STATISTICAL ANALYSIS PLAN

A Phase 1, Double-Blind, Placebo-Controlled, Single Ascending Dose Study in Healthy Volunteers to Evaluate the Safety, Tolerability, and Pharmacokinetics of RGLS8429

SAP Version 1.0
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for

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Submitted to:
Regulus Therapeutics Inc.
4224 Campus Point Court
Suite 210
San Diego, CA 92121
United States

Prepared by:
ICON Clinical Research, LLC
820 West Diamond Avenue, Suite 100
Gaithersburg, MD 20878
Telephone: (301) 944-6800
Fax: (215) 699-6288

SIGNATURES

ICON Early Phase Services, LLC Signature Page

ICON Author:

Siva Durga Prasad Sattu
Siva Durga Prasad Sattu
19 Sep 2022 21:14:35 PDT (-07:00)
REASON: I approve this document as author.
d46665cd-bd3c-4abf-9de6-c3fbe2e057ae

Siva Durga Prasad Sattu, M.Sc. Date
Senior Statistician II, Biostatistics

ICON Reviewers:

Shekar Sunkoju
Shekar Sunkoju
19 Sep 2022 03:01:15 PDT (-07:00)
REASON: I approve this document
d4744496-6a55-4b4f-98f6-4abf57c4707c

Shekar Sunkoju Date
Senior Manager, Biostatistics

Rakesh Govindaraj
Rakesh Govindaraj
20 Sep 2022 04:26:34 PDT (-07:00)
REASON: I approve this document
35960b85-4e6f-4d93-9975-f7e48313876f

Rakesh Govindaraj Date
Principal SAS Programmer, IEP SAS Programming

Laurie Reynolds
Laurie Reynolds
21 Sep 2022 06:44:02 PDT (-07:00)
REASON: I approve this document
d6ad1f2c-7b30-4b99-ad94-1e44ce82e6be

Laurie Reynolds. Date
Sr. PK Scientist, Quantitative Pharmacology &
Pharmacometrics

CLIENT SIGNATURE PAGE

Client Approvals:

Rekha Garg
Rekha Garg
23 Sep 2022 07:06:37 PDT (-07:00)

REASON: I approve this document

c465b3ea-142a-4c12-a06c-8863f5f6a2ed

Rekha Garg, MD, MS — Date
Clinical Development and Regulatory Consultant

Claire Padgett
Claire Padgett
21 Sep 2022 09:07:20 PDT (-07:00)

REASON: I approve this document

4b0a0d9e-bf5c-4fae-b106-69a797d0235a

Claire S. Padgett, PhD — Date
Clinical Consultant

LIST OF ABBREVIATIONS

Abbreviation	Explanation
Ae	Total amount of unchanged RGLS8429 excreted in the urine
AE	Adverse event
Alb	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the curve
AUC_{0-24}	Area under the concentration–time curve up to 24 hr post dose
AUC_{0-t}	Area under the concentration–time curve up to the last quantifiable concentration
AUC_{inf}	Area under the concentration–time curve extrapolated to infinity
BLQ	Below limit of quantitation
BMI	Body mass index
B2M	beta-2 microglobulin
BP	Blood pressure
BUN	Blood Urea Nitrogen
CL/F	Apparent clearance
CL_R	Renal Clearance
CLU	Clusterin
cm	centimetre
C_{max}	Maximum observed concentration
CT-proAVP	Copeptin
CRA	Clinical research associate
CRF	Case report form
CRO	Clinical research organization
CS	Clinically significant
CSR	Clinical study report
CV%	Coefficient of variation

Abbreviation	Explanation
DMP	Data Management Plan
DLT	Dose-limiting toxicity
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
EOS	End of study
EPQT	Early Precision QT
Fe	Fraction of unchanged RGLS8429 excreted in the urine
GCP	Good clinical practice
GGT	Gamma-glutamyl transferase
Hb	Hemoglobin
Hct	Hematocrit
HIV	Human immunodeficiency virus
hr	Hour
HR	Heart rate
ICH	International Conference on Harmonisation
IGFALS	Insulin-like Growth Factor Binding Protein Acid Labile Subunit
INR	International normalized ratio
kg	Kilogram
KIM-1	Kidney injury molecule 1
LDH	Lactate dehydrogenase
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCP-1	Monocyte chemoattractant protein-1 (MCP-1)
MCV	Mean corpuscular volume
MedDRA	Medical dictionary for regulatory activities
MTD	Maximum tolerated dose
NGAL	Neutrophil gelatinase-associated lipocalin
OTC	Over-the-counter

Abbreviation	Explanation
PC1	Polycystin 1
PC2	Polycystin 2
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PR (interval)	Interval measured from the beginning of the P wave to the beginning of the QRS complex in the heart's electrical cycle as measured by electrocardiogram
PT	Preferred Term
QRS	The interval between the Q wave and the S wave in the heart's electrical cycle as measured by electrocardiogram; principal deflection in the electrocardiogram
QT	Measure of time between the start of the Q wave and the end of the T wave in the heart's electrical cycle
QTcB	QT correction with Bazett formula
QTcF	QT interval corrected by Fridericia
Q1	First Quartile
Q3	Third Quartile
RBC	Red blood cells
RR	Respiratory rate
SAD	Single Ascending Dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SARA	Scale for the Assessment and Rating of Ataxia
SC	Subcutaneous
SD	Standard deviation
SOC	System Organ Class
$t_{½}$	Terminal half-life
TEAE	Treatment emergent adverse event
T_{max}	Time to maximum observed concentration
V_z/F	Apparent Volume of distribution
WBC	White blood cells
WHO	World Health Organization

