Protocol No. RVP-101-22

A Single Center, Double-Blinded Phase 1a Randomized Study to Evaluate Safety and Pharmacokinetics of a New Manganese Based Magnetic Resonance Imaging (MRI) Contrast Agent, RVP 001, in Healthy Adult Subjects

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Clinical Study Protocol

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RVL-101-22
Amendment 1

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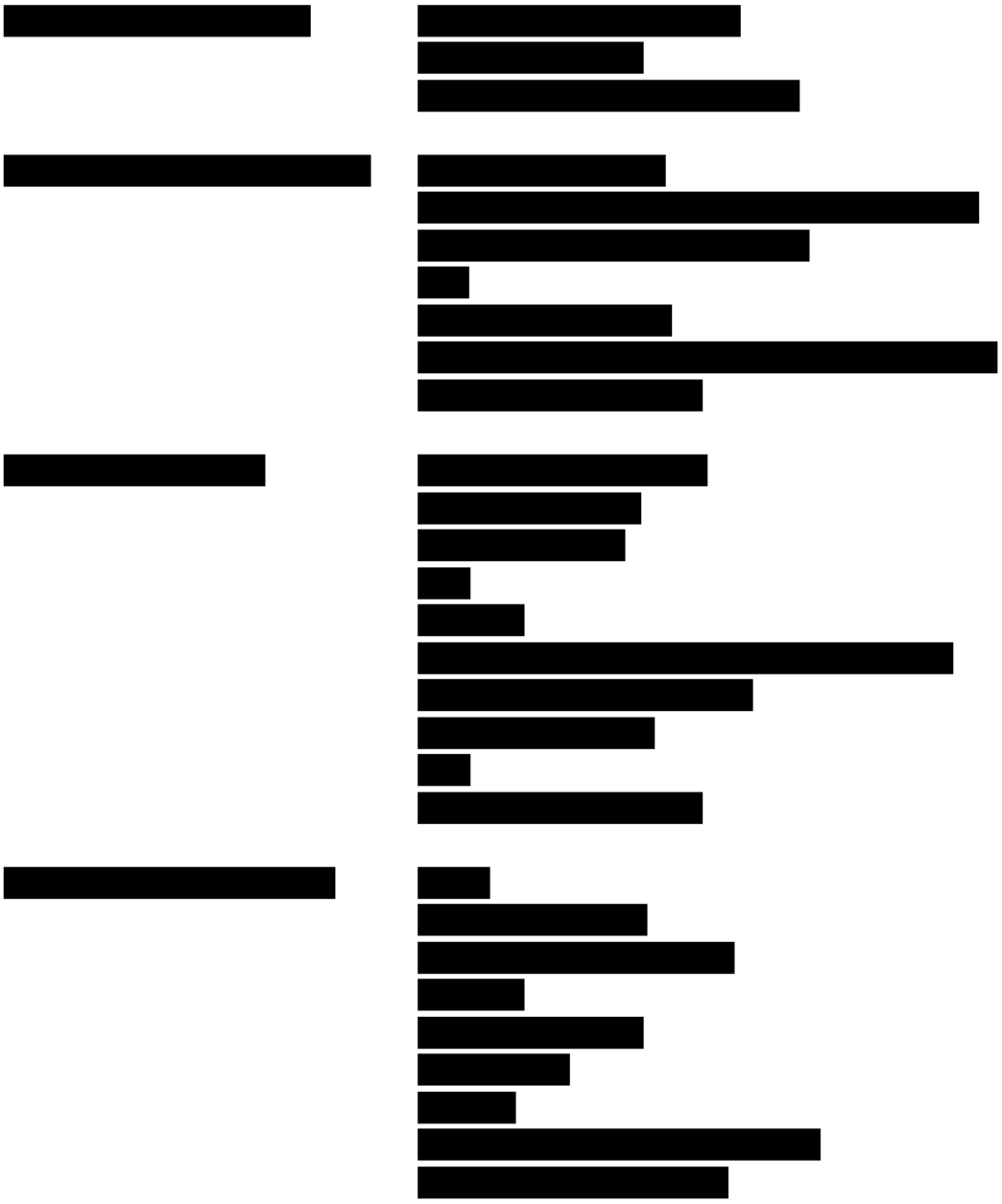


1 TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

2 ABBREVIATIONS

ADR	Adverse drug reaction
AE	Adverse Event
ALT	Alanine Aminotransferase
Anti-HBsAg	Antibody HBsAg
Anti-HCV	Antibodies against Hepatitis C virus
APTT	Activated partial thromboplastin time
AST	Aspartate Aminotransferase
AUC	Area under the concentration-time curve
BMI	Body Mass Index
BUN	Blood urea nitrogen
BP	Blood pressure
CDER	Center for Drug Evaluation and Research
CE	Contrast-enhanced
CFR	Code of Federal Regulations
CI	Confidence internval
CIOMS	Council for International Organizations of Medical Sciences
CK	Creatine Kinase
C_{max}	Maximum serum concentration
CNS	Central Nervous System
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CSPM	Clinical sample processing manual
CYP	cytochrome P450
CRO	Clinical Research Organization
CSR	Clinical study report
CTCAE	NCI Common Terminology Criteria Adverse Events
CV	Cardiovascular
CV%	Coefficient of variation
DDI	Drug-drug interaction
DMP	Data management plan

DNA	Deoxyribonucleic Acid
DSMP	Data Safety Monitoring Plan
eCRF	Electronic Case Report Forms
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
FDA	Food and Drug Administration
FIH	First In Human
FSH	Follicle stimulating hormone
GBCA	Gadolinium-Based Contrast Agent
GCP	Good Clinical Practice
Gd	Gadolinium
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HBsAg	Surface antigen of the hepatitis B virus (HBV)
hCG	Human Chorionic Gonadotropin
HCV	Hepatitis C virus
HCVAb	Hepatitis C virus antibodies
hERG	Human Ether-a-go-go-related Gene
HIV	Human immunodeficiency virus
HR	Heart rate
IB	Investigator's Brochure
IC_{50}	Half maximal inhibitory concentration
ICF	Informed consent form
ICH	International Conference on Harmonization
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IEC	International Ethics Committee
IND	Investigational New Drug Application
INR	International Normalized Ratio
IP	Investigational Product
IRB	Institutional Review Board
ISF	Investigator Site File

IV	Intravenous
Kg	Kilogram
LC	Liquid Chromatography
LLN	Lower limit of normal
LLOQ	lower limit of quantitation
MedDRA	Medical Dictionary for Regulatory Activities
mL	Milliliter
Mn	Manganese
Mn-DPDP	Mangafodipir trisodium
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MS	Mass Spectrometry
msec	Millisecond
MSSD	Maximum Safe Starting Dose
NOAEL	No-Observed-Adverse-Effect-Level
NSF	Nephrogenic Systemic Fibrosis
OAT3	Organic anion transporter 3
OATP1B1	Organic anion transporting polypeptide 1B1
P-gp	P-glycoprotein
PI	Principal Investigator
PK	Pharmacokinetic(s)
PT	Preferred term
Pt	Prothrombin time
QC	Quality Control
QTcF	Fridericia-corrected QT interval
RBC	Red blood cell
RNA	Ribonucleic Acid
SAD	Single Ascending Dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2; the virus that causes COVID-19

SD	Standard deviation
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
SRC	Safety Review Committee
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBD	To be determined
US	United States
ULN	Upper Limit of Normal
U.S.	United States
WBC	White blood cell
WHO	World Health Organization
WHO-DD	World Health Organization drug dictionary
WOCBP	Women of Childbearing Potential

3 PROTOCOL SUMMARY

3.1 SYNOPSIS

Title:	A Single Center, Double-Blinded Phase 1a Randomized Study to Evaluate Safety and Pharmacokinetics of a New Manganese Based Magnetic Resonance Imaging (MRI) Contrast Agent, RVP-001, in Healthy Adult Subjects
Objectives:	Primary objectives: • To evaluate the safety and tolerability of single ascending doses (SADs) of RVP-001 in healthy adult volunteers. Secondary objectives: • To determine the pharmacokinetics and elimination of RVP-001 in healthy adult volunteers.
Endpoints:	Safety: Safety will be evaluated throughout the study by assessing the following parameters: adverse events, physical examinations, injection site monitoring, electrocardiograms (ECGs), vital signs, clinical laboratory evaluations, medical history, and prior and concomitant medications. Pharmacokinetics and Elimination: Plasma and urine will be analyzed by high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) to measure the concentration of intact RVP-001 and to assess plasma pharmacokinetics. Urine and feces will be analyzed by inductively coupled plasma mass spectrometry (ICP-MS) for total manganese (Mn) content to quantify elimination of injected Mn and fractional excretion. Additional assessments of biotransformation products and metabolism may be performed.
Study Design:	This is a Phase 1a, single center, randomized, double-blinded, SAD-cohort study to assess the safety, tolerability, pharmacokinetics, and elimination of RVP-001 at four doses: [redacted]

[redacted]

During Phase 1a, no medical imaging will be performed.

Subjects will be confined to the Phase 1 facility from 24 hours prior to dose administration (Day -1) until 120 hours (Day 6) following dose administration. The plan is to enroll 4 dose cohorts of 8 healthy volunteers each, with two subjects receiving placebo and six subjects receiving RVP-001. For all cohorts, a staggered dosing schedule will include 2 sentinel subjects (1 active, 1 placebo) dosed initially, with the remaining 6 subjects dosed at least 24 hours later.

If this initial dose is safe and well tolerated, as defined in the criteria to proceed to the next dose level, then the second dose cohort group will be administered. This cycle will proceed as described to each successive dose level.

Depending on safety and tolerability data as well as on available pharmacokinetic (PK) data up to Day 6 (120 hours), the escalation scheme may be modified such that another intermediate dose level or a repeat dose is administered. This additional cohort will be optional and may be added depending on the outcome of the safety, tolerability, and PK assessments in the previous cohorts.

Subjects who withdraw or are withdrawn from the study after dosing for reasons other than safety and tolerability may be replaced after consultation between the Safety Committee members. In such case, the total number of subjects dosed will remain within a maximum of 10 subjects per cohort. Subjects withdrawn due to a study drug -related adverse event (AE) will not be replaced.

Inclusion/Exclusion

Subjects enrolled in this study will be from the community at large. The recruitment advertisements may use various media types (e.g., radio, newspaper, Clinical Research Organization [CRO] Web site, CRO volunteer database).

Subjects must meet all of the following criteria to be included in the study:

1) Male or female
2) Non-smoker (no use of tobacco products, e-cigarettes or nicotine replacement products within 12 months prior to screening)
3) ≥ 18 and ≤ 55 years of age at the time of signing informed consent

4) Body Mass Index (BMI) > 18.0 and < 32.0 kg/m^2 at screening
5) Body weight ≥ 55.0 kg for males and females at screening
6) Subject must be in good general health condition (i.e., full physical examinations, medical history, vital signs, 12-lead ECG, and clinical laboratory tests performed at screening) as determined by the investigator. Normal ECG is defined as normal cardiac conduction parameters including resting heart rate (HR) between 50 and 100 bpm, Fridericia-corrected QT interval (QTcF) ≤ 450 ms, and QRS interval < 120 ms
7) Subject shows normal biochemistry test results (within normal range or considered not clinically significant by the clinical investigator) at screening or admission, including items listed below:
    a. Blood urea nitrogen (BUN), and creatinine
    b. Albumin and total protein
    c. Alkaline phosphatase, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and total bilirubin
    d. Human immunodeficiency virus (HIV), Antibody HBsAg (anti-HBsAg) and antibodies against Hepatitis C virus (HCV) (anti-HCV).
8) Subject shows normal hematology test results (within normal range or considered not clinically significant by the clinical investigator) at screening or admission including items listed below:
    a. Red blood cell (RBC) count
    b. White blood cell (WBC) count with differential
    c. Hemoglobin and hematocrit
    d. Platelet count
9) Subject shows normal urinalysis test results (within normal range or considered not clinically significant by the clinical investigator) at screening or admission including items listed below:
    a) pH and specific gravity (refer to Section 9.2)
    b) blood, leukocyte, glucose, protein, ketones, and nitrite
    c) Drug and alcohol abuse screening test (drugs of abuse are listed in Section 9.1.5).
10) Subject shows normal coagulation test results (within normal range or considered not clinically significant by the clinical investigator) at screening or admission including prothrombin time (Pt), International Normalized Ratio (INR) and activated partial thromboplastin time (APTT).
11) Subject must agree to use an adequate method of contraception (as defined in Section 6.5)

12) Subject must be able to understand a written informed consent, which must be obtained prior to initiation of study procedures
13) Subject must be willing and able to comply with all study requirements.

Exclusion Criteria

Subjects to whom any of the following applies will be excluded from the study:

1) Any clinically significant abnormality or abnormal laboratory test results found during medical screening; positive test for hepatitis B, hepatitis C, or HIV found during medical screening
2) History of clinically significant cardiovascular (CV), renal, hepatic, chronic respiratory or gastrointestinal disease (except cholecystectomy); neurological or psychiatric disorder, as judged by the investigator
3) Evidence of clinically significant hepatic impairment at screening and admission including any of:
    a) Albumin above 1.3 × the upper limit of normal (ULN) or below 0.7 x the lower limit of normal (LLN)
    b) ALT above 1.5 × ULN
    c) AST above 2.5 × ULN
    d) total bilirubin above 2.0 × ULN (total bilirubin accepted up to 2 × ULN if direct bilirubin is within normal limits)
4) Evidence of clinically significant renal impairment at screening including estimated glomerular filtration rate (eGFR) of <60 mL/min or creatinine greater than 1.5 mg/dL. (The eGFR calculations will be performed using the CKD-EPI 2021 Refit Equation)
5) Clinically significant cardiac findings at screening or admission including resting HR below 50 or above 100 bpm, or ECG abnormality or QTcF above 450 ms
6) Known allergies to any component of RVP-001
7) Known drug allergies or sensitivities which have resulted in Emergency Room visits, hospitalizations, or need to carry rescue Epipens as a matter of routine practice; moderate or severe allergies to any intravenous (IV) contrast agents; seasonal allergies requiring medication
8) History of asthma
9) Subjects who have taken strong CYP inhibitors 14 days prior to first dose of study A list of these inhibitors is provided in Section 11.4 CYP

Inhibitors (Food and Drug Administration [**FDA] Table of Substrates, Inhibitors, and Inducers 2020**).

10) Subjects with diets that are high in Mn content (>2.5 mg/day). Foods known to have high Mn content, including shellfish (i.e., mussels, clams, oysters), hazelnuts, pecans, blueberries, pineapple, and/or supplements containing Mn.

11) Vital sign abnormalities at screening or admission (systolic blood pressure [BP] lower than 90 or over 140 mmHg, diastolic BP lower than 50 or over 90 mmHg)

12) History of significant alcohol abuse within two years prior to screening

    a) or regular use of alcohol within six months prior to the screening visit (in males more than 21 units per week and females more than 14 units per week (1 unit = 12 oz 1 bottle/can of beer, 1 oz 40% spirit, or 5 oz glass of wine)

    b) or a confirmed positive alcohol urine test at screening or admission.

13) History of significant drug abuse or use of soft drugs or hard drugs (drugs of abuse are listed in Section 9.1.5) within 1 year prior to screening.

14) A confirmed positive urine cotinine test at screening or admission.

15) Subjects who have received any investigational product (IP) in a clinical research study within 5 half-lives or within 30 days prior to first dose. However, in no event shall the time between last receipt of IP and first dose be less than 30 days.

