.2).

**Part 1 (SAD)**

Fifty-six participants will be randomized, 8 per cohort. For all dose levels, a sentinel group of 2 participants will be randomized as 1 on active treatment and 1 on placebo and the remaining participants will be randomized as 5 on active treatment and 1 on placebo at the same dose level at least 24 hours later. If participants in the FE cohort do not agree to perform the fed part, additional participants will be recruited.

**Part 2 (MAD)**

Up to 80 participants will be randomized with a minimum of 6 active treatment and 2 placebo participants in each dose level with the option to expand up to a maximum of 12 active treatment and 4 placebo participants per dose level. All dose levels will be assigned to active treatment or placebo using a 3:1 ratio. Criteria for expansion of cohorts are outlined in Section 4.1.5.4.

*Part 3 (DDI)*

*Sixteen participants will be enrolled to have at least 12 evaluable participants complete study Part 3. All participants will receive active treatment.*

### 6.3.2 Blinding and Emergency Unblinding

The study is Investigator-/subject-blind. This means that the participant, the Investigator, and all individuals in direct contact with the participant at the site will be blinded to the individual treatment assignment except the pharmacist handling the study drug. To allow informed recommendations or decisions regarding dosing in this study, an integrated assessment of the safety, tolerability, and available PK data will be made prior to each dose-escalation decision. Members of the project and study team who are not in direct contact with the participants are blinded with the following exceptions: The Clinical Pharmacologist and the Clinical Pharmacology Scientist who perform this assessment — together with the Statistician, Data Acquisition Specialist, and Clinical/Statistical Programmer — will be unblinded with regard to the treatment allocation of participants. PK/PD data can be received and cleaned on an ongoing basis. The data will be handled and cleaned in a secure area that is not accessible by any blinded Study Management Team member. Likewise, the Bioanalytical Manager, Bioanalyst, and the Pharmacometrician will be unblinded. Other members of the Sponsor's project and study teams who do not have direct contact with the participant may be unblinded, at the Clinical Pharmacologist`s discretion. The Clinical Pharmacologist or Clinical Pharmacology Scientist may share mean reports (e.g., tabular summaries or mean graphs by treatment group) with other individuals (e.g., Drug Safety Physician, Principal Investigator) involved in the dose-decision process, but they should not disclose individual treatment assignment. In order to maintain the blind, the unblinded pharmacist at the site will be responsible for the dispensation of all study treatment. The randomization list will be made available to the pharmacist preparing the study treatment. A sealed envelope that contains the treatment assignment for each participant will be provided to the Investigator. The sealed envelope will be retained by the Investigator (or representative) in a secured area.

As per Health Authority reporting requirements, the Sponsor will break the treatment code for all unexpected SAEs (see Appendix 2) that are considered by the Investigator to be related to study treatment. If possible, the Sponsor must be notified before the blind is broken, unless identification of the study treatment is required for medical emergency in which the knowledge of the specific blinded treatment will affect the immediate management of the participant's conditions (e.g., antidote is available). In this case, the Sponsor must be notified within 24 hours after breaking the blind, where possible. The date and reason for breaking the blind must be recorded, and the name of all the person(s) who had to be unblinded in the source documentation and eCRF, as applicable. Once the study is complete, all envelopes (sealed and opened) must be inventoried and returned to the Sponsor.

## 6.4 TREATMENT COMPLIANCE

The qualified individual responsible for dispensing the study treatment will prepare the correct dose according to the randomization schedule. This individual will write the date dispensed and participant number on the study treatment bottle, label and on the Drug Accountability Record. This individual will also record the study treatment number received by each participant during the study.

## 6.5 CONCOMITANT THERAPY

Any medication or vaccine (except COVID-19 vaccines) (including OTC or prescription medicines, approved dietary and herbal supplements, nutritional supplements) used by a participant from 4 weeks prior to screening until the follow-up visit must be recorded along with reason for use, dates of administration (including start and end dates), and dosage information (including dose and frequency).

Participants are allowed to receive a dose of COVID-19 vaccine at least 21 days prior to the first dose of study treatment or a dose of COVID-19 vaccine at least 10 days after the last dose of study treatment.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

All concomitant medications should be reported to the Investigator and recorded on the Concomitant Medications eCRF and any non-pharmacological interventions (e.g., individual psychotherapy, cognitive behavioral therapy, smoking cessation therapy, and rehabilitative therapy) as appropriate on the eCRF.

All medication administered to manage adverse events should be recorded on the AE eCRF.

### 6.5.1 Permitted Therapy

Participants must abstain from taking prescription or nonprescription drugs (including vitamins and dietary or herbal supplements) within 30 days or 5 half-lives (whichever is longer) before the start of study intervention until completion of the follow-up visit, unless, in the opinion of the Investigator and Sponsor, the medication will not interfere with the study.

Other concomitant medication may be considered on a case-by-case basis by the Investigator in consultation with the Medical Monitor if required.

Acetaminophen/paracetamol is allowed up to a maximum dose of 2 g/day up to 48 hours prior to dosing and after the in-house treatment, but dosage should not exceed 4 g total during the week prior to dosing.

### 6.5.2 Prohibited Therapy

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

As a general rule, no concomitant medication will be permitted, with the exception of medications to treat AEs, unless the rationale for exception is discussed and clearly documented between the Investigator and the Sponsor.

Use of the following therapies is prohibited during the study and for at least 30 days, or at least 5 half-lives of the medication, prior to initiation of study treatment (whichever is longer), unless otherwise specified below:

- Any prescribed or OTC medication (including herbal products, vitamin, mineral, dietary supplements, melatonin, and melatonin agonists).
- Any known inhibitor of cytochrome P450 (CYP) 3A4 or CYP2C19 taken within 4 weeks prior to start of administration of study drug (Day 1) or within 5 times the elimination half-life of the medication prior to start of study drug intake (whichever is longer), including but not limited to the following drugs: ritonavir, troleandomycin, telaprevir, danoprevir, elvitegravir, saquinavir, lopinavir, indinavir, nelfinavir, boceprevir, voriconazole, mifepristone, posaconazole, telithromycin, ketoconazole, itraconazole, fluconazole, erythromycin, clarithromycin, mibefradil, ceritinib, ribociclib, idelalisib, conivaptan, nefazodone, diltiazem, verapamil, cimetidine, fluvoxamine, fluoxetine, ASP8477, and ticlopidine.
- Any known inducer of CYP3A4 or CYP2C19 taken within 4 weeks prior to start of administration of study treatment (Day 1), including but not limited to the following drugs: rifampicin, rifabutin, glucocorticoids, carbamazepine, oxcarbazepine, phenytoin, phenobarbital, St. John's Wort extract, mitotane, avasimibe, rifapentine, ivosidenib, carbamazepine, lumacaftor, apalutamide, enzalutamide, and ritonavir.
- Use of drugs acting on platelets such as aspirin, clopidogrel, dipyridamole, ticlopidine, or with anticoagulant effect like heparin or warfarin.

## 6.6 DOSE MODIFICATION

In Part 1 it is anticipated that solubility may limit absorption at higher doses (> 400 mg); therefore, in order to explore higher exposures in the Part 1, doses may be split over the day (i.e., given BID).

Depending on the safety and/or PK characteristics of RO6953958, a BID regimen may be used instead of QD dosing regimen in Part 2. A detailed SoA in Part 2 if BID dosing will be provided in this case.

In Part 2 or Cohort 7 (Part 1), the timing of dosing may be changed for participants (Cohort 7 only) or additional participants in a cohort at a specific dose level, in order to explore the effect of time of dosing on the pharmacokinetics.

## 6.7 TREATMENT AFTER THE END OF THE STUDY

The Sponsor does not intend to provide RO6953958 or other study interventions to participants after conclusion of the study or any earlier participant withdrawal.

# 7. DISCONTINUATION OF STUDY, STUDY TREATMENT AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

An excessive rate of withdrawals (either participants discontinuing study treatment or withdrawing from the study) can render the study non-interpretable. Therefore, unnecessary withdrawal of participants should be avoided, and efforts should be taken to motivate participants to comply with all the study-specific procedures as outlined in this protocol.

Details on study and site closures are provided in Appendix 1 Study Governance Considerations Study.

## 7.1 DISCONTINUATION OF STUDY TREATMENT

For data to be collected at the time of treatment discontinuation and follow-up and for any further evaluations that need to be completed, see the SoA (Section 1.3).

Reasons for discontinuation of study treatment (or withdrawal from the study) may include, but are not limited to, the following:

- Participant withdrawal of consent at any time.
- Any medical condition that the Investigator or Sponsor determines may jeopardize the participant's safety if he or she continues in the study.
- Investigator or Sponsor determination that treatment discontinuation is in the best interest of the participant.
- Any event that meets stopping rules defined in Section 4.1.6.2.

Every effort should be made to obtain information on participants who withdraw from the study but have not withdrawn consent. Participants who discontinue study treatment prematurely will be asked to return to the clinic for a study completion/early termination visit (see Section 8.11.3) and may undergo follow-up assessments (see Section 8.11.4), unless the participant withdrew consent. The primary reason for premature study treatment discontinuation should be documented on the appropriate eCRF. Participants who discontinue study treatment prematurely may be replaced (Section 7.2).

