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Title Page

Protocol Title: A Phase 2 randomized, placebo-controlled, double-masked proof-of-concept study to investigate the efficacy and safety of runcaciguat (BAY 1101042) in patients with moderately severe to severe non-proliferative diabetic retinopathy

Protocol Number: 20739

Compound Number: BAY 1101042 / Runcaciguat

Short Title: Non-proliferative diabetic retinopathy treated with runcaciguat

Acronym: NEON-NPDR

Sponsor Name: Non-US: Bayer AG

US territory: Bayer HealthCare Pharmaceuticals Inc.

Legal Registered Address: Non-US: 51368 Leverkusen, Germany

US territory: 100 Bayer Boulevard, P.O. Box 915, Whippany NJ 07981-0915, USA

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The approval of the Statistical Analysis Plan is documented in a separate signature document.

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Version History

This Statistical Analysis Plan (SAP) for study 20739 is based on the clinical study protocol (CSP) (Version 5.0, Amendment number 4, dated 09 FEB 2023).

SAP Version	Date	Section	Change	Rationale
1.0	31 AUG 2023	Not applicable	Not applicable	Original version
2.0	17 NOV 2023	1.1 Objectives and Endpoints 4.2.2 Main Analytical Approach	The estimands framework was added for objectives and endpoints related to the DRSS.	To make the best use of all available data, including data from participants who discontinue the study early, the estimands framework was applied to the DRSS endpoints.
		4.3.1 Secondary Endpoints	The scale for DRSS across both eyes was changed to the for persons scale and the analysis was adapted accordingly.	Based on feedback from HAs the primary endpoint for Phase III studies in NPDR will be the DRSS for persons scale (see Section 6.6). Accordingly, the DRSS endpoints across both eyes was adapted to gain insights and allow comparability.
		1.1 Objectives and Endpoints 4.6.3 Biomarkers	The list of biomarker endpoints was adapted, and more comprehensive analyses of biomarkers were added.	The study team decided to include the analysis of biomarkers within the CSR, and not write a separate BER. Furthermore, see Section 4.8.
		Further sections	Other minor corrections (e.g., typos) not affecting the final analysis.	
3.0	03 MAY 2024	4.3.2 Subgroup Analyses	Definition of an additional subgroup	An improvement from PDR to NPDR is extremely unlikely. The subgroup was defined to only include participants without PDR at baseline.
		Further sections	Minor corrections and additions	

List of Abbreviations and Definitions of Terms

ACIR	Assessment criteria and identification requirements
AE(s)	Adverse event(s)
AESI(s)	Adverse event(s) of special interest
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BCVA	Best corrected visual acuity
BM	Biomarker
BMI	Body mass index
CEC	Clinical endpoint committee
CFP	Color fundus photography
CI	Confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
COVID-19	Coronavirus disease of 2019
CRF	Case report form
CRT	Central retinal thickness
CSP	Clinical study protocol
CV	Coefficient of variation
DBP	Diastolic blood pressure
DMC	Data Monitoring Committee
DME	Diabetic macular edema
dpt	Diopter
DR	Diabetic retinopathy
DRSS	Diabetic Retinopathy Severity Scale
EC	Ethics committee
ECG(s)	Electrocardiogram(s)
eCRF	Electronic case report form
(e)GFR	(Estimated) glomerular filtration rate
EoS	End of study
EoT	End of treatment
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	Fluorescein angiography
FAS	Full analysis set
HbA1c	Hemoglobin A1c (glycated hemoglobin)
HDL	High density lipoprotein
HLGT	High-level group term
ICF	Informed consent form
INR	International normalized ratio

IQR	Interquartile range
IRB	Institutional review board
LDL	Low density lipoprotein
LLOQ	Lower limit of quantification
LOCF	Last observation carried forward
MedDRA	Medical dictionary for regulatory activities
MH	Medical history
NPDR	Non-proliferative diabetic retinopathy
OCT	Optical coherence tomography
OCT-A	Optical coherence tomography angiography
PD	Pharmacodynamics
PDR	Proliferative diabetic retinopathy
PK	Pharmacokinetics
PKS	Pharmacokinetic analysis set
PoC	Proof-of concept
Pop-PK	Population pharmacokinetics
PPS	Per protocol set
PT	Preferred term
QD	*Quaque die*, daily
SAC	Statistical analysis center
SAE(s)	Serious adverse event(s)
SAF	Safety analysis set
SAP	Statistical Analysis Plan
SAS	Statistical analysis system
SBP	Systolic blood pressure
SCr	Serum creatinine
SD	Standard deviation
sGC	Soluble guanylyl cyclase
SoA	Schedule of activities
SOC	System organ class
TEAE(s)	Treatment emergent adverse event(s)
TLF specification	Table, listing and figure specification
ULN	Upper limit of normal
ULOQ	Upper limit of quantification
VTC	Vision threatening complications
WHO-DD	World Health Organization Drug Dictionary

1. Introduction

Runcaciguat (BAY 1101042) is a sGC activator that improved function and retinal structure in pre-clinical DR models.

This PoC study includes a subpart with PK/PD assessments (Part 1). Part 1 enrolls a subset of patients at a more intense investigation in specific study sites. Part 2 is the main PoC part, to be conducted in ophthalmology sites, that enrolls the majority of participants. PoC will be based on the evaluation of outcomes in participants from both parts, which will be running in parallel.

The present study is to be conducted to obtain information on sGC activators by investigating

1. whether runcaciguat improves the diabetic retinopathy score in patients with moderately severe to severe NPDR

   and to

2. explore the PK/PD relationship of runcaciguat on retinal perfusion

This SAP describes the final analysis of the study. No statistical interim analysis will be performed. TLF specifications are contained in a separate document. The analysis of Pop-PK data will not be described here but will be done in a separate analysis plan.

1.1 Objectives and Endpoints

The objectives and endpoints are summarized in Table 1-1.

Table 1-1: Study objectives and endpoints

Objectives	Endpoints
Primary objective: Efficacy Establish the PoC for the efficacy of the sGC activator runcaciguat in the treatment of NPDR.	**Primary endpoint:** • DRSS improvement ≥ 2 steps at 48 weeks of treatment in the study eye **Secondary endpoints for primary objective:** • VTC at 48 weeks of treatment in the study eye • DRSS improvement ≥ 2 steps at 24 weeks of treatment in the study eye • DRSS improvement ≥ 3 steps at 48 weeks of treatment on the scale for persons **Other endpoints (for primary objective):** • DRSS worsening of ≥ 3 steps at 48 weeks of treatment in the study eye • Change in visual acuity from baseline • Change in leakage area on FA • DRSS changes from baseline in the study and fellow eye • VTC in any eye up to 48 weeks of treatment
Secondary objective: Safety Investigate the safety and tolerability of runcaciguat in patients with NPDR	**Secondary endpoints for secondary objective:** • Frequency of TEAEs **Other endpoints (for secondary objective):** • Laboratory parameters • Vital signs • Electrocardiography
Other objectives Characterize PD effects of runcaciguat in patients with NPDR	**Other endpoints** • Central retinal thickness • HbA1c • Other biomarkers
Characterize PK of runcaciguat in patients with NPDR	• Pop-PK • Pre-dose (trough) plasma concentrations of runcaciguat by visit
Further investigate the study intervention and similar drugs (e.g., mode-of-action related effects, safety) and to further investigate pathomechanisms deemed relevant to NPDR, (cardio)vascular diseases and associated health problems	• Various biomarkers (e.g., diagnostic, safety, PD, monitoring, or potentially predictive biomarkers)

The primary estimand (analysis on PPS) is defined below:

Primary Objective: See above.

Estimand label: primary

Endpoint:

All DRSS derived efficacy endpoints

Population:

Adult patients with

- moderately severe to severe NPDR in the study eye (DRSS levels 47 or 53)
- Diabetes type 1 or 2
- BCVA ETDRS letter score in the study eye of ≥ 69 letters (approximate Snellen equivalent of 20/40 or better)
- Refraction with a spherical equivalent from -6 dpt to +5 dpt

Additionally, subpopulations as defined in Section 4.2.3 will be used for the analysis.

Treatment condition:

Runcaciguat vs. placebo

Population level summary:

The estimated difference in the proportion of responders between interventions.