TABLE OF CONTENTS

1. INTRODUCTION

This statistical analysis plan (SAP) is consistent with the statistical methods section of the final study protocol (Version 3.0 dated 06 June 2022) and includes additional detail of the safety, tolerability, and pharmacokinetics (PK) of RGLS8429 summaries to be included in the clinical study report (CSR).

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

The following are the study objectives.

2.1.1 Primary Objective

- To assess the safety and tolerability of single ascending subcutaneous (SC) doses of RGLS8429

2.1.2 Secondary Objectives

- To identify dose-limiting toxicity (DLT) and to determine the maximum tolerated dose (MTD) of a single SC dose of RGLS8429
- To characterize the PK properties of RGLS8429

2.1.3 Exploratory Objective

- To characterize multiple potential renal biomarkers

2.2 Endpoints

The following are the study endpoints.

2.2.1 Primary Endpoint

- Incidence of adverse events (AEs) and change in safety laboratory test results, vital signs, scale for the assessment and rating of ataxia (SARA), and electrocardiogram (ECG) parameters and Holter monitoring over time

2.2.2 Secondary Endpoints

- Incidence of DLT and determination of MTD
- PK parameters of RGLS8429 including:
  - Maximum observed concentration (C_{max})
  - Time to maximum observed concentration (T_{max})
  - Area under the concentration–time curve up to 24 hr post dose (AUC_{0-24})
  - Area under the concentration–time curve up to the last quantifiable concentration (AUC_{0-t})
  - Area under the concentration–time curve extrapolated to infinity (AUC_{inf})
  - Half-life ($t_{½}$)
  - Apparent clearance (CL/F)

- Apparent Volume of distribution (V_z/F)
- Fraction of unchanged RGLS8429 excreted in the urine (fe)
- Total amount of unchanged RGLS8429 excreted in the urine (Ae)

2.2.3 Exploratory Endpoints

- Characterize multiple potential renal biomarkers in urine (e.g., polycystin 1 [PC1], polycystin 2 [PC2], neutrophil gelatinase-associated lipocalin [NGAL], kidney injury molecule-1 [KIM-1], monocyte chemoattractant protein-1 [MCP-1], beta-2 microglobulin [B2M], and clusterin [CLU]) and in serum (e.g., Cystatin-C, Insulin-like Growth Factor Binding Protein Acid Labile Subunit [IGFALS], albumin [Alb], copeptin [CT-proAVP], N-acetyl-1-methylhistidine, and others).
- Renal Clearance (CL_R)

3 STUDY DESIGN

3.1 General

This is a Phase 1, randomized, double-blind, placebo-controlled, single ascending dose (SAD) study in healthy volunteers to evaluate the safety, tolerability, and PK of RGLS8429. A single ascending dose of RGLS8429 or placebo will be administered via SC injection to healthy volunteers.

Eight subjects will be randomized 3:1 to receive RGLS8429 or placebo in each cohort.

Cohort 1: 0.5 mg/kg RGLS8429 or placebo

Cohort 2: 1.0 mg/kg RGLS8429 or placebo

Cohort 3: 2.0 mg/kg RGLS8429 or placebo

Cohort 4: 4.0 mg/kg RGLS8429 or placebo

In each cohort, 6 subjects will be randomized to receive RGLS8429 and 2 will be randomized to receive placebo. Enrollment into subsequent cohorts will be initiated after review of safety (AEs and safety laboratory tests) from the prior cohort.

For Cohort 1, the first two subjects will receive RGLS8429 or placebo (randomized such that one subject receives RGLS8429 and one receives placebo) and this sentinel group will be monitored for 48 hrs prior to dosing the remaining subjects in the cohort.

Subjects will be admitted to the study center on Day -1, one day prior to the day of study drug administration (Day 1), and will be discharged on Day 2, no less than 6 hrs after the collection of the 24-hr PK sample. Subjects will be followed for a total of 4 weeks after dosing and will return to the site for follow-up visits weekly from Weeks 1 through 4. The sentinel subjects will remain in the clinic for 48 hrs after dosing.

Study procedures and assessments with their timing is summarized in Table 3-1.