Discontinuation of study intervention for abnormal liver function should be considered by the Investigator when a participant meets one of the conditions outlined (Appendix 3, Section 5) or if the Investigator believes that it is in best interest of the participant.

If a clinically significant finding is identified (including, but not limited to, changes from baseline in QTcF) after enrollment, the Investigator or qualified designee will determine if

the participant can continue in the study and if any change in participant management is needed. This review of the ECG printed at the time of collection must be documented. Any new clinically relevant finding should be reported as an AE.

## 7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY AND PARTICIPANT REPLACEMENT

Participants have the right to voluntarily withdraw from the study at any time for any reason.

In addition, the Investigator has the right to withdraw a participant from the study for medical conditions that the Investigator or Sponsor determines may jeopardize the participant's safety if the participant continues in the study.

If possible, information on reason for withdrawal from the study should be obtained. The primary reason for withdrawal from the study should be documented on the appropriate eCRF. Participants will not be followed for any reason after consent has been withdrawn.

When a participant voluntarily withdraws from the study, or is withdrawn by the Investigator, samples collected until the date of withdrawal will be analyzed, unless the participant specifically requests for these to be discarded or local laws require their immediate destruction. However, if samples have been tested prior to withdrawal, results from those tests will be used as part of the overall research data.

Participants who withdraw from the study for safety reasons will not be replaced. Participants who withdraw from the study for other reasons may be replaced to ensure adequate numbers of evaluable participants for dose escalation. Replacement of participants for other reasons will be discussed between the Investigator and the Sponsor, based on existing data. Replacement participants will start the study at the dose level due to be received by the participant they are replacing.

For data to be collected at the time of study discontinuation and at safety and follow-up visits, and for any further evaluations that need to be completed, see the SoA (Section 1.3).

## 7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if the participant repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible, counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the participant. These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, the participant will be considered to have withdrawn from the study.

Discontinuation of sites or of study as a whole are handled as part of Appendix 1.

# 8. STUDY ASSESSMENTS AND PROCEDURES

Study procedures and their timepoints are summarized in the SoA (Section 1.3). Protocol waivers or exemptions are not allowed.

Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment.

Procedures conducted as part of the participant's routine clinical management (e.g., blood count) and obtained before signing of the ICF may be used for screening or baseline purposes if the procedure met the protocol-specified criteria and was performed within the time-frame defined in the SoA (see Section 1.3).

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

## 8.1 EFFICACY ASSESSMENTS

Not applicable.

## 8.2 SAFETY ASSESSMENTS

Planned timepoints for all safety assessments are provided in the SoA (see Section 1.3).

Safety assessments will consist of monitoring and recording AEs, including SAEs and non-serious AEs of special interest (NSAESI); 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.

### 8.2.1 Physical Examinations

A complete physical examination will be performed at screening and at the follow-up visit. The examination should include an evaluation of the head, eyes, ears, nose, and throat; and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, neurological, and genitourinary (at the Investigator discretion) systems. In addition, neck and lymph nodes will be examined in Part 2 (MAD). Body weight will be measured at screening and follow-up visit; height will be measured at screening and used to calculate the BMI. Further examination of other body systems may be performed in case of evocative symptoms at the Investigator's discretion.

A brief physical examination will be performed at other timepoints in the SoA (see Section 1.3) and include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen), as well as symptom-driven checks. Any abnormality identified at baseline should be recorded on the General Medical History and Baseline Conditions eCRF. Changes from baseline abnormalities should be recorded in subject notes. New or worsened clinically significant abnormalities should be recorded as AEs on the Adverse Event eCRF.

### 8.2.2 Vital Signs

Vital signs will include body temperature (tympanic), pulse rate, respiratory rate, and BP. Core body temperature will be collected as an exploratory measure during PSG assessment nights; see Section 8.7.12 for further details. Vital signs will be taken before blood collection and will be measured in a supine position after a resting period of at least 5 minutes at the timepoints specified in the SoA (Section 1.3).

BP and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available. When possible, the same arm should be used for all BP measurements.

When measuring BP, the participant's arm should be unconstrained by clothing or other material, and the participant should be comfortably supine, with the legs uncrossed, and the back and arm supported, such that the middle of the cuff on the upper arm is at the level of the right atrium (the mid-point of the sternum). The “ideal” cuff should have a bladder length that is 80% and a width that is at least 40% of arm circumference (a length-to-width ratio of 2:1).

#### 8.2.2.1 Orthostatic Challenge Testing

*This test will be performed in Part 1 and Part 2 only.* During a 10-minute period during which the participant will remain in a supine position, BP and pulse rate will be measured after approximately 8, 9, and 10 minutes. If the last BP measurement deviates from the previous measurement by more than 5 mmHg, a 5-minute extension period will be added. If an extension period is needed. BP and pulse rate will be obtained after approximately 13, 14, and 15 minutes in the supine position.

The participant will then be asked to move into a standing position, and BP and pulse rate will be assessed again after 3 minutes of standing.

Standing BP and pulse rates will be compared against the latest blood pressure and pulse rate values obtained in the supine position. Orthostatic hypotension is defined as a decrease in SBP by at least 20 mmHg and/or a decrease in DBP by at least 10 mmHg.

### 8.2.3 Electrocardiograms

At each timepoint (see Section 1.3) at which triplicate ECGs are required, 3 individual ECG tracings should be obtained as closely as possible in succession, but no more than

2 minutes apart. The full set of triplicates should be completed in less than 5 minutes. The average of the 3 readings will be used to determine ECG intervals (e.g., PR, QRS, QT).

To minimize variability, it is important that participants 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 meals and any scheduled vital sign measurements and blood draws. In case of an absolute QT corrected for heart rate (QTc) of > 500 ms and an increase from baseline QTc > 60 ms, another ECG triplicate must be recorded within the next 5 minutes. It may be appropriate to repeat abnormal ECGs to rule out improper lead placement potentially contributing to the ECG abnormality.

For safety monitoring purposes, the Investigator or designee must review, sign, and date all ECG tracings. Paper or electronic copies will be kept as part of the participant's permanent study file at the site. If considered appropriate by the Sponsor, ECGs may be analyzed retrospectively at a central laboratory and may be electronically captured.

ECG characteristics, including heart rate, QRS duration, and PR and QT intervals, will be recorded on the eCRF or loaded electronically. QTcF and RR will be calculated automatically and recorded on the eCRF or loaded electronically. Changes in T-wave and U-wave morphology and overall ECG interpretation will be documented on the eCRF or loaded electronically. T-wave information will be captured as normal or abnormal, U-wave information will be captured in 2 categories: absent/normal or abnormal.

#### 8.2.3.1 Holter ECG Monitoring

Digital continuous recordings (Holter) for the extraction of 12-lead ECG traces at predefined timepoints will be performed in *Part 1 and Part 2* of the study, excluding the fed part in the FE cohort in Part 1, as specified in the SoA (see Section 1.3). Holter recordings will be sent to a central ECG analysis laboratory for ECG extraction and potential retrospective expert review with estimation of ECG intervals. Replicate ECG tracings will be extracted from the continuous recording during a 5-minute time window immediately preceding the scheduled timepoint.

Replicate ECG tracing will be extracted at the timepoints specified in the SoA (see Section 1.3). At these specific timepoints, the participants should be at rest and in a supine position for ≥ 10 minutes before and remain in a supine position for ≥ 5 more minutes after the specified ECG extraction timepoints.

While the participants are on Holter ECG monitoring, the absence of any environmental distractions (e.g., television, radio, conversation, or phone calls) during the pre- and

post-ECG rest period, and at every ECG timepoints specified in SoA (see Section 1.3), must be emphasized. In particular, activities known to cause changes in heart rate should be avoided.

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

The 12-lead Holter and ECG equipment will be supplied and supported by ERT. All ECG data will be collected using a Global Instrumentation (Manlius, NY, USA) M12R ECG continuous 12-lead digital recorder. The continuous 12-lead digital ECG data will be stored onto SD memory cards.

The following principals will be followed in ERT's core laboratory:

- ECG readers are blinded to the participant, visit and treatment allocation.
- A limited number of readers will be employed for the study.
- Baseline and on-treatment ECGs for a particular subject will be over-read on the same lead and will be analyzed by the same reader.
- The primary analysis lead is lead II. If lead II is not analyzable, then primary lead of analysis will be changed to another lead for the entire participant data set.

The following is a brief description of ECG analysis methods used by ERT's core laboratory.

#### 8.2.3.1.1 TQT Plus ECG Extraction Technique

Ten 14-second digital 12-lead ECG tracings will be extracted from the continuous Holter recordings using the 'TQT Plus method', a computer assisted and statistical process used by ERT. The method enables extraction of ECGs with the lowest heart rate (HR) variability and noise within the protocol-specified extraction time window (e.g., the HR and QT changes from beat-to-beat in the range of $< 10\%$). At each protocol-specified timepoint, 10 ECG replicates will be extracted from a 5-minute "ECG window" (typically, the last 5 minutes of the 15-minute period when the participant is maintained in a supine or semi-recumbent quiet position).