Intercurrent events:

- Dropout due to a VTC in the study eye:
  composite variable strategy (analyze study participant as non-responder)
- Dropout …

  … before Visit 9 (week 12)
  composite variable strategy (analyze study participant as non-responder)

  … after Visit 9 (week 12) with EoT DRSS assessment performed within 4 weeks of treatment discontinuation
  while on treatment strategy (use the last available DRSS assessment (LOCF)

  … after Visit 9 (week 12) with EoT DRSS assessment performed more than 4 weeks after treatment discontinuation
  while on treatment strategy (use the last available DRSS assessment prior to treatment discontinuation (LOCF))
- Non-compliance with study intervention, defined as actual drug intake lower than 80% or higher than 120% of the planned drug intake per protocol:
  principal stratum strategy (exclusion from analysis, as an adequate imputation of response information is considered not possible)

The secondary estimand (analysis on FAS) is identical to the primary estimand (analysis on PPS) with the exception that the condition relating to compliance is not included.

The other estimand (analysis on PPS) is defined below:

Primary Objective: See above.
Estimand label: other
Endpoint: DRSS improvement ≥ 2 steps at 48 weeks of treatment in the study eye
Population: Adult patients with • moderately severe to severe NPDR in the study eye (DRSS levels 47 or 53) • Diabetes type 1 or 2 • BCVA ETDRS letter score in the study eye of ≥ 69 letters (approximate Snellen equivalent of 20/40 or better) • Refraction with a spherical equivalent from -6 dpt to +5 dpt
Treatment condition: Runcaciguat vs. placebo
Population level summary: The estimated difference in the proportion of responders between interventions.
Intercurrent events: • Dropout due to a VTC in the study eye: *composite variable strategy* (analyze study participant as non-responder) • Missing DRSS assessment on Visit 15 (week 48) due to early discontinuation: *hypothetical strategy* (multiple imputations will be performed for the responder criterium) • Non-compliance with study intervention, defined as actual drug intake lower than 80% or higher than 120% of the planned drug intake per protocol: *principal stratum strategy* (exclusion from analysis, as an adequate imputation of response information is considered not possible)

1.2 Study Design

This is a randomized, double-masked parallel study with two study arms enrolling N=98 patients with NPDR. The study is conducted as a placebo-controlled study. Total treatment duration is 48 weeks. The primary endpoint will be the number of participants with a ≥ 2-step improvement (reduction) in the DRSS after 48 weeks of treatment in the study eye.

A steering committee consisting of ophthalmologists and diabetologists (masked) is put in place to provide scientific and operational recommendations to the study protocol and may be consulted to support decisions in study conduct. An independent DMC is set up to provide recommendations based on unmasked review of data, if needed (see section 4.7.1).

This Phase 2 study is conducted to determine whether runcaciguat, administered at once daily oral doses of a modified-release formulation, is more effective in treating NPDR than placebo. The study is subdivided into two components, as shown in Table 1-2. Part 1 is conducted to provide data for PK/PD analyses in diabetic patients with NPDR and to explore the value of different OCT-A assessments as PD marker in DR. Part 2 is the main part of this PoC study and will be conducted in parallel. Both components follow a similar design – except for the randomization ratio and additional examinations in Part 1. Both study subparts will be evaluated together, to the extent that common data are available and at the same time.

Table 1-2: Overall study plan

Study part	Main purpose	Overall design
Part 1 PK/PD	PK/PD assessment	Placebo-controlled, double-masked, 2-arm parallel-group design with 2:1 randomization. Total n = 18 Intra-individual weekly dose escalation in 30-mg increments up to 120 mg once daily. Each participant will continue treatment at the individual maximum dose for a total treatment duration of 48 weeks.
Part 2 PoC	Demonstration of PoC based on efficacy outcome after 48 weeks of treatment	Placebo-controlled, double-masked, 2-arm parallel-group design with 1:1 randomization. n = 40 per treatment arm Intra-individual weekly dose escalation in 30-mg increments up to 120 mg once daily. Each participant will continue treatment at the individual maximum dose for a total treatment duration of 48 weeks.

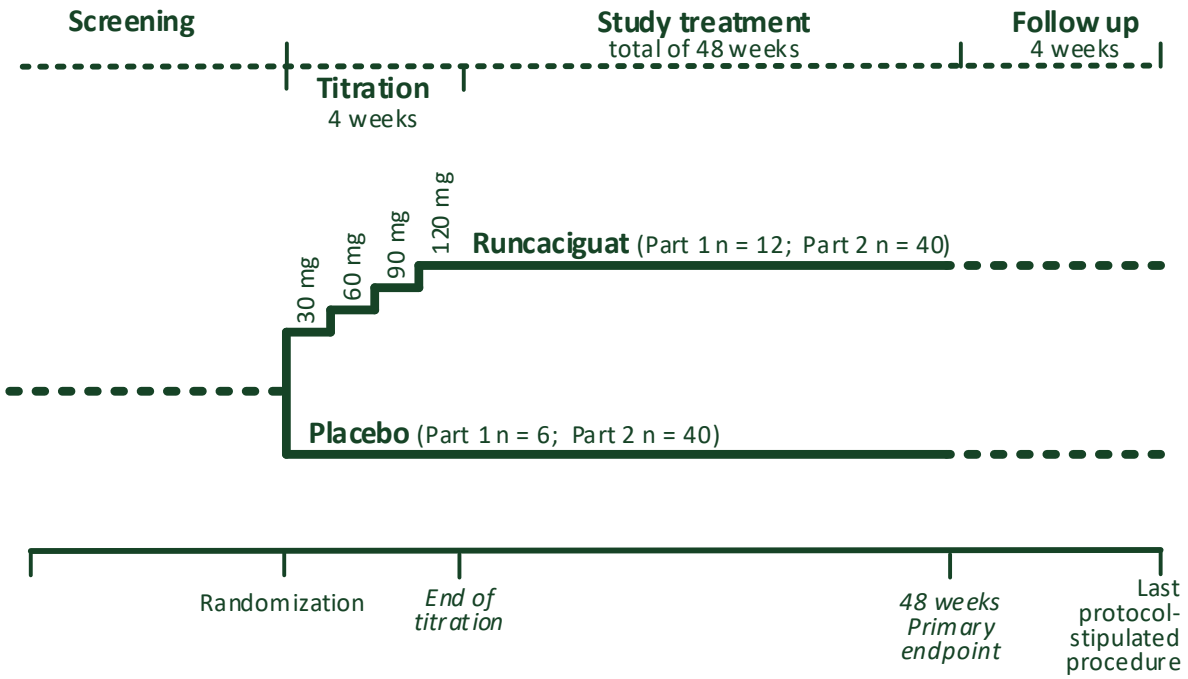
In Part 1, the randomized participants will be titrated at weekly visits in 30-mg increments to the target dose of 120 mg QD, or the maximum individually tolerated dose. After the titration period, the individually reached highest dose will be maintained until the EoT, for the total treatment duration of 48 weeks, followed by a 4-week safety follow-up period. A total of 18 participants are planned to be randomized into Part 1 (12 active; 6 placebo). A schematic study design for Part 1 is provided in Figure 1-1.

In Part 1, additional safety, PK and PD investigations will be performed during the titration phase. On Visits 2 to 6, participants will remain at sites for 4 hours post-dose. During this time, the pulse rate and the blood pressure will be checked, and OCT-A will be used to assess retinal perfusion as PD markers. Blood samples will be drawn for Pop-PK and for PK/PD analyses pre-dose and in two post-dose windows.

Recruitment, screening, and randomization of participants for Part 2 of the study is independent from Part 1. Part 2 of the study is planned to follow the same titration and dosing scheme as Part 1. In Part 2, the participants will be randomized to either placebo (n = 40) or runcaciguat (n = 40). The participants are planned to be titrated at 30-mg increments to the

maximum tolerated dose or sham titrated. Each participant will continue treatment for a total treatment duration of 48 weeks, followed by a 4-week follow-up period.

Figure 1-1: Schematic study design



To assess the efficacy, the retinal morphology will be investigated by 7-field CFP supported by 7-field FA images for the assessment of the DRSS by a central reading center. Two-step DRSS improvement at 48 weeks of treatment will be the primary efficacy endpoint. Additional DRSS assessments are done after 12 and 24 weeks of treatment. In addition, VTC will be recorded throughout the study and assessed as secondary efficacy endpoint.

During the study, 16 visits are planned, including the screening visit (Visit 1) and the end-of-study visit (Visit 16). For the SoA refer to Section 1.3 of the clinical study protocol.

Participants are randomly assigned to one of two study arms. Randomization will be done stratified by baseline DRSS (47 or 53). A combined total of approximately 98 participants for Part 1 and 2 will be randomly assigned for an estimated total of ≥ 40 participants per intervention group evaluable for primary endpoint assessment.

