NOVARTIS

Clinical Development

SEG101/Crizanlizumab

CSEG101A2203 / NCT04053764

A Phase II, multicenter, randomized, open label two arm study evaluating the effect of crizanlizumab + standard of care and standard of care alone on renal function in sickle cell disease patients ≥ 16 years with chronic kidney disease due to sickle cell nephropathy (STEADFAST)

Statistical Analysis Plan (SAP)

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Document History – Changes compared to previous final version of SAP

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
01-Nov-2019	Prior to FPFV	Creation of final version	N/A - First version	NA
16-Nov-2020 Amendment 1	After protocol amendment 01	Clarify that the test of the treatment effect will be based on the odds ratio estimated by the logistic regression model for the primary analysis	The null and alternative hypotheses were updated based on odds ratio estimated by the logistic regression model. It was added that the proportion of patients in each group with at least a 30% decrease in ACR will be presented along a 95% 2-sided confidence interval for the difference in proportions.	Section 2.5.2 Statistical hypothesis, model, and method of analysis
		To update the definition of CKD progression. As albuminuria also affects CKD outcomes, ACR should be incorporated into the definition of CKD progression.	The stratification factor was updated from "CKD stage" to "CKD risk category" to include albuminuria, as well as eGFR, in the analysis. The subgroup analysis based on CKD stage was changed to be based on CKD risk category.	Section 1.1 study design Section 2.5.2 Statistical hypothesis, model, and method of analysis
		To clarify Supportive analyses of the primary endpoint to provide	Supportive analyses of the primary endpoint to provide	Section 2.5.4 Supportive analyses

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
		additional information regarding the treatment effect	additional information regarding the treatment effect have been added, as have three new subgroup analyses to ascertain the treatment effect based on Hb, frequency of VOCs at baseline and chronic NSAID use.	Section 2.2.3 Subgroup of interest
		To strengthen the primary estimand framework by providing additional clarity regarding the handling of intercurrent events and clarifying the summary measure	Primary estimands section was added after Study objectives and related endpoints table 1-2. Initiation or discontinuation of L glutamine, intake of Voxelotor, blood transfusion or VOC occurred within 7 days of urine and blood sample collection were added as additional intercurrent events. The summary measure has been revised. The details of handling of the intercurrent events were provided. The imputation method has been revised from last on treatment	Section 1.2 Study objectives and endpoints Section 2.5.3 Handling of missing values/ censoring/discontinuations

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
			observation carried forward (LOCF) to multiple imputation (MI)	
		To update the analysis on Protein to creatinine ratio (PCR)	The analysis has been updated to be on combined PCR improvement and stable PCR data	Section 1.2 Study objectives and endpoints Section 2.7 Analysis of the key secondary objective
		To clarify the CKD progression analysis	The details of CKD progression analysis were added	Section 2.7.2 Statistical hypothesis, model, and method of analysis
		To strengthen the supplementary estimand framework by providing additional clarity regarding the handling of intercurrent events based on different rationale	The strategies of handling of the intercurrent events for supplementary estimands have been revised in Table 2-3	Section 2.5.3 Handling of missing values/ censoring/discontinuations Section 2.5.4 Supportive analyses
		To clarify on the models and statistical methods used for primary analysis, also to provide detailed instructions on how to do multiple imputation in SAS	The details for logistic regression, multiple imputation and SAS example code were added	Section 5.4.1 Primary analysis
		[redacted]	[redacted]	[redacted]

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
		[redacted]	[redacted]	
		Correct typo in test used in EAST software	Changed the sample size calculation to be based on a two-sample z test (and not a two sample t test)	Section 3 Sample size calculation
		To update baseline stratification factors, the discordances between the stratum recorded in IRT and the actual stratum recorded in the eCRF is not necessarily needed	Removed the analysis of discordances between the stratum recorded in IRT and the actual stratum in eCRF	Section 2.3 Patient disposition, demographics, and other baseline characteristics
		[redacted]	Claimed in the SAP that Pharmacokinetic endpoints [redacted] will not be reported in CSR according to Lean CSR principles, these analyses will be presented in separate reports as appropriate	Section 2.9 Pharmacokinetic endpoints [redacted]
		To update the word "subject" to "patient" to keep consistency through the document	Updated "subject" to "patient"	Through all the sections
16-Apr-2021	After protocol amendment 02	Due to global impact of the COVID-19 pandemic and long recruitment duration,	Reduced power from 85% to 80% resulting in sample	Section 3 Sample size calculation