Table 3-1: Schedule of Assessments and Procedures

	Screen [1]	Treatment													Follow-Up			
Week															1	2	3	4/EOS
Days	-28 to -2	-1	1											2	8	15	22	29
Hour			0 [2]	0.5	1	2	3	4	5	6	8	10	12	16	24				
Informed Consent	X																	
Demographics	X																	
Medical History	X																	
Physical Examination [3]	X		X											X	X	X	X	X
Weight and Height [4]	X	X													X			X
Vital Signs [5]	X	X	X		X	X		X			X			X	X	X	X	X
ECGs [6]	X		X					X		X			X	X				
Hematology [7]	X	X												X	X	X	X	X
Chemistry [8]	X	X												X	X	X	X	X
Coagulation [9]		X						X						X				X
Urinalysis [10]	X	X												X	X	X	X	X
Lipids [11]	X	X												X		X		X
Pregnancy Test [12]	X	X																X
Urine Drug and Alcohol Screen	X	X																X
HBsAg, HCV, HIV Screen	X																	
Inclusion/Exclusion Review	X	X																
Randomization		X																
SARA Assessment [13]			X						X					X				X
Adverse Events			X						X						X	X	X	X
Concomitant Medications	X	X	X						X						X	X	X	X
Study Drug Administration			X															
PK Plasma Collection [14, 19]			X			X		X		X	X		X	X		X		
24-Hr Urine Collection [15, 19]			X						X									
Serum biomarkers [16, 19]	X	X																
Urine biomarkers [17, 19]	X	X																
Holter monitoring [18]			X						X									
Admission to Clinic		X																
Discharge from Clinic														X				

Clinic Visit																X	X	X	X

Abbreviations: ECG, electrocardiogram; EOS, end of study or early termination visit; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; PK, pharmacokinetics; SARA=Scale for the Assessment and Rating of Ataxia.

1. Screening Period within 28 days prior to study drug administration (Baseline = Day -1 or Day 1 when test/procedure performed).
2. Ensure that all Day 1 procedures scheduled to be completed prior to dosing are completed prior to dosing.
3. Complete physical examination at Screening and Week 4/EOS Visit. Limited physical examination at other visits. The limited physical examination will be focused on general appearance, the respiratory, neurological, and cardiovascular systems, and subject-reported symptoms
4. Measure weight at all visits indicated. Height at Screening only. Calculate BMI at Screening Visit.
5. Systolic (SBP) and diastolic (DBP) blood pressure measurements, heart rate (HR), body temperature, and respiratory rate (RR).
6. Obtain a 12-lead ECG during Screening and print and review ECG tracings from the Holter monitor pre-dose and 4 hr, 6 hr, 12 hr, and 24 hr post-dose. Record new or worsening clinically significant abnormalities as an AE.
7. Full hematology panel including hemoglobin (Hb), hematocrit (Hct), red blood cell (RBC) count, red cell indices, white blood cell (WBC) count including absolute cell counts, with differential, and platelet count.
8. Fasting chemistry panel including total protein, glucose, uric acid, creatine kinase (CK), lactate dehydrogenase (LDH), sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine (Scr), and hepatic function panel (albumin, total bilirubin, direct bilirubin, ALT, AST, ALP, and gammaglutamyl transferase [GGT]).
9. Prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), and fibrinogen will be collected on Day -1; Day 1, 4 hr post-dose; and Day 2, 24 hr post-dose; and at Week 4/EOS.
10. Urinalysis panel including specific gravity, pH, protein, glucose, ketones, blood (Hb), leukocyte esterase nitrite, bilirubin, and urobilinogen. If protein, blood, or leukocyte esterase nitrate parameters are abnormal a microscopic examination will be completed.
11. Fasting lipid panel including total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL).
12. Only female subjects of childbearing potential. A serum pregnancy test will be performed at Screening and urine pregnancy tests will be performed at the remaining specified visits.
13. Perform the SARA assessment pre-dose on Day 1 and 4-6 hr post-dose on Day 1; on Day 2, 1 hr after the 24-hr PK sample collection; and at Week 4/EOS Visit.
14. Pre-dose plasma PK sample on Day 1 should be obtained within 60 minutes prior to dosing. Post-dose plasma samples should be obtained within the following time margins: collections at 2, 4, 6, and 8 hr ±10 minutes, 12 hr ±30 minutes, and 24 hr ±60 minutes.
15. 24-hr urine collection for PK analysis will begin immediately after study drug dosing on Day 1 (0-24 hr). The subject should void immediately before dosing (before the 24-hr urine collection is started). Last void will be at 24 hours post dose.
16. Serum sample for exploratory biomarkers (e.g., Cystatin-C, IGFALS, Alb, CT-pro-AVP, N-acetyl-1-methylhistidine, and others).
17. Collect at least 150 mL (**more if possible**) of mid-stream free-flow urine for analysis of PC1, PC2, NGAL, KIM-1, MCP-1, B2M, and CLU during Screening and on Day -1. These samples must NOT be the first urination of the day (to avoid too much debris in the sample).
18. Start Holter monitoring 1 hr before study drug administration on Day 1 and continue for 30 minutes after the 24-hr PK sample is obtained on Day 2.
19. Unused backup plasma, serum, and urine PK/biomarker samples may also be used for additional exploratory biomarkers analyses.

3.2 Study Population

32 healthy male or female volunteers 18 to 55 years old and with a body mass index (BMI) between 18 and 35 kg/m^2 will be randomized.