#### 8.2.3.1.2 Expert-Precision QT Analysis

Expert-precision QT analysis will be performed on all analyzable (non-artifact) beats in the 10 ECG replicates. Statistical quality control procedures are used to review and assess all beats and identify "high" and "low" confidence beats using several criteria, including:

- QT or QTc values exceeding or below certain thresholds (biologically unlikely)
- RR values exceeding or below certain thresholds (biologically unlikely)
- Rapid changes in QT, QTc or RR from beat to beat

Measurements of all primary ECG parameters (QT, QTc, RR) in all recorded beats of all replicates that are deemed “high confidence” will be performed using COMPAS software. All low confidence beats will be reviewed manually and adjudicated using pass-fail criteria. The final quality-control assessment will be performed by a cardiologist. The beats found acceptable by manual review will be included in the analysis. The median QT, QTc, and RR value from each extracted replicate will be calculated, and then the mean of all available medians from a nominal timepoint will be used as the participant’s reportable value at that timepoint.

Categorical T-wave morphology analysis and the measurement of PR and QRS intervals will be performed manually in 3 of the 10 ECG replicates at each timepoint. Each fiducial point (onset of P-wave, onset of Q-wave, offset of S-wave, and offset of T-wave) will be electronically marked.

For T-wave morphology and U-wave presence, treatment-emergent changes will be assessed (i.e., changes not present at baseline). For each category of T-wave morphology and of U-waves, the category will be deemed as present if observed in any replicate at the time point. For baseline, the category will be deemed as present if observed in any replicate from all time points that constitute baseline. The T-wave morphology categories are described in Table 15.

**Table 15 T-Wave Morphology and U-Wave Presence Categories (Assessed Manually)**

| Category | Description |
|---|---|
| Normal T-wave | Any positive T-wave not meeting any criterion below |
| Flat T-wave | T amplitude < 1 mm (either positive or negative) including flat isoelectric line |
| Notched T-wave (+) | Presence of notch(es) of at least 0.05 mV amplitude on ascending or descending arm of the positive T-wave |
| Biphasic | T-wave that contains a second component with an opposite phase that is at least 0.1 mV deep (both positive/negative and negative/positive and polyphasic T-waves included) |
| Normal T-wave (-) | T amplitude that is negative, without biphasic T-wave or notches |
| Notched T-wave (-) | Presence of notch(es) of at least 0.05 mV amplitude on descending or ascending arm of the negative T-wave |
| U-waves | Presence of abnormal U-waves |

### 8.2.4 Clinical Safety Laboratory Assessments

Normal ranges for the study laboratory parameters must be supplied to the Sponsor before the study starts. A list of clinical laboratory tests to be performed is provided in Appendix 4, and these assessments must be conducted in accordance with the separate laboratory manual and the SoA (see Section 1.3).

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.

- In the event of unexplained abnormal clinically significant laboratory test values, the tests should be repeated as soon as possible and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found.
- If such values do not return to normal/baseline within a period judged reasonable by the Investigator, the etiology should be identified and the Sponsor notified.
- If laboratory values from non-protocol-specified laboratory assessments performed at the local laboratory are considered clinically significant by the Investigator (e.g., SAE or AE or dose-modification) then, the results must be recorded in the eCRF.

Results of clinical laboratory testing will be received as electronically produced laboratory reports submitted directly from the local or central laboratory.

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 participant safety.

Where the clinical significance of abnormal laboratory results at screening is considered uncertain, screening laboratory tests may be repeated before randomization to confirm eligibility.

If there is an alternative explanation for a positive urine or blood test for drugs of abuse (e.g., previous occasional intake of a medication or food containing, for example, codeine, benzodiazepines or opiates) the test should be repeated to confirm washout.

### 8.2.5 Suicidal Risk Monitoring

RO6953958 is considered to be a CNS-active study treatment. There has been some concern that some CNS-active study treatments may be associated with an increased risk of suicidal ideation or behavior when given to some participants. Although this study treatment or other similar drugs in this class have not been shown to be associated with an increased risk of suicidal thinking or behavior when given to healthy participants, the Sponsor considers it important to monitor for such events before or during this clinical study.

Participants being treated with *multiple doses of* RO6953958 or placebo should be monitored appropriately and observed closely for suicidal ideation and behavior or any other unusual changes in behavior. Consideration should be given to discontinuing

RO6953958 or placebo in participants who experience signs of suicidal ideation or behavior.

Baseline assessment of suicidal ideation and behavior and/or treatment-emergent suicidal ideation and behavior will be monitored during the study using the Columbia-Suicide Severity Rating Scale (C-SSRS) “Baseline-Screening” version at screening and “Since Last Visit” version at subsequent visits. C-SSRS assessments will be completed by suitably trained staff.

### 8.2.6 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 participant within 30 days prior to the screening visit.

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

## 8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

The definitions of an AE or SAE can be found in Appendix 2. The NSAESI and disease-related events and/or disease-related outcomes not qualifying as AEs or SAEs are discussed in Sections 8.3.6.

The Investigator and any qualified designees are responsible for ensuring that all AEs (including assessment of seriousness, severity, and causality; see Appendix 2) are recorded on the AE eCRF and reported to the Sponsor in accordance with instructions provided in this section and in Appendix 2.

Procedures used for recording AEs are provided in Appendix 3.

### 8.3.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 2.

Investigators will seek information on AEs at each participant’s contact. All AEs, whether reported by the participant or noted by study personnel, will be recorded in the participant’s medical record and on the AE eCRF as follows:

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

**After initiation of study treatment**, all AEs, regardless of relationship to study treatment, will be reported until the last follow-up visit for each part.

**Post-Study AEs and SAEs:** The Investigator is not required to actively monitor participants for AEs after the end of the AE reporting period (i.e., the last follow-up visit for each part).

However, if the Investigator learns of any SAE (including a death) or other AEs of concern that are believed to be related to previous treatment with study treatment, at any time after a participant has been discharged from the study, and the Investigator considers the event to be reasonably related to the study treatment or study participation, the Investigator must promptly notify the Sponsor. For the procedure of reporting, see Appendix 2.

### 8.3.2 Method of Detecting Adverse Events and Serious Adverse Events

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

A consistent methodology of nondirective questioning should be adopted for eliciting AE information at all participant evaluation timepoints.

### 8.3.3 Follow-Up of Adverse Events and Serious Adverse Events

#### 8.3.3.1 Investigator Follow-Up

The Investigator should follow each AE until the event has resolved to baseline grade or better, the event is assessed as stable by the Investigator, the event is otherwise explained, the participant is lost to follow-up (Section 7.3), or the participant withdraws consent. Every effort should be made to follow all SAEs considered to be related to study treatment or study-related procedures until a final outcome can be reported.

During the study period, resolution of AEs (with dates) should be documented on the Adverse Event eCRF and in the participant'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 AE eCRF.

All pregnancies reported during the study should be followed until pregnancy outcome and reported according to the instructions provided in Section 8.3.5.

#### 8.3.3.2 Sponsor Follow-Up

For SAEs, NSAESIs, and pregnancies, the Sponsor or a designee may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional event details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) to perform an independent medical assessment of the reported event.

### 8.3.4 Regulatory Reporting Requirements for Serious Adverse Events

Prompt notification by the Investigator to the Sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC, and Investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.

An Investigator who receives an Investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then, file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate, according to local requirements.

For immediate and expedited reporting requirements from Investigator to Sponsor and from Sponsor to Health Authority, Investigators, IRB, and Ethics Committee (EC), see Appendix 2.

#### 8.3.4.1 Emergency Medical Contacts

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

### 8.3.5 Pregnancy

Male participants will be instructed through the ICF to immediately inform the Investigator if their partner becomes pregnant during the study or within 28 days after the final dose of study drug.

If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the pregnancy reporting process as detailed in Appendix 5.

Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs (Appendix 5).

### 8.3.6 Non-Serious Adverse Events of Special Interest

NSAESI 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 Appendix 2 for reporting instructions).

NSAESI for this study include the following:

- Cases of an elevated ALT or aspartate aminotransferase (AST) in combination with either an elevated bilirubin or clinical jaundice, as defined in Appendix 3.
- Suspected transmission of an infectious agent by the study treatment, 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 participant exposed to a medicinal product. This term applies only when a contamination of the study treatment is suspected.

## 8.4 TREATMENT OF OVERDOSE

Study treatment overdose is the accidental administration of a drug in a quantity that is higher than the assigned dose. An overdose or incorrect administration of study treatment is not an AE unless it results in untoward medical effects (see Sections 5 and 5.2 of Appendix 2 for further details).

Decisions regarding dose modifications, if applicable, will be made by the Investigator/treating physician in consultation with the Medical Monitor based on the clinical evaluation of the participant.

In the event of an overdose, the Investigator should:

1. Contact the Sponsor's Medical Monitor immediately.
2. Closely monitor the participant for AE/SAE and laboratory abnormalities until resolved. In case of AE/SAE, standard of care is to be used.
3. Obtain a blood sample for PK analysis if requested by the Medical Monitor (determined on a case-by-case basis).
4. Document the quantity of the excess dose, as well as the duration of the overdose, in the eCRF.

## 8.5 PHARMACOKINETICS

Concentrations of RO6953958 and its metabolites, RO7021594 and RO7045755, will be measured in plasma, urine and CSF using separate specific and validated LC-MS/MS assays. The blood, urine and CSF samples will be taken as outlined in the SoA (see Section 1.3). The date and time of each sample collection will be recorded in the eCRF. During the course of the study, PK sampling timepoints may be modified on the basis of emerging data to ensure the PK of RO6953958 can be adequately characterized.