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
Amend ment 2		the feasibility of recruiting 170 patients was reconsidered.	size reduction from 170 to 148	
		To update eGFR criteria	The eGFR upper limit of 130 mL/min/1.73 m2 for women was increased to 140 mL/min/1.73 m2 consistent with the criteria for males as there is no confirmed gender specific correlation with hyperfiltration in SCD patients	Section 1.1 Study design and Section 2.2.3 Subgroup of interest
		To update eGFR calculation and use of the Creatinine-based “Bedside Schwartz” equation (2009). The creatinine-based “Bedside Schwartz” equation (2009) will be used to calculate eGFR for patients under the age of 18 at screening as this is the most accurate calculation of eGFR for adolescents and will reduce the risk of overestimation of eGFR in adolescents	Included “Bedside Schwartz” equation 2009 for eGFR calculation for patients under the age of 18	Section 1.1 study design, Section 2.7 Analysis supporting secondary objectives
		Renal replacement therapy (i.e. hemodialysis, peritoneal dialysis, hemofiltration and	Included the intercurrent event renal replacement therapy (i.e. hemodialysis,	Section 1.2.1 Primary estimands, Section 2.5 Analysis

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
		kidney transplantation) is a newly identified intercurrent event	peritoneal dialysis, hemofiltration and kidney transplantation). Handling strategies of this intercurrent event was also added depending on different scenarios.	supporting primary objectives
		To align with the updated SOC CRF page	dose reduction and dose increase were deleted from summary table for SOC as such information will not be collected in CRF. The number of patients with dose change will be presented instead.	Section 2.4.1 Study treatment/compliance
		Analysis on missing data not caused by intercurrent events is added	Missing data not caused by intercurrent events will be handled by multiple imputation by assuming missing at random.	Section 2.5.3 Handling of intercurrent events of primary estimand
		To delete subgroup analysis on NSAID use as there's no clear definition	Deleted the subgroup analysis on chronic NSAID use.	Section 2.2.3 Subgroup of interest
		To delete the individual subject reticulocyte count plot	Deleted the figure 'Individual subject reticulocyte count plot for subjects with potential severe drug induced liver	Section 2.8.3 Laboratory data

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
			injury' as it's not applicable.	
		Clarification of 105-day-follow-up period. Patients who will receive crizanlizumab approximately 4 weeks after their last dose of study treatment, do not need to complete the 105-day follow-up. The end of the safety follow-up period for these patients will be the 30-day follow-up. All other patients are required to complete the 105-day follow-up.	Updated language to clarify on-treatment and post-treatment period based on 105-day follow-up change	Section 2.1.1 General definitions, Section 1.1 study design
		To clarify assessment for patients who discontinue study treatment early	In order to provide robust data, a mandatory Week 53 urine ACR/PCR visit was added to ensure this data was collected for patients who discontinue study treatment early.	Section 1.1 study design
		[redacted]	[redacted]	

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
		[redacted]	[redacted]	[redacted]
		To update the listing of Prophylactic Anticoagulants to general Anticoagulants as anticoagulants at therapeutic doses is allowed	Prophylactic anticoagulants updated to anticoagulants	Section 2.4.2 Prior, concomitant and post therapies
		To update the title in alignment with protocol amendment	Title updated from "Analysis of the primary objective(s)" to "Analysis supporting primary objectives" Title updated from "Analysis of the secondary objective(s)" to "Analysis supporting secondary efficacy objectives" Title updated from "Handling of missing values/censoring/discontinuations" to "Handling of intercurrent events of primary estimand"	Section 2.5 Analysis supporting primary objectives, Section 2.7 Analysis supporting secondary objectives Section 2.5.3 Handling of intercurrent events of primary estimand

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
		To delete the unnecessary listings based on "lean principal" and in alignment with Protocol amendment	Removed the listings for alcohol history, smoking history, hepatitis, and human immunodeficiency virus (HIV) screen, pregnancy test, immunogenicity results and analysis set. Clarified that only the listing for notable vital sign values and notable ECG values will be presented, the full listings on all vital sign assessments and ECG assessments will not be provided.	Section 2.3 Patient disposition, demographics and other baseline characteristics, section 2.8.4 Other safety data,
		To update the "supportive analyses" to "sensitivity analyses" in alignment with protocol amendment	Title updated from "supportive analyses" to "Sensitivity analyses"	Section 2.5.4 Sensitivity analyses
		Updates to consider COVID-19 pandemic situation	Text added specifying summary of protocol deviations and other issues related to COVID-19	Section 2.3 Disposition
			Added text describing analysis of COVID-19 related AEs	Section 2.8.1 Adverse events
		Some lab parameters have no normal	Updated the shift tables will be using	Section 2.8.3 Laboratory data

