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

INVESTIGATIONAL PRODUCT (IP)	**RGLS8429**
PROTOCOL NUMBER:	**RGLS8429-01 v3.0**
PROTOCOL DATE:	**6 June 2022**
IND NUMBER:	**158708**
SPONSOR NAME / ADDRESS:	**Regulus Therapeutics Inc.** **4224 Campus Point Court** **Suite 210** **San Diego, CA 92121** **United States**

DOCUMENT HISTORY

Version	Version Date	Summary of Changes and Rationale
Version 1.0	9 March 2022	Not Applicable
Version 2.0	09 May 2022	The protocol is being amended to: 1. NCI CTCAE Version 5.0 scale to rate adverse event severity in this healthy volunteer study, is replaced with the Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September 2007) for all the parameters except for serum creatinine. The KDIGO Clinical Practice Guideline for Acute Kidney Injury (https://kdigo.org/wp-content/uploads/2016/10/KDIGO-2012-AKI-Guideline-English.pdf) will be used to rate serum creatinine adverse event severity. Add AKI criteria to Section 3.2, Dose Escalation and Stopping Rules. 2. Increase the sampling window for the collection of post-dose pharmacokinetic plasma samples when multiple procedures are being performed at the same nominal timepoints. 3. Clarify that the NuvaRing is an acceptable depot contraceptive. 4. Allow the pre-dose ECG tracing to be obtained and the SARA test to be performed, prior to the start of Holter monitoring at the 60 minute pre-dose timepoint on Day 1.
Version 3.0	6 June 2022	The protocol is being amended to: 1. Change the RGLS8429 dose for cohort 4 from ≤ 5.0 mg/kg to 4.0 mg/kg. The dose of 4mg/kg has been selected for cohort 4 and is within the dose range supported by non-clinical toxicology and

Version	Version Date	Summary of Changes and Rationale
		pharmacokinetic studies, where RGLS8429 demonstrated dose-proportional exposure, and low variability. Hence, an interim PK analysis is not needed for dose level selection for cohort 4. 2. Correct minor typographical errors and clarify the description of the collection of potential renal biomarkers in urine

SPONSOR SIGNATURE PAGE

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Signature of Sponsor Lead Physician **DATE**

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SITE PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Signature of Site Principal Investigator	**DATE**
Printed Name of Site Principal Investigator **Institution Name:** _____ By my signature, I agree to personally supervise the conduct of this study at my study site and to ensure its conduct is in compliance with the protocol, informed consent, Institutional Review Board (IRB)/Ethics Committee (EC) procedures, instructions from Regulus representatives, the Declaration of Helsinki, International Conference on Harmonisation (ICH) Good Clinical Practices Guidelines, and local regulations governing the conduct of clinical studies.	

PROTOCOL SYNOPSIS

Study Title:

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

Protocol Number: RGLS8429-01 (IND 158708)

Indication: Treatment of Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Study Type: Phase 1, dose range-ranging study in healthy volunteers

Study Population: Approximately 32 healthy volunteers

Study Sites: Phase 1 Clinical Research Unit in the USA

Sponsor: Regulus Therapeutics Inc.

Study Drugs:

RGLS8429 injection will be provided in 2 mL clear glass vials containing sufficient volume to extract the labeled volume of 1 mL of 150mg/mL of RGLS8429 in 0.3% saline.

Placebo injection will be provided in 2 mL clear glass vials containing sufficient volume to extract the labeled volume of 1 mL of 1.5 µg/mL of riboflavin in 0.9% sodium chloride.

Both RGLS8429 and placebo solutions are clear and colorless to pale yellow.

Study Rationale:

ADPKD is a monogenic systemic disease characterized by slowly progressive, bilateral enlargement of fluid-filled polycystic kidneys where increase in total kidney volume occurs long before detectable decline of renal function. Fifty percent of ADPKD patients develop end-stage renal disease (ESRD) by the age of 60, accounting for 5% of all ESRD patients in the United States (US) requiring renal replacement therapy such as dialysis or transplant.

RGLS8429 is a potent and selective anti-miR-17 oligonucleotide that dose-dependently inhibits the function of miR-17 in kidney cells in vitro and in animal models of polycystic kidney disease (PKD) in vivo. The expression of the miR-17 family of microRNAs has been shown to be increased in kidney samples from both mouse ADPKD models and patients with ADPKD. Kidney-specific knockdown of the miR-17~92 cluster attenuates disease progression and increases overall survival in multiple transgenic mouse models of PKD and subcutaneous (SC) dosing with RGLS8429 resulted in improvement in two different mouse models of PKD. In the *Pkd1*$^{F/RC}$ mouse model, RGLS8429 significantly reduced kidney weight-to-body weight ratio, serum blood urea nitrogen (BUN), kidney cyst index, and kidney cyst proliferation. In the *Pcy*/DBA mouse model, RGLS8429 significantly reduced kidney weight-to-body weight ratio, Taken together, these studies demonstrate that miR-17 is a promising therapeutic target for the treatment of ADPKD.

Study Objectives:

Primary Objective

- To assess the safety and tolerability of single ascending doses of RGLS8429

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 pharmacokinetic (PK) properties of RGLS8429

Exploratory Objective

- To characterize multiple potential renal biomarkers

Study Endpoints:

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

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)
    - 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)

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)

Study Design and Methodology:

In this randomized, double-blind, placebo-controlled Phase 1 study, a single ascending dose of RGLS8429 or placebo will be administered via SC injection to healthy volunteers to evaluate the safety, tolerability, and PK of RGLS8429.

Eight subjects will be randomized 3:1 RGLS8429:Placebo into each of 4 sequential cohorts (32 subjects in total).

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 hr prior to dosing the remaining subjects in the cohort.

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 randomization procedures.

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 hr 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 hr after dosing.

Dose Escalation and Stopping Rules:

Prior to dose escalation, the Sponsor and the Investigator will perform a blinded review of the safety data (e.g., AE and safety laboratory tests through Day 15) for all subjects at the current dose level to make a dose escalation decision.

If 2 or more subjects at the same dose level experience the same or similar Grade ≥ 2 AEs (including clinically significant laboratory AEs) or if 1 subject experiences a Grade ≥ 3 AE or a serious adverse event (SAE) judged to be related to study drug, the Sponsor's Lead Physician will be unblinded to the treatment. Also, if 2 subjects develop stage 1 AKI or 1 subject develops stage 2 AKI the Sponsor's Lead Physician will be unblinded to the treatment.

The Sponsor's Lead Physician will determine whether the nature, severity, or number of event(s) warrants:

1. Continuing with the study as planned
2. Continuing with the study, but add additional safety evaluation(s)
3. Suspend dose escalation and enroll additional subjects at the same dose level
4. Add an intermediate-dose cohort or adjust the next dose level
5. Suspend or terminate the study

Study Population:

Approximately 32 healthy volunteers

Inclusion Criteria

1. Male or female, 18 to 55 years of age
2. Continuous nonsmoker who has not used nicotine-containing products for at least 3 months prior to dosing
3. Body mass index (BMI) 18 to 35 kg/m^2
4. Medically healthy, with no clinically significant medical history in the opinion of the Investigator
5. Estimated glomerular filtration rate (eGFR) ≥ 90 mL/min/1.73 m^2 (see Section 2.3.3)
6. Platelet count within the normal limits
7. Hemoglobin concentration within the normal limits
8. Liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], and bilirubin [total and direct]) must be ≤ the upper limit of normal (ULN)
9. Female subjects of childbearing potential must not be lactating and must have no plans to become pregnant during the course of the study through 28 days after the administration of study drug. Female subjects of childbearing potential who are heterosexual must agree to use one of the following methods of contraception considered to be highly effective (i.e., results in <1% failure rate when used consistently and correctly) from Screening through 28 days after the administration of study drug
    a. Intrauterine device (IUD) or intrauterine system (IUS) in place for at least 3 months prior to study drug administration
    b. Partner has had a vasectomy. Vasectomy in the partner is considered to be highly effective only if the partner is the sole sexual partner of the female subject of childbearing potential and had his vasectomy performed 6 months or more prior to randomization.
    c. Stable hormonal contraception associated with inhibition of ovulation (with approved oral, transdermal, or depot [e.g., NuvaRing, depot injection, etc.] regimen) for at least 3 months prior to study drug administration
10. A female subject of non-childbearing potential must have undergone one of the following sterilization procedures at least 6 months prior to the administration of study drug:
    a. Hysterectomy
    b. Bilateral oophorectomy
    c. Bilateral tubal occlusion
    d. Bilateral salpingectomy

    or be postmenopausal with no menstrual periods for at least 1 year prior to the administration of study drug.
11. Non-vasectomized heterosexual male subjects must agree to use a condom with spermicide or abstain from sexual intercourse from Day 1 until 28 days after the administration of study drug. (No restrictions are required for a vasectomized heterosexual male provided his vasectomy was performed 6 months or more prior to study start. A heterosexual male who has been

vasectomized less than 6 months prior to study start must follow the same restrictions as a non-vasectomized heterosexual male.)

12. Male and female subjects must agree not to donate sperm or preserve eggs (ova), respectively, from Day 1 until 28 days after the administration of study drug
13. Must agree not to donate blood in the 28 days prior to randomization or plasma in the 7 days prior to randomization through the end of study (EOS) visit
14. Seated blood pressure (systolic/diastolic) is ≥ 90/40 mmHg and ≤ 140/90 mmHg
15. Seated heart rate is ≥ 40 bpm and ≤ 99 bpm
16. Fridericia-corrected QT (QTcF) interval is < 460 msec (males) or < 480 msec (females) and without any ECG findings deemed clinically significant by the Investigator
17. Must understand and consent to the study procedures explained in the informed consent form (ICF) and be willing and able to comply with the protocol

Exclusion Criteria

1. Subject is mentally incapacitated or has significant emotional problems
2. Any medical condition or social circumstance that, in the opinion of the Investigator, may make the subject unlikely to complete the study or comply with study procedures and requirements, or may pose a risk to the subject's safety
3. History of malignancy, except for successfully treated squamous or basal cell carcinoma skin cancer
4. History or presence of opportunistic infection, in the opinion of the Investigator
5. History or presence of any clinically significant local infection (e.g., cellulitis, abscess) or systemic infection (e.g., septicemia) within 3 months prior to Screening
6. Fever (body temperature ≥ 100.4°F) or symptomatic viral or bacterial infection within 14 days prior to Screening, at Screening, at Day -1 check-in, or at Day 1 prior to dosing
7. History of any clinically significant reaction to previous injection of an oligonucleotide product in the opinion of the Investigator
8. History or presence of alcoholism or drug abuse within the past 2 years prior to Screening
9. Positive urine drug or alcohol results
10. Has ≥ 1 protein on urine dipstick
11. Positive results at Screening for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C virus (HCV) antibody
12. Positive urine cotinine
13. Unable to refrain from, or anticipates the use of, any drug, including prescription and non-prescription medications and herbal remedies, from 14 days prior to dosing until completion of the EOS visit, except as approved by the Investigator
14. History of any major surgical procedure within 3 months prior to dosing (excluding minor cosmetic surgery or minor dental procedures)

15. Subjects with tattoo(s) or scarring at or near the site of SC injection or any other condition that may interfere with injection site examination(s), in the opinion of the Investigator
16. Participation in another clinical trial and/or exposure to any investigational drug or approved therapy for investigational use within 28 days or 5 half-lives of the investigational drug's dosing, whichever is longer, prior to dosing. The 28-day and 5-half-life windows will be calculated from the date of the last dosing in the previous study to Day 1 of the current study.