3.3 Randomization and Treatment Assignments

This study will be double-blind and placebo-controlled. There will be 4 SAD cohorts. Eight subjects will be enrolled into each of the 4 dosing cohorts. Subjects in each cohort will be randomized at a ratio of 3:1 to receive RGLS8429 or placebo. In each cohort, 6 subjects will be randomized to receive RGLS8429 and 2 will be randomized to receive placebo.

ICON will provide a randomization schedule and eligible subjects will be randomized sequentially to receive the study drug assigned to the corresponding randomization number. Subjects may be randomized once all Screening and Day -1 procedures have been completed and eligibility has been confirmed by the Investigator. Details regarding randomization procedures will be provided in the study reference manual.

Enrollment into subsequent cohorts will be initiated after review of safety (AEs and safety laboratory tests) from the prior cohort. The investigator or a designee will assign sequential subject identification numbers to the subjects as they are screened and enrolled in the study.

An unblinded pharmacist will be responsible for preparing RGLS8429 or placebo and providing the study drug to blinded study personnel for administration according to the Pharmacy instructions within the Study Reference Manual.

3.4 Determination of Sample Size

Formal sample size calculations were not performed for this study. The dose cohort size is 8 subjects randomized 3:1 to RGLS8429 versus placebo. The size of each dose cohort was chosen to provide sufficient information to allow assessment of the safety and tolerability of RGLS8429.

4 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSIS

All analyses specified in this SAP are consistent with the final study protocol (Version 3.0, dated 06 June 2022). Any changes in the analyses provided or any additional analyses performed will be documented in the clinical study report (CSR).

5 QUALITY CONTROL AND QUALITY ASSURANCE METHODS FOR DATA ANALYSIS

Case report forms will be reviewed and processed according to the ICON Study Specific Procedure SSP DM-38770009.01 Data Management Plan (DMP). The DMP describes eCRF data processing, edit checks, data query management, medical dictionary coding, SAE reconciliation, data transfers, and data quality review through database lock or any necessary reopening of the database. After database lock, the data will be retrieved from the database using SAS version 9.4 or higher. Independent programming will be performed for the analysis. Statistical review should be conducted on an ongoing basis as each output passes validation after lead programmer review or at one-time point only when all outputs have been validated. When statistical review is performed and completed, the Lead Statistician, Programming lead and Project lead will sign a Biostatistics and Programming Validation and Release Form (refer to PRG001-SOP) to verify that all applicable procedures and review steps have been performed and authorizing the release of TLFs.

6 PHARMACOKINETIC AND PHARMACODYNAMIC ASSESSMENTS

6.1 Pharmacokinetic Assessment

Plasma concentrations of unchanged RGLS8429 at different time-points and urine concentrations collected over the specified interval will be used to derive the following PK parameters using non-compartmental methods (Phoenix™ WinNonlin®, Version 8.0 or later higher, Certara, Princeton, NJ)

The following PK parameters will be reported:

Variable	Definition
AUC_{0-24}	Area under the plasma concentration-time curve from time =0 to 24 estimated using the linear/log trapezoidal rule
AUC_{0-t}	Area under the plasma concentration-time curve from time =0 to the last quantifiable concentration estimated using the linear/log trapezoidal rule.
AUC_{inf}	Area under the plasma concentration-time curve extrapolated to infinite time calculated as: $$AUC_{inf} = AUC_{0-t} + \frac{C_{last}}{\lambda_z}$$ Where C_{last} is the last observed quantifiable concentration, and λ_z is the terminal rate constant for elimination.
C_{max}	Maximum observed plasma concentration.
T_{max}	Time to C_{max}. If the maximum observed concentration value occurs at more than 1 time point, T_{max} is defined as the first time point with this value.
$t_{½}$	Terminal elimination half-life in plasma computed as: $$t_{½} = \frac{\ln(2)}{\lambda_z}$$ Where ln(2) is the natural logarithm of 2.
CL/F	Apparent total clearance of the drug from plasma calculated as: $$CL/F = \frac{Dose}{AUC_{inf}}$$
V_z/F	Apparent volume of distribution during the elimination phase calculated as: $$V_z/F = \frac{Dose}{\lambda_z \cdot AUC_{inf}}$$

Variable	Definition
Ae_{0-24}	Total amount of unchanged RGLS8429 excreted in the urine after the first dose of RGLS8429 (Day 1): $Ae_{t_1-t_2} = Cu_{t_1-t_2} \cdot Vu_{t_1-t_2}$ Where Cu_{t1-t2} is the concentration in urine collected over the interval from time 0 to 24 hours and Vu_{t1-t2} is the volume of urine collected over the interval from time 0 to 24 hours.
fe_{0-24}	Fraction of the unchanged drug excreted in urine over the interval from time t_1 to time t_2 computed as: $fe_{0-24} = \frac{Ae_{0-24}}{Dose}$
CL_R	Renal Clearance computed as: $CL_R = \frac{Ae_{0-24}}{AUC_{0-24}}$

6.1.1 Treatment of Outliers

Individual concentration-time points, if considered anomalous, may be excluded from the analysis at the discretion of the pharmacokineticist following a review of the available documentation. Any such exclusion will be discussed with the sponsor and clearly outlined in the study report.