In a cohort, CSF sampling will be done predose in 4 participants and in the other 4 participants at approx. 5 to 7 h after dosing of RO6953958 (around $T_{max}$ of the M3

metabolite) for determination of RO6953958, M3 (RO7021594) and M1 (RO7045755) concentrations in CSF (i.e., one CSF sample per participant per cohort). A corresponding blood sample will be taken at the same timepoint. As an alternative to CSF sampling on Day 7, it may also be performed on Day 6 or Day 8. The time points of CSF sampling in a participant may be adjusted with emerging PK or CSF samples from preceding cohorts. In addition, these results might also lead to the conclusion to stop CSF sampling in subsequent cohorts.

Lumbar punctures will be performed by an experienced anesthesiologist using sterile techniques and local anesthesia, such as lidocaine. Subjects will be required to remain supine for up to 2 hours after the procedure. The Investigator may prescribe analgesics as needed for headache or pain in the lumbar puncture area.

*Blood samples for the determination of plasma concentrations of midazolam will be collected as detailed in the SoA (Section 1.3). Plasma concentrations of midazolam will be measured by a specific and validated LC-MS/MS method.*

A decision to stop PK sampling earlier or to collect more samples than the currently proposed scheduled times will be based on the PK profile of RO6953958 from preceding dose groups. Up to 3 additional PK samples may be taken per participant, and the timing of PK sampling may be changed based on emerging PK results after agreement with the Sponsor Clinical Pharmacologist and the Investigator without exceeding the total number of scheduled (+3 additional) PK samples.

Any changes in the timing or addition of timepoints for any planned study assessments must be documented and approved by the relevant study team member and then archived in the Sponsor and site study files, but this will not constitute a protocol amendment.

Remaining PK plasma, urine and CSF samples may be used for exploratory biomarker profiling, identification, assay development purposes, and assay validation during the development of study or compound related assays after the mentioned intended uses. The PK plasma, urine and CSF samples may be destroyed 6 months after the date of finalization of the bioanalytical report only after Sponsor’s approval or shipped to Roche. In the latter case, the PK plasma, urine and CSF samples may be stored up to 5 years after the final CSR has been completed unless the participant gives specific consent for his or her leftover samples to be stored in the RBR for optional exploratory research.

Details on sampling procedures, sample storage and shipment are given in the sample documentation. Drug concentration information that may unblind the study will not be reported to investigative sites or blinded personnel until the study has been unblinded.

- Other metabolites may be measured using a specific validated or qualified LC-MS/MS assay, as appropriate.

- PK samples from participants treated with placebo may not be analyzed but retained for subsequent analysis if appropriate. At a minimum, the $T_{max}$ sample will be analyzed for placebo participants.

## 8.6 IMMUNOGENICITY ASSESSMENTS

Not applicable.

## 8.7 PHARMACODYNAMICS AND BIOMARKER ANALYSES

### 8.7.1 Genetic and Genomic Analyses

#### 8.7.1.1 Clinical Genotyping

A mandatory whole blood sample will be collected for DNA extraction according to the SoA (see Section 1.3). If, however, the genetic blood sample is not collected during the scheduled visit, it may be collected at any time during the conduct of the clinical study.

The DNA may be used to determine if genetic variants (e.g., at drug metabolizing enzymes, transporters, receptors) affect the PK, PD, efficacy, and/or safety of RO6953958. This may include genome sequencing to detect these variants as well as, genotyping the AVPR1A gene including promoters. *DNA may also be used to detect whether genetic variants in genes controlling circadian rhythms and sleep are associated with the sleep-related measurements.*

Data arising from all biosamples, including samples for analyses of inherited DNA, will be subject to the confidentiality standards described in as described in Section 1.4 of Appendix 1. For participants who consent to research biosample repository (RBR), leftover samples will be transferred to RBR (see Section 8.9).

### 8.7.2 Pittsburgh Sleep Quality Index

The PSQI questionnaire will be recorded according to the SoA (see Section 1.3). The PSQI assesses sleep quality during the previous month (Buysse et al 1989). It consists of 19 self-rated questions. A wide variety of factors relating to sleep quality are assessed, including estimates of sleep duration and latency and the frequency and severity of specific sleep-related problems. These 19 items are grouped into seven component scores, each weighted equally on a 0–3 scale. The global PSQI score has a range of 0–21 and higher scores indicate worse sleep quality.

### 8.7.3 Patient-Reported Outcomes Measurement Information System Sleep Questionnaires

The PROMIS sleep questionnaire will be recorded according to the SoA (see Section 1.3).

The PROMIS Sleep Disturbance Short Form (Yu et al 2011; Buysse et al 2010) assesses self-reported perceptions of sleep quality, sleep depth, and restoration associated with sleep. This includes perceived difficulties and concerns with getting to

sleep or staying asleep, as well as perceptions of the adequacy of and satisfaction with sleep. This questionnaire assesses sleep disturbance over the past 7 days.

The PROMIS Sleep-Related Impairment Short Form (Buysse et al 2010) focuses on self-reported perceptions of waking alertness, sleepiness, and function within the context of overall sleep-wake function. This questionnaire assesses sleep-related impairment over the past 7 days.

### 8.7.4 Karolinska Sleepiness Scale (KSS)

The KSS will be recorded according to the SoA (see Section 1.3). The KSS measures the subjective level of sleepiness at a particular time during the day. On this scale, participants indicate which level best reflects the psycho-physical state experienced in the last 5 minutes.

The KSS (version b) is a 9-point scale (with descriptions from 1 = extremely alert to 9 = extremely sleepy – fighting sleep).

### 8.7.5 Epworth Sleepiness Scale (ESS)

The Epworth Sleepiness Scale (ESS; Johns 1991, Johns 1992) will be completed according to the SoA (see Section 1.3). The ESS is a brief, self-administered eight-item questionnaire that measures daytime sleepiness in adults. The participant will be asked to rate on a scale of 0 to 3 the chances that, “over recent times the past month” he/she would have dozed in eight specific situations that are commonly met in daily life (0 = would never doze; 3 = high chance of dozing). Thus, the participant is asked to characterize, retrospectively, part of his usual behavior in a variety of situations that are more or less soporific. The ESS score is the sum of eight item-scores and can range from 0 to 24.

This assessment takes approximately 5 minutes to perform.

### 8.7.6 Morningness-Eveningness Questionnaire (MEQ)

The morningness–eveningness questionnaire (MEQ; Horne et al 1976) is a self-assessment questionnaire that will be completed according to the SoA (see Section 1.3). The MEQ consists of 19 items, and was developed to assess individual differences in morningness and eveningness – the degree to which respondents are active and alert at certain times of day. Its main purpose is to measure whether a person's circadian rhythm (biological clock) produces peak alertness in the morning, in the evening, or in between.

### 8.7.7 Sleep Mat

A sleep mat that will be placed under the participant’s mattress and contains a vibration sensor to detect breathing and heart rate signals will be used to collect data on the participant’s sleep behavior according to the SoA (see Section 1.3).

Test data for the sleep mat will be collected from four participants during Part 1 (SAD) to ensure functioning of equipment for Part 2 (MAD).

### 8.7.8 Polysomnography

Polysomnography will be recorded according to the SoA (see Section 1.3) and used to assess the effect of RO6953958 on sleep. Specific endpoints will include but are not limited to total sleep time, sleep latency, wake after sleep onset, distribution of sleep stages (in minutes and/or %), amount of slow waves, density, amplitude and frequency of sleep spindles and sleep efficiency. A detailed description of PSG procedures will be provided separately.

### 8.7.9 Platelet Aggregation Assay

Inhibition of vasopressin-induced platelet aggregation will be tested on blood samples drawn at the indicated timepoints (see Section 1.3). This assay assesses the response to AVP EC80, which is the necessary concentration of vasopressin to be added to blood samples to provoke 80% platelet aggregation in absence of RO6953958. It is expected that the response to AVP EC80 will be increasingly inhibited by increasing concentration of RO6953958 in the blood of participants dosed with the drug. Participants must have a positive PAA at baseline for further sampling.

### 8.7.10 Facial Expression Recognition Task

The FERT will be recorded according to the SoA (see Section 1.3). The FERT is an emotion-related behavioral test designed to measure biases in emotional processing that are thought to be core components of affective disorders (Harmer et al 2008; Harmer et al 2009a; Harmer 2009b; Harmer et al 2010). The FERT assesses the interpretation of facial expressions. Faces with 6 different basic emotions (happiness, fear, anger, disgust, sadness, surprise) are displayed on a screen and participants are required to indicate the expression of the face. Different intensity levels of each emotion are presented, which increases the ambiguity of the facial expression and the sensitivity of the task.

### 8.7.11 *Circadian* Sleep Biomarkers

Serum cortisol and melatonin in both saliva and serum will be measured according to the SoA (see Section 1.3). Timings and number of samples (to a maximum of 12 samples per matrix) may be adjusted based on DLMO protocol. Full details on the timing of assessments will be detailed in a separate document.

### 8.7.12 Core Body Temperature Assessment

Core Body Temperature will be measured according to the SoA (see Section 1.3).