Statistical Methods:

Tabular summaries and analysis results will be generated by dose level for RGLS8429 and placebo (pooled across all cohorts). Descriptive statistics by dose level and treatment group will be tabulated by visit.

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.

Safety Analysis

Safety data (e.g., frequencies of treatment-emergent adverse events [TEAEs] and SAEs, changes from baseline in SARA test scores, safety laboratory test results, vital signs, ECG parameters and Holter monitoring) will be summarized descriptively by dose level and treatment group, as appropriate. No formal statistical tests will be conducted to assess the safety or tolerability of RGLS8429.

Pharmacokinetic Analysis

PK samples will be analyzed for RGLS8429 concentration using a validated, sensitive, specific bioanalytical method. The laboratory analyzing the PK samples will be unblinded, so PK analysis is performed only on subjects receiving RGLS8429. Placebo samples will be analyzed if necessary.

Using non-compartmental methods, the plasma and urine concentration versus time data will be used to derive the following PK parameters: C_{max}, T_{max}, $AUC_{0\text{-}24,}$ $AUC_{0\text{-}t}$, AUC_{inf}, $t_{½}$, CL/F, V_z/F, fe, and Ae.

Dose proportionality will be explored using the power model for C_{max}, $AUC_{0\text{-}24,}$ $AUC_{0\text{-}t}$, and AUC_{inf} s data permit.

Biomarker Analysis

Descriptive statistics will be used to characterize urine (e.g., PC1, PC2, NGAL, KIM-1, MCP-1, B2M, and CLU) and serum (e.g., Cystatin-C, IGFALS, Alb, CT-proAVP, N-acetyl-1-methylhistidine, and others) biomarkers in all subjects.

Table 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
Concomitant Medications	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																
Serum biomarkers [16, 19]	X	X																	
Urine biomarkers [17, 19]	X	X																	
Holter monitoring [18]			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 gamma-glutamyl 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 ±15 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 exploratory biomarkers (e.g., 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 analysis.

TABLE OF CONTENTS

LIST OF TABLES

1. INTRODUCTION

1.1. Disease Background

ADPKD, the most common form of polycystic kidney disease (PKD), is a monogenic systemic disease driven mostly by mutations in either *PKD1* or *PKD2* genes, leading to reduced expression and/or function of their encoded-proteins polycystin-1 (PC1) and/or polycystin-2 (PC2), excessive proliferation of the renal tubular epithelium and formation of multiple cysts (Torres, 2009; Chapman, 2015). Indeed, PC1 and PC2 levels in patients' urinary exosomes were shown to correlate inversely with the height-adjusted total kidney volume (Hogan, 2015). ADPKD is characterized by slowly progressive, bilateral enlargement of the fluid-filled polycystic kidneys, with kidney volume increasing long before detectable decline of kidney function. In addition to renal cysts, extrarenal cysts can also develop in the liver, pancreas, seminal vesicles, and arachnoid. Typical symptoms include flank pain, hematuria, proteinuria, renal colic, urinary tract infection, hypertension, and intracranial aneurysms. Fifty percent of ADPKD patients develop end-stage renal disease by the age of 60, accounting for 5% of all end-stage renal disease patients in the United States (US) requiring renal replacement therapy such as dialysis or transplant (Chebib, 2016).

1.2. miR-17 and ADPKD

PKD1 and *PKD2* genes, which are central to ADPKD pathogenesis, are both direct target genes of miR-17 (Patel, 2013). Increased miR-17 expression has been detected in kidney samples from both mouse ADPKD models and human ADPKD patients (Hajarnis, 2017). Kidney-specific overexpression of miR-17~92 repressed *Pkd1* and *Pkd2* expression and produced kidney cysts in mice (Patel, 2013), whereas genetic knockdown of miR-17~92 attenuated disease progression in multiple mouse models of PKD (Patel, 2013 and Hajarnis, 2017). Importantly, inhibition of miR-17 has been shown to increase PC1 and PC2 levels and reduce cyst growth in primary human ADPKD cyst cultures (Hajarnis, 2017 and Lee, 2019), whereas re-expression of PC1 and PC2 has been shown to rapidly reverse total kidney volume, cell proliferation, fibrosis, and inflammation in mouse models of ADPKD (Dong, 2021). Therefore, preferential targeting of the miR-17 family of miRNAs in the kidney is an attractive therapeutic approach to treat ADPKD.

Nonclinical pharmacology studies using anti-miR-17 oligonucleotides including the first-generation investigational product, RGLS4326, demonstrated that miR-17 inhibition confers therapeutic efficacy in multiple mouse models of PKD *in vivo* and human ADPKD donor primary cyst culture samples *in vitro* (Hajarnis, 2017 and Lee, 2019). Moreover, miR-17 inhibition has been shown to improve expression of pathogenic gene networks and pathways in PKD, including de-repression of messenger RNA (mRNA) of *Pkd1* and *Pkd2* genes in mouse models of ADPKD. The coordinated alteration of the polycystic kidney transcriptome towards a normal kidney profile following miR-17 inhibition exemplifies the potential of an anti-miR-17 oligonucleotide as a disease-modifying treatment for ADPKD (Lee, 2019). Importantly, recent data from the first cohort of a Phase 1b clinical trial (NCT04536688) demonstrated clinical evidence that treatment with RGLS4326 increased urinary PC1 and PC2 level (Lee, 2021) in

patients with ADPKD, likely through inhibition of miR-17 in the kidney. These results imply ongoing cis-repression of PC1 and PC2 by the overexpressed miR-17 in ADPKD patients prior to treatment with anti-miR-17, further validating miR-17 as a therapeutic target for ADPKD.

1.3. Nonclinical Pharmacokinetics, Pharmacology, and Toxicology

The following is a brief summary of the Nonclinical information. Please see the RGLS8429 Investigator's Brochure for a more detailed presentation of this information.

In nonclinical ADPKD efficacy studies, RGLS8429 treatment reduced disease progression in both *Pkd1*$^{F/RC}$ and *Pcy*/DBA mouse models. In the *Pkd1*$^{F/RC}$ mouse model, RGLS8429 caused a significant reduction in kidney weight-to-body weight ratio, serum blood urea nitrogen (BUN), kidney cyst index, and kidney cyst proliferation. In the *Pcy*/DBA mouse model, RGLS8429 reduced kidney weight-to-body weight ratio significantly. In addition, the combination of RGLS8429 and tolvaptan demonstrated a greater reduction in disease progression than either drug alone in the *Pcy*/DBA mouse model.

Based on in silico bioinformatics analysis and in vitro receptor binding studies, RGLS8429 appears to have low potential for off-target effects towards ribonucleic acid (RNA) and receptor targets. RGLS8429 had no effects on cardiovascular, respiratory, and central nervous system function at supraclinical concentrations and dose levels in animals. RGLS8429 had no meaningful effect on the human ether-à-go-go-related gene (hERG) potassium channels in vitro.

In nonclinical PK and toxicokinetic studies, SC administration of RGLS8429 demonstrated rapid absorption (time to maximum concentration [T_{max}] 1-2 hr) followed by relatively rapid plasma clearance. The plasma $t_{1/2}$ in mice (~ 2 hr) whereas $t_{1/2}$ ranged between 6 -7 hr in monkeys.

Plasma exposure increased in a generally dose-proportional manner across all dose levels, and no meaningful accumulation was observed in mice or monkeys. RGLS8429 is highly bound to plasma protein in mice, monkeys and humans, monkeys, and mice (79.1% ,86.6%, and 81.6 %, respectively). RGLS8429 distributes rapidly to the kidney and liver, is metabolically stable with only trace amounts of metabolites measured, and is excreted rapidly in the urine, primarily in the first 24 hours after dosing. Of note, the decreased renal function present in patients with ADPKD is expected to decrease the renal clearance of RGLS8429, and therefore, will be monitored closely in clinical trials.

Based upon *in vitro* studies, RGLS8429 has little potential for PK interactions with other drugs via either cytochrome P450 enzymes or transporters. In *Pcy*/DBA mouse model, co-administration of RGLS8429 and tolvaptan had no significant effect (defined as <2-fold change) on tolvaptan PK in plasma and RGLS8429 PK in kidney and liver.

Toxicity studies of RGLS8429 completed to date demonstrate that RGLS8429 is not acutely toxic at up to 2000 mg/kg in mice.

In a 13-week Good Laboratory Practice (GLP) toxicity study in CD-1 mice evaluating 60, 300 and 600 mg/kg weekly of RGLS8429, the no observed adverse effects level (NOAEL) was determined to be 60 mg/kg, with dose-limiting hepatotoxicity at higher dose levels. Minimal single-cell hepatocellular necrosis with 2 to 3-fold elevations in ALT and AST were observed at 600 mg/kg; in addition, one

mouse at 300 mg/kg had single-cell hepatocellular necrosis in the absence of elevations in ALT or AST at that dose level. Other findings at 300 mg/kg included: decreased thymic lymphocytes with corresponding decreased thymus size and weight, and mild decreased red cell mass with compensatory hematopoiesis in the spleen and bone marrow, both of which were considered to be anti-miR-17 effects. Additional changes at 300 mg/kg included transient nose/muzzle swelling post-dose, decreased serum cholesterol, karyomegaly and increased mitotic figures of hepatocytes in the liver (correlating with increased liver weight), and increased foamy macrophage infiltration in the injection site(s), all of which were considered non-adverse.

In a 13-week GLP toxicity study in cynomolgus monkeys evaluating 15, 75 and 150 mg/kg weekly of RGLS8429, highest dose of RGLS8429 (150 mg/kg) was well tolerated and was determined to be the NOAEL. The following findings, all of which were considered to be non-adverse, include: minimal increases in fibrinogen, minimal but transient increases in activated thromboplastin time (APTT), dose-related dark red discoloration at the injection site with microscopic findings of fibrosis, macrophage infiltration, perivascular inflammation, and hemorrhage, vacuolated macrophages in the lymph nodes, and basophilic granules within Kupffer cells in the liver.