16) Use of medication other than topical products without significant systemic absorption:

    a) prescription medication within 14 days prior to (the first) dosing

    b) over-the-counter products including natural health products (e.g., food supplements and herbal supplements) within 14 days prior to (the first) dosing, with the exception of the occasional use of acetaminophen (up to 2 g daily)

    c) a depot injection or an implant of any drug (including contraception) within 3 months prior to (the first) dosing

    d) Coronavirus Disease 2019 (COVID-19) vaccines are accepted concomitant medications.

17) Donation of plasma within 7 days prior to dosing. Donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to the first dosing.

18) Positive highly sensitive pregnancy test at screening or at admission for women of childbearing potential (WOCBP).
19) Have poor venous access that limits phlebotomy at screening and admission
20) Evidence of current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; the virus that causes COVID-19) infection
21) Male subjects with pregnant or lactating partners
22) Subjects who are, or are immediate family members of, a study site or sponsor employee
23) Any reason which, in the opinion of the Principal Investigator, would prevent the subject from participating in the study.

Phase:	1a
Study Population:	Heathy adult male and female volunteers will be enrolled in this study.
Number of subjects	Thirty-two (32) subjects (+ 8 subjects for potential replacement if applicable) will be enrolled in this study in 4 dose cohorts of 8 subjects each (6 RVP-001, 2 placebo per cohort). Depending on safety and tolerability as well as on available PK data, the escalation scheme may be modified such that an additional intermediate dose level or a repeat dose is administered. This additional cohort will be optional and may be added depending on the outcome of the safety, tolerability, and PK assessments in the previous cohorts. Subjects who withdraw or are withdrawn from the study after dosing for reasons other than safety and tolerability may be replaced after consultation between the Safety Committee members. In such case, the total number of subjects dosed will remain within a maximum of 10 subjects per cohort. Subjects withdrawn due to a study drug-related AE will not be replaced. A sample size of 8 subjects per cohort randomized in a 3:1 ratio to the study drug vs placebo represents a typical panel for a first-in-human study. This number of subjects is judged adequate to achieve the study objectives.
Description of Sites/Facilities Enrolling Participants:	This study will be conducted at a single site.

Study Drug, Dose, and Mode of Administration:	[redacted] At the clinical site a predetermined dose of the drug product will be drawn from the vial into a syringe affixed with a needle, for use as an IV bolus, based on the directions in the protocol.
Placebo, Dose, and Mode of Administration:	RVP-001 is a hypertonic solution that is clear, colorless to pale yellow, and is intended to be administered through the IV route via bolus injection. Given the lack of excipients in the formulation, normal saline will be used as an appropriate placebo. Amber tinted syringes and IV tubing as well as power injectors will be used to preserve blinding amongst the study staff, by concealing slight color and ease of injectability differences between the active pharmaceutical and placebo (saline). Weight based dosing will be used by pharmacy staff to prepare syringes containing RVP-001 or placebo for IV administration by the study staff. Power injectors will be used to standardize injection times of the bolus and the saline flush that follows.
Study Duration:	This study is estimated to take approximately 9 months from first-subject-in to the completion of the clinical study report (CSR).
Participant Duration:	Each subject will be confined to a Phase 1 facility from 24 hours (Day -1) prior to dose administration until 120 hours following dose administration. Subjects will be required to participate in a follow-up phone call on Day 8 to monitor for adverse events, and to self-report any AEs up to Day 30. Overall duration of individual subject participation (including screening) will be up to 58 days.

3.2 SCHEDULE OF ACTIVITIES (SOA)

Assessment	Screening	Admission	Days						Post-Treatment Phase Follow-up Phone Call	Subjects to Self-report AEs
Day	**-28 to -2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6[1,2]**	**8[3]**	**9-30[4]**
Informed Consent[5]	X									
Eligibility criteria review (Inclusion/Exclusion)	X	X[6]								
Demographics	X									
Medical History	X									
Admission to clinical site[7]		X								
Randomization			X[8]							
Vital signs[9]	X	X	X	X	X	X	X	X		

AE = adverse event

[1] **Discharge from unit will occur after the 120-hour PK blood sample (Day 6)**

[2] **In the event of early termination, all Day 6 assessments should be performed prior to discharge.**

[3] Post-treatment phase follow-up phone call can be performed in a window of Day 8 +1 day

[4] Adverse events (AEs) will be captured for 30 days post-final dose (subjects will be asked to self-report when the 30 days exceeds last follow-up). If emerging or ongoing AEs are reported at the follow-up phone call or after follow-up, additional visits and phone calls will be arranged as unscheduled visits

[5] **The latest version of the Informed Consent form must be signed prior to subject's inclusion (prior to the study drug administration on Day 1)**

[6] This is an update only of changes since screening.

[7] Subjects will arrive at the clinical site at approximately 24 hours prior to drug administration

[8] To be performed immediately prior to dosing

AE = adverse event; BMI = Body Mass Index; **HBsAg =** surface antigen of the hepatitis B virus (HBV); **HCVAb =** hepatitis C virus antibodies

[9] Vital signs include BP, HR, respiration rate, pulse oximetry (SpO2), and body temperature. Vital signs will be taken pre-dose (within 12 hours), at 6, 15, 30, 45, 60 minutes post dose and 2, 4, 8, 12, 24, 48, 72, 96, 120 hours post-dose for data collection. HR and pulse ox to be monitored continuously for 4 hours post-dose with any abnormal values to be followed as AEs.

Assessment	Screening	Admission	Days						Post-Treatment Phase Follow-up Phone Call	Subjects to Self-report AEs
Day	-28 to -2	-1	1	2	3	4	5	6[1,2]	8[3]	9-30[4]
Body Weight[10]	X	X						X		
Height	X									
Vein assessment	X	X								
BMI	X									
Physical Examination[11]	X	X						X		
Neurological assessment[12]	X	X						X		
Clinical laboratory tests[13]	X	X	X	X	X	X	X	X		
Serology (HIV-1/HIV-2 antibodies, HBsAg and HCVAb)	X									
Urinalysis[14]	X	X	X	X	X	X	X	X		
Single 12-lead safety electrocardiogram[15]			X	X						
Triplicate 12-lead safety electrocardiogram[16]	X	X	X	X	X	X	X	X		

[10] Body weight will be assessed at screening. Body weight will be also assessed at admission, and prior to discharge. Weight at admission will used for dosing calculation.

[11] A complete physical examination will be performed at screening, admission, and prior to discharge (Day 6).

[12] A complete neurological examination will be performed at screening, admission and prior to discharge (Day 6).

[13] Clinical laboratory tests (hematology, biochemistry [including lipid profile], coagulation) will be performed at screening, on admission; at 2 hours, 8 hours, and approximately 24 hours post-dose; 48, 72 and 96 hours and before discharge (120 hours).

AE = adverse event; FSH = follicle stimulating hormone; HIV = Human immunodeficiency virus

[14] Spot urinalysis samples will be taken at screening and upon admission. Subsequent small spot urine samples will be obtained pre-dose and at 24, 48, 72, 96, and 120 hours post dose. 24 hour urine Cr will also be measured from each of the 24-hour collection samples as described below under urine and feces.

[15] **Single recording ECG readings will be taken 1, 3 and 8 minutes post dose.**

[16] Triplicate ECG readings to be taken pre-dose (within 2 hours), 20, 40, 60 minutes and 2, 4, 8, 12, 24, 48, 72, 96, 120 hours post dose.

Assessment	Screening	Admission	Days						Post-Treatment Phase Follow-up Phone Call	Subjects to Self-report AEs
Day	-28 to -2	-1	1	2	3	4	5	6[1,2]	8[3]	9-30[4]
Alcohol, cotinine and drugs of abuse screen	X	X								
Pregnancy test[17]	X	X						X		
FSH[18]	X									
RVP-001 administration			X							
Injection site monitoring[19]			X	X	X	X	X	X		
Blood PK samples[20]			X	X	X	X	X	X		
		X	X	X	X	X	X	X		
Urine and Feces samples[21, 22]										

[17] A highly sensitive urine pregnancy test will be performed at screening for all female subjects. For women of childbearing potential, a highly sensitive urine pregnancy test will also be performed before discharge. A highly sensitive serum pregnancy test will be performed at admission.

[18] Post-menopausal females only.

AE = adverse event

[19] Injection site monitoring daily until discharge.

[20] Blood samples for PK assessment will be collected prior (within 2 hours prior to dose) and at 2, 5, 10, 20, and 30 minutes after the end of the bolus dose and at 1, 2, 4, 8, 12, 24, 48, 72, 96, and 120 hours post-dose (6 mL for each sampling time).

[21] All urine and feces will be collected for PK and elimination studies and be retained in 24-hour bins. Urine samples will be collected in the following approximate time intervals: -24-0 hours, 0-24 hours, 24-48 hours, 48-72 hours, 72-96 hours, 96-120 hours. Feces samples (entire stool) will be collected in the following approximate time intervals: -24-0 hours, 0-24 hours, 24-48 hours, 48-72 hours, 72-96 hours, 96-120 hours.

[22] Pre-dose 24 hour urine collection may be less than 24 hrs depending on time subject enters CSU to time of dosing.

Assessment	Screening	Admission	Days						Post-Treatment Phase Follow-up Phone Call	Subjects to Self-report AEs
Day	-28 to -2	-1	1	2	3	4	5	6[1,2]	8[3]	9-30[4]
Fluid Monitoring[23]		X	X	X	X	X	X	X		
Discharge from clinical site								X		
Adverse Event Monitoring	X	X	X	X	X	X	X	X	X	X
Concomitant Medication Recording	X	X	X	X	X	X	X	X	X	

[23] Fluid In/Out will be measured q8 hours.

4 INTRODUCTION

4.1 STUDY RATIONALE

Contrast-enhanced (CE) Magnetic Resonance Imaging (MRI) is a vital tool in diagnostic medicine. RVP-001 is being developed as a gadolinium (Gd)-free alternative to currently available general purpose MRI contrast agents, all of which belong to a class of compounds known as gadolinium-based contrast agents (GBCAs). General purpose MRI contrast agents are non-specific in their biodistribution and distribute in the blood pool and in the extracellular fluid (Aime & Caravan 2009). They are essential for routine diagnostic purposes and are used to help detect and stage a wide range of diseases, as well as to monitor disease progression and response to treatment (Klein et al. 2018). However, serious but rare side effects can occur with GBCAs, limiting their use. The United States (U.S.) Food and Drug Administration (FDA) advises that all GBCAs increase the risk for nephrogenic systemic fibrosis (NSF) among patients with impaired renal elimination of the drugs and requires a 'black box warning" directing physicians to avoid the use of GBCAs in these patients if possible; FDA later required other label updates to minimize the risk of NSF (FDA Drug Safety Communication 2010). Safety concerns continue to emerge as more becomes known about the long-term effects of GBCAs. It is established that residual Gd, in some chemical form, is retained in the brain and body for long periods (years) after administration of GBCAs; FDA has reacted with additional warnings required on drug labels (FDA Drug Safety Communication 2018). The indispensable role of GBCAs is reflected in the fact that they are administered over 8 million times per year in the U.S. (FDA MIDACM 2017).

Manganese (Mn), unlike Gadolinium, is a normal elemental component of the human body. Therefore, RVP-001 was designed to act in a functionally similar manner to GBCAs but to contain the biocompatible essential trace element Mn and not contain the gadolinium ion.

This highlights the high unmet medical need for a Gd-free alternative that can be used to provide important CE diagnostic MRI scans. Instead of Gd, RVP-001 utilizes chelated manganese ion (Mn^{2+}) to produce contrast in MRI scans. By avoiding Gd altogether in the contrast agent, safety concerns based upon Gd would be precluded.

The goal of this first clinical trial, RVL--22, is to evaluate the safety, tolerability, pharmacokinetics, and elimination of single ascending doses (SADs) of RVP-001 when injected as a single intravenous (IV) bolus administration in normal, healthy adults.

4.2 BACKGROUND

RVP-001 is a low molecular weight chelate of Mn^{2+} that was designed and developed to provide a Gd-free alternative to GBCAs for IV use in CE MRI. RVP-001, also known in the scientific literature as Mn-PyC3A, comprises a rationally designed hydrophilic, anionic hexadentate chelator complexed to divalent manganese (Mn^{2+}). This results in a chelate that simultaneously provides high relaxivity, is largely

confined to the extracellular space, and is highly resistant to Mn dissociation (Gale et al. 2018, Erstad et al. 2019).

The mechanism of action of MRI contrast agents is to reduce the relaxation times, T_1 and T_2, of water molecules in tissue. For example, in T_1-weighted MR images, tissue that has a shorter T_1 value has higher signal and appears brighter compared to tissue that has a longer T_1. Paramagnetic species such as Gd^{3+} (in GBCAs) or Mn^{2+} strongly reduce the T_1 of water molecules in close proximity to the metal ion, and as a result increase the MRI signal in a T_1-weighted image. The extent to which a contrast agent can change the relaxation time is characterized by its relaxivity and is denoted r_1 or r_2 depending on whether it refers to changing the T_1 time or T_2 time. Relaxivity is an inherent property of the contrast agent. Contrast agents with a higher r_1 relaxivity will cause a larger T_1 change at a given concentration than a contrast agent with a lower relaxivity. The amount of signal change generated by a contrast agent is proportional to both its relaxivity and its concentration within an anatomical region. The presence or absence of signal enhancement is used by physicians to identify whether pathology is absent or present (Klein et al. 2018). The most widely used indication for MRI contrast agents is for the detection of central nervous system (CNS) lesions. For example, many primary and metastatic brain tumors cause disruption of the blood-brain barrier, resulting in concentration of the contrast agent within the lesion bed. This results in local enhancement making the tumor highly conspicuous on MRI scans (Klein et al. 2018).

The MR contrast effects of an Mn^{2+} based contrast agent are exemplified in the experience with mangafodipir trisodium (Mn-DPDP; Teslascan®), an agent that was FDA-approved for use in contrast enhanced hepatobiliary MR imaging. In humans and animals, Mn-DPDP dissociates in the body immediately after IV administration, with about 80% of the administered Mn being released from its chelator in humans (Toft et al. 1997), whereas RVP-001 was designed and has been demonstrated to remain intact and to be excreted intact. This strong Mn^{2+} chelating property of RVP-001 versus Mn-DPDP is reflected in conditional stability constants for the 2 compounds at pH 7.4: RVP-001 utilizes a chelator that has over 100,000 times higher affinity to Mn^{2+} than Mn-DPDP (Gale et al. 2015). This lower stability of Mn-DPDP leads to greater Mn^{2+} release and consequently to greater retention of injected Mn in the body, with 15% of the injected Mn from Mn-DPDP remaining in rats 5 days after IV administration (Grant et al. 1994) compared to 0.058 ± 0.051% of Mn from IV injected RVP-001 remaining in rats at 7 days after injection (Erstad et al. 2019). Unlike Mn-DPDP which dissociates upon injection, RVP-001 was designed to be chemically stable and not release free Mn^{2+} ion in the body. Bioanalytical studies showed that RVP-001 is rapidly eliminated intact without undergoing metabolism or degradation in rats, mice, and monkeys.

4.2.1 NON-CLINICAL STUDIES

Nonclinical pharmacology data indicate that RVP-001 is a unique, effective imaging agent based on MRI studies in mice and baboons. The effectiveness of RVP-001 in these studies was generally equivalent to that of comparator GBCAs. The acute safety of RVP-001 was assessed in a standard Good Laboratory Practices (GLP)-compliant battery of safety pharmacology studies of CNS, respiratory, and CV function. RVP-001 is the N-methyl-D-glucamine (meglumine) salt of the Mn complex. Some early studies used the sodium salt form which is denoted RVP-001-30.