For measurements of core body temperature the Equivital EQ-02+ LifeMonitor in combination with the Philips VitalSense core body temperature pill will be used. Once the activated Core Body Temperature Pill is swallowed, the body worn sensor

(LifeMonitor) acts as antenna for picking up the core pill readings. The transit time through the body of the core pill varies between individuals, and normally ranges between 24 and 48 h. Additional details will be provided in a separate document.

### 8.7.13 Wake EEG

Wake EEG will be measured according to the SoA with the same EEG equipment used in PSG nights with a full 10-20 electrode montage (see Section 1.3).

### 8.7.14 *4β-Hydroxycholesterol and Total Cholesterol*

*Blood samples for the determination of plasma concentrations of 4βHC and total cholesterol will be collected from participants in Part 3 of the study as detailed in the SoA (Section 1.3).*

## 8.8 PHARMACODYNAMICS AND BIOMARKER SAMPLES

The samples may also be used for research purposes to identify biomarkers useful for predicting and monitoring response to RO6953958, identifying biomarkers useful for predicting, and monitoring RO6953958 safety and assessing PD effects of RO6953958. Additional markers may be measured in the case that a strong scientific rationale develops.

Samples should be collected as specified in the SoA (see Section 1.3).

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

The residual PD samples (including blood, serum, plasma, saliva, and derivatives such as DNA samples) will be destroyed within 5 years after the date of final clinical study report (CSR), unless the participant gives specific consent for the remainder of the PD samples to be stored for potential exploratory research within the RBR. If the participant provides consent for RBR, the samples will be stored and used not longer than 15 years after the date of final CSR. Any remaining PD samples (including blood, serum, plasma, and saliva) after the specified analyses may also be used for additional (assay) validation experiments, for research to develop methods, assays, prognostics, and/or companion diagnostics related to vasopressin, sleep disorders, ASD, or other related pathways and conditions.

Details on processes for collection and shipment of these samples will be described in a separate sample documentation.

## 8.9 SAMPLES FOR RESEARCH BIOSAMPLE REPOSITORY

### 8.9.1 Overview of the Research Biosample Repository

The Roche RBR is a centrally administered group of facilities for the long-term storage of human biologic samples, including body fluids, solid tissues, and derivatives thereof

(e.g., DNA, RNA, proteins, peptides). The collection, storage and analysis of the RBR samples will facilitate the rational design of new pharmaceutical agents and the development of diagnostic tests, which may allow for individualized drug therapy for patients in the future.

Residual samples for RBR (from participants who give specific consent to participate in this optional RBR) will be used to achieve the following objectives:

- To study the association of biomarkers with efficacy or progressive disease
- To identify safety biomarkers that are associated with susceptibility to developing AEs or can lead to improved AE monitoring or investigation
- To increase knowledge and understanding of disease biology and drug safety
- To study treatment response, including drug effects and the processes of drug absorption and disposition
- To develop biomarker or diagnostic assays and establish the performance characteristics of these assays

### 8.9.2 Sample Collection

The following samples will be stored in the RBR and used for research purposes, including, but not limited to, research on biomarkers related to study treatment or diseases:

- Residual plasma samples
- Residual CSF samples
- Residual saliva samples
- Residual urine samples
- Residual whole blood samples
- Residual samples prepared from whole blood (e.g., DNA, RNA)
- Residual samples from additional safety monitoring, if applicable

The sample collected for DNA extraction includes, but is not limited to, genomic analysis, and may be sent to one or more laboratories for analysis of germline or somatic mutations via whole genome sequencing, whole exome sequencing, next-generation sequencing, or other genomic analysis methods.

Genomics is increasingly informing researcher's understanding of disease pathobiology. Whole genome sequencing provides a comprehensive characterization of the genome and, along with clinical data collected in this study, may increase the opportunity for developing new therapeutic approaches. Data will be analyzed in the context of this study but will also be explored in aggregate with data from other studies. The availability of a larger dataset will assist in identification of important pathways, guiding the development of new targeted agents.

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

RBR samples will be stored and used not longer than 15 years after the date of final CSR. The RBR storage period will be in accordance with the IRB/EC‑approved ICF and applicable laws (e.g., Health Authority requirements).

The repository samples will be subject to the confidentiality standards (as described under Section 1.4 Confidentiality in Appendix 1).

## 8.10 MEDICAL RESOURCE UTILIZATION AND HEALTH ECONOMICS

Medical resource utilization and health economics parameters are not evaluated in this study.

## 8.11 TIMING OF STUDY ASSESSMENTS

### 8.11.1 Screening and Pretreatment Assessments

Written informed consent for participation in the study must be obtained before performing any study-specific screening tests or evaluations. ICFs for enrolled participant and for participants who are not subsequently enrolled will be maintained at the study site.

All screening, and all pretreatment assessments (related to entry criteria), must be completed and reviewed to confirm that participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure.

### 8.11.2 Assessments during Treatment

Under no circumstances will participants 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 (see Section 1.3). Assessments scheduled on the day of study treatment administration should be performed prior to administration of study treatment, unless otherwise noted in the SoA.

### 8.11.3 Assessments at Study Completion/Early Termination Visit

Participants who complete the study (as defined in Section 4.4) or discontinue from the study early (as described in Section 7) will be asked to return to the clinic after the final dose of study drug for a follow‑up visit (see SoA in Section 1.3 and Section 8.11.4).

### 8.11.4 Follow-Up Assessments

Assessments at the follow-up/early termination visit will be performed as indicated in the SoA (Section 1.3).After the study completion/early termination visit, AEs should be followed as outlined in Sections 8.3.1 and 8.3.3.

### 8.11.5 Assessments at Unscheduled Visits

Activities that are required to be performed in the case of an unscheduled visit triggered by an AE are guided by the nature of the AE and refer to further diagnostics and optimization of any therapy as needed.

## 9. STATISTICAL CONSIDERATIONS

## 9.1 STATISTICAL HYPOTHESES

See Section 9.4.3.2.

## 9.2 SAMPLE SIZE DETERMINATION

### 9.2.1 *Part 1 (Single Ascending Doses) and Part 2 (Multiple Ascending Doses)*

*For the SAD and MAD parts,* the number of participants to be randomized was chosen based on practical clinical judgment. The current planned study design and sample size complies with standard safety review rules applied in SAD and MAD studies.

### 9.2.2 *Part 3 (Drug-Drug Interaction)*

*A formal sample size calculation was performed to determine the number of participants required for midazolam drug-drug interaction part.*

*With a 20% drop out rate, 16 participants will be enrolled to obtain at least 12 participants who complete Part 3. With information from previous midazolam DDI studies (WP29393, BP41361, and BP40387), the intra-subject coefficient of variation (CV) for $AUC_{inf}$ and $C_{max}$ following administration of RO6953958 are assumed to be $\leq 21\%$.*

*Assuming a CV of 21%, a sample size of 12 evaluable participants will ensure the 2-sided 90% CI to not extend more than 1.33-fold above and not less than 0.75-fold below the true geometric mean ratio with 80% probability.*

## 9.3 POPULATIONS FOR ANALYSES

For purposes of analysis, the following populations are defined in Table 16.

### Table 16 Analysis Populations

| Population | Description |
|---|---|
| Safety | All participants randomized to study treatment and who received at least one dose of the study treatment, whether prematurely withdrawn from the study or not, will be included in the safety analysis. |
| Pharmacokinetic | All participants who have received active (RO6953958) treatment will be included in the PK analysis population. Participants will be excluded from the PK 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 PK analysis. Excluded cases will be documented together with the reason for exclusion. All decisions on exclusions from the analysis will be made prior to database closure. |
| Pharmacodynamic | Analyses of PD parameters will include all participants who received at least one dose of study drug treatment, are evaluable and have no major protocol violations. A participant is considered evaluable if at least one analysis parameter can be derived and subject did not vomit within 4 hours of drug administration. 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. |

## 9.4 STATISTICAL ANALYSES

### 9.4.1 Demographics and Baseline Characteristics

Demographic and other baseline characteristics of the safety analysis population will be listed and summarized with descriptive statistics.

### 9.4.2 Safety Analyses

All safety analyses will be based on the safety analysis population (see Table 17).

**Table 17 Safety Statistical Analysis Methods**

| Endpoint | Statistical Analysis Methods |
|---|---|
| Adverse events | The original terms recorded on the eCRF by the Investigator for adverse events will be coded by the Sponsor.<br>AEs will be summarized by mapped term and appropriate thesaurus level. |
| Clinical laboratory tests | All clinical laboratory data will be stored on the database in the units in which they were reported. Laboratory test values will be presented in International System of Units (SI units; Système International d'Unités) by individual listings with flagging of abnormal results. |
| Vital signs | Vital sign (supine and orthostatic) data will be presented by individual listings with flagging of values outside the normal ranges and flagging of abnormalities. In addition, tabular summaries will be used, as appropriate. |
| ECG data analysis | ECG data will be presented by individual listings. In addition, tabular summaries will be used, as appropriate.<br>The relationship between QTcF prolongation and the plasma concentration of RO6953958 may be investigated in an exploratory manner. |
| Concomitant medications | The original terms recorded on the participants' eCRFs by the Investigator for concomitant medications will be standardized by the Sponsor by utilizing a mapped term and appropriate drug dictionary level.<br>Concomitant medications will be presented in summary tables and listings. |
| Suicidal risk monitoring | Individual data on C-SSRS will be presented by listings. |

### 9.4.3 Pharmacokinetic Analyses

Analyses will be carried out on the PK analysis population. All PK parameters will be presented by listings and descriptive summary statistics (mean, standard deviation, coefficient of variation, median, minimum, and maximum) separately by group or cohorts.