SC administration of the clinical formulation (150 mg/kg in water for injection) was well tolerated in rabbits. RGLS8429 has shown no potential for genotoxicity in two *in vitro* studies (Ames test and micronucleus assay).

RGLS8429 did not cause overt cytotoxicity and showed no evidence of mitochondrial toxicity. Although certain oligonucleotides have exhibited the propensity to cause thrombocytopenia in animals and/or humans, *in vitro* studies suggest that RGLS8429 has minimal potential for adverse effects on platelets, which is consistent with the absence of adverse effects in mice and monkeys.

In addition, RGLS8429 did not induce genes involved in inflammation, consistent with the low pro-inflammatory effects observed.

1.4. Dose Rationale

The starting dose and dose levels selected for first-in-human dosing are based on the NOAELs and toxicity profiles in the GLP 13-week toxicity studies of RGLS8429. The NOAELs in mice and monkeys were 60 mg/kg and 150 mg/kg, respectively, for human equivalent doses (HED) of 5 and 48, respectively. Based on the HED in the more sensitive species (mouse) and using a safety factor of approximately 10 (in accordance with FDA Guidance for Industry, *Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers* [July 2005]), the starting dose in this study is 0.5 mg/kg and maximum dose will not exceed 5 mg/kg.

2. STUDY OBJECTIVES, ENDPOINTS, AND ASSESSMENTS

2.1. Study Objectives

Primary Objective

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

Secondary Objectives

- To identify DLT and to determine the MTD of a single SC dose of RGLS8429
- To characterize the PK properties of RGLS8429

Exploratory Objective

- To characterize multiple potential renal biomarkers

2.2. Study Endpoints

Primary Endpoint

- Incidence of adverse events (AEs) and change in safety laboratory test results, vital signs, SARA, and ECG parameters and Holter monitoring over time

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)
  - 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)

Exploratory Endpoints

- Characterize multiple potential renal biomarkers in urine (e.g., PC2, NGAL, KIM-1, MCP-1, B2M, and CLU) and in serum (e.g., Cystatin-C, IGFALS, Alb, CT-proAVP, N-acetyl-1-methylhistidine, and others)

2.3. Study Assessments and Procedures

Informed consent will be obtained prior to performing any study procedures.

2.3.1. Demographics, Medical History and Concomitant Medications

Subject demographic data collection, a review of each subject's medical history, and prior/current concomitant medications and medical procedures will be conducted according to the Schedule of Assessments and Procedures (see Table 1) to ensure all study entry criteria are met.

2.3.2. Physical Examinations

Physical examinations may be conducted by a physician or another medically qualified individual such as a physician's assistant or a nurse practitioner according to the Schedule of Assessments and Procedures (see Table 1). All physical examinations must include a complete neurologic exam. A full physical examination will include general appearance, head, ears, eyes, nose, mouth, skin, heart, lung, lymph nodes, and the gastrointestinal, musculoskeletal, respiratory, and neurological systems. The limited physical examination will be focused on general appearance, the respiratory, the neurological, and cardiovascular systems, and subject-reported symptoms.

2.3.3. eGFR Calculation

The Investigator will calculate the estimated glomerular filtration rate (eGFR) using the 2021 (chronic kidney disease epidemiology collaboration equation) CKD-EPI Creatinine, Age, Sex Equation (Inker, 2021) during Screening:

$$142 \times \min(Scr/k,1)^{\alpha} \times \max(Scr/k,1)^{-1.200}\ 0.9938^{age} \times 1.012\ [\text{if female}]$$

Scr is serum creatinine

k is 0.7 for females and 0.9 males

α is -0.241 for females and -0.302 for males

min indicates the minimum of Scr/k or 1

max indicates the maximum of Scr/k or 1

2.3.4. Height and Weight Measurements

Height will be recorded only during Screening.

Weight will be measured according to the Schedule of Assessments and Procedures (see Table 1). For measuring weight, a scale with appropriate range and resolution should be used and must be placed on a stable, flat surface. Subjects must remove shoes, bulky layers of clothing, and jackets so that only light clothing remains. Subjects must also remove the contents of their pockets and should remain still during measurement of weight.

2.3.5. Vital Signs

Body temperature, seated systolic and diastolic blood pressure (BP), heart rate (HR), and respiratory rate (RR) will be measured at times specified in the Schedule of Assessments and Procedures (see Table 1).

Body temperature may be measured orally, via the external auditory canal, or via the temporal artery (forehead). Temperature should be measured in a consistent manner for each subject throughout the entire study.

The use of an automated device for measuring BP and HR is acceptable. For BPs, a properly sized and calibrated BP cuff should be used for each measurement. Subjects should be seated for approximately 5 minutes before the BP is measured. The BP should be measured with the subject's feet flat on the floor, back supported, and arm supported at the level of the heart. The BP should be recorded to the nearest millimeters of mercury (mmHg).

When the timing of these measurements coincides with a blood sample collection, obtain the vital signs, and then collect the blood sample.

2.3.6. ECGs and Holter Monitoring

ECGs and Holter monitoring should be obtained at times specified in the Schedule of Assessments and Procedures (see Table 1).

The Investigator or a medically qualified designee should review all ECGs obtained during the study. Consultation with a cardiologist should be obtained if necessary. Record new or worsening clinically significant abnormalities as an AE. Post-treatment ECG results should be compared with the pre-dose ECG assessments.

Holter monitoring should be performed by the Investigator or a medically qualified designee at times specified in the Schedule of Assessments and Procedures (see Table 1). The Holter data will be analyzed centrally, and the independent central reader will remain blinded to treatment group. Throughout the duration of Holter monitoring, other procedures and SARA assessment may occur. At the specified timepoints, the procedures will be performed in this order: ECG, vital signs, PK and/or other blood collection, and then followed by the SARA assessment. Details and procedures will be provided in the Holter Monitoring Manual.

2.3.7. Safety Laboratory Tests

The Safety Laboratory Tests listed in Table 2: should be obtained at the times specified in the Schedule of Assessments and Procedures (see Table 1). Detailed collection, processing, storage, and shipment instructions will be provided in a Laboratory Manual.

Table 2: Safety Laboratory Tests

Hematology – Complete Blood Count	
• Hemoglobin (Hb) • Hematocrit (Hct) • Red Blood Cell (RBC) Count • Mean Corpuscular Volume (MCV) • Mean Corpuscular Hemoglobin (MCH) • Mean Corpuscular Hemoglobin Concentration (MCHC)	• Platelet Count • White Blood Cell (WBC) Count and Differential • Absolute Neutrophil Count • Absolute Lymphocyte Count • Absolute Monocyte Count • Absolute Eosinophil Count • Absolute Basophil Count
Chemistry – Metabolic Panel	
• Glucose • Calcium • Albumin (Alb) • Total Protein • Sodium • Potassium • Bicarbonate (Total CO_2) • Chloride • Blood Urea Nitrogen (BUN) • Uric Acid • Creatine kinase (CK)	• Phosphate • Lactate Dehydrogenase (LDH) • Blood Urea Nitrogen (BUN) • Creatinine (Scr) • Alkaline Phosphatase (ALP) • Alanine Aminotransferase (ALT) • Aspartate Aminotransferase (AST) • Gamma-Glutamyl Transferase (GGT) • Total Bilirubin • Direct Bilirubin
Urinalysis [a]	
• Specific Gravity • pH • Protein • Glucose • Ketones	• Blood (Hemoglobin) • Leukocyte Esterase Nitrite • Bilirubin • Urobilinogen • Microscopic Examination [b]
Coagulation	
• International Normalized Ratio (INR) • Prothrombin Time (PT)	• Activated Partial Thromboplastin Time (aPTT) • Fibrinogen
Lipids	
• Total cholesterol • Triglycerides	• High-density Lipoprotein (HDL) • Low-density Lipoprotein (LDL)

Unscheduled safety laboratory assessments may be obtained at any time during the study to assess potential safety concerns.

a Urinalysis testing will be conducted using standard commercial test strips (urine dipstick).

b Only if urine dipstick is positive for blood, protein, or leukocyte esterase nitrate.

2.3.8. Pregnancy Test

For female subjects of childbearing potential, a serum pregnancy test will be performed at Screening. Urine pregnancy tests will be performed at the remaining specified visits according to the Schedule of Assessments and Procedures (see Table 1). A positive urine pregnancy test will be confirmed with a serum pregnancy test.

2.3.9. Urine Drug and Alcohol Screening Test

The drug and alcohol screen will include measurement of opiates, cocaine, heroin, phencyclidine, amphetamines (including ecstasy), barbiturates, benzodiazepines, cannabinoids, and alcohol according to the Schedule of Assessments and Procedures (see Table 1).

2.3.10. HBsAg, HCV Antibody, and HIV-1/2 Antibodies Screening Tests

All subjects will be tested for HBsAg, HCV antibody, and HIV-1/2 antibodies at Screening.

2.3.11. Scale for the Assessment and Rating of Ataxia (SARA)

The SARA test is used in this study to detect possible central nervous system impairment by the study drug and should be performed according to the Schedule of Assessments and Procedures (see Table 1). When timepoints to perform the SARA assessment are near timepoints for other procedures, the procedures will be performed in this order: ECG, vital signs, PK and/or other blood collection, and then followed by the SARA assessment. The test should be performed by an Investigator who has been trained to conduct neurologic evaluations and should ideally be performed by the same individual during the study to minimize the variability in how the test is performed and scored. The scale is presented in Appendix B.

2.3.12. Pharmacokinetic Samples

Plasma PK samples will be collected at the times specified in the Schedule of Assessments and Procedures (see Table 1).

All efforts should be made to obtain PK samples at the specified nominal time relative to dosing. 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 ±15 minutes, 12 hr ±30 minutes, and 24 hr±60 minutes. The exact date and time of collection for each sample should be noted on the source document and in the case report form (CRF). Detailed collection, processing, storage, and shipment instructions for PK samples will be provided in the Laboratory Manual.

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) and the last void will be timed for 24 hours post-dose. Detailed collection, processing, storage, and shipment instructions for PK samples will be provided in the Laboratory Manual.

2.3.13. Serum Biomarkers

Collect serum samples during Screening and on Day -1 according to the Schedule of Assessments and Procedures (see Table 1) for exploratory biomarker analysis (e.g., Cystatin-C, IGFALS, Alb, CT-proAVP, N-acetyl-1-methylhistidine, and others). Detailed collection, processing, storage, and shipment instructions for serum biomarker samples will be provided in the Laboratory Manual.