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
		range defined by central lab	the pre-specified thresholds if to compare baseline to worst on-treatment value	
		Summary statistics will be provided for concentration at each time point, and this analysis will be included in CSR	Deleted "Data from Pharmacokinetic endpoints will not be reported in the CSR but will be reported in a separate report, as appropriate"	Section 2.9 Pharmacokinetic endpoints
06-June-2022	Prior to Snapshot for DMC	Amendment 03	To clarify that any AEs and ConMeds which are continuing as per data cut-off will be shown as 'ongoing' and when an AE has a start date before cut-off and an end date post cut-off, the outcome should be reported as unknown. (This update was missed in SAP amendment 4 so adding in amendment 5)	Sections 5.1.2
			Added a baseline stratification (This was also not added in SAP amendment 4)	Section 2.3
18-May-2023	After Protocol amendment v03, dated 02-Dec-2021	Changes in Protocol	Updated latest protocol version 03	Section 1 Introduction

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
			Updated study objective based on the protocol update	Section 1.1 Study Design
			Updated study objective based on the protocol update	Section 1.2 Study objectives and endpoints
			Updated "primary clinical question of interest "	Section 1.2.1 Primary estimands
			Removed comparison	Section 2.5 Analysis supporting primary objectives
			Removed comparison	Section 2.5.1 Primary endpoint
			Removed comparison and hypotheses	Section 2.5.2 Statistical hypothesis, model, and method of analysis
			Removed sensitivity analysis as per protocol amendment	Section 2.5.4 Sensitivity analyses
			Removed word compare and change to describe	Section 2.7 Analysis supporting secondary objective(s)
			Removed comparison from baseline	Section 2.7.1 Secondary endpoints
			1. Added "summarized descriptively" and removed word "analyzed". 2. Removed comparison from	Section 2.7.2 Statistical hypothesis, model, and method of analysis

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
			endpoint "Proportion of patients with ≥ 30% decrease in ACR at 6 months" 3. Removed comparison from objective "Proportion of patients with PCR improvement and stable PCR (improvement: ≥ 20% decrease in PCR from baseline; stable: within ± 20% change from baseline) at 12 months"	
			Removed comparison	Section 2.11 Resource utilization
			[redacted]	[redacted]

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
[redacted]	[redacted]	[redacted]	Updated Sample size section	Section 3 Sample size calculation

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List of abbreviations

ACE	Angiotensin-converting Enzyme
ACR	Albumin to Creatinine Ratio
ACS	Acute Chest Syndrome
ADA	Anti-drug Antibody
AE	Adverse Event
AESI	Adverse Event of Special Interest
AKI	Acute Kidney Injury
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
APC	Acute Pain Crisis
ARB	Angiotensin-Receptor Blocker
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Classification
C5b-9	Complement Membrane Attack Complex
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CI	Confidence Interval
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of Variance
DI	Dose Intensity
DMC	Data Monitoring Committee
ECG	Electrocardiogram
[redacted]	[redacted]
eCRF	Electronic Case Report/Record Form
eGFR	Estimated Glomerular Filtration Rate
EOT	End of Treatment
ER	Emergency Room
ET-1	Endothelin
FAS	Full Analysis Set
GGT	Gamma-glutamyltransferase
Hb	Hemoglobin
Hbs	Hhemoglobin S
HbSβ^0-thal	Hemoglobin S with β-thalassemia
HbSS	Homozygous Hemoglobin S (sickle cell anemia)
HC	Hydroxycarbamide
HIV	Human Immunodeficiency Virus
HU	Hydroxyurea
INR	International Normalized Ratio
IRT	Interactive Response Technology
i.v.	Intravenous

KDIGO	Kidney Disease Improving Global Outcomes
KIM-1	Kidney injury molecule-1
LLOQ	Lower Limit Of Quantification
[redacted]	[redacted]
[redacted]	[redacted]
MCS	Mental Component Summary
MedDRA	Medical Dictionary for Drug Regulatory Affairs
mg	Milligram(s)
mL	Milliliter(s)
MRI	Magnetic Resonance Imaging
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
NSAID	Non-Steroidal Anti-Inflammatory Drug
[redacted]	[redacted]
PCR	Protein to Creatinine Ratio
PD	pharmacodynamic(s)
PK	pharmacokinetics
[redacted]	[redacted]
PT	Prothrombin Time
[redacted]	[redacted]
RDI	Relative Dose Intensity
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCD	Sickle Cell Disease
SD	Standard deviation
[redacted]	[redacted]
SOC	Standard of Care
TFLs	Tables, Figures, Listings
[redacted]	[redacted]
ULN	Upper Limit of Normal
VOC	Vaso-Occlusive Crisis
WHO	World Health Organization

1 Introduction

This statistical analysis plan (SAP) provides detailed statistical methodology for the analyses of data which will be used for preparation of the CSEG101A2203 clinical study report (CSR) and is based on the study amended protocol v03, dated 02-Dec-2021.