During the initial 4 weeks, treatment will be titrated to the target dose of 120 mg runcaciguat or matching placebo. Treatment will continue at the highest tolerated dose until completion of 48 weeks of treatment if tolerated, or until development of a VTC or development of PDR.

An EoS visit will be performed 4 weeks after EoT. This includes both participants who complete the planned treatment period, and participants who discontinue treatment early including treatment discontinuation due to VTC.

The study population is characterized by the following main inclusion criteria:

- Age ≥ 18 years
- Moderately severe to severe NPDR in the study eye: DRSS levels 47 or 53
- Diabetes type 1 or 2

- BCVA ETDRS letter score in the study eye of ≥ 69 letters (approximate Snellen equivalent of 20/40 or better)
- Refraction with a spherical equivalent from -6 dpt to +5 dpt

2. Statistical Hypotheses

The primary endpoint is the proportion of patients with "≥ 2-step improvement in DRSS at 48 weeks of treatment in the study eye". This proportion will be denoted as π_{ACT} within the runcaciguat treatment arm and as π_{PLC}within the placebo treatment arm.

The study will be evaluated using a Bayesian approach with non-informative prior distribution. Using this approach for inference it is possible to base decisions directly on the posterior probabilities of the hypotheses of interest. Therefore, in a Bayesian setting it is not necessary to specify an alternative hypothesis.

The hypotheses of interest are the following:

- Hypothesis of clinical activity: $\pi_{ACT} > \pi_{PLC}$
- Hypothesis of clinical relevance: $\pi_{ACT} > 0.25$

2.1 Multiplicity Adjustment

Not applicable.

3. Analysis Sets

Final decisions regarding the assignment of participants to analysis sets will be made during the review of study data and documented in the final list of important deviations, validity findings and assignment to analysis sets.

For the purposes of analysis, the following analysis sets are defined in Table 3-1. The same definitions apply for Part 1 and Part 2.

Table 3-1: Analysis sets

Participant analysis set	Assignment rule
Full analysis set (FAS)	All participants of the respective study part randomized to treatment who fulfilled in/exclusion criteria.
Per protocol set (PPS)	All FAS participants of the respective study part without validity findings.
Pharmacokinetic analysis set (PKS)	All participants of the respective study part with PK information not affected by relevant validity findings and with at least one valid post-dose plasma concentration.
Safety analysis set (SAF)	All participants of the respective study part who took at least one dose of study intervention.

It is planned to pool the data of participants from Part 1 and Part 2 for analysis, without further stratification by analysis parts, considering that selection criteria and recruitment time periods were identical and all study procedures in Part 2 were performed also in participants enrolled in Part 1. Participants will be analyzed by their actual treatment to which they were treated for most of the time (i.e., placebo or runcaciguat, irrespective of the planned dose they were planned to be titrated to).

The detailed definitions of validity findings and the assignment of participants to the analysis sets will be based on the ACIR document.

4. Statistical Analyses

4.1 General Considerations

4.1.1 General Principles

The SAP will be finalized prior to unmasking.

The statistical evaluation will be performed by using the software SAS (release 9.4 or higher; SAS Institute Inc., Cary, NC, USA) and R (version 3.5.2 or higher; R Foundation for Statistical Computing, Vienna, Austria).

All data will be listed and summarized in tables. Graphical illustrations will be provided by treatment group (runcaciguat, placebo) where appropriate. Summary statistics will be presented for the participants treated with runcaciguat and for the participants treated with placebo. Descriptive statistics by visit will be provided for the original data as well as for change from baseline, where applicable. Additional summary statistics per dose of runcaciguat administered will be presented, where meaningful. Doses may be grouped to reach more meaningful sample sizes. Frequency tables will be provided for qualitative data.

Continuous variables assumed to be normally distributed will be summarized using the number of participants, mean, standard deviation, minimum, lower quartile, median, upper quartile and maximum. The geometric mean and SD will be provided additionally for the variables where log-normal distributions are assumed.

Generally, data will be analyzed comparing the two intervention groups against each other.

Furthermore, for the runcaciguat treatment group, summary statistics by visit for continuous variables will be further broken down by the actual maintenance dose level, i.e., the dose level reached after the end of the titration phase (at Visit 6) (in case a sufficient number of patients pertains to this dose level).

Data from unscheduled measurements will be listed, but not included in summary statistics or frequency tables.

Listings will be provided sorted by treatment group (runcaciguat, placebo), DRSS at baseline (47, 53) and participant identifier.

Baseline is defined as the last pre-dose assessment before first study intervention.

4.1.2 End of Treatment

Beside the Visit 15 EoT, the EoT values will also be derived to account for eCRF entry errors. The EoT value is the value of the visit at the last treatment day. In case no visit occurred at the last treatment day, the EoT value is the value of the first visit after last treatment.

4.1.3 Missing Data

For handling of missing data for the primary efficacy endpoint, see Section 5.3.2.

Statistical analysis will be based on the available data. Analysis will be performed considering all valid data observed for the respective analysis sets. All missing or partial data will be presented in the subject data listing as they are recorded on the CRF.

Date of first dose of study intervention is defined as the date of first dose from the appropriate CRF page capturing the study intervention (runcaciguat or placebo) if this date is complete. Date of last dose of study intervention is defined as the date of last dose from the appropriate

CRF page capturing the study intervention if this date is complete. If the first and the last dose dates are missing or incomplete, we will consider the following scenarios for imputation of missing dates:

First dose date is missing or incomplete	Imputation rule
Only day of first dose date is missing	Impute the first dose date as maximum of the date of randomization and first day of month.
Month and day of first dose date are missing	Impute the first dose date as randomization date.
First dose date is completely missing but last dose date is available	Impute the first dose date as randomization date but no later than the date of last dose.
First dose date and last dose date are missing	Impute the first dose date as randomization date if tablet boxes were dispensed and if these tablet boxes were not returned unused.
Last dose date is missing or incomplete	**Imputation rule**
Only day of last dose date is missing	1. Impute the last dose date using last day of the month. 2. Derive last dose date as minimum of (imputed last dose date, the date of death, the last visit date before follow-up) but no earlier than the date of first dose.
Month and day of last dose date is missing	1. Impute the last dose date using last day of the year. 2. Derive last dose date as minimum of (imputed last dose date, the date of death, the last visit date before follow-up) but no earlier than the date of first dose.
Last dose date is completely missing but first dose date is available or can be imputed	Impute last dose date as minimum of (the date of death, or the last visit date) but no earlier than the date of first dose.

4.1.4 Discontinuations

Participants who discontinue the study prematurely (i.e., dropout) will not be replaced. A temporary discontinuation exceeding 14 consecutive days or 21 days overall results in a permanent discontinuation. If unexpectedly high dropout rates occur (e.g., due to COVID-19), the number of randomized participants may be increased by up to 20%. For further details with respect to the analysis of dropouts refer to the Section 4.1.3 on handling of Missing Data.

A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, or compliance reasons. This includes cases where, in the investigator's opinion, continued participation in the study would be harmful to the participant's well-being. This is expected to be uncommon. At the time of discontinuing from the study, if possible (if the participant is not lost to follow-up and if the health status of the participant allows), Visit 15 should be conducted as early discontinuation visit, followed by safety follow-up (Visit 16). Data collected at early discontinuation visits will be handled as follows: If a valid DRSS assessment is available from the early discontinuation visit, this value will be considered for analyses for the respective visit. Other variables assessed at early discontinuation visits will be listed for individual follow-up but not be considered for summary statistics.

Participants who permanently discontinue study treatment are invited to continue to attend all protocol-specified study visits. Values assessed at visits after discontinuation from study treatment will be listed for individual follow-up but not be considered for summary statistics. All values assessed after study treatment discontinuation will be flagged in the listings.

4.1.5 Adverse events

For the purpose of date imputation, the treatment follow-up period date is defined as the last available visit date including unscheduled visits after the EoS visit.

If the start or stop time of the AE is missing completely, the duration of the AE will be calculated based on the start and stop date. If only the start or stop minutes are missing, the duration will be calculated based on the dates and hours. If the start date is partially missing, the first day of that month will be used when only the day is missing. If the stop date is partially missing, the last day of the month will be used when only the day is missing. If both the day and the month are missing, the date will be missing.

Imputed dates will only be used for summary tables. Listings will contain the original entries.

TEAEs are AEs that occurred after first dosing and existing pre-dose AEs that worsened in severity post-dose during the main treatment period until 28 days after the last dose of the study drug.