2.3.14. Urine Biomarkers

Collect at least 150 mL (**more if possible**) of mid-stream free-flow urine for analysis of exploratory biomarkers (PC1, and PC2, NGAL, KIM-1, MCP-1, B2M, CLU) during Screening and on Day -1 according to the Schedule of Assessments and Procedures (see Table 1). These samples must NOT be the first urination of the day (to avoid too much debris in the sample). Detailed collection, processing, storage, and shipment instructions for urine biomarker samples will be provided in the Laboratory Manual.

2.3.15. Exploratory Biomarkers

Residual plasma, serum, and urine samples (e.g., unused or backup) not utilized in required testing of PK/biomarkers will be retained and used to measure the concentrations of multiple potential exploratory renal function biomarkers in healthy volunteers. Subjects will provide consent for the use of these residual samples for exploratory tests.

3. STUDY DESIGN

3.1. Overview of Study Design

In this randomized, double-blind, placebo-controlled Phase 1 study, a single ascending dose of RGLS8429 or placebo will be administered via SC injection to healthy volunteers to evaluate the safety, tolerability, and PK of RGLS8429. 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 hr prior to dosing the remaining subjects in the cohort.

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 randomization procedures.

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 hr 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 hr after dosing.

3.2. Dose Escalation and Stopping Rules

Prior to dose escalation, the Sponsor and the Investigator will perform a blinded review of the safety data (e.g., AE and safety laboratory tests through Day 15) on all subjects at the current dose level to make a dose escalation decision.

If 2 or more subjects at the same dose level experience the same or similar Grade ≥ 2 AEs (including clinically significant laboratory AEs) or if 1 subject experiences a Grade ≥ 3 AE or a SAE judged to be related to study drug, the Sponsor's Lead Physician will be unblinded to the treatment. Also, if 2 subjects develop stage 1 AKI or 1 subject develops stage 2 AKI the Sponsor's Lead Physician will be unblinded to the treatment.

The Sponsor's Lead Physician will determine whether the nature, severity, or number of event(s) warrants:

1. Continuing with the study as planned
2. Continuing with the study, but add additional safety evaluation(s)
3. Suspend dose escalation and enroll additional subjects at the same dose level
4. Add an intermediate-dose cohort or adjust the next dose level
5. Suspend or terminate the study

3.3. Study Duration for Subjects

Subjects will participate in the study for up to 57 days (Screening up to 28 days, Treatment 1 day, Follow-up 28 days).

3.4. End of Trial

The End of Trial is defined as either the date of the last visit for the last subject to complete the post-treatment follow-up, or the date of receipt of the last data point from a subject, whichever is the later date.

4. STUDY POPULATION

4.1. Number of Subjects

Approximately 32 healthy volunteers will be enrolled. Subjects may be replaced at the discretion of the Sponsor (e.g., subject noncompliance with the protocol, early withdrawal, missing data, etc.).

4.2. Inclusion Criteria

Subjects must meet the following criteria to be eligible for study participation:

1. Male or female, 18 to 55 years of age
2. Continuous nonsmoker who has not used nicotine-containing products for at least 3 months prior to dosing
3. Body mass index (BMI) 18 to 35 kg/m^2
4. Medically healthy, with no clinically significant medical history in the opinion of the Investigator.
5. Estimated glomerular filtration rate (eGFR)≥ 90 mL/min/1.73 m^2 (see Section 2.3.3)
6. Platelet count within the normal limit
7. Hemoglobin concentration within the normal limits
8. Liver function tests (ALT, AST, ALP, and bilirubin [total and direct]) must be ≤ the ULN
9. Female subjects of childbearing potential must not be lactating and must have no plans to become pregnant during the course of the study through 28 days after the administration of study drug. Female subjects of childbearing potential who are heterosexual must agree to use one of the following methods of contraception considered to be highly effective (i.e., results in <1% failure rate when used consistently and correctly) from Screening through 28 days after the administration of study drug
    a. Intrauterine device (IUD) or intrauterine system (IUS) in place for at least 3 months prior to study drug administration
    b. Partner has had a vasectomy. Vasectomy in the partner is considered to be highly effective only if the partner is the sole sexual partner of the female subject of childbearing potential and had his vasectomy performed 6 months or more prior to randomization.
    c. Stable hormonal contraception associated with inhibition of ovulation (with approved oral, transdermal, or depot [e.g., NuvaRing, depot injection, etc.] regimen) for at least 3 months prior to study drug administration
10. A female subject of non-childbearing potential must have undergone one of the following sterilization procedures at least 6 months prior to administration of study drug:
    a. Hysterectomy
    b. Bilateral oophorectomy
    c. Bilateral tubal occlusion

d. Bilateral salpingectomy

or be postmenopausal with no menstrual periods for at least 1 year prior to administration of study drug.

11. Non-vasectomized heterosexual male subjects must agree to use a condom with spermicide or abstain from sexual intercourse from Day 1 until 28 days after the administration of study drug. (No restrictions are required for a vasectomized heterosexual male provided his vasectomy was performed 6 months or more prior to study start. A heterosexual male who has been vasectomized less than 6 months prior to study start must follow the same restrictions as a non-vasectomized heterosexual male.)
12. Male and female subjects must agree not to donate sperm or preserve eggs (ova), respectively, from Day 1 until 28 days after the administration of study drug
13. Must agree not to donate blood in the 28 days prior to randomization or plasma in the 7 days prior to randomization through the EOS visit
14. Seated blood pressure (systolic/diastolic) is ≥ 90/40 mmHg and ≤ 140/90 mmHg
15. Seated heart rate is ≥ 40 bpm and ≤ 99 bpm
16. Fridericia-corrected QT (QTcF) interval is < 460 msec (males) or < 480 msec (females) and without any ECG findings deemed clinically significant by the Investigator
17. Must understand and consent to the study procedures explained in the informed consent form (ICF) and be willing and able to comply with the protocol

4.3. Exclusion Criteria

Subjects with any of the following will be excluded from participation in the study:

1. Subject is mentally incapacitated or has significant emotional problems
2. Any medical condition or social circumstance that, in the opinion of the Investigator, may make the subject unlikely to complete the study or comply with study procedures and requirements, or may pose a risk to the subject's safety
3. History of malignancy, except for successfully treated squamous or basal cell carcinoma skin cancer
4. History or presence of opportunistic infection, in the opinion of the Investigator
5. History or presence of any clinically significant local infection (e.g., cellulitis, abscess) or systemic infection (e.g., septicemia) within 3 months prior to Screening
6. Fever (body temperature ≥ 100.4°F) or symptomatic viral or bacterial infection within 14 days prior to Screening, at Screening, at Day -1 check-in, or at Day 1 prior to dosing
7. History of any clinically significant reaction to previous injection of an oligonucleotide product in the opinion of the Investigator
8. History or presence of alcoholism or drug abuse within the past 2 years prior to Screening
9. Positive urine drug or alcohol results
10. Has ≥ 1 protein on urine dipstick

11. Positive results at Screening for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C virus (HCV) antibody
12. Positive urine cotinine
13. Unable to refrain from, or anticipates the use of, any drug, including prescription and non-prescription medications and herbal remedies, from 14 days prior to dosing until completion of the EOS visit, except as approved by the Investigator
14. History of any major surgical procedure within 3 months prior to dosing (excluding minor cosmetic surgery or minor dental procedures)
15. Subjects with tattoo(s) or scarring at or near the site of SC injection or any other condition that may interfere with injection site examination(s), in the opinion of the Investigator
16. Participation in another clinical trial and/or exposure to any investigational drug or approved therapy for investigational use within 28 days or 5 half-lives of the investigational drug's dosing, whichever is longer, prior to dosing. The 28-day and 5-half-life windows will be calculated from the date of the last dosing in the previous study to Day 1 of the current study.

5. STUDY VISITS

Study visits and procedures will be performed as outlined in Table 1. Additional details on the study assessments and procedures are given in Section 2.3. The study will consist of Screening, Treatment, and Follow-up periods. Additional visits may be scheduled to evaluate an abnormal laboratory value or reported AE. For each study subject, written informed consent will be obtained prior to any protocol-related activities. After signing of the ICF, completion of all Screening assessments, and confirmation of eligibility, subjects will be randomized for the study.

5.1. Screening Period (Day -28 to Day -1)

Subjects will be screened during the 28 days prior to administration of study drug (Day 1) to confirm that they meet the entry criteria for the study. Some of the procedures must occur on Day -1 after the subject is admitted to the clinic. Subjects who fail Screening (i.e., do not meet entry criteria) may be rescreened with the approval of the Sponsor's Lead Physician.

The following procedures are to be performed during Screening and/or Day -1:

- Informed consent
- Demographics (date of birth [ddmmmyyyy], sex, race, and ethnicity)
- Medical history
- Complete physical examination; must include a complete neurologic examination
- Weight (Screening and Day -1) and height (Screening); calculate BMI using Screening values; Day -1 weight will be used for dosing
- Vital signs (Screening and Day -1)
- 12 -lead ECG (Screening); Print and review
- Safety laboratory tests (Screening and Day -1):
  - Hematology
  - Chemistry
  - Urinalysis
  - Lipids
- Coagulation tests (Day -1)
- Pregnancy test, if applicable (serum at Screening; urine at and Day -1)
- Urine drug and alcohol screen (Screening and Day -1)
- HBsAg, HCV, HIV-1/2 (Screening)
- Serum biomarker sample collection (Screening and Day -1)
- Urine biomarker sample collection (Screening and Day -1)
- Inclusion/exclusion criteria review (Screening and Day -1)
- Randomization after enrollment confirmed (Day -1) (see Section 6.4)

- Concomitant medications and procedures (Screening and Day -1)
- Admission to clinic (Day -1)

5.2. Treatment Period (Day 1 and Day 2)

The following procedures are to be performed on Day 1 and/or Day 2:

- Limited physical examination (pre-dose Day 1 and 24 hr post-dose Day 2)
- Vital signs (Day 1 pre-dose and 1, 2, 4, and 8 hr post-dose; Day 2, 24 hr post-dose)
- Holter monitoring should be initiated 1 hr pre-dose and continue for approximately 30 minutes after the 24-hr PK sample is obtained (Day 1 and Day 2).
- Print and review ECG tracings pre-dose and 4, 6, 12, and 24 hr post-dose.
- SARA assessment (Day 1 pre-dose and 4-6 hr post-dose; Day 2, 24 hr post-dose)