Entire individual treatment profiles for a subject may be excluded following review of the available documentation and discussion with the sponsor. However, results of analysis with and without the excluded profiles may be presented in the study report. Any such exclusion will be clearly listed in the study report along with justification for exclusion.

6.1.2 Non-Quantifiable Concentrations

All concentration values reported as no results (not collected or not determined) values will be treated as missing. For the calculation of concentration summaries, all concentrations below the quantifiable limit (BLQ) will be treated as 0. For the purpose of calculating PK parameters and plotting mean and individual concentration-time profiles, BLQ values will be treated as 0 prior to the first measurable concentration. After the first measurable concentration, subsequent BLQ values (or mean concentration values that are BLQ) are treated as missing.

6.2 Biomarker Assessment

Collect mid-stream free-flow urine for analysis of urine exploratory biomarkers (e.g., PC1, PC2, NGAL, KIM-1, MCP-1, B2M and CLU) during Screening and Day -1 and collect serum samples during Screening and Day -1 for serum exploratory biomarkers (e.g., Cystatin-C, IGFALS, Alb, CT-pro-AVP, N-acetyl-1-methylhistidine, and others).

7 STATISTICAL METHODS

7.1 General

The statistical analysis will be conducted following the principles specified in the International Council for Harmonisation (ICH) Topic E9 Statistical Principles for Clinical Trials (CPMP/ICH/363/96).

All statistical analyses will be performed using the statistical software SAS Grid/ SAS Linux - SAS®, Version 9.4 or newer and any exceptions will be detailed in the CSR.

All results collected in the database will be presented in listings. All continuous data will be listed with the same precision as presented in the database.

Unless otherwise noted, continuous variables will be summarized descriptively using the number of non-missing observations (n), arithmetic mean (mean), standard deviation (SD), coefficient of variation (%CV), first (Q1) and third quartiles (Q3), median, minimum, and maximum; categorical variables will be summarized using the frequency count and the percentage of subjects in each category.

In the data listings, study day relative to dose of study drug will be presented. Study day relative to dose will be calculated as event date – dose date (+1 if event date ≥ dose date).

Baseline will be the latest available measurement (scheduled or unscheduled assessment) prior to the study drug administration in the study.

Subjects will be analyzed based on the actual treatment received.

For numerical variables, change from baseline will be calculated as (Post baseline value – Baseline value).

For safety summaries, the unscheduled and repeat assessments will not be included in descriptive statistics; however, all results will be included in the data listings.

All data will be summarized by cohort and treatment group, pooled RGLS8429, and pooled placebo unless otherwise specified.

7.2 Handling of Dropouts or Missing Data

There will be no imputations for missing data other than that implicit by ignoring missing data when using the trapezoidal rule for the calculation of AUCs. For all variables, only the observed data from the subjects will be used in the by-visit summaries.

The following imputation dates for AE and concomitant medication will only be applied for determination of TEAEs and prior/concomitant medication.

7.2.1 Handling of missing/ incomplete dates for Adverse Event

Imputation rules for missing or partial AE start date are defined below:

a) If only Day of AE start date is missing:

If the start date has month and year but day is missing, the first day of the month will be imputed.

- If this date is earlier than the first dose date, then the first dose date will be used instead.
- If this date is later than the stop date (possibly imputed), then the stop date will be used instead.

b) If Day and Month of AE start date are missing:

If the start date has year, but day and month are missing, the 1st of January will be imputed.

- If this date is earlier than the first dose date, then the first dose date will be used instead.
- If this date is later than the stop date (possibly imputed), then the stop date will be used instead.

c) If Year of AE start date is missing:

If the year of AE start is missing or AE start date is completely missing then imputation will not be done and this AE will be considered as TEAE.

7.2.2 Handling missing or partial Prior/Concomitant Medication Dates

Missing or partial medication start date:

a. If only Day is missing, the first day of the month will be assumed.
b. If Day and Month are both missing, the first day of the year will be assumed.
c. If Day, Month and Year are all missing, the day before the first dose date will be assumed.

Missing or partial medication stop date:

a. If only Day is missing, the last day of the month will be assumed.
b. If Day and Month are both missing, the last day of the year will be assumed.
c. If Day, Month and year are all missing, 'continuing' status to stop date will be assigned.

7.3 Multiple Comparisons and Multiplicity

There will be no multiple comparison adjustment for various analyses.

No formal statistical tests will be conducted to assess the safety or tolerability of RGLS8429.

7.4 Adjustments for covariates

Not applicable.

7.5 Multicenter Studies

This is a single-center study.

7.6 Examination of Subgroups

No subgroup analyses are planned.

7.7 Coding dictionaries

Medical history, AEs and medical procedures will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, Version 24.0 or higher). Medications will be coded using the World Health Organization dictionary of medical codes (WHO Global B3 format - March 2021).

7.8 Analysis Populations

7.8.1 Screened Population

Screened population includes all subjects who signed the informed consent form

7.8.2 Randomized Population

Randomized population includes all subjects who are randomized into the study.