To determine if an AE is treatment-emergent the following rules will be applied considering a worst-case scenario:

- If the date and time of AE start are completely missing, the AE will be considered as treatment-emergent unless the stop date and time suggest otherwise
- If the time of AE start is missing, but the start date (day, month, and year) of the AE is known, the AE will be considered as treatment-emergent if the AE start date is between the date of treatment start and the date of treatment end plus 28 days (both included), unless the stop date and time of the AE (or other information) suggest otherwise
- If the time and day of AE start are missing, but the month and year is known, the AE will be considered as treatment-emergent if the month of AE start is between the month of treatment start and the month of treatment end date plus 28 days (both included), unless the stop date and time of the AE (or other information) suggest otherwise
- If the time, day, and month of AE start are missing, but the year is known, the AE will be considered as treatment-emergent if the year of AE start is between the year of treatment start and the year of treatment end date plus 28 days (both included), unless the stop date and time (or other information) suggest otherwise.

4.1.6 Medical history

Completely or partially missing end dates will not be imputed. For imputation of missing diagnosis date, the following rules will be applied:

MH start date	Imputation rule
Only the day is missing	Impute the date as minimum of first day of the month and treatment start date - 1
Month and day are missing	Impute the date as minimum of July 1st of the year and treatment start date - 1
Start date is completely missing but parts of the stop date are available, or medication is ticked as ongoing	Impute the start date as minimum of (July 1st of the year of the initial visit and the treatment start date - 1)

4.1.7 Prior and concomitant medication

Generally, partially missing prior and concomitant medication start dates will be set to the earliest logically possible date. Partially missing stop dates will be set to the latest logically possible date. For prior and concomitant medication, the following imputation rules will be applied:

Start of medication is missing	Imputation rule
Only the day is missing	Impute the date as first day of the month.
Month and day are missing	Impute the date as January 1st of the year.
Start date is complete missing but parts of the stop date are available, or medication is ticked as ongoing	Impute the start date as minimum of (January 1st of the year of the initial visit and the previous or concomitant medication stop date), since information is available that the patient took previous or concomitant medication, and it can be assumed the patient was on medication already at study start.
Stop of medication is missing	**Imputation rule**
Only the day is missing	Impute the date as minimum of date of death, date of last contact and day of incomplete date replaced by last day of the month.
Month and day are missing	Impute the date as the minimum of date of death, date of last contact and day and month of incomplete date replaced by December 31st.
Stop date is complete missing but medication is ticked as ongoing	Impute the date as the minimum of date of death and date of last visit.

4.1.8 Laboratory and Biomarker values

Regarding the imputation of PK concentrations below the LLOQ see Section 4.6.1.

Descriptive statistics at any time will only be calculated if at least 2/3 of the individual data were measured and were above the LLOQ and below the ULOQ. For the calculation of descriptive statistics, a data point below LLOQ will be substituted by one half of this limit, a data point above ULOQ will be substituted by ULOQ.

In tables showing descriptive statistics, where values below LLOQ or above ULOQ are included, these statistics will be marked. Differences (i.e., changes from baseline) will not be calculated if both measurements are substituted.

Values incorporated as '< x' into the database will be substituted by x/2, values incorporated as '> y' will be substituted by y itself for calculation of summary statistics.

In case that more than one normal range is available for a laboratory parameter, all normal ranges will be provided in the tables and the widest normal range will be displayed in the figures. No normalization of laboratory values will be performed.

If not stated otherwise, summary statistics (minimum and maximum excluded) (and figures) focused on mean values will be provided if evaluable data of ≥ 3 participants are available. Minimum and maximum will be provided if at least one evaluable data point exists.

4.1.9 Data Rules

Summary statistics of continuous variables assumed as normally distributed will be provided including *absolute* changes from baseline as described in Section 4.1.1. Absolute changes from baseline will be calculated as post-dose value minus baseline value unless noted otherwise. In case of continuous variables assumed to be log-normally distributed summary statistics will comprise *relative* changes from baseline. Relative changes from baseline will be calculated as post-dose divided by baseline value unless noted otherwise. Baseline is defined as the last pre-dose assessment before first study intervention.

In case of replicate measurements for one visit mean values of the unrounded original values will be calculated by participant and visit. Summary statistics of these mean values will be provided.

Box plots will be displayed with whiskers of 1.5 times the interquartile range (i.e., third quartile minus first quartile) above the third quartile, and a distance of 1.5 times the interquartile range below the first quartile. All data points above or below the whiskers will be printed separately.

4.2 Primary Endpoint Analysis

4.2.1 Definition of Endpoint

The primary endpoint is '≥ 2-step improvement in DRSS at 48 weeks of treatment in the study eye'.

The proportion of patients with '≥ 2-step improvement' will be denoted as π_{ACT} within the runcaciguat treatment arm and as π_{PLC} within the placebo treatment arm.

To assess the efficacy, the retinal morphology will be investigated by 7-field CFP used for assessment of the DRSS by a central reading center. The images of the screening visit of both eyes will be used as part of the eligibility assessment regarding the severity of DR.

4.2.2 Main Analytical Approach

For the primary analysis, the last observation before missing DRSS values will be carried forward (LOCF) to impute missing values if the participant dropped out after visit 9 (week 12) and the EoT DRSS assessment was performed within 4 weeks of treatment discontinuation (*while on treatment strategy*). If the last measured DRSS score already showed an improvement by ≥ 2 steps at the last measurement prior to the missing assessment, the participant will be considered a responder for the primary endpoint. Otherwise, the participant will be considered a non-responder. If the participant dropped out after visit 9 (week 12) and the EoT DRSS assessment was performed more than 4 weeks after treatment discontinuation the last available DRSS assessment prior to treatment discontinuation will be used (LOCF) (*while on treatment strategy*). Participants who dropped out before visit 9 (week 12), or who had a VTC in the study eye prior to the missing assessment will be considered non-responders regardless of the DRSS level (*composite variable strategy*).

Treatment groups will be runcaciguat and placebo, actually administered dose levels will not be considered for this analysis.

Primary efficacy analyses will be performed on the PPS and will include only study intervention compliant participants (*principal stratum strategy*). In addition, sensitivity analyses will be performed on the FAS. Further analyses may include a separate analysis for each runcaciguat maintenance dose level that was reached during titration (on the PPS). Participants will be analyzed according to the treatment (i.e., runcaciguat or placebo) they actually received.

The study will be analyzed using Bayesian inference with non-informative priors. PoC shall be defined as fulfillment of the following criteria:

- $P(\pi_{ACT} - \pi_{PLC} > 0 \mid \text{data}) \geq 0.9$
  i.e., the posterior probability that the difference between π_{ACT} and π_{PLC} is greater than 0 (clinical activity) given the data observed in the study needs to be 90% or more,

and

- $P(\pi_{ACT} > 0.25 \mid \text{data}) \geq 0.5$
  i.e., the posterior probability that π_{ACT} is greater than 0.25 (clinical relevance) given the data observed in the study needs to be 50% or more.

95% credible intervals will also be presented for π_{ACT} and for π_{PLC}

The response criterion is assumed to be binomially distributed within each treatment arm. For the Bayesian evaluation, a Jeffrey's prior (i.e., a Beta (0.5,0.5)-prior) will be used. Under these assumptions, the posterior distribution for the parameter π will be a Beta (k+0.5, n-k+0.5)-distribution, where

- k is the number of patients with DRSS ≥ 2-step improvement within the respective study arm and
- n is the number of patients within the respective study arms.

In order to demonstrate the clinical activity, the posterior distribution of $\pi_{ACT} - \pi_{PLC}$ will be used to establish the posterior probability that $\pi_{ACT} - \pi_{PLC} > 0$ by using a Monte-Carlo-approach.

The posterior distribution for clinical relevance can be directly evaluated from the Beta (k+0.5, n-k+0.5)-distribution in the runcaciguat arm.

The Bayesian posterior probabilities $P(\pi_{ACT} - \pi_{PLC} > 0 \mid \text{data})$ and $P(\pi_{ACT} > 0.25 \mid \text{data})$ will be calculated using a Monte Carlo Approach: Within each arm, 100000 samples will be obtained from the posterior distribution (i.e., a Beta (k+0.5, n-k+0.5)-distribution). As a seed for random number generation, a fixed seed will be used. This seed has to be included into the program output, in order to ensure that the analysis can be repeated.