  Note: The pre-dose ECG tracing and SARA procedures may be performed prior to the start of Holter monitoring at the 1 hr pre-dose timepoint on Day 1.
- Safety laboratory tests (Day 2, 24 hr post-dose):
  - Hematology
  - Chemistry
  - Urinalysis
  - Lipids
- Coagulation test (Day 1, 4 hr post-dose and Day 2, 24 hr post-dose)
- AE monitoring (Day 1 through Day 2)
- Concomitant medications and procedures (Day 1 through Day 2)
- Study drug administration (Day 1 at Time Zero)
- PK plasma samples (Day 1 pre-dose and 2, 4, 6, 8, and 12 hr post-dose; Day 2, 24 hr post-dose. 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±15 minutes, 12 hr±30 minutes, and 24 hr±60 minutes.
- 24-hr urine collection on Day 1 and Day 2 for PK analysis will begin immediately after study drug dosing on Day 1 (0-24 hr). The subject should void immediately before dosing and at the end of the 24-hr collection period.
- Discharge from clinic (Day 2) no less than 6 hr after the 24-hr PK sample collection

5.3. Follow-Up Period (Day 3 to Day 29)

5.3.1. Follow-Up Visit (Day 8)

The following procedures are to be performed on Day 8:

- Limited physical examination; must include a complete neurologic examination

- Weight
- Vital signs
- Safety laboratory tests:
  - Hematology
  - Chemistry
  - Urinalysis
- AEs since the last visit
- Concomitant medications and procedures since last visit

5.3.2. Follow-Up Visit (Day 15)

The following procedures are to be performed on Day 15:

- Limited physical examination; must include a complete neurologic examination
- Vital signs
- Safety laboratory tests:
  - Hematology
  - Chemistry
  - Urinalysis
  - Lipids
- AEs since the last visit
- Concomitant medications and procedures since last visit
- Plasma PK sample

5.3.3. Follow-Up Visit (Day 22)

The following procedures are to be performed on Day 22:

- Limited physical examination; must include a complete neurologic examination
- Vital signs
- Safety laboratory tests:
  - Hematology
  - Chemistry
  - Urinalysis
- AEs since the last visit
- Concomitant medications and procedures since last visit

5.3.4. Follow-Up / End of Study [EOS] Visit (Day 29)

The following procedures are to be performed on Day 29; or if the subject withdraws from the study early, these procedures should be performed:

- Complete physical examination; must include a complete neurologic examination
- Weight
- Vital signs
- Safety laboratory tests
    - Hematology
    - Chemistry
    - Coagulation
    - Urinalysis
    - Lipids
- Urine pregnancy test, if applicable
- Urine drug and alcohol screen
- SARA Assessment
- AEs since the last visit
- Concomitant medications and procedures since the last visit

5.4. Unscheduled Visits

Unscheduled visits may be conducted at the Investigator's discretion and/or when repeat of laboratory assessments is needed. In addition, the following procedures will occur at any unscheduled visits:

- Adverse event evaluation
- Concomitant medications and procedures since last visit will be reviewed
- Additional assessments may be performed, as determined by the Investigator

6. STUDY DRUG

6.1. RGLS8429

RGLS8429 injection will be provided in 2 mL clear glass vials containing sufficient volume to extract the labeled volume of 1 mL of 150 mg/mL of RGLS8429 in 0.3% saline. The solution is clear and colorless to pale yellow.

6.2. Placebo

Placebo injection will be provided in 2 mL clear glass vials containing sufficient volume to extract the labeled volume of 1 mL of 1.5 µg/mL of riboflavin in 0.9% sodium chloride. The solution is clear and colorless to pale yellow.

6.3. Storage of Study Drug

Site staff must ensure that all study drug is stored in a secured area with controlled access under required storage conditions and in accordance with applicable regulatory requirements. To ensure that the investigative staff are not unblinded, only unblinded pharmacy staff and an unblinded clinical research associate should have access to the unblinded study drug. The clinic and non-pharmacy staff should be provided only with blinded syringes for injection to subjects.

Study drug vials should be stored in their original containers and in accordance with the labels. Study drug must be stored in a freezer at -20° ± 5°C. The study drug should be stored in its carton to protect it from light until it is removed from the carton to prepare for administration.

The site must be capable of measuring and documenting the daily minimum and maximum temperatures for all freezers used to store study drug. Temperature monitoring information should be captured from the time of study drug receipt throughout the duration of the study. A site procedure that ensures active evaluation for temperature excursions should be in place and appropriate documentation of temperature monitoring must be available. The minimum and maximum temperature should be checked each business day to confirm that no excursion occurred, and the site should have the capability to view the minimum/maximum temperature for all nonworking days upon return to normal operations. The temperature monitoring device and freezer used to store study drug should be inspected regularly to ensure that they are maintained in working order.

Any excursions from the labeled storage conditions should be reported to Regulus or designee upon discovery. In the event of an excursion, the site should ensure that the product is returned to the storage conditions described in the labeling as soon as possible. Deviations from the storage requirements, including any actions taken, must be documented, and reported to Regulus or designee.

Receipt of study drug, opening and closing the freezer, and other routine handling operations where the study drug is briefly out of the temperature range described in the labeling will not be considered excursions.

6.4. Subject Identification and Randomization

The Investigator or a designee will assign sequential Screening identification numbers to the subjects as they are screened for the study. Subjects will then be assigned different sequential subject identification numbers as they are enrolled in the study. Details regarding subject identification procedures will be provided in the Study Reference Manual.

Regulus or designee 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.

6.5. Preparation and Dispensing

Study drug will be prepared by an unblinded pharmacist or other qualified and trained personnel at the site as delegated on the delegation log. The blinded study drug will be provided to the blinded study personnel for administration according to the randomization procedures.

The study drug vials are for single use only. The Study Reference Manual will provide detailed instructions on how to prepare the study drug for administration.

6.6. Administration

Study drug will be administered via SC injection as a bolus in the anterior abdominal wall in accordance with the standard-of-care procedures at the site. Study drug should be administered by the Investigator, or another qualified and trained site staff member as delegated on the delegation log. Because the volume of study drug will vary considerably depending on the dose level, the following guideline must be followed: the maximum volume for each injection must not exceed 2 mL (e.g., a 6-mL dose would require three 2 mL injections in the abdomen). The Study Reference Manual will provide detailed instructions on how to prepare and administer the study drug.

6.7. Study Drug Accountability

The site must maintain adequate records documenting the receipt, preparation, use, loss, or other disposition of the study drug. All study drug will be accounted for using a drug accountability form/record.

6.8. Destruction of Study Drug Supplies

Regulus or designee will provide guidance on the destruction of unused study drug at the site. The Investigator must ensure that the materials are destroyed in compliance with applicable environmental regulations, institutional policy, and any special instructions provided by Regulus. All study drug destruction must be documented adequately.

7. CONCOMITANT MEDICATIONS AND PROCEDURES

7.1. Permitted Concomitant Medications and Procedures

A concomitant medication is any drug or substance other than study drug, including over-the-counter medications, herbal medications, and vitamin supplements, administered during the subject's participation in this study. A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy, physical therapy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed during the subject's participation in this study.

Concomitant medications (and dosing adjustments) and procedures to treat new or existing medical conditions or AEs will be permitted.

All medications and procedures used in the 3 months before Randomization and the subsequent concomitant medications and procedures received/performed during the study must be recorded.

7.2. Prohibited Concomitant Medications and Procedures

Not applicable as long as guidelines for permitted concomitant medications and procedures are followed.

8. DATA ANALYSIS / STATISTICAL METHODS

8.1. General Considerations

The primary objective of this study is to assess the safety and tolerability of RGLS8429. Secondary objectives include characterizing the PK of RGLS8429 and to characterize several exploratory urine and serum biomarkers.

Tabular summaries and analysis results will be generated by dose level for RGLS8429 and placebo (pooled across all cohorts). Descriptive statistics by dose level and treatment group will be tabulated by visit. For continuous endpoints, the descriptive statistics will include the number of observations, mean, median, standard deviation, coefficient of variation (%CV), first and third quartiles, minimum and maximum. Geometric mean and geometric %CV will be presented where appropriate. For categorical endpoints, the number and percentage of subjects will be summarized by dose level and treatment group.

Details of all statistical methods will be provided in the Statistical Analysis Plan.

8.2. Sample Size Determination

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.

8.3. Analysis Populations, Subgroups, and Covariates

8.3.1. Analysis Populations

Safety Population

The Safety Population consists of all subjects who received a dose of study drug.

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 level received.

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 level received.

Biomarkers Population

The PD Population consists of all randomized subjects who have at least one Screening and/or pre-dose value for the biomarker parameters.

8.4. Final Analysis

The final analysis of the study will be performed when all subjects have received study drug and been followed for 4 weeks.

8.4.1. Disposition

The number and percent of subjects enrolled, receiving assigned treatment, completed assigned treatment, and completing study will be tabulated by treatment cohort. Subjects who withdraw from study drug and/or study prematurely will be summarized by treatment cohort with the reason for early discontinuation.

8.4.2. Demographic and Baseline Characteristics

Subject demographics (age, sex, race, ethnicity) and baseline characteristics will be summarized descriptively by treatment cohort and for all subjects based on the Safety Population.

8.4.3. Safety Analysis

The Safety Population will be used for all safety analyses. No formal statistical tests will be conducted to assess the safety or tolerability of RGLS8429.

Subject incidence of all TEAEs, all TEAEs related to study drug, and all SAEs will be tabulated by system organ class and preferred term. All fatal adverse events, SAEs, and TEAEs leading to withdrawal from study drug will also be listed.

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

The analyses of safety laboratory tests, vital signs, and ECGs will include summary statistics over time tabulated by cohort. Similarly, changes from baseline will be summarized. Shifts based on normal ranges between the baseline and the worst on-study value will be tabulated by dose level and treatment group.

The results from the central reader assessment of ECGs extracted from the Holter data will be used in all statistical analysis. The following intervals will be summarized by treatment group: PR, QRS, RR, HR, uncorrected QT, QTcB, and QTcF. Details of additional analyses will be provided in the Statistical Analysis Plan.

The total dose of study drug and the proportion of subjects receiving each dose level will be summarized using descriptive statistics.

Changes from baseline in the SARA test scores will be summarized descriptively over time and by treatment cohort for each category and the total score. The SARA is a tool for assessing ataxia. It has eight categories with accumulative score ranging from 0 (no ataxia) to 40 (most severe ataxia). When completing the outcome measure, each category is assessed and scored accordingly. Scores for the eight items range as follows:

1. Gait (0-8 points),
2. Stance (0-6 points),
3. Sitting (0-4 points),
4. Speech disturbance (0-6 points),
5. Finger chase (0-4 points),
6. Nose-finger test (0-4 points),
7. Fast alternating hand movement (0-4 points),

8. Heel-shin slide (0-4 points).

Once each of the 8 categories has been assessed, the total is calculated to determine the severity of ataxia.