#### 9.4.3.1 Pharmacokinetic Parameters

Individual and mean plasma and urine concentrations of RO6953958 and its metabolites RO7021594 and RO7045755 *(all parts) and midazolam (Part 3)* versus time data will be tabulated and plotted by dose level. Graphical displays of PK data may also be provided. The plasma, urine, and CSF PK of RO6953958 and its metabolites RO7021594 and RO7045755 *(all parts) and midazolam (Part 3)* will be tabulated and summarized as appropriate. Inter−participant variability and drug accumulation will be evaluated. Calculation of urine PK parameters (fractional excretion, renal clearance) will also be performed. *PK parameters will be read directly from the plasma concentration-time profiles or calculated using standard non-compartmental methods.* Parameters calculated will include but not be limited to the following:

**Part 1 (SAD/FE)**

- $C_{max}$, $t_{max}$, $C_{last}$, $t_{last}$, z, $t_{1/2}$, $AUC_{0–12h}$, $AUC_{0–last}$, $AUC_{inf}$, CL/F
- V/F, Ae, Fe, and CLR

**Part 2 (MAD)**

Day 1 and Day 10:

- $C_{max}$, $C_{avg}$, $t_{max}$, $AUC_{0-\tau}$, $\lambda_z$, $t_{½}$, CL/F, V/F, Ae, Fe, and CLR
- Steady-state achievement: $C_{trough}$
- Accumulation: RAUC, $RC_{max}$, $RC_{trough}$
- Molecular weight adjusted metabolite-to-parent ratio for $C_{max}$ and AUC parameters as appropriate.

***Part 3 (DDI)***

*RO6953958 and its metabolites RO7021594 and RO7045755(as appropriate):*

- *$C_{max}$, $C_{avg}$, $t_{max}$, $AUC_{0-\tau}$, $\lambda_z$, $t_{½}$, CL/F, V/F*
- *Steady-state achievement: $C_{trough}$ (Days 12-14)*
- *Accumulation: RAUC, $RC_{max}$, $RC_{trough}$*
- *Molecular weight adjusted metabolite-to-parent ratio for $C_{max}$ and AUC parameters as appropriate.*

*Midazolam:*

- *$T_{max}$, $C_{max}$, $t_{1/2}$, $AUC_{last}$, $AUC_{inf}$, $AUC_{\%extra}$, $\lambda z$, CL/F, V/F*
- *Accumulation: RAUC, $RC_{max}$, on days 1 and 2 vs. days 13 and 14*
- *CL: Total plasma clearance (IV midazolam only)*
- *F*
- *$V_{SS}$*

*Using data from all study parts,* additional PK analyses maybe be conducted as appropriate for RO6953958 and its metabolites RO7021594 and RO7045755. CSF/plasma concentration ratios will be calculated for RO6953958, M3, and M1, as applicable.

### 9.4.3.2 Statistical Analysis Methods

**Part 1 (SAD/FE)**

The following statistical analysis on the PK parameters ($AUC_{0-\infty}$ [or if cannot properly be determined $AUC_{0-t}$ or $AUC_{0-last}$] and $C_{max}$) will be performed after completion of the study

to explore dose proportionality. The following linear model will be applied to the log-transformed, dose-normalized PK study variables:

$y_{ij} \sim \mu + s_i + \lambda_j + \varepsilon_{ij}$ (i= 1,2,...,N; j=1,2,...,M)

where M is the number of different doses, N is the total number of participants, $\mu$ denotes the general mean of the transformed variables, $s_i$ is the random subject effect, $\lambda_j$ is the effect of the $j^{th}$ dose and $\varepsilon_{ij}$ is the residual error for subject i in the dose level j. The random effects are assumed to be independent and normally distributed with zero means and variances $\sigma^2_s$ and $\sigma^2_\varepsilon$ respectively.

Least square means with corresponding 90% confidence intervals (CI) will be derived for each dose level and plotted to evaluate if there are obvious trends for normalized PK parameters of decreasing/increasing with dose.

The following hypothesis will be tested (in an exploratory sense) for the primary PK parameters:

$H_0$: There is no deviation from dose proportionality (i.e., $\lambda_1 = \lambda_2 = \cdots = \lambda_M$)

versus

$H_1$: There is a deviation from dose proportionality ($\lambda_1 \neq \lambda_j$ for some i, j).

All p-values will be interpreted in an exploratory sense only.

A similar linear model will be applied to the log-transformed PK study variables to analyze FE:

$y_{ij} \sim \mu + \lambda_j + s_i + \varepsilon_{ij}$ (i= 1,2,…,N; j=1,2)

where $\mu$ denotes the general mean of the transformed variables, $\lambda_j$ is the effect of the feeding condition j (1=fed; 2=fasted), $s_i$ is the random subject effect and $\varepsilon_{ij}$ is the residual error for subject i in feeding condition j. The random effects are assumed to be independent and normally distributed with zero means and variances $\sigma^2_s$ and $\sigma^2_\varepsilon$ respectively.

Least square means with corresponding 90% CI will be derived to compare the relative bioavailability of the different feeding conditions.

Again, results of the statistical model will be interpreted in an exploratory sense.

**Part 2 (MAD)**

A similar model as for Part 1 will be applied to $AUC_{0\text{-}t}$ and $C_{max}$ in order to investigate possible deviations from dose proportionality.

The estimation of the time to attainment of steady-state in Part 2 will be based on the available trough concentrations ($C_{trough}$) from Day 1 to Day 10. It is postulated that a participant has reached steady-state as soon as $C_{24h}$ is at least 90% of its plateau value, i.e., 90% of the true $C_{24h}$ that this participant would reach after "infinitely" many doses. In order to estimate the time to steady-state of a participant with regression analysis, it is assumed that the true $C_{24h}$ versus time curve approaches its asymptotic value $C_{24h}$ (∞) (plateau) exponentially in time, i.e.,

$C_{24h}(t) = C_{24h}(\infty) \times (1-e^{-kt})$

Based on a statistical fit of this model to the measured concentrations one can estimate the earliest point in time where $C_{24h}$ reaches 90% of the curves maximum ($C_{24h}$ [∞]), the so called $t_{90\%}$. The analysis will be supported by appropriate graphical displays. If BID dosing is used in Part 2 (MAD) then $C_{12h}$ will be considered instead of $C_{24h}$.

The dose dependency of the accumulation ratio, defined as:

$$\frac{\mathrm{PK-Param_{Day_{10}}}}{\mathrm{PK-Param_{Day_1}}}$$

will be estimated for the log–transformed primary PK parameters based on an analysis of variance model with "log accumulation ratio" as dependent variable and "dose group" as factor.

***Part 3 (DDI)***

*The effect of RO6953958 on the PK of midazolam will be assessed. The effect of RO6953958 on the natural log-transformed $C_{max}$, $AUC_{inf}$ ($AUC_{last}$ if $AUC_{inf}$ cannot be derived) will be assessed with a linear mixed-effects model. Treatment will be used as fixed effect and participant will be used as a random effect. Point estimates for the means and corresponding 90% CI for the differences in means between the two treatments (RO6953958 plus midazolam as the test treatment, versus midazolam alone as the reference) will be obtained from the linear mixed effects model and exponentiated to obtain geometric means, geometric mean ratios, and respective 90% CI on the original scale. The model result will be derived separately for oral and IV midazolam.*

### 9.4.4 Cardiodynamic Electrocardiogram (ECG) Assessment

Cardiodynamic ECG evaluation (optional) will be performed for Part 1 (SAD) under fasted conditions and Part 2 (MAD) if necessary (based on observed PK and other project considerations). If this evaluation is performed, the primary analysis will be based on concentration-QTc modeling of the relationship between the plasma concentrations of RO6953958 and its metabolites RO7021594 and RO7045755 and change-from-baseline QTcF (ΔQTcF) with the intent to exclude an effect of placebo-corrected ΔQTcF (ΔΔQTcF) > 10 msec at clinically relevant plasma concentrations. The effect of

RO6953958 on the placebo-corrected ΔQTcF, ΔHR (heart rate), ΔPR, and ΔQRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) will also be evaluated at each post-dosing time point ('by-time point' analysis) using the Intersection Union Test. In addition, an analysis of categorical outliers will be performed for changes in HR, PR, QRS, QTcF, T-wave morphology, and U-wave presence.

Cardiodynamic ECG evaluation will be described in a separate statistical analysis plan.

### 9.4.5 Pharmacodynamic Analyses

All PD parameters will be presented by listings and descriptive summary statistics separately by dose.

Listings for the change from baseline (absolute and relative) and difference to placebo and the corresponding summary statistics may also be presented. Graphical displays may be used, as appropriate.

PD analysis may assess the correlation of PD baseline levels, peak concentrations after treatment or change from baseline with PD effects.

PKPD analysis may be performed for PD parameters if the data allow.

Further analysis may be described in a separate technical document if appropriate.

## 9.5 SUMMARIES OF CONDUCT OF STUDY

All protocol deviations will be listed. Data for study treatment administration and concomitant medication will be listed. The number of participants who were randomized, discontinued and completed the study will be summarized and listed.