From these random samples, the following quantities will be obtained:

- Estimation of $P(\pi_{ACT} - \pi_{PLC} > 0 \mid \text{data})$:
  - Difference $\delta^{(i)}$ between the i[th] sampled proportion in the active arm ($=\pi_{ACT}^{(i)}$) and the i[th] sampled proportion in the placebo arm ($=\pi_{PLC}^{(i)}$):
    $$\delta^{(i)} = \pi_{ACT}^{(i)} - \pi_{PLC}^{(i)}$$
  - Relative frequency of the event '$\delta^{(i)} > 0$' within the samples of $\delta^{(i)}$
- Estimation of $P(\pi_{ACT} > 0.25 \mid \text{data})$:
  - Relative frequency of the event '$\pi_{ACT}^{(i)} > 0.25$' within the samples of $\pi_{ACT}^{(i)}$

4.2.3 Sensitivity Analysis

For the sensitivity analysis, a multiple imputation with 100 repetitions will be performed to receive the responder criterium for participants with missing DRSS assessment on Visit 15 (week 48) due to early discontinuation (*hypothetical strategy*). The imputation model used to generate complete data sets will be a logistic regression for the responder criterium with stratum (baseline DRSS) and treatment group as effects. To use the regression method, the pattern of missingness need to be monotone.

Participants who had a VTC in the study eye prior to the missing assessment will be considered non-responders regardless of the DRSS level (*composite variable strategy*).

Treatment groups will be runcaciguat and placebo, actually administered dose levels will not be considered for this analysis.

The analyses will be performed on the PPS and will include only study intervention compliant participants (*principal stratum strategy*).

The analysis using Bayesian inference with non-informative priors, as described in the section above, will be repeated on the complete data sets obtained from the multiple imputation.

4.2.4 Subgroup Analyses

To build hypothesis on subpopulations with better treatment response and on parameters that may be useful as biomarkers for activity, the following subgroups may be assessed if of relevant size (at least 10 participants per arm), as the underlying characteristics may influence the primary efficacy endpoint:

DRSS at baseline

- 47
- 53

Sex

- Male
- Female

Diabetes

- Type 1
- Type 2

Age

- ≥ median age
- < median age

Hypertension at baseline

- Yes (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg)
- No (SBP < 140 mmHg and DBP < 90 mmHg)

Decrease in DBP (Visit 6 vs. Baseline)

- Yes: decrease ≥ 5 mmHg
- Yes: decrease ≥ 10 mmHg
- No

HbA1c at baseline

- ≤ 8.5%
- > 8.5%

Renal function at baseline by eGFR

- eGFR ≤ 60 mL/min
- eGFR ≤ 45 mL/min (only if ≥ 10 participants)

The primary analysis will be repeated by subgroups as defined above. This way it is possible to assess the treatment effect on DRSS improvement within subgroups. The analyses of subgroups will be performed on the PPS.

4.2.5 Descriptive Analysis

Descriptive statistics for DRSS including change from baseline will be provided. Graphs displaying DRSS versus time will be generated. In addition, individual plots by stratum (DRSS at baseline) and treatment group will be presented. Furthermore, graphs for changes of DRSS at 12, 24, 48 weeks of treatment versus baseline will be provided.

- Descriptive statistics of DRSS including change to baseline will also be broken down by the subgroups defined above and by country (US vs. Non-US).

4.3 Secondary Endpoint Analysis

4.3.1 Secondary Endpoints

4.3.1.1 Definition of Endpoints

'≥/< x steps improvement/deterioration in DRSS (for persons)' at 48 (resp. 12, 24) Weeks of treatment (in the study eye)

These endpoints will be assessed using the same methodology described for the primary efficacy endpoint (see Section 4.2.2).

- '≥ 2 steps improvement in DRSS' at 12, 24 Weeks of treatment in the study eye
- '< 2 steps deterioration in DRSS' at 12, 24, 48 Weeks of treatment in the study eye
- '≥ 1 step improvement in DRSS' at 12, 24, 48 Weeks of treatment in the study eye
- '≥ 3 step improvement in DRSS' at 12, 24, 48 Weeks of treatment in the study eye
- '≥ 3 step improvement in DRSS for persons' at 12, 24, 48 Weeks of treatment
- '< 3 step deterioration in DRSS' at 12, 24, 48 Weeks of treatment in the study eye
- '< 3 step deterioration in DRSS for persons' at 12, 24, 48 Weeks of treatment

DRSS for persons will be derived according to Table 6-2.

Responder and non-responder definition for the endpoints above:

- For '≥ x steps improvement in DRSS' endpoints
    - Participants with '≥ x steps improvement in DRSS' will be counted as *responder*
    - Participants with '< x steps improvement in DRSS' will be counted as *non-responder*
- For '< x steps deterioration in DRSS' endpoints
    - Participants with '< x steps deterioration in DRSS' will be counted as *responder*
    - Participants with '≥ x steps deterioration in DRSS' will be counted as *non-responder*

Vision-threatening complications at 12, 24, 48 Weeks of treatment in the study eye

Assessment of VTC as secondary endpoint is planned in addition, to correlate the DRSS-change with a clinical outcome parameter in support of the efficacy assessment in this study. VTC are defined as occurrence of any of the following AEs:

- PDR (DRSS ≥61)
- Any ocular neo-vascularization (retinal or anterior-segment neovascularization)
- Center-involved (central ETDRS subfield) DME
- Drop of BCVA of 10 letters or more from baseline

Study investigations may be continued after treatment discontinuation, after investigator assessment. Treatment of the VTC should follow local standards and guidelines, at the discretion of the investigator.

Number of participants with TEAEs

For the definition of TEAEs refer to section 4.1.5.

4.3.1.2 Main Analytical Approach

'≥ x steps improvement/deterioration in DRSS (for persons)' at 48 (resp. 12, 24) Weeks of treatment (in the study eye)

This endpoint will be assessed using the same methodology described for the primary efficacy endpoint (see Section 4.2.2) on the PPS and in addition, as a sensitivity analyses on the FAS.

95% credible intervals and point estimates for the difference between the active and placebo response rates will be provided.

Vision-threatening complications at 12, 24, 48 Weeks of treatment in the study eye

VTC will be recorded continuously during the study. Predefined timepoints for assessment are 12, 24, and 48 weeks of treatment. VTC are recorded separately for the study eye and the fellow eye. Separate assessments will be done for the study eye and across both eyes (timepoint when a VTC appears in any eye). These endpoints will be analyzed by the Kaplan-Meier-method on the PPS and in addition, as a sensitivity analyses on the FAS. Confidence intervals will be provided for probability of having a vision threatening event up to these time points. Participants dropping out from the study without a VTC will be censored at the last visit.

Furthermore, if deemed necessary, the endpoint VTC in any eye (yes/no) will be analyzed using the same Bayesian analysis described for the primary efficacy endpoint (see Section 4.2.2) on the PPS.

Number of participants with TEAEs

For analysis of AEs refer to Section 4.5.2.

4.3.2 Subgroup Analyses

The secondary analysis of DRSS for persons will be repeated for the following subgroup:

DRSS for persons at baseline

- ≤ 53 / 53 (scale step ≤ 11)

4.3.3 Supportive Secondary Endpoints

Supportive secondary endpoints will be analyzed descriptively for the PPS and the FAS and will include the following:

- Changes in DRSS from baseline in the study and fellow eye
- Changes in DRSS for persons from baseline

DRSS for persons will be derived according to Table 6-2.

Changes in DRSS from baseline in the study and fellow eye

Frequencies for changes of DRSS versus baseline in the study and fellow eye will be provided. Furthermore, frequencies for changes of DRSS for persons versus baseline will be provided. In addition, graphs for changes of DRSS for persons at 12, 24, 48 weeks of treatment versus baseline will be provided.

4.4 Other Endpoint Analysis

If not mentioned otherwise, these endpoints will be analyzed by descriptive statistics for continuous variables and frequency tables for categorical variables. Furthermore, these endpoints will be analyzed separately for study eye and fellow eye, if not mentioned otherwise.

4.4.1 Best corrected visual acuity

Change in BCVA from baseline: BCVA will be assessed using a modified ETDRS protocol starting 4 meters (AREDS, 1999). Descriptive statistics for visual acuity including change from baseline will be provided using geometric and arithmetic means as defined in Section 4.1.

4.4.2 Fluorescein angiography

Change in leakage area on FA: The anatomical state of the retinal vasculature of the study eye (transit eye) and the fellow eye (non-transit eye) will be evaluated by FA. The angiographic images will be evaluated by a study ophthalmologist for individual safety decisions. Fluorescein leakage area will be evaluated using FA. Descriptive statistics including box plots will be provided for the change in quantitative FA assessments from baseline to Week 24 and 48 by treatment group using geometric and arithmetic means as defined in Section 4.1.