For motor activities of the four extremities (Items 5-8), assessments are performed bilaterally, and the mean values are used to obtain the total score.

8.4.4. Pharmacokinetic Analysis

PK samples will be analyzed using a validated, sensitive, specific method. The laboratory analyzing the PK samples will be unblinded to ensure that PK analysis is performed only on subjects receiving RGLS8429. Placebo samples will be analyzed if necessary. Descriptive statistics for plasma concentrations by time point and by cohort will be tabulated. Descriptive statistics for PK parameters will be summarized by cohort.

Concentration data will be presented and summarized for the PK Full population.

Using non-compartmental methods, the plasma and urine concentration data will be used to derive the following PK parameters: C_{max}, T_{max}, AUC_{0-24}, AUC_{0-t}, AUC_{inf} (where calculable), $t_{½}$, CL/F, V_z/F, fe, and Ae. Additional PK parameters may be calculated as appropriate. The PK Evaluable Population will be used for these analyses.

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

8.4.5. Biomarker Analysis

Descriptive statistics will be used to characterize urine (e.g., PC1, PC2, NGAL, KIM-1, MCP-1, B2M, and CLU) and serum (e.g., Cystatin-C, IGFALS, Alb, CT-proAVP, N-acetyl-1-methylhistidine, and others) biomarkers in all subjects. The analysis of urine and serum biomarkers is exploratory. The PD Population will be used for these analyses.

9. ADVERSE EVENTS

9.1. Monitoring, Recording and Reporting of Adverse Events

An adverse event (AE) is any noxious, unintended, or untoward medical occurrence that may appear or worsen in a subject during a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject's health, including laboratory test values (as specified by the criteria in Section 9.3), regardless of etiology. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE. A diagnosis or syndrome should be recorded on the AE page of the CRF rather than the individual signs or symptoms of the diagnosis or syndrome.

Overdose (accidental or intentional), abuse, withdrawal, sensitivity, or toxicity to a study drug should be reported as an AE. If an overdose is associated with an AE, the overdose and AE should be reported as separate terms. Any sequelae of an accidental or intentional overdose of a study drug should be reported as an AE on the AE CRF. If the sequelae of an overdose are a SAE, then the sequelae must be reported on the AE CRF and marked as serious. The overdose resulting in the SAE should be identified as the cause of the event on the CRF but should not be reported as an SAE itself.

In the event of overdose, the subject should be monitored as appropriate and should receive supportive measures, as necessary. There is no known specific antidote for RGLS8429 overdose. Actual treatment should depend on the severity of the clinical situation and the judgment and experience of the treating physician. (Please refer to the RGLS8429 Investigator's Brochure, Summary of Data and Guidance for the Investigator for additional information.)

All subjects will be monitored for AEs during the study. Assessments may include monitoring of any or all the following parameters: the subject's clinical symptoms, laboratory, pathological, radiological, or surgical findings, physical examination findings, or findings from other tests and/or procedures.

All AEs and SAEs will be recorded by the Investigator with the start of study drug administration until EOS Visit. All SAEs made known to the Investigator at any time thereafter that are suspected of being related to study drug will also be recorded. AEs and SAEs will be recorded on the AE page of the CRF and in the subject's source documents.

9.2. Evaluation of Adverse Events

A qualified Investigator must evaluate all adverse events.

9.2.1. Seriousness

An SAE is any AE occurring at any dose that:

- Results in death.
- Is life-threatening (i.e., in the opinion of the Investigator, the subject is at immediate risk of death from the AE).
- Requires inpatient hospitalization or prolongation of existing hospitalization (hospitalization is defined as an inpatient admission, regardless of length of stay).
- 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. Important medical events are defined as those occurrences that may not be immediately life-threatening or result in death, hospitalization, or disability, but may jeopardize the subject or require medical or surgical intervention to prevent one of the other outcomes listed above. Medical and scientific judgment should be exercised in deciding whether such an AE should be considered serious.

Events **not considered** to be SAEs are hospitalizations for:

- A standard procedure for protocol therapy administration. However, hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as an SAE.
- Routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
- The administration of blood or platelet transfusion as routine treatment of a concurrent medical condition. However, hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable SAE.
- A procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). However, hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable SAE.
- Hospitalization or prolongation of hospitalization for technical, practical, or social reasons, in absence of an AE.
- A procedure that is planned (i.e., planned prior to start of treatment on study); must be documented in the source document and the CRF. Hospitalization or prolonged hospitalization for a complication remains a reportable SAE.
- An elective treatment of or an elective procedure for a pre-existing condition, unrelated to the studied indication that has not worsened from baseline.
- Emergency outpatient treatment or observation that does not result in admission, unless fulfilling other seriousness criteria above.

If an AE is considered serious, the AE page/screen of the CRF must be completed and marked as serious.

For each SAE, the Investigator will provide information on severity, start, and stop dates, relationship to the study drug, action taken regarding the study drug, and outcome.

9.2.2. Severity/Intensity

For both AEs and SAEs, the Investigator must assess the severity/intensity of the event.

The Investigator will assess the grade of the AE according to the FDA's Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September 2007) except for AEs associated with abnormal serum creatinine level. The Investigator will use the KDIGO Clinical Practice Guideline for Acute Kidney Injury (https://kdigo.org/wp-content/uploads/2016/10/KDIGO-2012-AKI-Guideline-English.pdf) to grade the severity of an AE associated with abnormal serum creatinine level. Toxicities that are not specified in these guidances will be defined as follows:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2: Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
- Grade 4: Life-threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

The term "severe" is often used to describe the intensity of a specific event (as in mild, moderate, or severe myocardial infarction); however, the event itself may be of relatively minor medical significance (such as severe headache). This criterion is *not* the same as "serious", which is based on subject/event *outcome* or *action* criteria associated with events that pose a threat to a subject's life or functioning.

Seriousness, not severity, serves as a guide for defining regulatory obligations.

9.2.3. Causality

The Investigator must determine the relationship between the study drug and the occurrence of an AE/SAE. This relationship is indicated by as "unrelated" or "related" in response to the question: Is there a reasonable possibility that the event may be caused by the study drug. In addition to a temporal relationship, the Investigator should consider the following when determining the relationship between an AE and study drug: whether an alternative etiology has been identified, mechanism of action of the study drug, and/or the biological plausibility of a relationship.

9.2.4. Duration

For both AEs and SAEs, the Investigator will provide a record of the start and stop dates of the event. In all cases, the AE must have the same verbatim term throughout. Within the duration of the SAE or AE, the maximum grade should be used to categorize severity.

9.2.5. Action Taken

The Investigator will report the action taken with study drug as a result of an AE or SAE, as applicable and report if concomitant and/or additional treatments were given for the event.

9.2.6. Outcome

Subjects will be followed until AEs have either resolved, returned to baseline status, or are deemed stable or commensurate with ongoing disease processes, per the Investigator's judgment.

One of the four outcomes listed below must be recorded:

- **Resolved** – The subject has recovered fully from the event with no residual effects observable or returned to baseline status.
- **Resolved with sequelae** – The subject has recovered from the event with some residual effect(s) observable. The residual effect(s) will be recorded in the CRF.

- **Ongoing** – Effects of the event are still present, regardless of whether the effect is changing or stable and persistent.
- **Fatal outcome** (for serious adverse events only)

9.3. Abnormal Laboratory Values

An abnormal laboratory value is an AE if the abnormality:

- Results in discontinuation from the study.
- Requires treatment, stopping or holding study drug, or any other therapeutic intervention.
- Is judged to be clinically significant, e.g., one that indicates a new disease process and/or organ toxicity; or is an exacerbation or worsening of an existing condition.

Regardless of severity grade, only laboratory abnormalities that fulfill a seriousness criterion need to be documented as a serious adverse event.

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page/screen of the CRF. If the abnormality was not a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE. If possible, the laboratory abnormality should be recorded as a medical term and not simply as an abnormal laboratory result (e.g., record thrombocytopenia rather than decreased platelets).

9.4. Pregnancy

All pregnancies or suspected pregnancies occurring in either a female subject or female partner of a male subject are immediately reportable events.

In the event that a pregnancy or suspected pregnancy (including elevated βhCG or positive pregnancy test in a female subject) should occur while the subject is in study, or within 90 days of the subject's dose of study drug, it will be considered an immediately reportable event. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the study pharmacovigilance group immediately by email using the Pregnancy Reporting Form.

The female subject may be referred to an obstetrician-gynecologist or another appropriate healthcare professional for further evaluation.

The Investigator will follow the female subject until completion of the pregnancy and must notify the study pharmacovigilance group immediately about the outcome of the pregnancy (normal or abnormal outcome) using the Pregnancy Follow-up Report Form.

If the outcome of the pregnancy was abnormal (e.g., spontaneous abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE to the study pharmacovigilance group within 24 hr of the Investigator's knowledge of the event.

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in-utero exposure to the study drug should also be reported to the study pharmacovigilance group within 24 hr of the Investigator's knowledge of the event.

9.4.1. Male Subjects

If a female partner of a male subject taking study drug becomes pregnant, the male subject taking study drug should notify the Investigator, and the pregnant female partner should be advised to call their healthcare provider immediately.

9.5. Reporting of Serious Adverse Events

All AEs that meet criterion for an SAE must be entered on the AE CRF and marked as serious in the clinical database. All SAEs must be reported to the study pharmacovigilance group within 24 hr of knowledge of the event.

The Investigator is required to ensure that the data on these forms is accurate and consistent. This requirement applies to all SAEs (regardless of relationship to study drug) that occur during the study (from the administration of study drug until the EOS Visit) or any SAE made known to the Investigator at any time thereafter that are suspected of being related to study drug.

The SAE report should provide a detailed description of the SAE and include a concise summary of hospital records and other relevant documents. If a subject died and an autopsy has been performed, copies of the autopsy report and death certificate are to be sent to the study PV mailbox as soon as these become available. Any follow-up data should be detailed in the AE CRF.

Where required by local legislation, the Investigator is responsible for informing the Institutional Review Board/Ethics Committee (IRB/EC) of the SAE and providing them with all relevant initial and follow-up information about the event. The Investigator must keep copies of all SAE information on file including correspondence with the study pharmacovigilance group and the IRB/EC.

9.6. Safety Queries

Queries pertaining to SAEs will be communicated from the study pharmacovigilance group to the site via electronic mail. The response time is expected to be no more than five (5) business days. Urgent queries (e.g., missing causality assessment) may be handled by phone.