In nonclinical studies RVP-001 was shown to be chemically stable with a relaxivity comparable to GBCAs, and to provide effective contrast enhancement in animal models. RVP-001 has the desired pharmacokinetic (PK) attributes of rapid elimination, low tissue retention and resistance to metabolic degradation (Gale at al. 2015, Gale at al. 2018, Erstad et al. 2019, Zhou et al. 2021). RVP-001 was shown to undergo mixed hepatobiliary and renal elimination in mice, baboons, and rats (Gale at al. 2018, Erstad et al. 2019, Zhou et al. 2021), with increased fractional hepatobiliary elimination in a rat model of renal impairment (Zhou et al. 2021).

[redacted]

Refer to the Investigator Brochure for details of all non-clinical studies to date. The proposed Phase 1a study will constitute the first in human (FIH) study of RVP-001 to assess safety and PK using a randomized SAD scheme.

4.3 RISK/BENEFIT ASSESSMENT

4.3.1 RISK ASSESSMENT

RVP-001 has not yet been evaluated in humans.

In nonclinical toxicology studies in rats (Studies 1737-19008 and GD64LN) and cynomolgus monkeys (Studies 1737-19021 and MJ95QX), RVP-001 was found to be safe and well tolerated at projected clinically useful doses. [redacted]

4.3.2 KNOWN POTENTIAL BENEFITS

As the study treatment is not being given to subjects to treat any symptoms or illness, there will be no direct medical benefit from participation in this trial.

4.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Mn has a well understood toxicity profile. Known effects from Mn toxicity inform potential safety concerns for the entire class of Mn-containing MRI contrast agents include the possibilities of Mn retention in the brain subsequent to chronic overexposure, hypersensitivity reactions, lowering of seizure threshold, and transient changes in CV function as a consequence of acute overexposure to free Mn^{2+}. To mitigate risk from Mn exposure, RVP-001 was designed to stably chelate Mn^{2+}, to remain intact while in the body, and to be excreted intact. Nonetheless FDA has requested Reveal submit expedited IND safety reports for any reports of seizure, stroke, cerebral venous thrombosis, QTc >480 msec, or QTc increase of 60 msec over baseline, regardless of assessment of causality.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events (AEs) of RVP-001 may be found in the investigator's brochure (IB).

4.4 STUDY OBJECTIVES

The objectives of this study are as follows:

Primary objective:

- To evaluate the safety and tolerability of SADs of RVP-001 in healthy adult volunteers.

Secondary objective:

- To determine the pharmacokinetics and elimination of RVP-001 in healthy adult volunteers.

4.5 STUDY ENDPOINTS

Safety:

Safety will be evaluated throughout the study by assessing the following parameters: AEs, physical examinations, injection site monitoring, ECGs, vital signs, clinical laboratory evaluations, medical history, and prior and concomitant medications.

Pharmacokinetics and Elimination:

Plasma and urine will be analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) to measure the concentration of intact RVP-001 and to assess plasma pharmacokinetics. Urine and feces will be analyzed by inductively coupled plasma mass spectrometry (ICP-MS) for total Mn content to quantify elimination of injected Mn via the urine and feces. Additional assessments of biotransformation products may be performed.

5 STUDY DESIGN

5.1 OVERALL DESIGN

This is a Phase 1a, single center, randomized, double-blinded, SAD-cohort study to assess the safety, tolerability, pharmacokinetics, and elimination of RVP-001 at four doses: [redacted]

During Phase 1a, no medical imaging will be performed.

Subjects will be confined to the Phase 1 facility from 24 hours prior to dose administration (Day -1) until 120 hours (Day 6) following dose administration. The plan is to enroll 4 dose cohorts of 8 healthy volunteers each, with two subjects receiving placebo and six subjects receiving RVP-001. For all cohorts, a staggered dosing schedule will include 2 sentinel subjects (1 active, 1 placebo) dosed initially, with remaining 6 subjects dosed at least 24 hours later.

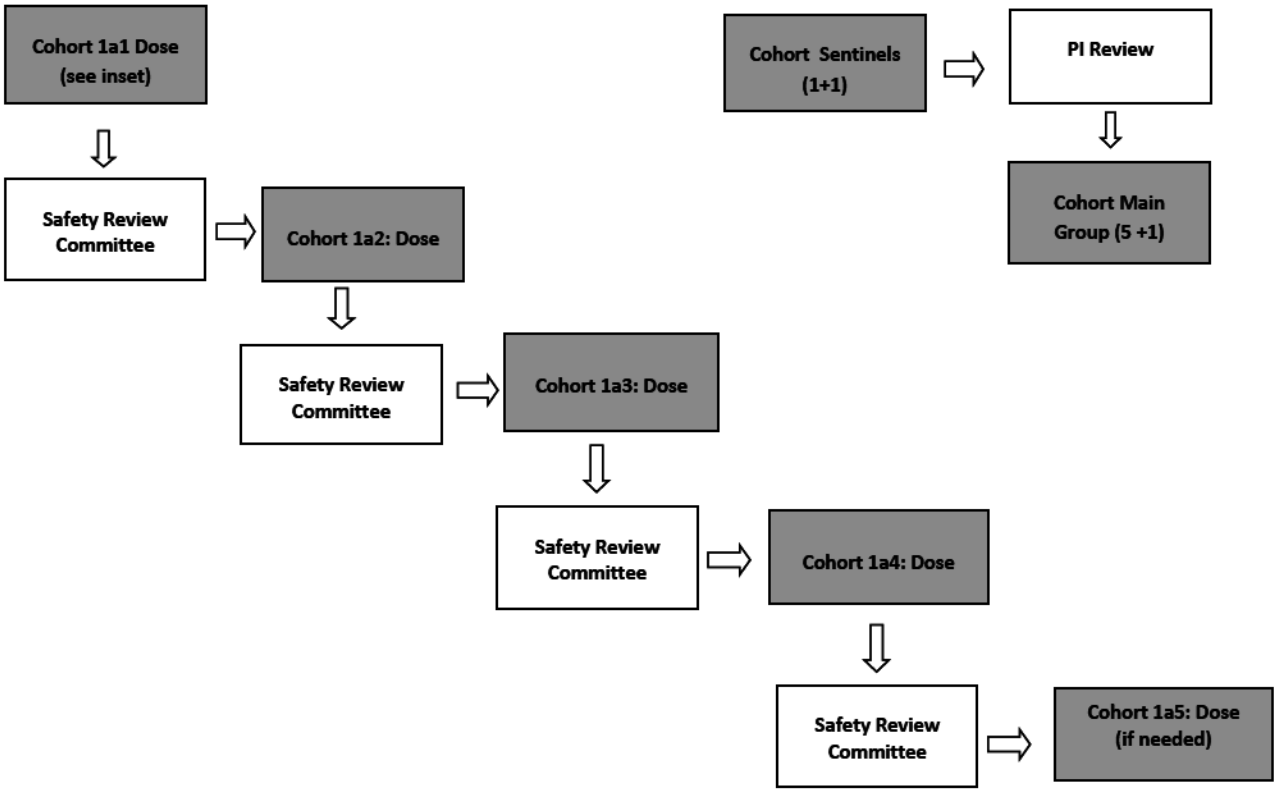
If this initial dose is safe and well tolerated, as defined in the criteria to proceed to the next dose level, then the second dose cohort group will be administered. This cycle will proceed as described to each successive dose level.

Depending on safety and tolerability as well as on available PK data up to Day 6 (120 hours), the escalation scheme may be modified such that another intermediate dose level or a repeat dose is administered. This additional cohort will be optional and may be added depending on the outcome of the safety, tolerability, and PK assessments in the previous cohorts.

Subjects who withdraw or are withdrawn from the study after dosing for reasons other than safety and tolerability may be replaced after consultation between the Safety Committee members. In such case, the total number of subjects dosed will remain within a maximum of 10 subjects per cohort. Subjects withdrawn due to a study drug-related AE will not be replaced.

The dose escalation schema is shown in Figure 1.

Figure 1 Dose Escalation Schema



5.2 JUSTIFICATION FOR DOSE

Phase 1a will be an escalating single-dose, safety, tolerance, and pharmacokinetic study of RVP-001 in dose cohort groups of healthy adult male and female volunteers (Table 1). The starting clinical dose is based on nonclinical safety, toxicity, and pharmacokinetic data, in accordance with FDA guidance (Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, FDA 2005) and in conjunction with preclinical imaging data generated with RVP-001. [redacted]

Table 1 Phase 1a Planned Dose Escalation

Cohort	Dose (mg Mn/kg)	Number of Subjects (drug + placebo)	Process (sentinel +remaining)	Route of Administration
1a.1	2.0	6 + 2	2 + 6	IV injection
1a.2	4.0	6 + 2	2 + 6	IV injection
1a.3	7.0	6 + 2	2 + 6	IV injection
1a.4	12.0	6 + 2	2 + 6	IV injection
1a.5	TBD	6 + 2	2 + 6	IV injection

IV = intravenous; TBD = to be determined

5.3 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 3.2.

The end of the study is defined as completion of the last visit or procedure shown in the SoA in the trial for the last participant enrolled in the study.

6 STUDY POPULATION

6.1 JUSTIFICATION FOR THE STUDY POPULATION

This is a FIH study. A healthy volunteer population has been selected for the study because healthy subjects with no concomitant diseases and using no concomitant medications represent a homogeneous population allowing for proper evaluation of the safety, tolerability, and PK of a drug without confounding factors.

Since the risk to the developing human fetus following exposure to RVP-001 is unknown at this time, female subjects will be enrolled in this trial only if they meet all inclusion criteria and none of the exclusion criteria. To ensure that female subjects are not pregnant or likely to become pregnant during the study, confirmation will be obtained by serum and urine tests prior to study drug administration. In addition, female subjects of childbearing potential and non-surgically sterile male subjects will be required to use effective contraceptive methods for an appropriate duration (refer to Section 6.5 for acceptable contraceptive methods)

6.2 SAMPLE SIZE

Thirty-two (32) subjects (+ 8 subjects for potential replacement if applicable) will be enrolled in this study in 4 dose cohorts of 8 subjects each (6 RVP-001, 2 placebo).

Depending on safety and tolerability as well as on available PK data, the escalation scheme may be modified such that an additional intermediate dose level or a repeat dose is administered. This additional cohort will be optional and may be added depending on the outcome of the safety, tolerability, and PK assessments in the previous cohorts.

Subjects who withdraw or are withdrawn from the study after dosing for reasons other than safety and tolerability may be replaced after consultation between the Safety Committee members. In such case, the total number of subjects dosed will remain within a maximum of 10 subjects per cohort. Subjects withdrawn due to a study drug-related AE will not be replaced.

A sample size of 8 subjects per cohort randomized in a 3:1 ratio to the study drug vs placebo represents a typical panel for a first-in-human study. That number of subjects is judged adequate to achieve the study objectives.

6.3 INCLUSION CRITERIA

Subjects enrolled in this study will be from the community at large. The recruitment advertisements may use various media types (e.g., radio, newspaper, Clinical Research Organization [CRO] Web site, CRO volunteer database).

Subjects must meet all of the following criteria to be included in the study:

1) Healthy adults
2) Non-smoker (no use of tobacco products, e-cigarettes or nicotine replacement products within 12 months prior to screening)
3) ≥ 18 and ≤ 55 years of age at the time of signing informed consent
4) Body Mass Index (BMI) > 18.0 and < 32.0 kg/m^2 at screening
5) Body weight ≥ 55.0 kg for males and females at screening
6) Subject must be in good general health condition (i.e., full physical examinations, medical history, vital signs, 12-lead ECG, and clinical laboratory tests performed at screening) as determined by the investigator. Normal ECG is defined as normal cardiac conduction parameters including resting HR between 50 and 100 bpm, Fridericia-corrected QT interval (QTcF) ≤ 450 ms, and QRS interval < 120 ms
7) Subject shows normal biochemistry test results (within normal range or considered not clinically significant by the clinical investigator) at screening or admission including items listed below:
    a. Blood urea nitrogen (BUN), and creatinine
    b. Albumin and total protein
    c. Alkaline phosphatase, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and total bilirubin
    d. Human immunodeficiency virus (HIV), Antibody HBsAg (anti-HBsAg) and antibodies against Hepatitis C virus (HCV) (anti-HCV)
8) Subject shows normal hematology test results (within normal range or considered not clinically significant by the clinical investigator) at screening or admission including items listed below:
    e. Red blood cell (RBC) count
    f. White blood cell (WBC) count with differential
    g. Hemoglobin and hematocrit
    h. Platelet count
9) Subject shows normal urinalysis test results (within normal range or considered not clinically significant by the clinical investigator) at screening or admission including items listed below:
    a) pH and specific gravity (refer to Section 9.2)
    b) blood, leukocyte, glucose, protein, ketones, and nitrite
    c) Drug and alcohol abuse screening test (drugs of abuse are listed in Section 9.1.5)
10) Subject shows normal coagulation test results (within normal range or considered not clinically significant by the clinical investigator) at screening or admission, including prothrombin time (Pt), International Normalized Ratio (INR), and activated partial thromboplastin time (APTT).
11) Subject must agree to use an adequate method of contraception (as defined in Section 6.5)
12) Subject must be able to understand a written informed consent, which must be obtained prior to initiation of study procedures
13) Subject must be willing and able to comply with all study requirements

6.4 EXCLUSION CRITERIA

Subjects to whom any of the following applies will be excluded from the study:

1) Any clinically significant abnormality or abnormal laboratory test results found during medical screening; positive test for hepatitis B, hepatitis C, or HIV found during medical screening
2) History of clinically significant CV, renal, hepatic, chronic respiratory or gastrointestinal disease (except cholecystectomy); neurological or psychiatric disorder, as judged by the investigator
3) Evidence of clinically significant hepatic impairment at screening and admission including any of:
    a) Albumin above 1.3 × the upper limit of normal (ULN) or below 0.7 x the lower limit of normal (LLN)
    b) ALT above 1.5 × ULN
    c) AST above 2.5 × ULN
    d) total bilirubin above 2.0 × ULN (total bilirubin accepted up to 2 × ULN if direct bilirubin is within normal limits)
4) Evidence of clinically significant renal impairment at screening including estimated Glomerular filtration rate (eGFR) of <60 mL/min or creatinine greater than 1.5 mg/dL. (The eGFR calculations will be performed using the CKD-EPI 2021 Refit Equation)
5) Clinically significant cardiac findings at screening or admission including resting HR below 50 or above 100 bpm, or ECG abnormality or QTcF over 450 ms
6) Known allergies to any component of RVP-001
7) Known drug allergies or sensitivities which have resulted in Emergency Room visits, hospitalizations, or need to carry rescue Epipens as a matter of routine practice; moderate or severe allergies to any IV contrast agents; seasonal allergies requiring medication
8) History of asthma
9) Subjects who have taken strong CYP inhibitors 14 days prior to first dose of study A list of these inhibitors is provided in Section 11.4 CYP Inhibitors (FDA Table of Substrates, Inhibitors, and Inducers 2020).
10) Subjects with diets that are high in Mn content (>2.5 mg/day). Foods known with high Mn content include shellfish (i.e., mussels, clams, oysters), hazelnuts, pecans, blueberries, pineapple, and/or supplements containing Mn
11) Vital sign abnormalities at screening or admission (systolic BP lower than 90 or over 140 mmHg, diastolic BP lower than 50 or over 90 mmHg)
12) History of significant alcohol abuse within two years prior to screening
    a) or regular use of alcohol within six months prior to the screening visit (in males over 21 units per week and females over 14 units per week [1 unit = 12 oz 1 bottle/can of beer, 1 oz 40% spirit, or 5 oz glass of wine])
    b) or a confirmed positive alcohol urine test at screening or admission
13) History of significant drug abuse or use of soft drugs or hard drugs (drugs of abuse are listed in Section Drug, Alcohol and Cotinine Screen 9.1.5) within 1 year prior to screening
14) A confirmed positive urine cotinine test at screening or admission.