7.8.3 Safety Population

The Safety Population consists of all subjects who received a dose of study drug. Subjects will be analyzed based on treatment received.

7.8.4 Pharmacokinetic Full Population

The PK Full Population consists of all subjects who received a dose of RGLS8429 and have at least 1 concentration data point. Subjects will be analyzed based on dose received.

7.8.5 Pharmacokinetic Evaluable Population

The PK Evaluable Population consists of all subjects who received a dose of RGLS8429 and have at least one PK parameter that can be reliably estimated. Subjects will be analyzed based on dose received.

7.8.6 Biomarkers (Pharmacodynamic (PD)) Population

The Biomarker (PD) Population consists of all screened subjects who have at least one screening and/or pre-dose value for the biomarker parameters.

7.9 Subject Accountability

Summaries of analysis populations and subject disposition will be presented by cohort and overall. It will contain the following information:

- Number of subjects screened
- Number of subjects randomized
- Number and percent of subjects who completed the study
- Number and percent of subjects who discontinued early and reason for early discontinuation
- Number and percent of subjects in the Safety, PK Full, PK Evaluable, and PD Populations

Percentage will be calculated based on number of subjects in Randomized Population except for number of subjects screened.

Subject disposition data, exclusions from the analysis populations, eligibility criteria satisfaction and consent information will be presented in listings for the Randomized Population.

7.10 Protocol Deviations

All protocol deviations will be identified prior to database lock. Major protocol deviations will be listed. In addition, a separate listing of all inclusion and exclusion deviations will be provided.

7.11 Subject Demographics and Baseline Characteristics

Subject demographics (age, sex, race, and ethnicity) and baseline characteristics (height, weight, BMI, and eGFR) will be summarized descriptively based on the Randomized Population. Subject demographics and baseline characteristics will be listed.

7.12 Medical and Surgical History

The medical and surgical history data will be coded using the MedDRA, Version 24.0 or higher. and will be listed and summarized using the Randomized Population.

7.13 Prior and Concomitant Medications

All prior and concomitant medications will be coded using the WHO Global B3 format - March 2021. Concomitant Medications will be listed and summarized using the Safety Population. Prior Medications will be listed only for Randomized Population. A separate listing will be provided for concomitant procedures for Safety population.

7.14 Measurements of Treatment Compliance

A listing of treatment compliance will be provided for eCRF collected exposure data e.g expected dose, actual dose, number of injections etc. for the Randomized Population.

7.15 Pharmacokinetic Analysis

The PK Full Population will be used for all concentration (plasma and urine) listings, summary tables, and graphs. The PK Evaluable Population will be used for PK parameters listing (plasma and urine), PK parameters summary (plasma and urine), and statistical analysis.

The individual sampling and subject concentration-time for plasma drug concentrations of RGLS8429 will be listed and displayed graphically on linear and semi-log scales. The drug concentrations will be summarized descriptively by RGLS8429 cohort and nominal time point in tabular and graphical formats (linear and semi-log scales). Linear and semi-logarithmic plots of the individual plasma concentration by actual sampling time for each RGLS8429 cohort will be provided by subject (one subject per page). Overlay of individual plasma concentration profiles over time will be provided by RGLS8429 cohort. Plots of mean (SD) plasma concentration profiles versus time will be presented with all cohorts superimposed on the same plot.

All PK parameters will be listed. Summary statistics will be calculated for the plasma RGLS8429 PK parameters for each dose cohort using descriptive statistics to include, as appropriate, n, mean, SD, CV, Q1 and Q3, minimum, median, maximum, geometric mean and geometric %CV. For T_{max}, summary statistics will be described by n, minimum, median, and maximum only.

The total amount of RGLS8429 excreted in urine will also be assessed, where feasible. Urine pharmacokinetic parameters (CL_R., fe, Ae) will be listed for each subject by RGLS8429 cohort and summarized using descriptive statistics. Individual urine concentrations and volumes will be listed. In addition, urine samples will be qualitatively screened for potential RGLS8429 catabolites.

Additional PK parameters may be estimated as appropriate. The parameters stated above will be reported if they are estimable and will be discussed in the CSR. When necessary, interpolation or λ_z extrapolation may be used to estimate partial AUCs. The value of $AUC\%_{extrap}$ will be less than or equal to 20% for the AUC_{inf} to be considered well estimated. If this proportion is > 20%, then the values of AUC_{inf} will be treated with caution. When AUC_{inf} is calculated and tabulated, the $AUC\%_{extrap}$ will also be tabulated. Where $AUC\%_{extrap}$ >20%, AUC_{inf} and all related parameters (i.e. CL/F and Vz/F) will be presented but excluded from the calculation of summary statistics. All values excluded from the summaries should be flagged in the individual listings with an explanation for the exclusion.