9.7. Expedited Reporting of Adverse Events

For the purpose of regulatory reporting, the Sponsor's Lead Physician will determine the expectedness of events suspected of being related to study drug based on information in the Reference Safety Information section of the Investigator Brochure.

All suspected unexpected serious adverse reactions (SUSARs) will be reported in an expedited manner in accordance local and FDA (21 CFR 312.32) regulations.

The study pharmacovigilance group shall notify the Investigator of the following information:

- Any AE suspected of being related to the use of study drug in this study or in other studies that is both serious and unexpected (i.e., SUSAR).
- Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Where required by local legislation, the Investigator shall notify his/her IRB/EC promptly of these new serious and unexpected AE(s) or significant risks to subjects.

The Investigator must keep copies of all pertinent safety information on file including correspondence with Regulus and the IRB/EC. (See Section 13.3 for record retention information).

9.8. Study Pharmacovigilance Group Contact Information

See Study Reference Manual

10. DISCONTINUATIONS

The following events are considered valid reasons for discontinuing a subject from the study drug and/or from the study:

- Protocol violation
- Non-compliance
- Adverse event
- Withdrawal by subject
- Subject lost to follow-up
- Sponsor decision
- Investigator decision
- Pregnancy or pregnancy of partner
- Death
- Other

The reason for discontinuation should be recorded in the CRF and in the source documents.

The decision to discontinue a subject remains the responsibility of the treating physician, which will not be delayed or refused by the Sponsor. However, prior to discontinuing a subject, the Investigator should (if possible) contact the Sponsor's Lead Physician and forward appropriate supporting documents (if possible) for review and discussion.

11. EMERGENCY PROCEDURES

11.1. Emergency Contact

In emergency situations, the Investigator should provide for appropriate emergency care without delay and contact the Sponsor's Lead Physician or designee by telephone at the number(s) listed in the Study Reference Manual.

12. REGULATORY CONSIDERATIONS

12.1. Good Clinical Practice

The procedures set out in this study protocol pertaining to the conduct, evaluation, and documentation of this study are designed to ensure that the Sponsor, its authorized representative, and Investigator abide by Good Clinical Practice (GCP), as described in International Conference on Harmonisation (ICH) Guideline E6 (R2) and in accordance with the general ethical principles outlined in the Declaration of Helsinki. The study will receive approval from an IRB/EC prior to commencement. The Investigator will conduct all aspects of this study in accordance with applicable national, state, and local laws of the pertinent regulatory authorities.

12.2. Investigator Responsibilities

Investigator responsibilities are set out in the ICH Guideline for Good Clinical Practice E6 (R2) and in the local regulations. Regulus staff or an authorized representative will evaluate and approve all Investigators who in turn will select their staff.

The Investigator should ensure that all persons assisting with the study are adequately informed about the protocol, amendments, study treatments, as well as study-related duties and functions, including obligations of confidentiality of Regulus information. The Investigator should maintain a list of Sub-Investigators and other appropriately qualified persons to whom he or she has delegated significant study-related duties.

The Investigator is responsible for keeping a record of all subjects who sign an informed consent form (ICF) and are screened for entry into the study. Subjects who fail Screening must have the reason(s) recorded in the subject's source documents.

The Investigator, or a designated member of the Investigator's staff, must be available during monitoring visits to review data, resolve queries and allow direct access to subject records (e.g., medical records, office charts, hospital charts, and study-related charts) for source data verification. The Investigator must ensure timely and accurate completion of CRFs and queries.

The information contained in the protocol and amendments (except for the information provided by Regulus on public registry websites) is considered Regulus confidential information. Only information that is disclosed previously by Regulus on a public registry website may be freely disclosed by the Investigator or its institution, or as outlined in the Clinical Trial Agreement. The Regulus protocol, amendments, and IB information are not to be made publicly available (for example on the Investigator's or their institution's website) without express written approval from Regulus. Information proposed for posting on the Investigator's or their institution's website must be submitted to Regulus for review and approval, providing at least 5 business days for review.

At the time results of this study are made available to the public, if requested, Regulus will provide Investigators with a summary of the results that is written for the lay person. The Investigator is responsible for sharing these results with the subject and/or their caregiver as agreed by the subject.

12.3. Subject Information and Informed Consent

The Investigator must obtain informed consent of a subject and/or a subject's legal representative prior to any study-related procedures.

Documentation that informed consent occurred prior to the study subject's entry into the study and of the informed consent process should be recorded in the study subject's source documents including the date. The original ICF signed and dated by the study subject and by the person consenting the study subject prior to the study subject's entry into the study, must be maintained in the Investigator's study files and a copy given to the study subject. In addition, if a protocol is amended and it impacts on the content of the informed consent, the ICF must be revised. Study subjects participating in the study when the amended protocol is implemented must be re-consented with the revised version of the ICF. The revised ICF signed and dated by the study subject and by the person consenting the study subject must be maintained in the Investigator's study files and a copy given to the study subject.

12.4. Confidentiality

Regulus affirms the subject's right to protection against invasion of privacy and to follow ICH and other local regulations (whichever is most stringent). Regulus requires the Investigator to permit Regulus representatives and, when necessary, representatives from regulatory authorities, to review and/or copy any medical records relevant to the study in accordance with local laws.

Should direct access to medical records require a waiver or authorization separate from the subject's signed ICF, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

12.5. Protocol Amendments

Any amendment to this protocol must be approved by the Sponsor and submitted to the IRB/EC for written approval. Written approval must be obtained before implementation of the amended version occurs. The written signed approval from the IRB/EC should specifically reference the Investigator name, protocol number, study title, and amendment number(s) that are applicable. Amendments that are administrative in nature do not require IRB/IEC approval but will be submitted to the IRB/IEC for information purposes.

12.6. Institutional Review Board/Independent Ethics Committee Review and Approval

Before the start of the study, the study protocol, ICF, and any other appropriate documents will be submitted to the IRB/EC with a cover letter or a form listing the documents submitted, their dates of issue, and the site (or region or area of jurisdiction, as applicable) for which approval is sought. If applicable, the documents will also be submitted to the authorities in accordance with local legal requirements.

RGLS8429 can be supplied to an Investigator by Regulus or its authorized representative only after documentation on all ethical and legal requirements for starting the study has been received by Regulus or its authorized representative. This documentation must also include a list of the members of the IRB/EC and their occupation and qualifications. If the IRB/EC will not disclose the names, occupations, and qualifications of the committee members, it should be asked to issue a statement confirming that the composition of the committee is in accordance with GCP. For example, the IRB General Assurance Number may be accepted as a substitute for this list. Formal approval by the IRB/EC should mention the protocol title, number, amendment number (if applicable), study site (or region or area of jurisdiction, as applicable), and any other documents reviewed. It must mention the date on which the decision was made and must be officially signed by a committee member. Before the first subject is enrolled in the study, all ethical and legal requirements must be met.

The IRB/EC and, if applicable, the authorities, must be informed of all subsequent protocol amendments in accordance with local legal requirements. Amendments must be evaluated to determine whether formal approval must be sought and whether the ICF should also be revised.

The Investigator must keep a record of all communication with the IRB/EC and, if applicable, between a Coordinating Investigator and the IRB/EC. This statement also applies to any communication between the Investigator (or Coordinating Investigator, if applicable) and regulatory authorities.

Any advertisements used to recruit subjects for the study must be reviewed by Regulus and the IRB/EC prior to use.

12.7. Ongoing Information for Institutional Review Board/ Ethics Committee

If required by legislation or the IRB/EC, the Investigator must submit to the IRB/EC:

- Information on serious or unexpected adverse events as soon as possible
- Periodic reports on the progress of the study
- Deviations from the protocol or anything that may involve added risk to subjects in accordance with IRB/EC guidelines

12.8. Termination of the Study

Regulus reserves the right to terminate this study at any time for reasonable medical or administrative reasons. Any premature discontinuation will be documented appropriately according to local requirements (e.g., IRB/EC, regulatory authorities, etc.).

In addition, the Investigator or Regulus has the right to discontinue a single site at any time during the study for medical or administrative reasons such as:

- Unsatisfactory enrollment
- GCP noncompliance
- Inaccurate or incomplete data collection
- Falsification of records
- Failure to adhere to the study protocol

13. DATA HANDLING AND RECORDKEEPING

13.1. Data/Documents

The Investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of the study drug are complete, accurate, filed, and retained. Examples of source documents include hospital records; clinic and office charts; laboratory notes; memoranda; subject's diaries or evaluation checklists; dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies; microfiche; x-ray film and reports; and records kept at the pharmacy, and the laboratories, as well as copies of CRFs.

13.2. Data Management

Data will be collected via CRF, and data entry will be performed through username- and password-protected access to a secure database. These data will be verified electronically through use of programmed edit checks specified by the Sponsor. Discrepancies in the data will be brought to the attention of the Sponsor and investigational site personnel, as necessary. Resolutions to these issues will be reflected in the database. An audit trail within the system will track all changes made to the data.

13.3. Record Retention

Essential documents must be retained by the Investigator according to the period outlined in the Clinical Trial Agreement. The Investigator must retain these documents for the time period described above or according to local laws or requirements, whichever is longer. Essential documents include, but are not limited to, the following:

- Signed ICFs for all subjects
- Subject identification code list, Screening log (if applicable), and enrollment log
- Record of all communications between the Investigator and the IRB/EC
- Composition of the IRB/EC
- Record of all communications between the Investigator, Regulus, and their authorized representative(s)
- List of Sub-Investigators and other appropriately qualified persons to whom the Investigator has delegated significant study-related duties, together with their roles in the study, curriculum vitae, and their signatures
- RGLS8429 accountability records
- Record of any body fluids or tissue samples retained
- All other source documents (subject records, hospital records, laboratory records, etc.)
- All other documents as listed in Section 8 of the ICH consolidated guideline on GCP (Essential Documents for the Conduct of a Clinical Trial)

The Investigator must notify Regulus if he/she wishes to assign the essential documents to someone else, remove them to another location, or is unable to retain them for a specified period. The Investigator must obtain approval in writing from Regulus prior to destruction of any records. If the Investigator is

unable to meet this obligation, the Investigator must ask Regulus for permission to make alternative arrangements. Details of these arrangements should be documented.

All study documents should be made available if required by relevant health authorities. Investigator or institution should take measures to prevent accidental or premature destruction of these documents.

14. QUALITY CONTROL AND QUALITY ASSURANCE

All aspects of the study will be monitored carefully by Regulus or its authorized representative for compliance with applicable government regulations with respect to current GCP and SOPs.