15) Subjects who have received any investigational product (IP) in a clinical research study within 5 half-lives or within 30 days prior to first dose. However, in no event shall the time between last receipt of IP and first dose be less than 30 days.

16) Use of medication other than topical products without significant systemic absorption:

    a) prescription medication within 14 days prior to (the first) dosing
    b) over-the-counter products including natural health products (e.g., food supplements and herbal supplements) within 14 days prior to (the first) dosing, with the exception of the occasional use of acetaminophen (up to 2 g daily)
    c) a depot injection or an implant of any drug (including contraception) within 3 months prior to (the first) dosing
    d) Coronavirus Disease 2019 (COVID-19) vaccines are accepted concomitant medications

17) Donation of plasma within 7 days prior to dosing; donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to the first dosing

18) Positive highly sensitive pregnancy test at screening or admission for WOCBP.

19) Have poor venous access that limits phlebotomy at screening and admission

20) Evidence of current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; the virus that causes COVID-19) infection

21) Male subjects with pregnant or lactating partners

22) Subjects who are, or are immediate family members of, a study site or sponsor employee

23) Any reason which, in the opinion of the Principal Investigator, would prevent the subject from participating in the study.

6.5 CONTRACEPTION AND RESTRICTIONS

Male Subjects with Partners of Childbearing Potential

Male subjects who are sexually active with a partner of childbearing potential must use, with their partner, a condom plus an approved method of highly effective contraception from the time of informed consent until 97 days after last study drug administration. This is based on 90 days (one cycle of spermatogenesis) plus 5 half-lives of the IP. The half-life of the RVP-001 is unknown in humans. Therefore, the duration from final dose to the last follow-up visit/phone call will be used.

The following methods are acceptable:

- Partner's use of combined (estrogen and progestogen-containing) hormonal contraception associated with inhibition of ovulation:
  - oral
  - intravaginal
  - transdermal
- Partner's use of progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral
  - injectable/implantable
  - intrauterine hormone-releasing system
- Partner's use of intrauterine device
- Vasectomized
- Partner's bilateral tubal occlusion

Males with Partners of Non-childbearing Potential

There is a significant risk of drug exposure through the ejaculate (which also applies to vasectomized males) that might be harmful to sexual partners. Therefore, even if a male is sexually active with a partner of non-childbearing potential, they will be required to use a condom from first administration of study drug until the follow-up visit/phone call.

Female Subjects of Childbearing Potential

Female subjects who are sexually active and of childbearing potential must use, with their partner, an approved method of highly effective contraception from the time of informed consent until 37 days after last study drug administration. This has been calculated based on 30 days (one female menstrual cycle) plus 5 half-lives of the study drug. The half-life of the RVP-001 is unknown in humans. Therefore, the duration from final dose to the last follow-up visit/phone will be used.

The following highly effective methods are acceptable:

- Intrauterine device
- Vasectomized partner
- Bilateral tubal occlusion

Female subjects are not required to use any of the above contraceptive methods if their sexual partner is female.

All Subjects (Male and Female Subjects of Childbearing Potential)

Alternatively, sexual abstinence is considered a highly effective method only if defined as refraining from intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

Females of Non-Childbearing Potential

Female subjects who are not of childbearing potential do not need to use any methods of contraception. A woman is considered a WOCBP unless post-menopausal or permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy. A post-menopausal state is defined as no menses for 12 months without an alternative medical cause and confirmed by a FSH result of ≥40 IU/L.

6.5.1 SPERM DONATION

Male subjects should not donate sperm for the duration of the study and for 97 days after last study drug administration.

6.5.2 EGG DONATION

Female subjects should not participate in egg donation for the duration of the study and for at least 97 days after last study drug administration.

6.5.3 PREGNANCY

Subjects will be instructed that if they/their partner becomes pregnant during the study this should be reported to the investigator. The investigator should also be notified of pregnancy occurring during the study but confirmed after completion of the study. In the event that a subject/subject's partner is subsequently found to be pregnant after the subject is included in the study, then consent will be sought from the subject/partner and, if granted, any pregnancy will be followed and the status of mother and/or child(ren) will be reported to the sponsor after delivery. Any subject reporting a pregnancy during the study will be discontinued from the study treatment and every reasonable effort will be made by Quotient to follow up the pregnancy until delivery.

A pregnancy notification form and follow-up will be completed.

6.6 LIFESTYLE CONSIDERATIONS

During this study, participants are asked to abstain from:

- food containing poppy seeds during the 48 hours prior to screening and the 48 hours prior to admission until discharge from the clinical site
- food or beverages containing xanthine derivatives or xanthine-related compounds or energy drinks from 48 hours prior to the first dosing until discharge from the clinical site
- food or beverages containing grapefruit, starfruit, pomegranate, pineapple, pomelo, and blueberries from 24 hours prior to the first dosing until discharge from the clinical site
- taking part in any unaccustomed strenuous exercise from 72 hours before the screening visit and then from 72 hours prior to admission until discharge from the study
- donating blood or plasma (outside of this study) from clinical unit admission throughout the study duration and for at least 30 days following last dose of study medication

- Consumption of alcoholic products: this will be prohibited from 24 hours prior to screening and 24 hours prior to admission until discharge from the clinical unit

The additional restrictions above are not exclusion criteria; if non-compliance occurs, the sponsor will be contacted, and each deviation will be assessed on a case-by-case basis.

6.7 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study.

The reason(s) for the screen failure will be documented and may include demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

6.8 STRATEGIES FOR RECRUITMENT AND RETENTION

The investigator or delegated, qualified study staff member will explain and review the Institutional Review Board (IRB) approved informed consent form (ICF) with each subject. Subjects will then be given the opportunity to ask questions and will be informed of their right to withdraw from the study without prejudice. After this explanation and before any study specific procedures are performed, the subject will voluntarily sign and date the IRB approved ICF.

7 STUDY INTERVENTION

7.1 RVP-001

The clinical formulation of RVP-001 will be prepared for IV injection. At the clinical site a predetermined dose of the drug product will be drawn from the vial into a syringe affixed with a needle, for use as an IV bolus, based on the directions in the protocol.

This product is for single-use only and will not contain a preservative.

Study drug preparations, methods of administration, and final volumes will be detailed in the study-specific pharmacy manual.

7.1.1 PLACEBO

A volume of normal saline equal to the dose volume of RVP-001 will be administered IV and followed with the appropriate volume of saline flush. Given the lack of excipients in the formulation, normal saline is an appropriate placebo. RVP-001 is clear, colorless to pale yellow solution, and has a higher viscosity than normal saline. Therefore, all drug and placebo syringes and IV tubes used for administration will be amber tinted, and a power injector will be used to administer the IV bolus and saline chase to protect blinding.

7.1.2 ADMINISTRATION OF STUDY DRUG, MEALS, AND FLUIDS

All injectables will be prepared in the laboratory and administered using amber tinted syringes and IV tubing. Power injectors will be used to standardize injection times of the bolus and the saline flush that follows. Specific details of study drug and doses to be administered are provided in Section 7.2.3 and Section 5.2, respectively. Subjects will be dosed on the morning of Day 1.

The exact time of dosing will be decided based on logistics and will be documented in Electronic Case Report Forms (eCRF). Details of the procedures adopted for maintaining the study blind during dosing are provided in Section 7.3. If appearance matching of the placebo is not possible then masking and/or unblinded dosing teams will be used to preserve the study blind. Further details of these steps will be contained in the study specific blinding plan, which will be approved prior to dosing (see also Section 7.3). For each subject, all scheduled post dose activities and assessments will be performed relative to the time of study drug administration (see Section 9.5 for further details). Reference time for all post-dose activities will be time of beginning of bolus dose injection.

A staggered dosing schedule will be used. In each cohort 2 sentinel subjects (1 active and 1 placebo) will be dosed first and the remaining 6 subjects (5 active and 1 placebo) will be dosed 24 hours later. All cohorts will be dosed in a similar manner. See Section 7.3 for further details.

To assess tolerability of the IV administration, the first subject will be dosed at least 30 min prior to dosing the second subject. All subsequent dosing of the study drug will be staggered by at least 15 min. To allow

for potential interruptions and injector variations, up to a 20% difference in duration of the infusion will not be considered as a protocol deviation provided that full planned dose is administered. Any such minor discrepancies should not have a significant impact on the overall study objectives.

The planned injection time will be approximately 5-15 sec (depending upon patient weight/dose); the actual infusion start and stop time will be recorded in the source documents. In all cases, the study drug injection will be followed by 20 second injection of normal saline.

A 20 gauge IV will be started in the right antecubital fossa, where possible, and must demonstrate easy flush. An IV bolus injection of RVP-001 will be administered via a power injector at a flow rate of up to 2 mL/sec followed by bolus administration of up to 40 cc of normal saline also administered at up to 2 mL/sec. Precise volumes and rates of injection of both RVP-001 and saline flush will be recorded.

Further details of procedures for injection can be found in the study drug injection guidelines.

IV blood samples will be obtained from the opposite arm to the bolus, where possible.

7.1.3 MEALS AND FLUID

Subjects will be allowed water up to 1 hour before the scheduled dosing time. Subjects will be required to drink 250 mL of water at 1 hour post-dose to ensure adequate hydration and an additional 250 mL of water 1-2 hours post dose. Thereafter, water will be allowed ad libitum. Subjects will be strongly encouraged to drink allowed fluids throughout study. Decaffeinated fluids will be allowed ad libitum from lunch time on the day of dosing.

The caloric/fat content of meals are not required to be controlled. Subjects will be provided with a standardized menu that does not contain foods high in Mn content.

Subjects will be provided with a light snack and then fast from all food and drink (except water) for a minimum of 8 hours prior to dosing until approximately 4 hours post-dose at which time lunch will be provided. An evening meal will be provided at approximately 10 hours post-dose and an evening snack at approximately 14 hours post-dose. On subsequent days, meals will be provided at appropriate times.

7.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

7.2.1 PACKAGING, LABELING AND DISPENSING

The Sponsor will be responsible for ensuring that the study drug is manufactured in accordance with applicable current Good Manufacturing Practice (GMP) regulations and requirements.

The study drug will be labeled according to the requirements of local law and legislation. The study drug will be prepared and dispensed according to the pharmacy manual by the clinical site's pharmacy.

Based on the subject selection list, the appropriate dose of RVP-001 or matching placebo will be prepared and dispensed by an unblinded pharmacist at the clinical site.

7.2.2 STORAGE AND HANDLING

At the study site, study drug must be kept in a secure, locked area or locked cabinet with access restricted to designated study site personnel. Vials should be stored at room temperature of 25°C (77°F), excursions permitted between 15°C to 30°C (59°F to 86°F) in a dry area, protected from light. The lot numbers and expiration dates (where available) of the study drugs supplied will be recorded in the final Clinical Study Report (CSR). Records will be made of the receipt and dispensing of the study drugs supplied. At the conclusion of the study any unused study drugs will be retained by CRO, returned to the Sponsor or designee, or destroyed, as per Sponsor instructions. If no supplies remain, this fact will be documented in the pharmacy product accountability records. All study drug will be transported, received, stored, and handled strictly in accordance with the container or product label, the instructions supplied to the clinical site and its designated pharmacy, the site's standard operating procedures (SOPs), and applicable regulations. Appropriate storage temperature and transportation conditions will be maintained for the study drug from the point of manufacture until delivery to the site. The pharmacy staff will examine the shipment to verify that the study drugs were received in acceptable condition. Once inspected, the study drugs will be stored in a secure area with access restricted only to authorized staff, under physical conditions consistent with the study drug requirements. The site pharmacist or delegate is responsible for ensuring that all study drugs received at the site are inventoried and accounted for throughout the study, according to the applicable regulations and the site's SOPs. Details of the receipt, storage, dispensing, and destruction will be recorded and maintained in the pharmacy logbook by the pharmacy staff. The Sponsor will provide written instructions regarding the final disposition of the unused study treatments. Copies of the study treatment accountability records will be provided to the Sponsor at the completion of the study and will be made available for review by the Site Monitor, if applicable, during the course of the study.

7.2.3 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The Sponsor will be responsible for ensuring that the study drug is manufactured in accordance with applicable currentGMP regulations and requirements.

The study drug will be prepared and dispensed in amber syringes according to the pharmacy manual by the clinical site's pharmacy, unless the Sponsor supplies the pharmacy with prelabeled individual dosing samples.

[redacted]

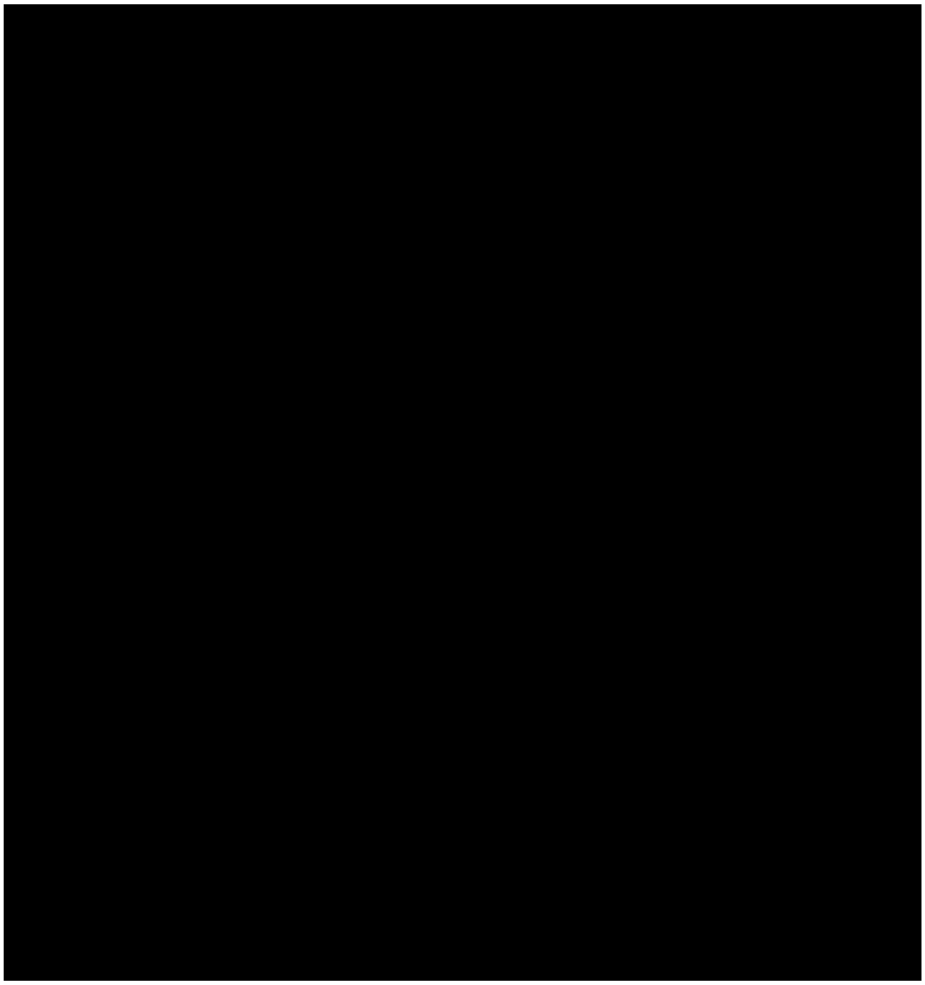
RVP-001 drug product is provided in clear glass vials (type 1 USP) with bromobutyl rubber stoppers and aluminum flip-and-tear type seal.