The shells for the in-text tables and figures as well as the post-text tables, figures and listings (TFLs) are in the TFL shells document. Programming specifications for datasets, including derivation of variables, are given in the programming data specifications (PDS) document.

All data will be analyzed by Novartis using Novartis clinical data standards (NCDS). Analysis data sets and statistical outputs will be produced using the SAS system Version 9.4 or higher (UNIX environment) in the global programming & statistical (GPS) environment.

1.1 Study design

This is a Phase II, multicenter, randomized two arm, open label study to evaluate descriptively the effect of crizanlizumab + standard of care and standard of care alone on renal function in sickle cell disease (SCD) patients with SCD-related chronic kidney disease (CKD).

The study will include patients ≥ 16 years of age with a confirmed diagnosis of SCD and SCD-related CKD. Homozygous HbS (HbSS sickle cell anemia) and HbSβ^0-thal SCD genotypes will be included. Patients eligible for the study will have:

- Estimated Glomerular Filtration Rate (eGFR) ≥45 to ≤ 140 mL/min/1.73 m2 based on the CKD/-EPI formula (in patients ≥ 18) and Creatinine-based "Bedside Schwartz" equation (2009) (in patients <18)
- Albumin to Creatinine Ratio (ACR) of ≥ 100 to < 2000 mg/g, despite standard of care treatment for SCD-related CKD

Eligible patients will be receiving standard of care drug(s) for SCD-related CKD according to institutional and local guidelines and the discretion of the physician. Any of the following drugs that the patient is receiving at study entry will be considered the patient's standard of care: hydroxyurea (HU)/ hydroxycarbamide (HC), angiotensin-converting enzyme (ACE) inhibitors, and/or angiotensin receptor blocker (ARB)s. The patient will continue to take their usual standard of care drug(s) during the study; thus, there may be some variation in the standard of care regimens used by the different patients in the study. Patients must have been receiving their standard of care drug(s) prior to study entry according to the dosing requirements (see amended protocol v03 Table 6-1) and must plan to continue the same dose and schedule until the patient has reached the end of the study. The screening assessments will be done within 1 to 28 days prior to baseline (Week 1 Day 1).

Overall, approximately 50 patients will be randomized 1:1 to receive either crizanlizumab (5 mg/kg) + standard of care or standard of care alone. Patients will be stratified at randomization based on CKD risk category (moderate risk or high/very high risk) and HU/HC prescription (Yes/No). The CKD risk categories used for stratification are based on both eGFR and albuminuria assessed by ACR as defined in Table 1-1:

Table 1-1 Prognosis of chronic kidney disease by glomerular filtration rate and albuminuria categories (chronic kidney disease risk categories)

	ACR (30-300 mg/g 3-30 mg/mmol) [1]	ACR (>300 mg/g > 30 mg/mmol)
eGFR ≥ 90 mL/min/1.73 m²	1	2
eGFR 60-89 mL/min/1.73 m²	1	2
eGFR 45-59 mL/min/1.73 m²	2	3

1 = moderately increased risk; 2 = high risk; 3 = very high risk
[1] ACR ranges included above are according to KDIGO (2013); Note that patients in this study will have eGFR ≥ 45 to ≤140 mL/min/1.73 m2 and ACR of ≥ 100 to < 2000 mg/g. Patients should be randomized based on ACR in mg/g.

Throughout this document, "study treatment" will refer to both treatment arms.

Patients randomized to crizanlizumab + standard of care will receive crizanlizumab by intravenous (i.v.) infusion over 30 minutes on Week 1 Day 1, followed by a second dose 14 days later (Week 3 Day 1), and then on Day 1 of every 4 weeks for a total on-study treatment period of 12 months in addition to their usual standard of care treatment. The visit for the assessment of the primary endpoint will take place on Week 53 Day 1. Patients will be followed up for two safety assessments 30 days (phone call) and 105 days (study visit) after the Week 51 Day 1 visit. Patients who will receive crizanlizumab approximately 4 weeks after their last dose of study treatment, do not need to complete the 105-day follow-up. The end of the safety follow-up period for these patients will be the 30-day follow-up. All other patients are required to complete the 105-day follow-up.