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## 11. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

The following section includes standard appendices such as Appendix 1 (For regulatory, ethical and study oversight considerations), Appendix 2 (For Adverse Events: Definitions and Procedures for Evaluating, Follow-up and Reporting) and Appendix 3 (Procedures for Recording Adverse Events), Appendix 4 (Clinical Laboratory Tests), Appendix 5 (Contraceptive Guidance and Collection of Pregnancy Information).

# Appendix 1
# Regulatory, Ethical, and Study Oversight Considerations

## 1. REGULATORY AND ETHICAL CONSIDERATIONS

### 1.1. COMPLIANCE WITH LAWS AND REGULATIONS

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

### 1.2. INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE

This protocol, the ICFs, any information to be given to the participant (e.g. advertisements, diaries etc), 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 participant 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 (Section 2.3.1 of this Appendix).

The Investigator should follow the requirements for reporting all adverse events to the Sponsor. 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.

### 1.3. INFORMED CONSENT

The Sponsor's Master Informed Consent Form (and ancillary sample ICFs 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. Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center. The Sponsor

or its designee must review and approve any proposed deviations from the Sponsor's sample ICFs 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. Participants must be re-consented to the most current version of the ICF(s) during their participation in the study. A copy of the ICF(s) signed by all parties must be provided to the participant or the participant's legally authorized representative.

### Consent to Participate in the Research Biosample Repository

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

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

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

### Approval by the Institutional Review Board or Ethics Committee

Collection, storage, and analysis of RBR samples is contingent upon the review and approval of the exploratory research and the RBR portion of the ICF by each site's IRB/EC and, if applicable, an appropriate regulatory body. If a site has not been granted approval for RBR sampling, this section of the protocol will not be applicable at that site

### Withdrawal from the Research Biosample Repository

Participants who give consent to provide samples for the RBR have the right to withdraw their samples at any time for any reason. If a participant wishes to withdraw consent to the testing of his or her samples, the Investigator must inform the Medical Monitor and Site Monitor in writing of the participant's wishes using the RBR Withdrawal Form and, if the study is ongoing, must enter the date of withdrawal on the RBR Withdrawal of Informed Consent eCRF. The participant will be provided with instructions on how to withdraw consent after the study is closed. A participant's withdrawal from Study BP41695 does not, by itself, constitute withdrawal of samples from the RBR. Likewise, a participant's withdrawal from the RBR does not constitute withdrawal from Study BP41695. Data already generated before time of withdrawal of consent to RBR will still be used.

## 1.4. CONFIDENTIALITY

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.

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

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

### Confidentiality for Research Biosample Repository

Data generated from RBR samples must be available for inspection upon request by representatives of national and local Health Authorities, and the Sponsor's monitors, representatives, and collaborators, as appropriate.

Participant medical information associated with RBR samples is confidential and may only be disclosed to third parties as permitted by the ICF (or separate authorization for use and disclosure of personal health information) signed by the participant, unless permitted or required by law.

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

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

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

### Monitoring and Oversight Research Biosample Repository

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

## 1.5. 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., last participant, last visit).

# 2. DATA HANDLING AND RECORD

## 2.1. DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

### 2.1.1. Data Quality Assurance

All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

The Sponsor or designee is responsible for the data management of this study including quality checking of the data.

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

### 2.1.2. Source Data Records

Source documents (paper or electronic) are those in which participant 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, COAs (paper or eCOA), 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, participant files, and records kept at pharmacies, laboratories, and medico-technical departments involved in a clinical study.

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 below.

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.

### 2.1.3. 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.

## 2.2. RETENTION OF RECORDS

Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the Investigator for at least 15 years after study 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 destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

The Sponsor will retain study data for 25 years after the final study results have been reported or for the length of time required by relevant national or local health authorities.

## 2.3. STUDY RECORDS

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully reconstructed, including but not limited to the protocol, protocol amendments, ICFs, and documentation of IRB/EC and governmental approval.

Roche shall also submit an Annual Safety Report once a year to the IEC and CAs according to local regulatory requirements and timelines of each country participating in the study.

### 2.3.1. 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 participants or any non-substantial changes, as defined by regulatory requirements.

### 2.3.2. Publication Policy and Protection of Trade Secrets

Regardless of the outcome of a trial, the Sponsor is dedicated to openly providing information on the trial to healthcare professionals and to the public, both at scientific congresses and in peer-reviewed journals. The Sponsor will comply with all requirements for publication of study results. For more information, refer to the Roche Global Policy on Sharing of Clinical Trials Data at the following Web site:

www.roche.com/roche_global_policy_on_sharing_of_clinical_study_information.pdf

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 for approval 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.

### 2.3.3. Dissemination of Clinical Study Data

The results of the study may be communicated in scientific meetings and/or peer-reviewed literature. In addition, results of the study will also be included in submissions to regulatory agencies and other health authorities.

### 2.3.4. Management of Study Quality

The Sponsor will implement a system to manage the quality of the study, focusing on processes and data that are essential to ensuring participant safety and data integrity. Prior to study initiation, the Sponsor will identify potential risks associated with critical trial processes and data and will implement plans for evaluating and controlling these risks. Risk evaluation and control will include the selection of risk-based parameters (e.g., adverse event rate, protocol deviation rate) and the establishment of quality tolerance limits for these parameters prior to study initiation. Detection of deviations from quality tolerance limits will trigger an evaluation to determine if action is needed. Details on the establishment and monitoring of quality tolerance limits will be provided in a Quality Tolerance Limit Management Plan.

### 2.3.5. Site Inspections

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

## 3. ADMINISTRATIVE STRUCTURE

The Sponsor of the study 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.

## 4. STUDY AND SITE CLOSURE

The Sponsor (or designee) has the right to close the study site or 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 participants.
- Participant enrollment is unsatisfactory.

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

Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The Investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local Health Authorities, the Sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of participants by the Investigator.
- Discontinuation of further study treatment development.

# Appendix 2
# Adverse Events: Definitions and Procedures for Evaluating, Follow-up and Reporting

## 1. DEFINITION OF ADVERSE EVENTS

According to the E2A ICH guideline for Good Clinical Practice, an **adverse event** is any untoward medical occurrence in a participant or clinical investigation participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An adverse event can therefore be:

- 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.

**Events Meeting the AE Definition:**

- Deterioration in a laboratory value (hematology, clinical chemistry, or urinalysis) 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 treatment (see Appendix 3, Section 4).
- Exacerbation of a chronic or intermittent preexisting condition, including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study.
- 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).

**Events NOT Meeting the AE Definition:**

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of preexisting disease(s) or condition(s) present or detected at the start of the study that do not worsen.

## 2. DEFINITION OF SERIOUS ADVERSE EVENTS

If an event is not an AE per definition above, then it cannot be a serious adverse event (SAE) even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A serious adverse event is defined as any untoward medical occurrence that at any dose:

- **Results in death.**
- **Is life-threatening.**

  The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- **Requires inpatient hospitalization or prolongation of existing hospitalization** (see Appendix 3).

  In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

  Hospitalization for elective treatment of a preexisting condition that did not worsen from baseline is not considered an AE.
- **Results in persistent or significant disability/incapacity**

  Disability means substantial disruption of the participant's ability to conduct normal life functions.

  This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
- **Is a congenital anomaly/birth defect**.
- **Other significant events**:

  Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

# 3. RECORDING OF ADVERSE EVENT AND/OR SERIOUS ADVERSE EVENT

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.

The Investigator will then record all relevant AE/SAE information in the CRF.

It is **not** acceptable for the Investigator to send photocopies of the participant's medical records to Medical Monitor in lieu of completion of the eCRF.

There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to the Sponsor.

The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

## 3.1. ASSESSMENT OF SEVERITY

The Investigator will make an assessment of severity for each AE and SAE reported during the study and assign it to one of the categories provided in Table 1 (as a guidance for assessing adverse event severity).

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 predefined grading criteria [e.g., National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE] criteria); 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.

**Table 1 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 above).

## 3.2. ASSESSMENT OF CAUSALITY

Investigators should use their knowledge of the participant, 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 treatment, indicating "yes" or "no" accordingly. The following guidance should be taken into consideration:

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

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

## 4. FOLLOW-UP OF AES AND SAES

The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

If a participant dies during participation in the study or during a recognized follow-up period, the Investigator will provide the Sponsor with a copy of any post-mortem findings including histopathology.

New or updated information will be recorded in the originally completed eCRF.

The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

# 5. 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 treatment:

- Serious adverse events
- Non-serious adverse events of special interest (NSAESI)
- Pregnancies (see Section 8.3.5)

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.

## 5.1 REPORTING REQUIREMENTS OF SERIOUS ADVERSE EVENTS AND NON-SERIOUS ADVERSE EVENTS OF SPECIAL INTEREST

**Events that Occur prior to Study Treatment Initiation**

After informed consent has been obtained but prior to initiation of study treatment, 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 Serious Adverse Event Responsible immediately (i.e., no more than 24 hours after learning of the event).

**Events that Occur after Study Treatment Initiation**

For reports of serious adverse events and non-serious adverse events of special interest (Section 8.3.6) that occur after initiation of study treatment (Section 8.3.1), 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 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 Serious Adverse Event 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.