[redacted]

7.2.4 DOSE PREPARATION

Dosing will be weight-based, with a dose chart provided in study protocols that prescribes the precise amount of RVP-001 in ML to be dispensed at each dose level. The pharmacist will draw the appropriate volume of RVP-001 into a syringe, which may require use of multiple bottles of RVP-001.

[redacted]



Prepared syringes may be stored at room temperature prior to dosing for up to 60 minutes. A similar volume of normal saline will be dispensed for subjects receiving placebo. All syringes and IV tubing will be amber tinted to protect blinding. Further information will be provided in the pharmacy manual.

Further information will be provided in the Pharmacy Manual.

7.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Subjects eligible for participation will be randomized to receive either RVP-001 or the placebo immediately prior to dosing.

A subject selection list will be produced prior to dosing using the randomization schedule and will be retained in the Investigator Site File (ISF).

For all cohorts, a sentinel group of 2 subjects will be dosed ahead of the remaining subjects in each cohort. For each cohort in which sentinel dosing is or may be used, using a computer-generated randomisation schedule, the first 2 subject numbers will be allocated to active or placebo in a 1:1 ratio (i.e., 1 subject will be randomly assigned to receive RVP-001 and 1 subject will be randomly assigned to receive placebo). The remaining 6 subject numbers will be allocated to active or placebo in a 5:1 ratio (i.e., 5 subjects will be randomly assigned to receive RVP-001 and 1 subject will be randomly assigned to receive placebo).

This is a double-blind study. Subjects and the clinical personnel involved in the collection, monitoring, revision, or evaluation of AEs, personnel of the bioanalytical department, and personnel who could have an impact on the outcome of the study will be blinded with respect to the subject's treatment assignment (RVP-001 or placebo). Blinding will be maintained at least until the clinical phase of this part of the study is completed (i.e., when investigation and reporting of all AEs has been completed for all cohorts).

The randomization schedule and disclosure envelopes will be generated by an unblinded statistician at Quotient according to Quotient's SOPs. The unblinded statistician will not be involved in any decisions relating to populations for analysis prior to unblinding. Prior to database lock and unblinding, all original randomization materials, including the original final signed and dated randomization schedule, will be held by the Quality Assurance (QA) department at Quotient. The Data Sciences department will not have access to the randomization schedule before database lock and unblinding.

Interim PK parameter estimations will be performed using bioanalytical data applied with subject aliases in order to maintain the study blind.

There may be instances where interim PK data have the potential to be treatment revealing (e.g., missed blood sampling occasions). In these cases, every effort will be made by the pharmacokineticist to maintain the study blind by appropriate presentation of data to the study team. Data demonstrating extremes of exposure will always be presented, regardless of the potential to reveal the study blind. To permit selection of the dose level, individual data may be made available in full if judged necessary by the investigator or sponsor regardless of the potential to reveal study blind. Where possible this should be to a sub-group of the review committee who are responsible for dose level selection.

Designated pharmacy personnel at the clinical sites not directly involved with the clinical aspects of the trial will prepare and dispense the study medication and will be aware of the randomization code. A copy of the randomization schedule or corresponding disclosure envelopes will also be made available to the pharmacovigilance provider for analysis of pharmacovigilance.

The study drug and placebo will have the same visual appearance in order to avoid compromising the study blinding. If appearance matching of the placebo is not possible then use of masking and/or unblinded dosing teams will be used to preserve the study blind. Further details of these steps will be contained in the study specific pharmacy manual which will be approved prior to dosing.

In the event of an emergency, or replacement of subject, an envelope for each subject containing his/her treatment assignment will be available from clinical personnel involved with the preparation of the study

medication. Two sets of disclosure envelopes (i.e., sealed envelopes containing individual subject randomization details) will be provided. One set will be held in the clinical area and the other retained in the ISF.

These may be used in the event of an emergency by the investigator. Any request for information on the randomization schedule after initial issue must be made using a randomization disclosure form, except in the case of emergency unblinding, which must be recorded on the emergency unblinding form. Access to study drug assignment will be immediately available if the investigator deems it necessary to break the study blind in the interest of a subject's medical safety, in case of a medical emergency, or if warranted during scheduled safety reviews. The medical monitor must be contacted within 24 h following disclosure of study drug assignment.

Details of any disclosure of the randomization schedule will be documented and retained in the ISF. The sponsor will be notified if the study blind is broken.

The study blind will be broken after the study database has been locked and the safety population has been defined. Any subsequent request for issue of the randomization schedule prior to unblinding must be made using a randomization disclosure form.

The randomization code will not be available to the bioanalytical lab prior to the analysis of the plasma samples from each cohort.

7.3.1 SUBJECT NUMBERS

Subjects will be assigned a three-digit screening number after informed consent is obtained (e.g., 001, 002, 003, etc.). Four-digit subject numbers will be allocated on the morning of dosing according to the code 1001 to 1040 using the lowest number available. Replacement subjects will be allocated subject numbers 9001 to 9040, where the last three digits are the same as those of the original subject (e.g., if Subject 1005 withdraws, the replacement will have Subject Number 9005 and will receive the same regimen as Subject 1005 with the exception of any study drugs dosed in a previous regimen that have been deemed sub-optimal).

Subject numbering by cohort is provided in Table 3.

Table 3 Subject Numbers by Cohort

Cohort	Subject Numbers	Replacement Numbers
1a.1	1001-1008	9001-9008
1a.2	1009-1016	9009-9016
1a.3	1017-1024	9017-9024
1a.4	1025-1032	9025-9032
1a.5 (if needed)	1033-1040	9033-9040

7.4 STUDY INTERVENTION COMPLIANCE

During all clinical phases of the study, subjects will be observed by study staff to assure compliance to all study procedures, including dose administration.

The IV dose will be administered by trained staff to ensure dosing compliance.

The date and time that each subject is dosed will be recorded in the subject's eCRF. Any violation of compliance will require evaluation by the investigator and sponsor to determine if the subject can continue in the study.

7.5 CONCOMITANT THERAPY

Prescription and over-the-counter medications will be prohibited within 14 days prior to (the first) dosing and throughout the study. Over-the-counter products including natural health products (e.g., food supplements and herbal supplements) within 14 days prior to (the first) dosing, with the exception of the occasional use of acetaminophen (up to 2 g daily) are also prohibited.

The metabolic pathway of RVP-001 is not fully understood, and no formal drug-drug interaction (DDI) studies have been conducted with RVP-001. Therefore, in an effort to prevent the occurrence of any DDIs, the use of cytochrome P450 (CYP) inhibitors is prohibited for use up to 14 days prior to use of RVP-001 as stated in exclusion criteria (see Section 6.4). Also a table listing additional CYP inhibitors is provided in Section 11.4 CYP Inhibitors (FDA Table of Substrates, Inhibitors and Inducers 2020).

Subjects also cannot have depot injection or an implant (including contraception) of any drug within 3 months prior to (the first) dosing.

COVID-19 vaccines are accepted concomitant medications.

All concomitant medication use will be documented.

Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications, and supplements. Medications will be coded using World Health Organization (WHO) Drug Dictionary Global Drug Reference.

8 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

8.1 BEFORE FIRST TREATMENT ADMINISTRATION

Before the first treatment administration, inclusion/exclusion criteria will govern the subjects to be dosed. Subjects withdrawn before first treatment administration will not be followed up and will not undergo End-of-Study/Early Termination assessments.

Subjects are free to withdraw their consent to participate in the study at any time, without prejudice. The reason for their withdrawal or for deciding to end their participation will be documented.

8.2 AFTER FIRST TREATMENT ADMINISTRATION

Subjects may, at any time, voluntarily withdraw from the study or be removed from the study at the discretion of an investigator or the sponsor. An investigator may withdraw a subject at any time if it is determined that continuing the study would result in a safety risk to the subject or if their behavior is deleterious to the study environment.

If such withdrawal occurs, or if the subject fails to return for visits, an investigator should determine the primary reason for a subject's premature withdrawal from the study and record the reason in the subject's study documents. Attempts should be made to have the subject complete the End-of-Study/Early Termination assessments.

An investigator may remove a subject from the study, following consultation with the sponsor due to an unanticipated event that could result in an inadequately characterized PK profile, such as a missed blood draw or urine collection, an AE, meal deviation or concomitant medication intake.

Attempts should be made to have such subjects complete the End-of-Study/Early Termination assessments. End-of-Study/Early Termination assessments should be performed as soon as possible after the last study treatment administration.

Details of reasons for removal of subjects will be recorded, reported to the Sponsor and documented in the CSR.

Early withdrawal is defined as the date of the decision to withdraw the subject from the study. Subject completion is defined as the date of the last procedure conducted or last contact (e.g., phone call or unscheduled visit) for that subject.

8.3 STOPPING RULES

8.3.1 INDIVIDUAL SUBJECT STOPPING RULES

Participation in the clinical study may be discontinued by the physician in charge of the study or by the sponsor for any of the following reasons, but not limited to:

1) AEs (including if a subject develops any significant illness or needs to undergo any major surgery during course of the study including seizure, stroke, cerebral venous thrombosis)
2) Subject non-compliance (including any violation of protocol requirements which may affect the study outcome)
3) Subjects will be discontinued from the clinical study for any of the following reasons, but not limited to:
    a) Meets either of the following criteria based on ECG readings:
        1) QTcF > 480 msec
        2) QTcF change from baseline >60 msec
4) A 7-day or 15-day IND Safety report will be submitted for any treatment emergent case of seizure, stroke, cerebral venous, thrombosis, QTc greater than 480 msec or QTC increase of more than 60 msec over baseline

For the purpose of withdrawal criteria, baseline will be considered as the last available assessment prior to first dose.

8.3.2 COHORT STOPPING RULES

If any of the following safety concerns are observed, dosing of all subjects at the given dose level or higher will be suspended/halted and all available data will be evaluated by the Safety Review Committee (SRC).

1) If one (1) RVP-001-related SAE occurs in a cohort
2) Moderate or severe AEs in 50% of the subjects in the cohort or more
3) Clinically significant laboratory abnormalities of the same character in 3 or more subjects (e.g., ALT >3 ULN)
4) Clinically significant changes in vital signs of the same character in 3 or more subjects: BP consistently greater than 160 mmHg associated with an increase from baseline greater than 20 mmHg
5) Clinically significant changes in ECGs of the same character in 3 or more subjects: QTcF greater than 480 msec with an increase from baseline greater than 60 msec

Dose continuation (remaining subjects within a cohort or remaining dosing for a subject) or escalation decision will be made following evaluation of the event by the SRC.

Prior to starting a new dose/cohort, dose escalation will be discussed during the SRC meeting and may be modified or stopped depending on the investigator's or sponsor's decision, based on but not limited to the following:

1) New clinically significant abnormalities in physical examination, 12-lead ECG or vital signs in 2 or more subjects
2) Overall pattern of clinical changes or symptoms that may have appeared minor in terms of an individual AE or subject, but which collectively present a safety concern
3) Clinically significant changes in organ-specific laboratory parameters (e.g., liver function enzymes, renal function studies in one or more subjects)
4) Pattern of laboratory changes (e.g., a consistent increase or decrease within 2 or more subjects or within or across dosing groups) which might indicate an overall safety concern

In the event that a cohort is stopped due to non-drug-related events, a new cohort of additional subjects may be enrolled to replace the stopped cohort.

8.3.3 TRIAL STOPPING RULES

Dosing and/or recruitment will be interrupted until the SRC has completed a thorough review of all available data, for any of the following reasons, but not limited to:

- If 1 subject experiences an SAE

Clinical trial stopping rules:

- If 2 RVP-001 related SAEs occur in a cohort
- If 2 subjects experience severe RVP-001 related AEs
- Occurrence of an RVP-001 related AE or SAE which results in death

Relatedness to study drug will be determined by the investigator.

If the study is halted, the IRB will be notified. The study may be resumed or terminated; however, it will not be resumed until a further notice to resume the study is submitted and approved by the IRB.

8.4 LOST TO FOLLOW-UP

For subjects who are lost to follow-up (i.e., those subjects whose status is unclear because they fail to be available for study visits without stating an intention to withdraw), an investigator should show "due diligence" by documenting in the source documents steps taken to contact the subject, e.g., dates of telephone calls, registered letters, etc.

9 STUDY ASSESSMENTS AND PROCEDURES

9.1 SAFETY ASSESSMENTS

9.1.1 SAFETY MONITORING

Subjects will be monitored throughout the study by clinical site staff for AEs. A physician will be responsible for the clinical aspects of the study and will be available at all times during the study. If necessary, a physician, either at the clinical site or in a nearby hospital will administer treatment for any AE(s).

Additional non-invasive procedures that are already specified in the protocol may be performed, if it is believed that an important effect of the IP(s) is occurring or may occur at a time when no measurements are scheduled, or if extra procedures are needed in the interests of safety.

Additional blood samples, urine samples or other diagnostic tests for safety assessments may be taken if required by the investigator or sub-investigator at any point.

9.1.2 VITAL SIGNS

BP, respiratory rate, HR, and body temperature will be measured in a supine position (except for safety reasons) at the time points described in Section 3.2 (S0A). HR and pulse oximetry will be monitored continuously for 4 hours post dose.

Subject should be in supine position at least 5 minutes prior to measurements.

The acceptable deviations from the nominal vital signs measurement time points are:

- The pre-dose vital signs measurements will be taken ≤2 h before dosing.
- Post-dose vital signs measurements will be taken ± 2 min from the nominal post-dose time points for the first hour. Post-dose vital signs measurements will be taken ± 15 min from all other nominal post-dose time points.
- Discharge vital signs measurements will be taken ± 1 h from the nominal time point.

If a subject shows an abnormal assessment at any stage, repeat measurements may be made and the abnormality followed to resolution if required. Additional measurements may be taken as deemed necessary by the investigator or sub-investigator.

Any clinically significant abnormality, including changes from baseline, must be reported as an AE.

9.1.3 ECG MEASUREMENTS

Supine (single) 12-lead ECGs will be taken at 1, 3 and 8 minutes post dose after the subject has been in the supine position for a minimum of 5 min. Supine triplicate 12-lead ECGs (3 ECGs taken approximately 1 minute apart) after the subject has been in the supine position for a minimum of 5 min will be performed at the nominal timepoints in Section 3.2 (SoA). For triplicate ECGs, the mean of the three recordings will be reported.

- The pre-dose ECG measurements will be taken ≤2 h before dosing
- Post-dose single ECG measurements (minutes 1, 3 and 8) will be taken ± 2 min from the nominal post-dose time point
- All other ECGs will be taken ± 10 min for first 3 hours (excluding above) post dose and ± 30 min thereafter.
- Discharge ECG measurements will be taken ± 1 h from the nominal time point.

If a subject shows an abnormal assessment at any stage, repeat measurements may be made and the abnormality followed to resolution if required. Additional measurements may be taken as deemed necessary by the investigator or sub-investigator.

Any clinically significant abnormality, including changes from baseline, will be reported as an AE.

9.1.4 PHYSICAL EXAMINATION INCLUDING NEUROLOGICAL ASSESSMENT

A complete physical examination will be performed at admission. Height and body weight will be measured, and BMI will be calculated at screening. A complete physical examination includes assessments of the following: head, eyes, ears, nose, throat, neck, chest, lungs, abdomen, extremities, musculoskeletal, dermatological, CV/peripheral vascular, and neurological examination.

A complete neurological exam (including mental status exam, cranial nerves, coordination, and peripheral motor, sensory and reflex examinations of all extremities) will be performed as indicated in Section 3.2 (SoA).

If a subject reports or daily examination identifies any treatment emergent moderate or severe neurological signs/symptoms, a complete neurological examination will be performed.