4.4.3 Optical coherence tomography and OCT-angiography

The following parameters will be assessed, as applicable:

- CRT
- OCT-A perfusion density, vessel density
- OCT-A size of foveal avascular zone in study eyes and across both eyes
- OCT-A-DART scans: assessment of vessel thickness

These will be analyzed separately by study part. Furthermore, only relative changes will be provided for the above-mentioned endpoints.

Change in central retinal thickness

CRT will be evaluated using OCT and will be collected at each visit. Data will be provided by the central reading center for central subfield thickness, center point thickness, and total macular volume. The absolute values and relative changes from baseline with 95% CI in these parameters at each visit will be summarized descriptively by treatment group.

Change in vessel density, perfusion density, size of foveal avascular zone

Vessel density (skeleton), perfusion density, size of foveal avascular zone will be measured by the central reading center for study eye and the fellow eye. Vessel density and perfusion density are evaluated for the different retinal layers assessed: Superficial Retinal Layer, Intermediate Retinal Layer and Deep Retinal Layer and Retina (cumulative, all layers combined).

Relative changes from baseline will be assessed for study eyes and across both eyes. Furthermore, sub-analyses of relative changes will be done for all (individual) eyes by DRSS at baseline. Parameters will be summarized using number of eyes/participants, arithmetic mean, arithmetic SD, arithmetic CV, minimum, median, maximum. In addition, relative change from baseline with 95% CI will be provided.

Change in retinal vessel flow

Retinal vessel flow will be measured by the central reading center from OCT-A DART scans for study eye and the fellow eye.

Relative changes from baseline will be assessed for study eyes and across both eyes. Furthermore, sub-analyses of relative changes will be done for all (individual) eyes by DRSS at baseline. Parameters will be summarized using number of eyes/participants, arithmetic mean, arithmetic SD, arithmetic CV, minimum, median, maximum. In addition, relative change from baseline with 95% CI will be provided.

4.4.4 Slit lamp biomicroscopy (anterior and posterior segment)

Abnormal findings will be recorded in the eCRF as either MH or AE, as applicable, and will be analyzed analogously.

4.4.5 Tonometry (intraocular pressure measurement)

Descriptive statistics for intraocular pressure including change from baseline will be provided using arithmetic means. The used methods will be listed only.

4.5 Other Safety Analyses

The secondary objective of the study is to investigate the safety and tolerability of runcaciguat in patients with NPDR. All safety analyses will be performed on the SAF, if not stated otherwise. Participants will be analyzed according to the treatment they actually received.

4.5.1 Treatment Compliance, Duration and Exposure

Tables will be presented for the FAS and PPS.

Frequency tables and summary statistics will be presented for total dose, treatment dose per week, and treatment duration (absolute duration and frequencies with weekly intervals). In addition, treatment compliance will be summarized presenting the percent of tablets that were actually taken out of the planned number of tablets. Frequencies of participants showing compliance <80%, 80-120%, and >120% of the planned tablets will be presented.

The frequencies of dose modifications will be presented by treatment group and visit, especially to assess the escalation, maintenance, reduction, and withdrawal of doses, including the reasons for maintenance, reduction, or withdrawal. Furthermore, the frequency of participants being treated on a certain dose level will be presented by treatment group, visit and dose level to assess the titration level being reached during up-titration and in the maintenance phase.

A listing with the actual dose levels or sham-titration levels for each visit will be presented to get an overview of the individual titration schemes.

4.5.2 Adverse Events

Individual listings of all AEs will be provided indicating whether the AE is treatment-emergent or treatment-emergent related to study intervention and including AE onset relative to start of treatment, the actual dose (or sham titration) level at the start of the AE, and the duration of the AE.

AEs will be summarized using the MedDRA terms by SOC, HLGT (if feasible) and PT.

The following AEs have been defined as AESIs and are recorded as such:

- AEs suggestive of hepatic injury,
- Syncope/loss of consciousness,
- Acute kidney injury suggested by 2.0-fold increase in serum creatinine or >50% decrease in eGFR.

The number of participants with at least one such event in the following TEAE categories will be given by treatment and total:

- Any AE
- any TEAE,
- any mild, moderate, severe TEAE (maximum intensity),
- any TEAE related to study intervention,
- any mild, moderate, severe TEAE related to study intervention,
- any TEAE related to study procedures,
- any treatment emergent SAE,
- any TEAE leading to death,
- any treatment emergent SAE related to study intervention,
- any TEAE leading to not up-titrating study intervention,
- any TEAE leading to dose reduction of study intervention,
- any TEAE leading to interruption of study intervention,

- any TEAE leading to permanent discontinuation of study intervention, and
- any treatment emergent AESI (also broken down by category of special interest as stated above).

These frequency tables will also be presented broken down by maximum intensity of the TEAEs, where appropriate.

Individual listings will be provided for serious TEAE, deaths, treatment emergent AESI, TEAE leading to study intervention interruption, TEAE leading to not up-titrating study intervention, TEAEs leading to study intervention reduction, and TEAEs leading to study intervention discontinuation.

Participants on active treatment are expected to be up titrated to different dose levels of runcaciguat. Thus, the frequencies of participants with TEAEs will also be presented for each dose level. This summary will be presented for 30, 60, 90, 120 mg of runcaciguat, and a combined placebo group (corresponding to a dose level of zero, i.e., sham titrations will not be handled as different placebo dose levels). Since the exposure times for each dose level will not be comparable, incidences will be calculated with respect to the exposure time in the respective dose level. An AE will be allocated to the dose level on which it started. The number of participants with AEs allocated to a certain dose level will be presented and divided by the exposure time of that dose level considering all participants on that dose level. The result will be multiplied by 100 to present incidences of participants with TEAEs per 100 participant-years. In addition, the analysis will be broken down by titration phase and maintenance phase to check whether TEAEs on a new higher dose level differ from TEAEs on maintenance treatment. The following time windows will be assigned to titration phase and maintenance phase for the respective runcaciguat dose levels:

- 30 mg: Titration phase: week 1 Maintenance phase: week 2-97
- 60 mg: Titration phase: week 2 Maintenance phase: week 3-97
- 90 mg: Titration phase: week 3 Maintenance phase: week 4-97
- 120 mg: Titration phase: week 4 Maintenance phase: week 5-97

The table will be repeated summarizing incidences of participants with treatment emergent AESIs.

AEs will be displayed graphically (if feasible) by HLGT, grouped by SOC.

4.5.3 Laboratory assessments

- HbA1c
- Triglycerides
- HDL- / LDL-/ total serum cholesterol
- Further parameters are detailed in Section 10.2 of the CSP

Continuous laboratory values will be summarized descriptively by parameter including absolute changes from baseline. Frequency tables will be provided for qualitative data.

In addition, the frequencies for liver abnormality criteria as defined in CSP Section 10.5 will be presented:

- ALT or AST >3 x ULN,
- ALT or AST >3 x ULN **and** Total bilirubin >2 x ULN,

- ALT or AST >3 x ULN **and** INR >1.5 x ULN,
- ALT or AST >5 x ULN for more than 2 weeks,
- ALT or AST >8 x ULN.

4.5.4 eGFR

eGFR will be calculated using CKD-EPI formula, using the serum creatinine (SCr) concentration measured at screening. The calculation will be done by the central laboratory using the formulas specified in Table 4-1.

Table 4-1: Formula for eGFR calculation

	SCr level at screening	Formula
Women	≤ 0.7 mg/dL	$\text{GFR} = 144 \times \left(\frac{\text{SCr}}{0.7}\right)^{-0.329} \times 0.993^{Age}$ [× 1.159 if black]
	≤ 62 µmol/L	$\text{GFR} = 144 \times \left(\frac{\text{SCr}}{62}\right)^{-0.329} \times 0.993^{Age}$ [× 1.159 if black]
	> 0.7 mg/dL	$\text{GFR} = 144 \times \left(\frac{\text{SCr}}{0.7}\right)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]
	> 62 µmol/L	$\text{GFR} = 144 \times \left(\frac{\text{SCr}}{62}\right)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]
Men	≤ 0.9 mg/dL	$\text{GFR} = 144 \times \left(\frac{\text{SCr}}{0.9}\right)^{-0.411} \times 0.993^{Age}$ [× 1.159 if black]
	≤ 80 µmol/L	$\text{GFR} = 144 \times \left(\frac{\text{SCr}}{80}\right)^{-0.411} \times 0.993^{Age}$ [× 1.159 if black]
	> 0.9 mg/dL	$\text{GFR} = 144 \times \left(\frac{\text{SCr}}{0.9}\right)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]
	> 80 µmol/L	$\text{GFR} = 144 \times \left(\frac{\text{SCr}}{80}\right)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]

GFR = glomerular filtration rate in mL/min/1.73 m^2, eGFR = Estimated glomerular filtration rate, SCr = Serum creatinine

The eGFR values based on serum creatinine are assumed to be log-normally distributed and will be summarized descriptively including relative changes from baseline with 95% CI. The eGFR values will be summarized using summary statistics as defined for log-normally distributed variables.