**Reporting of Post-Study Adverse Events and Serious Adverse Events**

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

## 5.2 REPORTING REQUIREMENTS FOR CASES OF OVERDOSE OR MEDICATION ERROR

Accidental overdose and medication error (hereafter collectively referred to as "special situations"), are defined as follows:

- Accidental overdose: accidental administration of a drug in a quantity that is higher than the assigned dose
- Medication error: accidental deviation in the administration of a drug. In some cases, a medication error may be intercepted prior to administration of the drug.

Special situations are not in themselves adverse events, but may result in adverse events. Each adverse event associated with a special situation should be recorded separately on the Adverse Event eCRF. If the associated adverse event fulfills seriousness criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event). For RO6953958 and placebo,

adverse events associated with special situations should be recorded as described below for each situation:

- Accidental overdose: Enter the adverse event term. Check the "Accidental overdose" and "Medication error" boxes.
- Medication error that does not qualify as an overdose: Enter the adverse event term. Check the "Medication error" box.
- Medication error that qualifies as an overdose: Enter the adverse event term. Check the "Accidental overdose" and "Medication error" boxes.

In addition, all special situations associated with RO6953958 and placebo, regardless of whether they result in an adverse event, should be recorded on the Adverse Event eCRF and should be recorded as described below:

- Accidental overdose: Enter the drug name and "accidental overdose" as the event term. Check the "Accidental overdose" and "Medication error" boxes.
- Medication error that does not qualify as an overdose: Enter the name of the drug administered and a description of the error (e.g., wrong dose administered, wrong dosing schedule, incorrect route of administration, wrong drug, expired drug administered) as the event term. Check the "Medication error" box.
- Medication error that qualifies as an overdose: Enter the drug name and "accidental overdose" as the event term. Check the "Accidental overdose" and "Medication error" boxes. Enter a description of the error in the additional case details.
- Intercepted medication error: Enter the drug name and "intercepted medication error" as the event term. Check the "Medication error" box. Enter a description of the error in the additional case details.

As an example, an accidental overdose that resulted in a headache would require the completion of two Adverse Event eCRF pages, one to report the accidental overdose and one to report the headache. The "Accidental overdose" and "Medication error" boxes would need to be checked on both eCRF pages.

## 6. EXPEDITED REPORTING TO HEALTH AUTHORITIES, INVESTIGATORS, INSTITUTIONAL REVIEW BOARDS, AND ETHICS COMMITTEES

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

To determine reporting requirements for single adverse event cases, the Sponsor will assess the expectedness of these events through use of the reference safety information in the RO6953958 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.

# Appendix 3
# Procedures for Recording Adverse Events

Investigators should use appropriate medical terminology and consider medical concepts and adverse event may fall into 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.

## 1. ADVERSE EVENTS OCCURRING SECONDARY TO OTHER EVENTS

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

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

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

## 2. PERSISTENT OR RECURRENT ADVERSE EVENTS

A persistent adverse event is one that extends continuously, without resolution, between participant evaluation timepoints. 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 participant evaluation timepoints and subsequently recurs. Each recurrence of an adverse event should be recorded separately on the Adverse Event eCRF.

## 3. 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 or treatment discontinuation).
- Results in a medical intervention (e.g., potassium supplementation for hypokalemia) or a change in concomitant therapy.
- Clinically significant in the Investigator's judgment.

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

If a clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 times the upper limit of normal [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.

## 4. 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 or treatment discontinuation).
- Results in a medical intervention or a change in concomitant therapy.
- Clinically significant in the Investigator's judgment.

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

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

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

## 5. 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.

The most appropriate diagnosis or (if a diagnosis cannot be established) the abnormal laboratory values should be recorded on the Adverse Event eCRF (see Appendix 2) and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event), either as a serious adverse event or a non-serious adverse event of special interest (see Section 8.3.6).

## 6. DEATHS

All deaths that occur during the protocol-specified adverse event reporting period (see Section 5 of Appendix 2), regardless of relationship to study treatment, must be recorded on the Adverse Event eCRF and immediately reported to the Sponsor.

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. 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. The term "sudden death" should not be used unless combined with the presumed cause of death (e.g., "sudden cardiac death").

## 7. 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”).

## 8. 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 Appendix 2), except as outlined below.

An event that leads to hospitalization under the following circumstances should not be reported as an adverse event or a serious adverse event:

- Hospitalization for respite care
- Planned hospitalization required by the protocol (e.g., for study treatment administration or insertion of access device for study treatment administration)
- Hospitalization for a preexisting condition, provided that all of the following criteria are met:

  The hospitalization was planned prior to the study or was scheduled during the study when elective surgery became necessary because of the expected normal progression of the disease.

  The participant has not suffered an adverse event.

An event that leads to hospitalization under the following circumstances is not considered to be a serious adverse event, but should be reported as an adverse event instead:

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

# Appendix 4
# Clinical Laboratory Tests

The tests detailed in Table 1 will be performed by the central laboratory.

Local laboratory results are only required in the event that the central laboratory results are not available in time for either study treatment administration and/or response evaluation. If a local sample is required, it is important that the sample for central analysis is obtained at the same time. Additionally, if the local laboratory results are used to make either a study treatment decision or response evaluation, the results must be captured in source documentation and entered as a comment into the eCRF.

Protocol-specific requirements for inclusion or exclusion of participants are detailed in Sections 5.1 and 5.2, respectively, of the protocol.

Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

## Table 1 Protocol-Required Safety Laboratory Assessments

All study-required laboratory assessments may be performed by a central laboratory.

| Laboratory Assessments | Parameters |
|---|---|
| Hematology | • Leucocytes, erythrocytes, hemoglobin, hematocrit, platelets, differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes). |
| Clinical Chemistry | • Sodium, potassium, chloride, bicarbonate ($CO_2$), glucose (fasting*), urea, creatinine, protein, albumin, phosphate, calcium, total and direct bilirubin, alkaline phosphatase, ALT, AST, uric acid, LDH gamma-glutamyl-transferase (GGT), creatine phosphokinase (CPK).<br>* In Part 2 *and Part 3* where participants are dosed in fed state, on Day 2, Day 5, Day 9; safety blood samples and PK samples will be taken at the same time (to avoid additional venepunctures) and blood glucose will not be fasted. |
| Coagulation** | • INR, aPTT.<br>**Coagulation samples taken for lumbar puncture are captured only for exclusion purposes and will not be recorded electronically. |
| Viral Serology | • HIV (specific tests HIV-1 antibody, HIV-1/2 antibody, HIV-2 antibody), hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody. |

| Laboratory Assessments | Parameters |
|---|---|
| Urinalysis | • Specific gravity.<br>• Dipstick: pH, glucose, protein, blood, nitrite, leukocyte.<br>• If there is a positive result (confirmed by a positive repeated sample), urine will be sent to the laboratory for microscopy and if necessary a urine culture. If there is an explanation for the positive dipstick results (e.g., menses), it should be recorded and there is no need to perform microscopy and culture.<br>• Microscopic examination (sediment, RBCs, WBCs, casts, crystals, epithelial cells, bacteria), if blood or protein is abnormal. |
| Other Screening Tests | • Urine drug screen (to include at minimum: cotinine, amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines).<br>• Urine alcohol test. |

The results of each test maybe entered into the eCRF or transferred electronically.

Investigators must document their review of each laboratory safety report.

## Additional Statistical Considerations for Clinical Laboratory Data

- Standard Reference Ranges and Transformation of Data

Potential analysis considerations for analyzing Laboratory data includes the use of Standard Reference Ranges and potential transformation of data for specific lab tests.

In this scenario, Roche standard reference ranges, rather than the reference ranges of the Investigator, can be used for specific parameters. For these 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.

- Definition of Laboratory Abnormalities

For all laboratory parameters included in the analysis described above, 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 participant listings of laboratory data.

In addition to the standard reference range, a marked reference range has been predefined by Roche for these laboratory parameters. 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 participant, the midpoint of the standard reference range will be used as the participant's baseline value for the purposes of determining marked laboratory abnormalities. Marked laboratory abnormalities will be labeled in the participant listings as "HH" for very high or "LL" for very low.

# Appendix 5
# Contraceptive Guidance and Collection of Pregnancy Information

## 1. DEFINITIONS

- Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile. The definition of childbearing potential may be adapted for alignment with local guidelines or requirements.

## 2. COLLECTION OF PREGNANCY INFORMATION

- **Male participants with partners who become pregnant**

The Investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study (see Section 8.3.5 Pregnancy). This applies only to male participants who receive study treatment.

Attempts should be made to collect and report details of the course and outcome of any pregnancy in the partner of a male participant exposed to study treatment. The Investigator will record pregnancy information on the Clinical Trial Pregnancy Reporting Form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy. When permitted by the site, the pregnant partner would need to sign an Authorization for Use and Disclosure of Pregnancy Health Information to allow for follow-up on her pregnancy. If the authorization has been signed, the Investigator should update the Clinical Trial Pregnancy Reporting Form with additional information on the course and outcome of the pregnancy when available. An Investigator who is contacted by the male participant 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. The female partner will be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Monitoring of the participant's partner should continue until conclusion of the pregnancy. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for procedure.

## 3. CONGENITAL ANOMALIES/BIRTH DEFECTS

Any congenital anomaly/birth defect in a child born to a female partner of a male participant exposed to study treatment 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).