Changes from baseline abnormalities will be recorded at each subsequent physical examination. New or worsened abnormalities should be recorded as AEs, if judged appropriate by the Principal Investigator or Medical Sub-Investigator.

9.1.5 DRUG, ALCOHOL AND COTININE SCREEN

A urine drug screen (Amphetamines [refers to a group of drugs that includes methamphetamine/MDMA {commonly known as Ecstasy}], Barbiturates, Benzodiazepines, Cocaine, Ethanol, Marijuana/Cannabis, Methadone, Morphine/Opiates, pH, Phencyclidine and Tricyclic Antidepressants) and an alcohol urine test and urine cotinine test will be performed at screening. A urine drug screen, alcohol test and cotinine test will also be performed at admission.

9.1.6 PREGNANCY TEST

A highly sensitive urine pregnancy test will be performed at screening for all female subjects. For WOCBP, a highly sensitive urine pregnancy test will also be performed before discharge. A highly sensitive serum pregnancy test will be performed at admission. The samples will be collected and processed as detailed in the clinical sample processing manual (CSPM).

9.1.7 FOLLICLE-STIMULATING HORMONE TEST

Serum FSH tests will be performed as detailed in Section 3.2 (SoA). The samples will be collected and processed according to the CSPM.

9.1.8 BODY WEIGHT, HEIGHT, AND BMI

The subject's body weight and height will be measured, and their BMI will be calculated according to the time schedule presented in Section 3.2 (SoA).

9.1.9 INJECTION SITE MONITORING

An assessment of each injection site will be made by a nurse, nurse practitioner, physician's assistant or physician according to the schedule in Section 3.2 (SoA).

The acceptable deviations from the nominal time points are:

- Post-dose (including discharge) examinations will be carried out ± 1 hour from the nominal post-dose time point

The injection site will be assessed and recorded according to the grading system in Table 4.

Table 4 Injection Site Examination

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Injection Site Pain or Tenderness *Report only one*	Pain or tenderness causing no or minimal limitation of use of limb	Pain or tenderness causing greater than minimal limitation of use of limb	Pain or tenderness causing inability to perform usual social & functional activities	Pain or tenderness causing inability to perform basic self-care function OR Hospitalization indicated
Injection Site Erythema or Redness[12] *Report only one* *> 15 years of age*	2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities	≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities	≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
≤ 15 years of age	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection Site Induration or Swelling *Report only one* *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*
≤ 15 years of age	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*
Injection Site Pruritus	Itching localized to the injection site that is relieved spontaneously or in < 48 hours of treatment	Itching beyond the injection site that is not generalized OR Itching localized to the injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA

[12] Injection Site Erythema or Redness should be evaluated and graded using the greatest single diameter or measured surface area.

NA = not applicable

9.2 LABORATORY ASSESSMENTS

Refer to CSPM for detailed instructions for all sample handling.

The acceptable deviations from the nominal blood sampling time points for biochemistry, hematology, serology and coagulation are:

- The pre-dose blood sample will be taken up to 2 hours before dosing
- *2* to 12 hour post-dose blood samples will be taken within ± 10 minutes of the nominal post-dose sampling time
- 24 to 120 hour post-dose blood samples will be taken within ± 30 minutes of the nominal post-dose sampling time

The acceptable deviations from the nominal urine sampling time points for urinalysis are:

- The pre-dose urine sample will be taken from the 24 hour (day-1) urine collection within 1 hour of nominal timepoint).
- Post-dose urine samples will be taken ± 1 hour from the nominal urine sampling time

The acceptable deviations for PK blood and bioanalytical urine samples can be found in Section 9.2.6.

In cases where laboratory findings are outside the normal range and the investigator believes that the results may be of clinical significance, repeat sampling may be requested as clinically indicated. If the abnormal finding is clinically significant, appropriate actions will be taken e.g., the subject will not be entered into the study, or the subject may be withdrawn from the study. The subject will be referred to their primary care provider or other appropriate provider for further care if necessary. The same will apply if the results of the HBsAg, HCV Ab, or HIV test are positive and in addition the investigator will ensure that adequate counselling is available if requested.

Abnormal results at follow-up assessments will also require repeat testing if the investigator believes the results may be of clinical significance.

Any clinically significant abnormality, including changes from baseline, must be reported as an AE.

Additional blood and/or urine samples may be taken for safety tests. Furthermore, additional assays outside those specified in the protocol may be performed for safety reasons as requested by the investigator.

9.2.1 BIOCHEMISTRY

Biochemistry will be performed at the time points specified in Section 3.2 (SoA). The following will be assessed: Alanine Aminotransferase (ALT), Albumin, Alkaline Phosphatase, Aspartate Aminotransferase (AST), Bilirubin (Total), Bilirubin (Direct), BUN, Calcium, Carbon Dioxide, Chloride, Creatine Kinase (CK), serum Creatinine, eGFR will be calculated at screening using CKP-EPI 2021 Refit equation for eligibility

purposes, Follicle Stimulating Hormone (FSH; post-menopausal female subjects only when indicated), Glucose (unscheduled time points), Glucose (Fasting), Human Chorionic Gonadotropin (hCG) (female subjects when indicated), Potassium, Phosphate (Inorganic), Protein (Total), Sodium, and Lipid profile (cholesterol, HDL, LDL, triglycerides).

9.2.2 SEROLOGY

Hepatitis B (HBsAg) antigen, HCV antibody, and HIV antibody detection will be performed at screening.

9.2.3 HEMATOLOGY

Hematology will be performed at screening, on admission, approximately at 24 hours post-dose, and before discharge. The following will be assessed: Basophils, Eosinophils, Hematocrit, Hemoglobin, Lymphocytes, Mean Cell Hemoglobin, Mean Cell Hemoglobin Concentration, Mean Cell Volume, Monocytes, Neutrophils, Platelet Count, RBC Count, Red Cell Distribution Width, Mean Platelet Volume, and WBC Count.

9.2.4 COAGULATION

Pt, INR, and APTT will be performed at screening, on admission, and approximately at 24 hours post-dose.

9.2.5 URINALYSIS

Urinalysis will be performed at screening, and on admission. Small spot urine sample collections for urinalysis will be collected at the end of each 24 hour urine collection interval for elimination studies. The following will be assessed: pH, specific gravity, protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, and leukocytes. Unless otherwise specified, microscopic examination will be performed on abnormal findings.

In addition, an aliquot of urine from each 24 hour collection will be analyzed for 24 hour urine creatinine

. The 24 hour urine creatinine, 24 hour urine volume, and corresponding serum creatinine will be used to calculate 24 hour urine creatinine clearance.

9.2.6 BIOANALYTICAL SAMPLE COLLECTION AND PROCESSING

Plasma and urine will be analyzed by high performance LC-MS/MS to measure the concentration of intact RVP-001 in order to assess plasma pharmacokinetics at Inotiv. Separate urine and feces samples will be analyzed by ICP-MS for total Mn content to quantify elimination of injected Mn and fractional excretion at Quotient Bioanalytical. Separate plasma and urine samples will be sent to Massachusetts General Hospital for exploratory studies.

A total of 16 blood samples will be drawn from each subject for PK analyses. Venous blood will be collected prior to (within 2 hours prior to dose) and at 2, 5, 10, 20, and 30 minutes after the end of the bolus dose and at 1, 2, 4, 8, 12, 24, 48, 72, 96, and 120 hours post-dose (6 mL for each sampling time).

The acceptable deviations from the nominal blood sampling times are as follows:

- The pre-dose samples will be taken up to 2 hours before dosing
- 0 to 1 hour post-dose samples will be taken within ± 2 minutes of the nominal post-dose sampling time
- 2 to 12 hour post-dose samples will be taken within ± 10 minutes of the nominal post-dose sampling time
- 24 to 120 hour post-dose samples will be taken within ± 30 minutes of the nominal post-dose sampling time

The total blood volume withdrawn from each subject will not exceed 550 mL in an 8 week period (based on a representative healthy subject weighing at least 110 pounds). The blood volume taken during the study is presented in Table 5. The number and timing of samples may be amended following any interim PK parameter estimations.

Table 5 Estimated Total Blood Volume

Study Visit	Sample Type	Number of Samples	Sample Volume	Total Volume
Screening	Safety laboratory assessments (biochemistry [including FSH], hematology, coagulation and serology)	1	22.7 mL	22.7 mL
Admission (Day -1)	Safety laboratory assessments (biochemistry [including pregnancy], hematology, and coagulation)	1	15.2 mL	15.2 mL
Day 1	Safety laboratory assessments (biochemistry, hematology, and coagulation)	2	14.2 mL	28.4 mL
Day 1 to Day 6	Pharmacokinetic assessments	16	6.0 mL	96.0 mL
Day 2 to Day 6	Safety laboratory assessments (biochemistry, hematology, and coagulation)	5	14.2 mL	71.0 mL
Total Blood Volume per Subject per Cohort:				233.3 mL

FSH = follicle stimulating hormone

Actual sampling times will be used for statistical analyses. Blood samples will be collected by direct venipuncture as per clinical site operational practice.

Deviations of blood volume will be reported only when the total volume of the whole study is exceeded.

Plasma samples will be collected and processed as per the agreed upon procedures of the bioanalytical lab(s). Each sample will be split into the following components: (i) PK evaluation to be analyzed [redacted], (ii) Developmental analysis by [redacted], (iii) Banking: Stored at -80 °C at [redacted] per GLP standards.

The acceptable deviations from the nominal urine sampling times are as follows:

- The -24 hours to 0 hours urine collection will be collected within -180 minutes to +60 minutes of the nominal dose sampling time

- The subsequent 24 hour urine collections will be collected within ± 60 minutes of the nominal post-dose sampling time

Urine samples will be collected as individual samples and combined in the following time intervals: -24-0 hours, 0-24 hours, 24-48 hours, 48-72 hours, 72-96 hours, 96-120 hours. Each sample will be weighed prior to combining. After final collection for each container, total sample will be weighed, and urine volume measured. Aliquots will be removed for urinalysis and additional aliquots will be sent for following analyses: (i) Elimination evaluation RVP-001: ship to [redacted] (i) Elimination evaluation total Mn: ship to [redacted] (iii) Developmental Analysis: [redacted], (iv) Banking: Stored at -80 °C at [redacted] per GLP standards.

Feces samples will be collected in the following intervals: -24-0 hours, 0-24 hours, 24-48 hours, 48-72 hours, 72-96 hours, 96-120 hours. Individual samples will be collected, labeled, and stored. Samples will be prepared as per the agreed upon procedures of the bioanalytical laboratory and CSPM, and then shipped to [redacted].

9.3 SCREENING

Within the 28 days preceding first dose, all subjects will be required to undergo a screening visit. Screening procedures will be carried out in accordance with the schedule of assessments in Section 3.2 (SoA).

If the start of the study is delayed for any reason so that the interval between screening and first dose exceeds 28 days, all or part of the screening procedures will be repeated at the discretion of the investigator.

Screening safety procedures such as safety bloods, ECGs, vital signs, urine alcohol, urine cotinine, urine drug screen and urinalysis can be repeated as clinically indicated under the discretion of the investigator or sub-investigator if there is a concern regarding a subject's safety or eligibility to participate in the trial.

9.3.1 RE-SCREENING

This study permits the re-screening of a subject who has discontinued the study as a pre-treatment failure (i.e., subject has not been randomized); the reason for failure must be temporary and expected to resolve. If re-screened, the subject must be re-consented.

9.4 ADMISSION AND PRE-DOSE PROCEDURES

The identity of the subjects will be confirmed at admission and pre-dose.

In addition, the ongoing eligibility of subjects will be re-assessed at admission/pre-dose, as described in Sections 6.3 and 6.4.

Admission/pre-dose safety procedures such as safety bloods, ECGs, vital signs, urinalysis, and drugs of abuse tests can be repeated as clinically indicated under the discretion of investigator or sub-investigator if there is a concern regarding a subject's safety or eligibility to participate in the clinical trial.

Alternative subjects for the first dose occasion, in any group, will not require admission procedures to be repeated if dosing is within 2 days.

If dosing is delayed, subjects who have completed admission procedures do not need admission procedures to be repeated if dosing is within 2 days and the subjects have remained resident in the clinical unit.

The subjects will be admitted to the clinical unit in the morning on the day before dosing (Day -1).

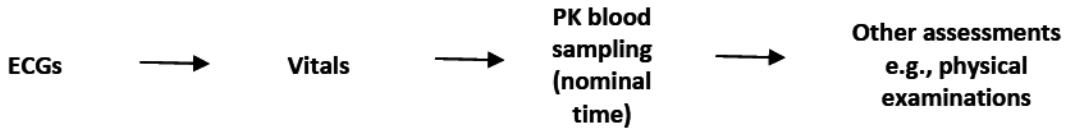
The admission and pre-dose procedures are presented in Section 3.2 (SoA).

9.5 TIMING OF PROCEDURES

There are times where the protocol requires more than one procedure to be completed at the same time point. In these instances, the following will apply to post-dose time points:

- Sequencing: ECGs should be taken prior to vital signs when both measurements are scheduled at the same time point.
- Priority for precise timing: PK samples should take priority (i.e., taken at the nominal time point) over other procedures scheduled at the same time point
- Other assessments, e.g., physical examinations, will be performed within the required time windows.

As guidance, the preferred order of assessments is:



All safety assessments will be timed and performed relative to the start of dosing.

9.6 DISCHARGE FROM THE CLINICAL UNIT

Subjects will be discharged from the clinical unit following completion of study-specific procedures at 120 h post-dose (Day 6). However, the investigator may ask a subject to remain domiciled for additional safety observation and monitoring (e.g., as indicated for an AE).

9.7 FOLLOW-UP VISIT

Subjects will receive a follow-up call 7 days following dose administration to assess for any new AEs since discharge. A questionnaire will be followed for the follow-up interview.

9.8 DATA COLLECTION AND EVALUATION

All clinical raw data will be recorded promptly, accurately, and legibly; either directly into the e-source data or indelibly on paper (e.g., ECG readings). A detailed list of the type (electronic or paper) and location for all source data will be included in the Trial Master File. All raw data will be conserved in order to maintain data integrity. A physician and/or the clinical staff will assume the responsibility of ensuring the completeness and accuracy of the clinical data.

9.9 SUBJECT WITHDRAWAL AND REPLACEMENT

Subjects will be advised that they are free to withdraw from the study at any time. Over the course of the study, the Sponsor and the Investigator(s) or a delegate may withdraw any subject from the study for non-compliance with protocol requirements or significant protocol deviation. Subjects may also be withdrawn by the Investigator(s) or a delegate for:

- Pregnancy *[if applicable]*
- Termination of the study by the sponsor, regulatory agency, or IRB
- Upon the subject's request (withdrawal of consent)
- Significant deviation from the protocol
- Significant concurrent illness or requirement for prohibited medication
- Evidence of current SARS-CoV-2 infection
- Subject non-compliance
- At the discretion of the investigator

Clinical laboratory results will be reviewed by a Medical Sub-Investigator prior to dosing, when applicable; subjects will be withdrawn from the study if it is deemed that the subject's safety may be at risk on the basis of these test results.

Subjects who withdraw or are withdrawn from the study after dosing will not be systematically replaced. However, at least 7 (out of 8) subjects from a same dose level should have completed the dosing period, and safety and tolerability data collected up to the follow up visit should be available for these subjects before the SRC can decide on dose escalation. Therefore, subjects who withdraw or are withdrawn from the study after dosing for reasons other than safety and tolerability may be replaced after consultation between the Safety Committee members. In such case, the total number of subjects dosed will remain within a maximum of 10 subjects per cohort.