Geometric mean eGFR including one SD range over time will be plotted. Individual plots of eGFR values will also be presented by stratum (DRSS at baseline) and treatment group.

A frequency table will be presented summarizing decreases in eGFR values from baseline by >50%, >40%, >30%, etc. including cumulative frequencies. Decrease in eGFR [%] will be calculated as 1- (eGFR post-dose / eGFR at baseline) *100.

4.5.5 Vital signs

- SBP, DBP
- Heart rate

The measurements at each visit during the titration phase will be summarized for each time point descriptively by parameter including absolute changes from baseline. Arithmetic mean plots including standard deviation over time will be presented.

Frequencies of participants with a decrease in SBP from the previous visit by >30 mmHg will be presented. In addition, frequencies for the following combinations of the above criteria will be summarized reflecting the up-titration criteria:

- ≥105 mmHg SBP *and* ≤30 mmHg decrease from previous visit (dose increase),
- ≥105 mmHg SBP *and* >30 mmHg decrease from previous visit
  or
  <105 mmHg *and* ≥90 mmHg SBP (dose maintenance),
- <90 mmHg SBP (dose decrease to previous level).

4.5.6 Additional Safety Assessments

ECG

During Part 1 up to study Visit 6 (inclusive), triplicate ECG tracings will be recorded. These triplicate tracings may be transferred to a central ECG reading center for later QT evaluation. The results of the QT evaluations may be reported separately. For part 2, single ECGs will be recorded and transferred to a central ECG reading center for evaluation.

ECG parameters (ventricular rate, PR interval, QRS-duration, and QT interval) will be determined and transferred to the database. The QT interval will also be corrected for the heart rate using Fridericia's method (QTcF), i.e., by dividing the observed QT interval by the cube-root of RR:

$$\mathrm{QTcF} = \frac{\mathrm{QT}}{\sqrt[3]{\mathrm{RR}}}$$

Corrected QT intervals and the heart rate will be provided by the central ECG lab and summarized descriptively by parameter including absolute changes from baseline.

A frequency table will be provided for the ECG findings, presence of sinus rhythm and atrial fibrillation and overall interpretation of ECGs (normal; abnormal, clinically insignificant; abnormal, clinically significant), by visit including any visit.

4.6 Other Analyses

4.6.1 Pharmacokinetics

All analyses of PK will be made on the PK analysis set.

The concentration data will be included in a Pop-PK model, and this will be described in a separate M&S Analysis Plan. The results will be reported in a separate M&S Report separately from the main clinical study report.

The concentration-time courses of runcaciguat will be tabulated separated by dose group and sampling time window. The following statistics will be calculated for each of the sampling windows: geometric mean, geometric SD (re-transformed SD of the logarithms) and CV, arithmetic mean, SD and CV, minimum, median, maximum values, and the number of measurements. These statistics (except for minimum and maximum) at any time will only be calculated if at least 2/3 of the individual data were measured and were above the LLOQ. For their calculation, a data point below LLOQ will be substituted by one half of this limit. Statistics will be marked in the tables if values below the LLOQ are included in their calculation.

Furthermore, the following parameters will be analyzed by the statistics mentioned above:

- C_{trough}
- $C_{1\text{-}2\ hours}$
- $C_{2\text{-}4\ hours}$

4.6.2 PK/PD by groups and by dose level maintained at least for 50% of the study

- CRT by C_{trough}
- Retina perfusion by C_{trough}

Scatter plots for change from baseline of CRT vs. C_{trough} and changes from baseline of retina perfusion vs. C_{trough} will be provided by dose level maintained at least for 50% of the study.

4.6.3 Biomarkers

All Biomarker analyses will be performed on the PPS. Biomarker analyses include the following endpoints:

- HbA1c
- Plasma cyclic guanosine monophosphate (cGMP)
- plasma kynurenine
- serum cystatin C (CysC)
- plasma norepinephrine (NE) (optional if measured)

Descriptive Statistics

Summary statistics including ratio to baseline and change to baseline will be presented (using arithmetic and geometric means) and frequency tables for qualitative data will be provided.

All biomarkers are assumed to be log-normally distributed and will be summarized using number of participants, geometric mean, geometric SD, arithmetic mean, arithmetic SD, minimum, median, IQR, maximum. In addition, ratios to baseline with 95% CI will be provided. These summary statistics will be presented at each planned time point by study intervention for the observed data.

In addition, geometric mean plots of biomarker absolute concentrations, geometric mean plots (including one standard deviation range) of biomarker ratio to baseline and arithmetic mean plots (including one arithmetic standard deviation range) of biomarker change to baseline over time will be presented for all biomarkers. Individual plots of biomarker concentrations will be presented for the absolute concentrations, the ratio to baseline, and change to baseline.

Inferential Statistics

Biomarkers will be assumed log-normally distributed. Therefore, biomarker values will be log-transformed for inferential analyses (unless specified otherwise). The change to baseline of log-transformed measurements is equivalent to the log-transformed ratio-to-baseline:

$$\boldsymbol{log(BM) - log(BM_{bl}) = log\left(\frac{BM}{BM_{bl}}\right)}$$

In order to analyze the impact of treatment on the log-transformed biomarker ratio-to-baseline levels over time ($\Delta BM._{time}$), a linear mixed model will be utilized with $\Delta BM._{time}$ levels post-dose as the dependent variable and corresponding (log-transformed) biomarker baseline level,

intervention group, visit and the interaction of intervention group and visit as the independent variables:

$$\Delta BM_{.time} \sim BM_{.BL} + intervention\ group + visit + intervention\ group * visit + \text{error(within subject)} + \text{error(residual)}$$

A similar linear mixed model will be applied as sensitivity analysis for the absolute difference to baselines of biomarkers without log-transformation.

The subject information will enter the model as a random effect, which will be modelled as an individual random intercept, to account for repeated measures per patient. Time related dependencies per participant will be modelled using an autoregressive correlation structure of order one [AR(1)]. The structure of the covariance matrix might be adapted, e.g., in case of convergence problems.

In addition, least-square means will be calculated and back-transformed (only if biomarkers are log-transformed) per intervention group as well as for treatment effect (difference between intervention group and placebo) and reported with 95% CI and p-value for each time points and across all time points.

Plots of least-square means (back-transformed, if required) for ratios and changes to baseline per intervention group, as well as the treatment effect, at each time point and 95% CI will be provided.

Further analyses may include a separate analysis for each runcaciguat maintenance dose level that was reached during titration.

Responder Analysis

Participants from the runcaciguat group will be classified as responders and non-responders based on the primary outcome definition: responders '≥ 2-step improvement in DRSS at 48 weeks of treatment in the study eye'.

To assess if biomarkers could predict responders vs non-responders at baseline, a univariate logistic regression will be applied for each of the biomarker *at baseline* (log-transformed, unless specified otherwise). The odds ratio (back-transformed, only if biomarkers were log-transformed) with 95% CI will be reported, as well as the p-value.

Furthermore, in order to analyze the impact of responder group (responders vs non-responders) on the log-transformed biomarker ratio-to-baseline levels over time, a linear mixed model including *all available visits* (after the first dose) will be applied. The corresponding (log-transformed) biomarker at baseline, responder group, visit, and the interaction of responder group and visit will be used as independent variables. Time related dependencies per participant will be modelled using an autoregressive correlation structure of order one [AR(1)]. The structure of the covariance matrix might be adapted, e.g., in case of convergence problems using a compound symmetry structure.

In addition, least-square means will be calculated and back-transformed (only if biomarkers are log-transformed) per responder group as well as for the responder effect (difference between responders and non-responders) and reported with 95% CI and p-value for each time points and across all time points.

Plots of least-square means (back-transformed, if required) for ratios to baseline per responder group, as well as the responder effect, at each time point and 95% CI will be provided.

A similar linear mixed model will be applied as sensitivity analysis for the absolute difference to baselines of biomarkers without log-transformation.

4.7 Interim Analyses

Not applicable.