The apparent terminal phase rate-constant (λ_z) will be estimated by log-linear regression of the concentration-time data associated with this phase. The decision as to which data points describe the terminal phase will be reached by inspecting the semi-logarithmic plot of the data, only considering concentrations at time points beyond T_{max}. A minimum of three data points will be used for the estimation of λ_z preferably covering a time span of at least 2 half-lives. Poorly estimated λ_z (i.e. if a time span of at least 2 half-lives is not used or $r^2_{adj} \leq 0.80$), the corresponding $t_{½}$ and other parameters derived using λ_z (i.e. AUC_{inf}, CL/F, V_z/F, and $t_{½}$) will be flagged in the report. Summary statistics of these parameters (i.e. AUC_{inf}, CL/F, V_z/F, and $t_{½}$)

will be reported with and without these outliers (i.e., with and without parameters where λ_z was poorly estimated).

Dose Proportionality Assessment:

Dose proportionality will be explored using the power model for C_{max}, AUC_{inf}, AUC_{0-24} and AUC_{0-t} as data permit for the PK evaluable population.

A power model will be fit separately to describe the relationship between pharmacokinetic parameter (C_{max}, AUC_{inf}, AUC_{0-24} and AUC_{0-t}) and actual dose in mg using the least-squares linear regression model as follows:

$\ln(\text{PK parameter}) = \alpha + \beta \ln(\text{dose}) + \varepsilon$.

This linear model will be used to provide a 95% confidence interval (CI) for β (slope).

From each pharmacokinetic parameter model, the intercept of the regression line α and the slope of the regression line β will be presented along with the 95% CI of the slope. Actual dose of drug and not dose level will be used in analysis. A minimum of 3 non-missing PK parameter values per dose cohort must be available to estimate dose proportionality with the power model.

7.16 Biomarker Analysis

The Biomarker (PD) Population will be used for all biomarker analyses. Baseline (the average of screening and Day -1 samples) biomarker data will be listed and summarized using descriptive statistics. Three summaries will be presented: Summary of Screening (Day -28 to Day-2), Summary of Day -1 and Summary of the average of Screening and Day-1 samples (Baseline).

7.17 Safety Analyses

All Safety analysis will be presented using the Safety Population. No formal statistical tests will be conducted to assess the safety or tolerability of RGLS8429.

Tables will be presented by cohort and treatment group, pooled RGLS8429 and pooled placebo.

7.17.1 Adverse Events

All AE summaries will include TEAEs. TEAEs or TESAEs are those which occur or worsen after the administration of study drug until the Day 29 or EOS Visit whichever occurs later.

All AEs will be classified by primary system organ class (SOC) and preferred term (PT) according to the MedDRA Version 24.0 or higher. Data collected in CRF along with duration of AE will be presented in listing. Duration of AE will be calculated as stop date – start date.

The overall incidence of TEAEs (number and percentage of subjects) as well as the number of events will be summarized. It includes severity, relationship to study drug of TEAE and SAEs, TEAEs leading to study discontinuation, life-threatening SAEs, and SAEs resulting in death.

The TEAEs will be summarized and tabulated at both the subject (n [%] of subjects) and event (number of events) level:

- TEAEs by SOC and PT
- TEAEs by SOC, PT, and maximum reported severity
- TEAEs by SOC, PT, and relationship to study drug
- TESAEs by SOC and PT

For the incidence at the subject level by SOC and PT, if a subject experiences more than 1 event within the same SOC and PT, only 1 occurrence will be included in the incidence.

For the incidence at the subject level by SOC, PT, and severity, if a subject experiences more than 1 event within the same SOC and PT, only the most severe occurrence will be included in the incidence.

For the incidence at the subject level by SOC, PT, and relationship to study drug; if a subject experiences more than 1 event within the same SOC and PT, only the most closely related occurrence will be included in the incidence.

The incidence of DLTs will be summarized by cohort and treatment group.

Any SAEs, AEs with outcome of death and AEs resulting in discontinuation of study will be listed separately.

7.17.2 Clinical Laboratory Assessments

Clinical laboratory data will be summarized using System International (SI) units and the Safety Population will be used.

Observed and change from baseline of continuous clinical laboratory values (serum chemistry, coagulation panel, hematology, lipids, and urinalysis) will be summarized for each parameter at each visit/timepoints using descriptive statistics.

Shift from baseline to worst post-baseline laboratory findings based on normal range criteria will also be summarized.

A listing of all clinical laboratory data for each subject at each visit will be presented. Laboratory values outside the reference range will be flagged with ‘L’ for low and ‘H’ for high in the data listings and a list of abnormal values will be presented.

For female subjects, serum/urine pregnancy test results during the study will be presented in the individual subject data listings.

7.17.3 Vital Signs

Observed and change from baseline vital signs values will be summarized at each timepoint. Vital signs data will be listed by subject at each timepoint.

7.17.4 Electrocardiograms

Shift tables will be presented that summarize the overall ECG interpretation as normal, abnormal not clinically significant, or abnormal clinically significant from baseline to each post-baseline time point.

Individual ECG data will be listed.

A separate statistical analysis plan will be prepared for the Holter monitoring data following an Early Precision QT (EPQT) analysis. This is not included within this statistical analysis plan.

7.17.5 SARA Assessment

Scale for the Assessment and Rating of Ataxia (SARA) scores for individual item will be summarized descriptively. Observed and change from baseline in SARA individual and total scores will be summarized at each timepoint.