Subjects withdrawn for safety reasons will be asked to remain at the clinic until the Investigator(s) or a delegate agrees that the subject is fine and can be discharged. As soon as subject withdrawal is confirmed, blood sampling will be stopped.

The minimum number of evaluable subjects is 7 per cohort. An evaluable subject is defined as a subject who has pharmacokinetic, safety, and tolerability data out to 120 hours post dose, for evaluation of the primary objective.

9.10 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

9.10.1 DEFINITION OF AN ADVERSE EVENT (AE)

An AE is any untoward medical occurrence in a subject who has been administered a pharmaceutical product or not, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding judged clinically significant), symptom, or disease temporally associated with the use of a medicinal (investigational) product (study drug) or clinical procedure, whether or not it is related to the medicinal (investigational) product (study drug) or clinical procedure. An adverse drug reaction (ADR) is any AE where a causal relationship with the study drug is at least a reasonable possibility (possibly related or probably related).

9.10.2 DEFINITION OF A SERIOUS ADVERSE EVENT (SAE)

A SAE is any AE that at any dose:

- Results in death
- Is life-threatening (i.e., in the opinion of the principal investigator (PI) the subject is at immediate risk of death from the AE)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (a substantial disruption of the subject's ability to conduct normal life functions)
- Results in a congenital anomaly/birth defect
- Constitutes an important medical event:
    - These may not result in death, be life-threatening, or require hospitalization, but may be considered serious when they jeopardize the health of the subject and require medical or surgical intervention to prevent one of the outcomes listed.
    - Any other event (e.g., allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse) thought to be serious by the Principal Investigator

Events not considered to be serious are hospitalizations that:

- Were planned before entry into the clinical trial
- Are for elective treatment of a condition unrelated to the studied indication or its treatment
- Occur on an emergency outpatient basis and do not result in admission (unless fulfilling other criteria above)
- Are parts of the normal treatment or monitoring of the studied indication and are not associated with any deterioration in condition

9.10.3 DEFINITION OF SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTIONS

Suspected Unexpected Serious Adverse ReactionS (SUSARs) are AEs that are believed to be related to a test product and are both unexpected (i.e., the nature or severity is not expected from the information provided in the IB) and serious. SUSARs are subject to expedited reporting to the IRB (see Section 9.10.8 for details on reporting SUSARs).

9.10.4 RECORDING OF ADVERSE EVENTS

AEs will be recorded and evaluated for their seriousness, severity, and relationship to the study treatment. AEs will be collected and documented from the time of providing written informed consent (screening) until 7 days after study drug administration. Subjects will be asked to self-report between day 8 (follow up call) and day 30 following drug administration. If emerging or ongoing AEs are reported at the follow-up phone call or after follow-up, additional visits and phone calls will be arranged as unscheduled visits. During each study visit the subject will be questioned directly regarding the occurrence of any adverse medical event according to the schedule in eCRF. All AEs, whether ascribed to study procedures or not, will be documented immediately in the subject's eCRF. AEs will be followed-up until complete resolution, or until the Investigator judges it safe to discontinue follow-up. The relationship to the study medication will be classified according to the categories described in Section 9.10.6. Classification of AEs will be performed by System Organ Class (SOC) and Preferred Term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA), version 24.0 or higher.

Concomitant medications will be coded using the World Health Organization drug dictionary (WHO-DDE March 2021 or later).

9.10.5 ADVERSE EVENTS SEVERITY

The severity of AEs will be graded according to criteria from the NCI Common Terminology Criteria Adverse Events (CTCAE) version 5.0. (Table 6).

The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Table 6 NCI Common Terminology Criteria Adverse Events (CTCAE) version 5.0. Severity Grade

GRADE	DESCRIPTION
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (instrumental activities of daily living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (self-care activities of daily living refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE. *Grade 5 (death) is not appropriate for some AEs and therefore is not an option.

AE = adverse event

9.10.6 ADVERSE EVENT CATEGORIES FOR DETERMINING RELATIONSHIP TO STUDY DRUG

PROBABLE (first three conditions must apply)

This category applies to those AEs that are considered, with a high degree of certainty, to be related to the study drug. An AE may be considered probable, if:

- It follows a reasonable temporal sequence from administration of the drug.
- It cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental, or toxic factors or other modes of therapy administered to the subject.
- It disappears or decreases on cessation or reduction of the dose. (There are important exceptions when an AE does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists, e.g., (1) bone marrow depression, (2) tardive dyskinesias.)
- It follows a known pattern of response to the suspected drug.
- It reappears upon re-challenge.

POSSIBLE (first two conditions must apply)

This category applies to those AEs in which the connection with the study drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if, or when:

- It follows a reasonable temporal sequence from administration of the drug.
- It may have been produced by the subject's clinical state, environmental or toxic factors or other modes of therapy administered to the subject.
- It follows a known pattern of response to the suspected drug.

REMOTE (first two conditions must apply)

In general, this category is applicable to an AE that meets the following criteria:

- It does not follow a reasonable temporal sequence from administration of the drug.
- It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the suspected drug.
- It does not reappear or worsen when the study drug is re-administered.

UNRELATED

This category is applicable to those AEs which are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment, etc.) and do not meet the criteria for study drug relationship listed under remote, possible, or probable.

Refer to Table 7 for determining relationship of AEs.

Table 7 Determining the Relationship of Adverse Events to the Study Drug

	Probable	Possible	Remote	Unrelated
Clearly due to extraneous causes	–	–	–	+
Reasonable temporal association with drug administration	+	+	–	–
May be produced by subject clinical state, etc.	–	+	+	+
Known response pattern to suspected drug	+	+	–	–
Disappears or decreases on cessation or reduction of the dose	+	–	–	–
Reappears on re-challenge	+	–	–	–

9.10.7 REPORTING SERIOUS ADVERSE EVENTS

Any SAE will be reported to the Sponsor via telephone, fax, e-mail, or in person, within 24 hours of knowledge by the Investigator. This will be managed according to the Safety Management Monitoring Plan. The notification must be directed to:

[redacted]

SAEs must be reported to the IRB within 24 hours of site awareness.

9.10.8 SUSPECTED, UNEXPECTED, SERIOUS ADVERSE DRUG REACTIONS (SUSAR)

It is the responsibility of the sponsor to determine whether a reported SAE fits the classification of a SUSAR and to notify the investigator of their decision as soon as possible.

The investigator and sponsor are responsible for notifying regulatory agencies of fatal or life-threatening serious, unexpected ADRs (by telephone, facsimile transmission or in writing) as soon as possible, but no later than 7 calendar days after becoming aware of the information.

Additionally, within 8 days after having informed the agency(ies), a complete report must be submitted, including an assessment of the importance and implication of any findings.

It is the responsibility of the investigator and sponsor to report SAEs to the IRB responsible for the study following the local procedure. The task of reporting other SUSARs may be delegated to the pharmacovigilance provider.

9.10.9 OTHER SUSPECTED, UNEXPECTED, SERIOUS ADVERSE DRUG REACTIONS

The Sponsor is responsible for notifying regulatory agencies of all other suspected, unexpected, serious ADRs that are neither fatal nor life-threatening as soon as possible, but no later than 15 calendar days after becoming aware of the information.

It is the responsibility of the investigator and sponsor to report to the IRB responsible for the study all other suspected, unexpected, serious ADRs that are neither fatal nor life-threatening, as soon as possible, but no later than 15 calendar days after first knowledge by the Investigator. The task of reporting other SUSARs may be delegated to the pharmacovigilance provider.

9.10.10 REPORTING OF URGENT SAFETY ISSUES

The sponsor is required to inform the appropriate regulatory authorities and investigators and the investigator is responsible for informing the IRB, within 3 calendar days of the urgent safety issue.

9.10.11 REPORTING OTHER SERIOUS IMPORTANT MEDICAL EVENTS

Report when the event does not fit the other outcomes, but the event may jeopardize the subject and may require medical or surgical intervention (treatment) to prevent one of the other outcomes. Examples include allergic bronchospasm (a serious problem with breathing) requiring treatment in an emergency room, serious blood dyscrasias (blood disorders), or seizures/convulsions that do not result in hospitalization. The development of drug dependence or drug abuse would also be examples of medical events.

9.10.12 POTENTIAL CLASS SPECIFIC EVENTS

Based on FDA review of available data, there are potential safety concerns for the entire class of Mn containing MRI contrast agents. These include the possibilities of Mn retention in the brain, hypersensitivity reactions, lowering of seizure threshold, and transient changes in CV function.

Therefore, sponsor will submit a 7- or 15-day IND safety report for any emergent treatment of seizure, stroke, cerebral venous thrombosis, QTc above 480 msec, or QTc increase of 60 msec over baseline, regardless of assessment of causality.

9.10.13 REPORTABLE DISEASE

In the case a subject has or manifested any clinical signs characteristic of a reportable disease (e.g., tuberculosis, SARS-CoV-2) it is the responsibility of the Medical Director to notify the Public Health authorities within 48 hours after becoming aware of the information or per local guidelines.

Additional details can be found in the safety management plan.

9.11 COVID RESPONSE PLAN

9.11.1 COVID-19 RELATED RISKS AND RISK MITIGATION MEASURES

The following risks and risk mitigating measures apply to the time in which the study is conducted during the COVID-19 pandemic.

9.11.1.1 STUDY DRUG RELATED RISK

Against the background of the COVID-19 pandemic, the potential risk of a subject developing COVID-19 has been considered in terms of the risk-benefit evaluation. RVP-001 is not biologically active. Based on available non-clinical and clinical data (including class effects), it is considered that a subject would not be at increased risk of either becoming infected with SARS-CoV-2 or experiencing a more severe illness. That is, the study drug has no known immunomodulatory effect that would confer an increased risk to healthy subjects enrolled in the study.

9.11.1.2 GENERAL COVID-19 RELATED RISKS AND RISK MITIGATION MEASURES

General risk mitigation against COVID-19 will be implemented in accordance with Quotient's monitoring and prevention control measures.

The risk mitigation measures, where applicable, will be amended based on emerging government guidance.

9.11.1.3 COVID-19 VACCINE-RELATED RISK

Approved (including health authority conditional marketing authorization) COVID-19 vaccines e.g., killed, inactivated, peptide, deoxyribonucleic acid (DNA), and riboneucleic acid (RNA) vaccines may be permitted according to the investigator's discretion and as per local guidance.

Due to lack of biological activity of RVP-001, there is no perceived impact on the safety of the study subjects or on the study objectives for subjects who may receive these vaccines (either first, second, or booster doses). It is also very unlikely that administration of the study drug would interfere with COVID-19 vaccination response; however, no specific pre-clinical or clinical investigations have been conducted at this point with RVP-001.

The emerging safety and efficacy data from millions of vaccinated people indicate that these vaccines have an excellent safety and efficacy record. In the broader interests of society and to limit the extent of the global pandemic, it is important that subjects should receive a vaccine when it is offered to them.

9.12 SAFETY REVIEW COMMITTEE (SRC)

9.12.1 DECISION POINTS

The following in-study decisions will be made during this study:

- Dose selection for Cohorts 2 to 4.
- Progression from sentinel group to main group
- Changes to safety and/or PK time points, if there is reason to believe that the change might improve the quality of the data as a consequence of review of emerging data.

9.12.2 CRITERIA FOR DOSE DECISIONS

Cohorts will be dosed sequentially in an ascending fashion.

Following completion of each SAD cohort, a SRC will convene to review all available blinded safety, tolerability and available PK/exposure assessments up to Day 6 (120 hours) performed for each SAD cohort, and together with attempts to correlate exposure levels with observed AEs to evaluate progression to the next dose cohort.

The SRC will be comprised of the Principal Investigator at the clinical site and the Sponsor's medical personnel, at a minimum.

Criteria for Sentinel Dosing Decision(s)

For all cohorts following a sentinel design the decision to proceed with the main group will be made by the investigator, based on safety data until 24 hours post-dose of the last sentinel subject. The investigator will inform the sponsor of any safety concerns.
For additional details refer to Data Safety Monitoring Plan (DSMP).

10 STATISTICAL CONSIDERATIONS

10.1 POPULATIONS FOR ANALYSES

Populations and analysis sets will be determined for safety and PK after database lock using the criteria defined in the statistical analysis plan (SAP); the SAP will be signed off prior to database lock. The safety population and safety analysis set will be defined after database lock but prior to study unblinding; all other populations will be defined after database lock when the relevant data are available.

10.1.1 SAFETY POPULATION

The safety population will include all subjects who received at least 1 dose of RVP-001 or placebo.

The number of subjects who were included, who discontinued, and who completed the study will be tabulated. The primary reasons for discontinuation will be provided.

10.1.2 PHARMACOKINETIC POPULATION

The PK population will consist of all treated subjects who have sufficient data to calculate at least one of the PK parameters. However, the PK analysis dataset will exclude data associated with protocol violations and/or major protocol deviations (subject should not have violated any major entry criterion likely to impact the PK analysis and should not have deviated significantly from the protocol between enrollment and successful study completion).

The PK population will be further described in a SAP.

Statistics for demographic and baseline data will be detailed in the SAP.

10.2 STATISTICAL ANALYSES

10.2.1 SAFETY ANALYSES

The clinical laboratory tests, measurements of vital signs, pulse oximetry, ECGs, physical examination, and other safety parameters will be used to perform the safety statistical analysis.

10.2.2 SAFETY ENDPOINTS

The safety endpoints are the frequency, type and severity of AEs, clinical laboratory results, vital signs, ECG, pulse oximetry, physical examination, and neurological assessment findings, and body weight.

10.2.3 SAFETY STATISTICAL METHODOLOGY

All safety data (AEs, clinical laboratory results, vital signs, pulse oximetry and ECGs) will be listed. Safety data will be summarized with descriptive statistics and frequency tables. If requested by the Sponsor and/or SRC additional analysis may be performed.

Statistics for summary of AEs and safety results will be detailed in a SAP.

10.2.4 BASELINE DESCRIPTIVE STATISTICS

Listings and descriptive summary statistics of demographic (age, height, weight, and BMI) and baseline data will be presented.

10.2.5 PLANNED INTERIM ANALYSES

Interim PK analysis will be performed after each cohort (dose selection and safety assessment).

The SAP will describe the planned interim analyses in greater detail.

10.2.6 PHARMACOKINETICS ANALYSIS CONCENTRATION DATA:

Plasma concentration data will be summarized by treatment and occasion (as applicable) using the following descriptive statistics: Sample size (N), minimum (min), arithmetic mean, median, maximum (max), standard deviation (SD), and coefficient of variation (CV%). Descriptive statistics will be calculated at each individual time point. Concentrations that are below the lower limit of quantitation (LLOQ) will be treated as zero for descriptive statistics.

The individual plasma concentration/time profiles will be presented using the actual sampling times whereas the mean plasma concentration-time profiles will be presented using the nominal sampling times. Concentration profiles will be presented on both linear and logarithmic scales.

10.2.7 PHARMACOKINETIC PARAMETERS:

Plasma RVP-001 concentration-time data will be analyzed by non-compartmental approach consistent with the IV route of administration using Phoenix WinNonlin v8.0 or higher. Nominal times may be used for interim analyses; however, actual sampling times will be used for final calculations.