14.1. Study Monitoring and Source Data Verification

Regulus or designee ensures that appropriate monitoring procedures are performed before, during, and after the study. All aspects of the study are reviewed with the Investigator and the staff at a study initiation visit and/or at an Investigators' Meeting. Prior to enrolling subjects into the study, a Regulus representative or designee will review the protocol, CRFs, procedures for obtaining informed consent, record keeping, and reporting of AEs/SAEs with the Investigator. Monitoring will include on-site visits with the Investigator and his/her staff as well as any appropriate communications by mail, email, or telephone. During monitoring visits, the facilities, study drug storage area, CRFs, subject's source documents, and all other study documentation will be inspected/reviewed by the Regulus representative.

Accuracy will be checked by performing source data verification that is a direct comparison of the entries made onto the CRFs against the appropriate source documentation. Any resulting discrepancies will be reviewed with the Investigator and/or his/her staff. Any necessary corrections will be made directly to the CRFs or via queries by the Investigator and/or his/her staff. Monitoring procedures require that informed consents, adherence to inclusion/exclusion criteria, and documentation of SAEs and their proper recording be verified. Additional monitoring activities may be outlined in a study-specific monitoring plan.

14.2. Audits and Inspections

In addition to the routine monitoring procedures, a representative of Regulus or designee may conduct audits of clinical research activities to evaluate compliance with Good Clinical Practice guidelines and regulations.

The Investigator is required to permit direct access to the facilities where the study took place, source documents, CRFs, and applicable supporting records of study subject participation for audits and inspections by IRB/ECs, regulatory authorities, and company-authorized representatives. The Investigator should make every effort to be available for the audits and/or inspections. If the Investigator is contacted by any regulatory authority regarding an inspection, he/she should contact Regulus or designee immediately.

15. PUBLICATIONS

As described in Section 12.2, all protocol- and amendment-related information, with the exception of the information provided by Regulus or designee on public registry websites, is considered confidential information and is not to be used in any publications without prior approval. Regulus protocol-related information proposed for use in a publication must be submitted to Regulus for review and approval and should not be utilized in a publication without express written approval from Regulus, or as described in the Clinical Trial Agreement.

Regulus will ensure Regulus-sponsored studies are considered for publication in the scientific literature in a peer-reviewed journal, irrespective of the results. At a minimum, this applies to results from clinical studies, and any other study results of significant medical importance.

Study results may also be presented at one or more medical congresses and may be used for scientific exchange and teaching purposes. Additionally, this study and its results may be submitted for inclusion in all appropriate health authority study registries, as well as publication on health authority study registry websites, as required by local health authority regulations.

Eligibility for external authorship, as well as selection of first authorship, will be based on several considerations, including, but not limited to, contribution to protocol development, study recruitment, data quality, participation in data analysis, participation in study steering committee (when applicable), and contribution to abstract, presentation, and/or publication development.

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APPENDIX A. TABLE OF ABBREVIATIONS

Abbreviation	Explanation
%CV	Coefficient of variation
ADA	Anti-drug antibodies
ADPKD	Autosomal dominant polycystic kidney disease
ADL	Activities of daily living
Ae	Total amount of unchanged RGLS8429 excreted in the urine
AE	Adverse event
Alb	Albumin
ALT	Alanine aminotransferase
AUC_{0-24}	Area under the concentration–time curve up to 24 h 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
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
BMI	Body mass index
B2M	beta-2 microglobulin
BP	Blood pressure
BUN	Blood urea nitrogen
CKD-EPI	Chronic kidney disease epidemiology collaboration equation
CL/F	Apparent clearance
C_{max}	Maximum observed concentration
CT-proAVP	
CK	Creatine kinase
CRF	Case report form
CRO	Clinical Research Organization
DLT	Dose-limiting toxicity
ECG	Electrocardiogram
EOS	End of study
fe	Fraction of unchanged RGLS8429 excreted in the urine
GCP	Good clinical practice

Abbreviation	Explanation
GGT	Gamma-glutamyl Transferase
GLP	Good Laboratory Practice GLP
Hb	Hemoglobin
Hct	Hematocrit
HED	Human equivalent dose
HIV	Human immunodeficiency virus
HR	Heart rate
IGFALS	Insulin-like Growth Factor Binding Protein Acid Labile Subunit
ICF	Informed consent form
ICH	International Conference on Harmonisation
INR	International normalized ratio
IUD	Intrauterine device
IUS	Intrauterine system
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
miRNA	MicroRNA
MTD	Maximum tolerated dose
ncRNA	Non-coding microRNA
mmHg	Millimeters of mercury
MRI	Magnetic resonance imaging
NOAEL	No-observed-adverse-effect Level
NGAL	Neutrophil gelatinase-associated lipocalin
PC1	Polycystin 1
PC2	Polycystin 2
PD	Pharmacodynamic
PK	Pharmacokinetic

Abbreviation	Explanation
PKD	Polycystic kidney disease
PT	Prothrombin time
Q2W	Every other week
RBC	Red blood cells
RR	Respiratory rate
SARA	Scale for the Assessment and Rating of Ataxia
SAE	Serious adverse event
SC	Subcutaneous
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
T_{max}	Time to maximum observed concentration
TEAE	Treatment emergent adverse event
$t_{½}$	Half-life
ULN	Upper limit of normal
V_z/F	Volume of distribution
WBC	White blood cells

APPENDIX B. SCALE FOR THE ASSESSMENT AND RATING OF ATAXIA (SARA)

Schmitz-Hübsch T, Tezenes du Montcel S, Baliko L, et al: Scale for the assessment and rating of ataxia: development of a new clinical scale. *Neurology* 66:1717-1720, 2006.

Rater: _____ date: _____ patient: _____

Scale for the assessment and rating of ataxia (SARA)

1) Gait

Proband is asked (1) to walk at a safe distance parallel to a wall including a half-turn (turn around to face the opposite direction of gait) and (2) to walk in tandem (heels to toes) without support.

- **0** Normal, no difficulties in walking, turning and walking tandem (up to one misstep allowed)
- **1** Slight difficulties, only visible when walking 10 consecutive steps in tandem
- **2** Clearly abnormal, tandem walking >10 steps not possible
- **3** Considerable staggering, difficulties in half-turn, but without support
- **4** Marked staggering, intermittent support of the wall required
- **5** Severe staggering, permanent support of one stick or light support by one arm required
- **6** Walking > 10 m only with strong support (two special sticks or stroller or accompanying person)
- **7** Walking < 10 m only with strong support (two special sticks or stroller or accompanying person)
- **8** Unable to walk, even supported

Score _____

2) Stance

Proband is asked to stand (1) in natural position, (2) with feet together in parallel (big toes touching each other) and (3) in tandem (both feet on one line, no space between heel and toe). Proband does not wear shoes, eyes are open. For each condition, three trials are allowed. Best trial is rated.

- **0** Normal, able to stand in tandem for > 10 s
- **1** Able to stand with feet together without sway, but not in tandem for > 10s
- **2** Able to stand with feet together for > 10 s, but only with sway
- **3** Able to stand for > 10 s without support in natural position, but not with feet together
- **4** Able to stand for >10 s in natural position only with intermittent support
- **5** Able to stand >10 s in natural position only with constant support of one arm
- **6** Unable to stand for >10 s even with constant support of one arm

Score _____

3) Sitting

Proband is asked to sit on an examination bed without support of feet, eyes open and arms outstretched to the front.

- **0** Normal, no difficulties sitting >10 sec
- **1** Slight difficulties, intermittent sway
- **2** Constant sway, but able to sit > 10 s without support
- **3** Able to sit for > 10 s only with intermittent support
- **4** Unable to sit for >10 s without continuous support

Score _____

4) Speech disturbance

Speech is assessed during normal conversation.

- **0** Normal
- **1** Suggestion of speech disturbance
- **2** Impaired speech, but easy to understand
- **3** Occasional words difficult to understand
- **4** Many words difficult to understand
- **5** Only single words understandable
- **6** Speech unintelligible / anarthria

Score _____

Rater: _____date: _____patient:_____

5) Finger chase

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Examiner sits in front of proband and performs 5 consecutive sudden and fast pointing movements in unpredictable directions in a frontal plane, at about 50 % of proband´s reach. Movements have an amplitude of 30 cm and a frequency of 1 movement every 2 s. Proband is asked to follow the movements with his index finger, as fast and precisely as possible. Average performance of last 3 movements is rated.

- **0 No dysmetria**
- **1 Dysmetria, under/ overshooting target <5 cm**
- **2 Dysmetria, under/ overshooting target < 15 cm**
- **3 Dysmetria, under/ overshooting target > 15 cm**
- **4 Unable to perform 5 pointing movements**

Score	Right	Left
mean of both sides (R+L)/2		

6) Nose-finger test

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to point repeatedly with his index finger from his nose to examiner's finger which is in front of the proband at about 90 % of proband's reach. Movements are performed at moderate speed. Average performance of movements is rated according to the amplitude of the kinetic tremor.

- **0 No tremor**
- **1 Tremor with an amplitude < 2 cm**
- **2 Tremor with an amplitude < 5 cm**
- **3 Tremor with an amplitude > 5 cm**
- **4 Unable to perform 5 pointing movements**

Score	Right	Left
mean of both sides (R+L)/2		

7) Fast alternating hand movements

Rated separately for each side
Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to perform 10 cycles of repetitive alternation of pro- and supinations of the hand on his/her thigh as fast and as precise as possible. Movement is demonstrated by examiner at a speed of approx. 10 cycles within 7 s. Exact times for movement execution have to be taken.

- **0 Normal, no irregularities (performs <10s)**
- **1 Slightly irregular (performs <10s)**
- **2 Clearly irregular, single movements difficult to distinguish or relevant interruptions, but performs <10s**
- **3 Very irregular, single movements difficult to distinguish or relevant interruptions, performs >10s**
- **4 Unable to complete 10 cycles**

Score	Right	Left
mean of both sides (R+L)/2		

8) Heel-shin slide

Rated separately for each side
Proband lies on examination bed, without sight of his legs. Proband is asked to lift one leg, point with the heel to the opposite knee, slide down along the shin to the ankle, and lay the leg back on the examination bed. The task is performed 3 times. Slide-down movements should be performed within 1 s. If proband slides down without contact to shin in all three trials, rate 4.

- **0 Normal**
- **1 Slightly abnormal, contact to shin maintained**
- **2 Clearly abnormal, goes off shin up to 3 times during 3 cycles**
- **3 Severely abnormal, goes off shin 4 or more times during 3 cycles**
- **4 Unable to perform the task**

Score	Right	Left
mean of both sides (R+L) / 2		