4.7.1 Data Monitoring Committee

An independent DMC will be in place to monitor the safety of the participants and PK throughout the trial based on unmasked data (refer to the DMC Charter for further details). There is no plan to perform interim efficacy analyses to terminate the study for early evidence of efficacy. The DMC will monitor outcomes only to determine the relative benefit/risk. In addition, the DMC will provide a recommendation to the Steering Committee to continue or stop recruitment in any treatment arm. The DMC will consist of a chair, and members who have appropriate qualifications for their respective professions (ophthalmologist, expert for hypertension and cardiovascular risks, and medical statistician); and who are independent from and external to the Sponsor, SAC, EC/IRB, Coordinating or Principal Investigator(s), site Investigator(s) or site Sub-Investigator(s) and Steering Committee membership, Advisory Board membership, CEC membership, if applicable or any other capacity related to study operations. DMC meetings will comprise an open and a closed session. Open sessions will describe the data in a manner which will not destroy the masking of the study. Closed sessions will present unmasked data. To keep the masked status for sponsor employees, an independent SAC will be in place to prepare unmasked tables and provide them to the DMC prior to each DMC meeting.

4.8 Changes to Protocol-planned Analyses

The exploratory biomarker serum lipoprotein was not considered necessary to be analyzed for NEON based on available data from the CONCORD study (BAY 1101042 / 18748) and was therefore removed from the SAP.

5. Sample Size Determination

For the evaluation, a Bayesian concept for inference will be used, which is based on the posterior probabilities that the research hypotheses are true. Clinically relevant benefit of runcaciguat in treatment of advanced NPDR will be considered established and will result in "PoC" if the following criteria are met:

- ≥ 90% posterior probability for the hypothesis of clinical activity
  (i.e., $P(\pi_{ACT} > \pi_{PLC}|data) \geq 0.9$)
- ≥ 50% posterior probability for the hypothesis of clinical relevance
  (i.e., $P(\pi_{ACT} > 0.25|data) \geq 0.5$).

The sample size has been chosen to provide at least 90% probability to obtain PoC under the following assumptions:

- True response probability for placebo: 10%
- True response probability for runcaciguat 120 mg: 35%

The response criterion is assumed to be binomially distributed. For the Bayesian evaluation, a Jeffries' prior (i.e., a Beta (0.5;0.5)-prior) has been used. Sample size was determined with a simulation approach using the R environment.

To be able to declare both clinical activity and clinical relevance ≥ 40 evaluable participants are needed in both groups.

98 participants will be randomly assigned to the study intervention such that approximately 80 evaluable participants complete the study. Of 98 participants, 18 participants will be randomized with a ratio of 2:1 to highest runcaciguat dose and placebo for Part 1. 80 participants will be randomized with a ratio of 1:1 to placebo and runcaciguat for Part 2 (Table 5-1).

Table 5-1: Planned sample size

	Target dose	Part 1	Part 2	Total
Randomized	Placebo	6	40	46
	30 mg	-	-	-
	60 mg	-	-	-
	90 mg	-	-	-
	120 mg	12	40	52
	Overall	18	80	98
Evaluable*				80

*Minimum evaluable for primary endpoint.

6. Supporting Documentation

6.1 Appendix 1: Participant disposition

Based on all participants enrolled, i.e., signed the ICF, an overview will be given of all participants who reached the following milestones:

- Screening
- Randomization
- Start of treatment
- Completion of 12 weeks of treatment (Visit 9)
- Completion of 24 weeks of treatment (Visit 12)
- Completion of 48 weeks of treatment (Visit 15, unless early discontinuation)
- Completion of the study (Visit 16, unless early discontinuation).

Participants will be considered as study completers if they completed the EoT Visit (week 48), irrespective of treatment discontinuations or interruptions. Participants will be considered as treatment completers if they were on treatment until the EoT Visit (week 48). Completion status of the study will be presented for all epochs: screening (all participants enrolled), treatment (i.e., titration and maintenance phase), follow-up (both based on all participants randomized), including reasons for not completing the respective epoch.

In addition, an overview of all participants in the different analysis sets will be provided together with reasons for restrictions of validity (based on all participants randomized).

6.2 Appendix 2: Baseline characteristics and demographics

Demographic data and baseline characteristics will be presented for total and each treatment group in the FAS, PPS, SAF, and PKS (if different). Summary statistics will be provided for quantitative demographic data and baseline characteristics, including

- Age
- Weight
- Height
- BMI
- HbA1c
- eGFR
- BCVA score
- OCT CRT

Frequency statistics will be provided for qualitative data, including

- DRSS of study eye (stratification criterion)
- Study eye
- Sex
- Race
- Ethnicity
- Smoking status,
- Alcohol consumption
- Caffeine consumption
- Country

Diabetic retinopathy history will be summarized separately:

- Duration of diabetic retinopathy (difference between the reported onset date and the patient's date of randomization).
- N (%) of patients reporting a history of type 1 and type 2 diabetes mellitus.
- Duration of diabetes mellitus type 1 and type 2 (difference between the reported onset date and the patient's date of randomization).
- N (%) of patients reporting a history of arterial hypertension, myocardial infarction, transient ischemic attacks, stroke, and diabetic nephropathy.

6.3 Appendix 3: Protocol deviations

The important protocol deviations will be listed and summarized in a frequency table (based on all participants randomized). COVID-19 pandemic associated reasons for discontinuation might be included in the table, i.e., the information whether decision for discontinuation was made by the participant, the physician or was due to logistical reasons.

Impact of COVID-19 may be presented. Descriptive tables displaying number of participants affected and number and type of COVID-19 related protocol deviations are added if needed. A separate listing displays all participants affected by the COVID-19 related study disruption by unique participant identifier and by investigational site.

The number of participants who prematurely discontinue study participation and those who discontinue study treatment permanently for any reason, as well as the respective reasons, will be reported. Plots for "Time to end of study" and "Time to permanent discontinuation of study treatment" will be provided for runcaciguat and placebo group in one plot.

6.4 Appendix 4: Medical history

MH findings will be summarized overall and differentiating between past and ongoing disorders using MedDRA terms using the most recent effective MedDRA version (based on the SAF population).

6.5 Appendix 5: Prior and concomitant medication

Prior and concomitant medication will be summarized, separately by WHO-DD using the latest effective version of the dictionary (based on the SAF population). Prior medication is medication that was terminated before first study intervention intake. Concomitant medication is medication that was not terminated before first study intervention intake or started after first study intervention intake.

6.6 Appendix 6: ETDRS final retinopathy severity scales

Table 6-1: ETDRS final retinopathy severity scale for individual eyes

Level	Severity	Scale step
10	DR absent	1
20	Microaneurysms only	2
35	Mild NPDR	3
43	Moderate NPDR	4
47	Moderately severe NPDR	5
53	Severe NPDR	6
61	Mild PDR	7
65	Moderate PDR	8
71	High-risk PDR	9
75	High-risk PDR	10
81	Advanced PDR: fundus partially obscured, center of macula attached	11
85	Advanced PDR: posterior fundus obscured, or center of macula detached	12
90	Cannot grade, even sufficiently for level 81 or 85	13

ETDRS = Early Treatment Diabetic Retinopathy Study; DR = diabetic retinopathy; NPDR = non-proliferative DR; PDR = proliferative DR

Table 6-2: Abbreviated Summary of ETDRS final retinopathy severity scale for persons

Level	Description	Scale step
10 / 10	No DR	1
20 / <20	Microaneurysms only, one eye	2
20 / 20	Microaneurysms only, both eyes	3
35 / <35	Mild NPDR, one eye	4
35 / 35	Mild NPDR, both eyes	5
43 / <43	Moderate NPDR, one eye	6
43 / 43	Moderate NPDR, both eyes	7
47 / <47	Moderately severe NPDR, one eye	8
47 / 47	Moderately severe NPDR, both eyes	9
53 / <53	Severe or very severe NPDR, one eye	10
53 / 53	Severe or very severe NPDR, both eyes	11
61 / <61	Mild PDR, one eye	12
61 / 61	Mild PDR, both eyes	13
65 / <65	Moderate PDR, one eye	14
65 / 65	Moderate PDR, both eyes	15
71+ / <71	High risk PDR, one eye	16
71+ / 71+	High risk PDR, both eyes	17+

Modified from N Engl J Med 2010; 363:233-244, supplementary appendix, Table 2.

7. References

Fisch R, Jones I, Jones J, Kerman J, Rosenkranz GK and Schmidli H. Bayesian Design of Proof-of-Concept Trials, Therapeutic Innovation & Regulatory Science. 2015; 49(1):155-162

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