The parameters presented in Table 8 will be determined, but not limited to, for each plasma concentration-time profile for those subjects that received treatment as the data allows:

Table 8 Pharmacokinetic Parameters of RVP-001 in Plasma

Parameter	Definition
C_{max}*	Maximum observed plasma concentration
t_{max}	Time of maximum observed plasma concentration; if it occurs at more than one time point, Tmax is defined as the first time point with this value
t_{lag}	Time from dosing to the first observed quantifiable concentration
t_{last}	Last observed quantifiable concentration
$AUC_{(0-24)}$	Area under the plasma concentration versus time curve from time 0 to 24 hours
$AUC_{(0-t)}$*	Area under the plasma concentration versus time curve from time 0 to the time of last measured concentration
$AUC_{(0-inf)}$	Area under the plasma concentration versus time curve from time 0 to infinity
$t_{1/2\ alpha}$	Distribution half-life
$t_{1/2}$	Terminal half-life, calculated as ln(2)/λz
λ_z	Elimination rate constant or Kel
Vd	Volume of distribution based on the terminal phase calculated using AUC(0-inf) after a single IV administration
CL	Total body clearance calculated after a single IV administration

AUC = area under the concentration-time curve; Cmax = maximum serum concentration; IV = intravenous
* Dose normalized $AUC_{(0-t)}$ and C_{max} will also be calculated.

Urinary concentrations and cumulative amounts of RVP-001 excreted will be determined and summarized by dose level and occasion.

[redacted]

[redacted]

Additional details can be found in the SAP.

10.2.8 PHARMACOKINETIC STATISTICAL METHODOLOGY

Listings and figures of individual RVP-001 plasma concentrations and summaries will be based on the randomized safety population. RVP-001 plasma concentrations will be summarized descriptively at each time point for each dose group using the randomized safety population. Pharmacokinetic metrics will be summarized descriptively for each dose group using the full analysis population. Cumulative amounts of RVP-001 excreted in urine by collection interval will be summarized descriptively.

10.2.9 DESCRIPTIVE STATISTICS

Descriptive statistics will be calculated for each treatment group. Plasma PK parameters will be summarized by treatment and occasion (as applicable) using the following descriptive statistics: N, min, arithmetic mean, geometric mean, median, max, SD, CV%, 95% confidence interval (CI) (geometric mean), and geometric mean CV%.

10.2.10 STATISTICAL ANALYSIS

Formal statistical analysis will be performed on the log-transformed PK parameters maximum serum concentration (C_{max}), area under the concentration-time curve $(AUC)_{(0-t)}$, and $AUC_{(0-inf)}$ to assess dose proportionality. This will be assessed using the following power model:

$$\log_e (AUC \text{ or } Cmax) = \mu + \beta x \log_e (Dose)$$

The estimate obtained for b is a measure of dose proportionality. The estimate of b together with its 90% CI (bl,bu) will be presented to quantify the degree of non-proportionality.

Further details relating to the statistical analysis will be included in the SAP including the following:

- Criteria to be used to define each of the populations and analysis sets
- Additional detail covering the analyses and/or description of primary and secondary analyses and safety data
- Handling of missing data, unused or spurious data
- Handling of data from withdrawn subjects
- Unblinding procedures and maintaining the blind

Further detail on the statistical analysis will be included in the SAP.

10.2.11 PLANNED INTERIM ANALYSES

Interim PK analysis will be performed after each cohort (dose selection and safety assessment).

The SAP will describe the planned interim analyses in greater detail.

10.2.12 DETERMINATION OF SAMPLE SIZE

This study is exploratory and therefore the sample size is not based on statistical considerations.The cohort size is a common size for phase 1 studies.

11 APPENDIX 1: SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

11.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

11.1.1 REGULATORY AND ETHICAL CONSIDERATIONS

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) international ethical guidelines
  - Applicable International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- Protocols and any substantial amendments to the protocol will require health authority approval prior to initiation except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), European Medical Device Regulation 2017/745 for clinical device research (if applicable), and all other applicable local regulations

11.1.2 FINANCIAL DISCLOSURE

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

11.1.3 INFORMED CONSENT PROCESS

11.1.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant, and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol:

- IRB approved Informed Consent

11.1.3.2 CONSENT PROCEDURES AND DOCUMENTATION

- The investigator or his/her representative will explain the nature of the study, including the risks and benefits, to the participant [or their legally authorized representative], and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants [or their legally authorized representatives] will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, privacy, and data protection requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be reconsented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant [or their legally authorized representative].

The ICF will contain a separate section that addresses the use of remaining mandatory samples for optional exploratory research. The investigator or authorized designee will explain to each participant the objectives of the exploratory research. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a participant's agreement to allow any remaining specimens to be used for exploratory research. Participants who decline to participate in this optional research will not provide this separate signature.

11.1.4 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the

Investigational New Drug (IND) sponsor, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants and the IRB, and sponsor will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

(Study may resume once concerns about safety, protocol compliance, and data quality are addressed and satisfy the sponsor, IRB, and/or FDA.)

11.1.5 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their representatives. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, regulatory agencies, sponsor, or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data which is collected for purposes of statistical analysis and scientific reporting will be transmitted to and stored at Quotient Sciences. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems

used by the clinical site and by Quotient research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at Quotient Sciences.

Personal data will be securely stored to prevent unauthorized access, disclosure, dissemination, alteration or loss of information and unauthorized personal data processing.

Access to personal information is restricted so that only personnel who are required to access personal data as part of their job role can do so. All personnel who access personal information are bound by a duty of confidentiality.

Technical arrangements surrounding the electronic storage and use of data are as follows:

- Computers storing electronic personal data are protected by antivirus software, and the networks on which computers are linked are protected by industry grade firewalls
- Off-site personnel can only access networked computers through a virtual private network
- Electronic access to data is limited according to user roles
- All data are stored on password protected computers

Organizational arrangements are as follows:

- All buildings are secured by key-card access
- Manual files of personal data are stored within restricted areas of the clinical unit that can only be accessed by authorized personnel
- Data security and/or confidentiality provisions are utilized in agreements with third parties
- Documented back-up and disaster recovery procedures are in place
- Internal audit and compliance functions provide regulatory oversight

The sponsor shall be the data controller in respect of the personal data of the study subjects collected in connection with the study and shall act in accordance with the relevant data protection laws in relation to the collection and processing of those personal data. Records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Anonymized personal data shall be collected and processed for the purposes of the study and may also be added to research databases and used in the future by the sponsor and its affiliates for certain additional clinical research, for product regulation and safety reporting purposes, and for ensuring compliance with legal requirements. The study subjects' anonymized personal data may be processed for such purposes by other parties including: the sponsor's affiliates and licensing partners; the sponsor's business partners; regulatory agencies and other health authorities; and IRB. The study subjects' authorization for such use and disclosure shall be obtained by the study subjects signing the ICF for the study.

Additionally, Quotient personnel are contractually bound by a duty of confidentiality and receive training on this matter.

11.1.6 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at Quotient Sciences. After the study is completed the de-identified, archived data will be transmitted to and stored at Quotient Sciences for use by other researchers including those outside of the study. Permission to transmit data to Quotient Sciences will be included in the informed consent.

With the participant's approval and as approved by local IRBs, de-identified biological samples may be utilized for further exploratory research. These samples may be held in storage by Quotient and Reveal Pharmaceuticals and their partners for up to 10 years.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the sponsor.

11.1.7 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Sponsor Medical Representative**
Maria Bermudez MD, CPI	[redacted]
Quotient Sciences	
3898 NW 7th Street Miami, FL 33126	
Phone Number: (day) 1-305-644-9903 ext. 115 (out of hours emergency) 1-305-644-9903	
Email: maria.bermudez@quotientsciences.com	

11.1.8 SAFETY OVERSIGHT

The principal investigator is responsible for the day-to-day safety oversight activities.

Refer to Section 8.3 Stopping Rules and Section 9.8 Safety Review Committee.

More details of safety monitoring of study can be found in the safety plan.

11.1.9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected; that the reported trial data are accurate, complete, and verifiable; and that the conduct of the

trial is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

The Sponsor or its representative may visit the study facilities at any time in order to maintain current and personal knowledge of the study through review of the records, comparison with source documents, and observation and discussion of the conduct and progress of the study. The clinical site will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s) by providing direct access to source data/documents.

11.1.10 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation, and completion. An individualized quality management plan will be developed to describe a site's quality management.]

Quality control (QC) procedures will be implemented beginning with the data entry system, and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written SOPs, the monitors will verify that the clinical trial is conducted, and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., GLP, GMP).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

11.2 DATA HANDLING AND RECORD KEEPING

11.2.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

11.2.1.1 CASE REPORT FORMS

The data required by the protocol is obtained in two ways. Source Documents are used in the clinic recording devices during procedures. The data are transcribed from source into the secure, validated eCRF for each subject included in a clinical trial (i.e., each subject who received a study drug treatment). Screen Failure data will also be transcribed into the eCRF and reported at the discretion of the Sponsor.

Data assembled outside the clinic source (i.e., safety lab data and PK concentration data) will be received from a specified external vendor via an electronic data file. The file will be received encrypted (or posted to a secure File Transfer Protocol) and stored in a secure folder on a server. The electronic data file(s) are independent of the eCRF data during the conduct of the study.

The eCRF cleaned data will be reviewed, approved, and electronically signed by the Principal Investigator or delegate. The eCRF data and external data files will be output in SAS® datasets. All data will be included with the final report to be provided to the Sponsor.

11.2.1.2 DATA MANAGEMENT AND PROCESSING

Data Management activities performed during the data management conduct of the study trial will be documented in the Data Management Plan (DMP). The eCRF system is the tool used to conduct all data cleaning activities, monitoring activities, and review/approval activities for clinic collected data and procedure data. The external data files are reconciled (to compare the external vendor data and eCRF sample collection data). Data Management activities are performed in accordance with the Data Management SOPs.

In addition to the cleaning activities, data entered in the eCRF database will be checked for accuracy via programmed and manual checks. When the database data is declared to be complete and accurate, the database will be locked, and all users write access removed.

11.2.1.3 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

All documentation and correspondence pertaining to the study (eCRF, raw data, letters etc.) will be kept in accordance with 21 CFR and the ICH guidelines for GCP: Guidance for Industry E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) 2018.

All study related documents will be retained for a period of at least 2 years following the date a marketing application is approved for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and regulatory authorities have been notified; or 8 years after completion of the study, whichever is longer. After this time, the sponsor will be contacted to ascertain whether continued storage or destruction is required in accordance with current regulations.

11.2.2 PUBLICATION AND DATA SHARING POLICY

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 before submission. This allows the sponsor to protect proprietary information and to provide comments.

The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this trial will be described in the Clinical Study Agreement.

11.3 REFERENCES

Aime S, Caravan P. Biodistribution of gadolinium-based contrast agents, including gadolinium deposition. *J Magn Reason Imaging*. 2009; 30:1259-1267.

Amos-Kroohs RM, Usach V, Piñero G, et al. Metal bashing: iron deficiency and manganese overexposure impact on peripheral nerves. *J Toxicol Environ Health A*. 2019; 82(2): 99-112. doi:10.1080/15287394.2019.1566105

Erstad D, Ramsay I, Jordan V, et al. Tumor contrast enhancement and whole-body elimination of the manganese-based magnetic resonance imaging contrast agent Mn-PyC3A. *Invest Radiol*. 2019. 54(11):697-703. doi: 10.1097/RLI.0000000000000593.

Food and Drug Administration (FDA). Drug Development and Drug Interactions | Table of Substrates, Inhibitors and Inducers. Updated March 2020. Available via: *https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers*.

FDA. Guidance for Industry: Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. Issued July 2005. Center for Drug Evaluation and Research (CDER).

FDA. Medical Imaging Drugs Advisory Committee Meeting. Briefing Document, September 8, 2017. Available via: *https://www.fda.gov/advisory-committees/advisory-committee-calendar/updated-start-time-and-public-participation-information-september-8-2017-meeting-medical-imaging*.

FDA Drug Safety Communication: New warnings for using gadolinium-based contrast agents in patients with kidney dysfunction. Originally posted September 9, 2010; updated February 6, 2018. Available via: https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-new-warnings-using-gadolinium-based-contrast-agents-patients-kidney.

FDA Drug Safety Communication: FDA warns that gadolinium-based contrast agents (GBCAs) are retained in the body; requires new class warnings. Originally posted December 19, 2017; updated May 16, 2018. Available via: *https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-fda-warns-gadolinium-based-contrast-agents-gbcas-are-retained-body*.

Gale EM, Atanasova IP, Blasi F, Ay I, Caravan P. A manganese alternative to gadolinium for MRI contrast. J Am Chem Soc. 2015; 137:15548-15557.

Gale E, Wey HY, Ramsay I, et al. A manganese-based alternative to gadolinium: contrast-enhanced MR angiography, excretion, pharmacokinetics, and metabolism. *Radiology*. 2018. 286:865-872. doi: 10.1148/radiol.2017170977.

Grant D, Zech K, Holtz E. Distribution and in vivo stability of manganese dipyridoxyl diphosphate in relation to imaging efficacy. *Invest Radiol*. 1994; 29:S2, S249-S250.

Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc Washington, DC: National Academy Press; 2001.

Klein JS, Brant WE, Helms CA, Vinson EN. Brant and Helms' Fundamentals of Diagnostic Radiology. 5th ed. Lippincott Williams & Wilkins; 2018.

MULTITRACE-5 (trace elements 5 injection) Prescribing Information. Shirley, NY: American Regent, Inc. Revised April 2019.

Radbruch A, Richter H, Bücker P, et al. Is Small Fiber Neuropathy Induced by Gadolinium-Based Contrast Agents?. *Invest Radiol*. 2020; 55(8): 473-480. doi:10.1097/RLI.0000000000000677.

Toft K, Hustvedt S, Grant D, et al. Metabolism and pharmacokinetics of MnDPDP in man. *Acta Radiologica*. 1997; 38:677-689. doi.org/10.1080/02841859709172400

Zhou IY, Ramsay IA, Ay I, et al. Positron Emission Tomography-Magnetic Resonance Imaging Pharmacokinetics, In Vivo Biodistribution, and Whole-Body Elimination of Mn-PyC3A. *Invest Radiol.* 2021; 56:261-270.

11.4 CYP INHIBITORS

Examples of strong and moderate clinical index inhibitors for P450-mediated metabolisms:

	Strong Index Inhibitors	Moderate Index Inhibitors
CYP1A2	Fluvoxamine [a]	--
CYP2B6 [b]	--	--
CYP2C8	Clopidogrel [c], gemfibrozil [d]	--
CYP2C9	--	Fluconazole [e]
CYP2C19	Fluvoxamine [a]	--
CYP2D6	Fluoxetine [f], paroxetine	Mirabegron
CYP3A	Clarithromycin [g], itraconazole [g]	Erythromycin, fluconazole [e], verapamil [g]

AUC = area under the concentration-time curve; CYP = cytochrome P450; DDI = drug-drug interaction; OATP1B1 = organic anion transporting polypeptide 1B1; OAT3 = organic anion transporter 3; P-gp = P-glycoprotein

a Strong inhibitor of CYP1A2 and CYP2C19, and moderate inhibitor of CYP2D6 and CYP3A.

b We currently do not have index inhibitors for CYP2B6.

c Strong inhibitor of CYP2C8, weak inhibitor of CYP2B6, and inhibitor of OATP1B1. The glucoronide metabolite is also an inhibitor for CYP2C8 and OATP1B1.

d Strong inhibitor of CYP2C8 and inhibitor of OATP1B1 and OAT3. The glucoronide metabolite is also an inhibitor for CYP2C8 and OATP1B1.

e Strong inhibitor of CYP2C19 and moderate inhibitor of CYP2C9 and CYP3A.

f Strong inhibitors of CYP2C19 and CYP2D6. (g) Inhibitor of P-gp (defined as those increasing AUC of digoxin to ≥1.25-fold).

Source: FDA Table of Substrates, Inhibitors and Inducers 2020

Signatures for Sponsor

Sponsor Study Number: RVL-101-22

Quotient Sciences Study Number: QSC206096

Date of Protocol: May 9, 2022

Version 2.0 (Amendment 1)

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