Total SARA score for each subject will be calculated including score for gait, stance, sitting, speech disturbance and mean score of finger chase, nose-finger test, fast alternating hand movements and heel-shin slide.

7.18 Planned Interim Analysis

There is no formal interim analysis planned.

However, ongoing assessments of the PK, safety and tolerability, will be performed for dose escalation meetings.

8 GENERAL CONVENTIONS FOR TABLES, LISTINGS, AND FIGURES

Tables and listings will be presented in landscape mode with minimum of 3/4" bound edge margin and 3/8" other margins on 8.5" x 11" paper.

Times new roman font size of no less than 8 point will be used for tables and listings.

A source line will be included on the bottom of each page of all tables and listings. It will contain the SAS code program name and the run date and time.

Each variable is recorded to a specific number of decimal places. If the raw data is presented with varying precision, then the least precise value will be considered as the data precision.

For summary tables, unless otherwise specified, the number of decimal places provided in the tables and listings will be based on the accuracy of the least accurate value in the raw data as follows:

n	Integer
Arithmetic mean	1 decimal place more than the least accurate number in the raw data
SD	2 decimal places more than the least accurate number in the raw data
%CV	2 decimal places
Geometric mean	1 decimal place more than the least accurate number in the raw data
Geometric CV	2 decimal places
Median	1 decimal place more than the least accurate number in the raw data
Quartiles (Q1 and Q3)	1 decimal place more than the least accurate number in the raw data
Minimum	same number of decimal places as raw data
Maximum	same number of decimal places as raw data
Confidence interval	same number of decimals as the associated statistic
Percentage	1 decimal place
Geometric mean ratio	2 decimal places

The sample column layouts are given below:

RGLS8429 0.5 mg/kg (N=xx)	RGLS8429 1.0 mg/kg (N=xx)	RGLS8429 2.0 mg/kg (N=xx)	RGLS8429 4.0 mg/kg (N=xx)	Pooled RGLS8429 (N=xx)	Pooled Placebo (N=xx)

- For PK tables placebo will not be presented

The listing layouts are given below:

Cohort/ Treatment	Subject Number/ Age/ Sex/ Race	xxxx	xxxx	xxxx
Cohort 1/ RGLS8429 0.5 mg/kg	XXXX-XXXX/ xx/ X/ X			
Cohort 1/ Placebo	XXXX-XXXX/ xx/ X/ X			
Cohort 2/ RGLS8429 1.00 mg/kg	XXXX-XXXX/ xx/ X/ X			
Cohort 2/ Placebo	XXXX-XXXX/ xx/ X/ X			
.....				

9 LIST OF TABLES, FIGURES, AND LISTINGS

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Table 14.1.2	Analysis Populations
Table 14.1.3	Demographic and Baseline Characteristics (Randomized Population)
Table 14.1.4	Medical and Surgical History Summary (Randomized Population)
Section 14.2	**Pharmacokinetic/Biomarker Data Summaries**
Section 14.2.1	**Pharmacokinetic Data Summaries**
Table 14.2.1.1	Summary of RGLS8429 Plasma Concentrations (units) by Timepoint (PK Full Population)
Table 14.2.1.2	Summary of RGLS8429 Plasma PK Parameters (PK Evaluable Population)
Table 14.2.1.3	Summary of RGLS8429 Plasma PK Parameters after excluding Outliers (PK Evaluable Population) Note: Outliers are included (e.g., estimates of T1/2 if a time span of at least 2 half-lives is not used or $r^2_{adj} \leq 0.80$)
Table 14.2.1.4	Summary of RGLS8429 Urine PK Parameters (PK Evaluable Population)
Table 14.2.1.5	Statistical Analysis of Dose Proportionality of Pharmacokinetic Parameters (PK Evaluable Population)
Figure 14.2.1.1	Mean (+/-SD) of Plasma Concentration of RGLS8429 -Time Profiles by Treatment Group (PK Full Population) Note: Present both linear and semi-log
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Table 14.3.5.1	Summary Statistics of Absolute and Change from Baseline for Clinical Laboratory Parameters (Safety Population) Note: continuous tests only
Table 14.3.5.2	Shift Table for Clinical Laboratory Parameters (Safety Population)
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Table 14.3.5.4	Shift Table for Electrocardiogram Interpretations (Safety Population)
Table 14.3.5.5	Summary Statistics of Absolute and Change from Baseline in Scale for the Assessment and Rating of Ataxia (SARA) (Safety Population)

Listing Number	**Listing Name**
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Listing 16.2.1.2	Subject Disposition (Randomized Population) Note: Randomization and study completion
Section 16.2.2	**Protocol Deviations**
Listing 16.2.2	Protocol Deviations (Safety Population)
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Figure 16.2.5.1	Individual RGLS8429 Plasma Concentration-Time (PK Full Population) Note: For each subject, present the individual concentration in linear and semi-log scale.
Figure 16.2.5.2	Spaghetti Plot of RGLS8429 Overlay of Individual RGLS8429 Plasma Concentrations-Time by Dose (PK Full Population) (Note: the individual concentration will be presented in the same plot for linear and semi-log scales.)
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