Patients in the standard of care alone arm will continue to receive their usual standard of care treatment and will attend study visits per the same schedule as the patients randomized to crizanlizumab + standard of care (i.e., visits on Week 1 Day 1, Week 3 Day 1, then every 4 weeks up to Week 51 Day 1, followed by a visit on Week 53 Day 1 and two safety follow-up assessments 30 days phone call and 105 days study visit after Week 51 Day 1 [as applicable]).

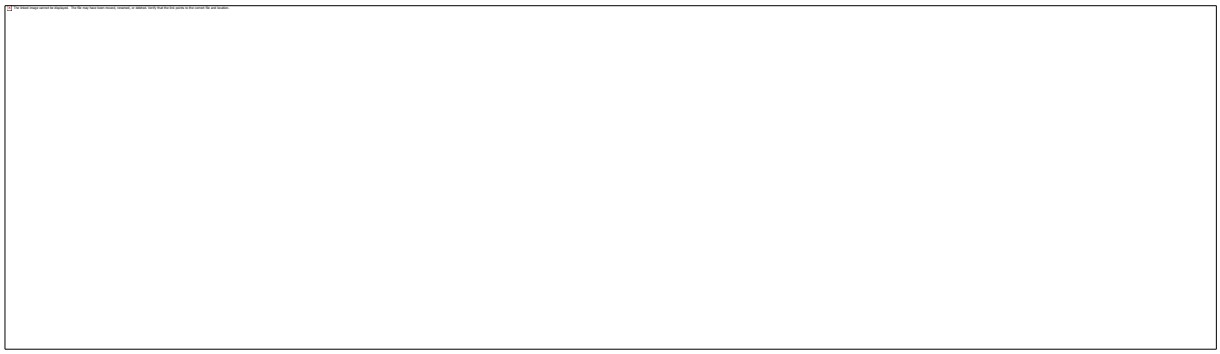
Patients will discontinue the study due to unacceptable toxicity, death, or if they are lost to follow-up, and may also be discontinued from the study at the discretion of the investigator or patient if the study treatment is discontinued (however, please see amended protocol v03 Section 6.5.1 and Section 9.1 for further details). All patients who discontinue the study treatment early will attend an end of treatment (EOT) visit (visit EOT; within 7 days of the last dose of the discontinued study treatment) and be followed in the mandatory safety follow-up period, which includes safety assessments at 30 days (phone call) and 105 days (study visit, as applicable) after the last dose of the discontinued study treatment. These patients will also return for a mandatory Week 53 urine ACR/PCR assessment. The Week 53 urine ACR/PCR assessment should occur 12 months after the first dose of study treatment.

AEs will be recorded throughout the study. All patients will have hematologic, clinical chemistry and coagulation assessments done according to amended protocol v03 Table 8-1. Efficacy assessments of albuminuria (urine ACR) (see amended protocol v03 Section 8.3.1),

protein to creatinine ratio (PCR) (amended protocol v03 Section 8.3.2), eGFR (amended protocol v03 Section 8.3.3), and progression of CKD (amended protocol v03 Section 8.3.4) will be assessed based on these laboratory assessments. [redacted] In addition, patients randomized to the crizanlizumab + standard of care arm will have immunogenicity and PK assessments at select time points.

The study design is provided in Figure 1-1.

Figure 1-1 Study design



No interim analysis will be conducted for this study.

The final analysis will be performed after final database lock when all patients have completed the study (including their safety evaluation 30 and 105 days [as applicable] after the last dose of study treatment) or they have prematurely discontinued.

1.2 Study objectives and endpoints

Objectives and related endpoints are provided in Table 1-2.

Table 1-2 Objectives and related endpoints

Objectives	Endpoints
Primary objective	**Endpoint for primary objective**
To evaluate descriptively the effect of crizanlizumab + standard of care and standard of care alone on albuminuria (ACR) decrease at 12 months	Proportion of patients with ≥ 30% decrease in ACR at 12 months from baseline
Secondary objective(s)	**Endpoint(s) for secondary objective(s)**
To evaluate descriptively the effect of crizanlizumab + standard of care and standard of care alone on change in albuminuria (ACR)	Mean change in ACR from baseline to 3, 6, 9, and 12 months of treatment
To evaluate descriptively the effect of crizanlizumab + standard of care and standard of care alone on albuminuria (ACR) decrease at 6 months	Proportion of patients with ≥ 30% decrease in ACR at 6 months from baseline
To evaluate descriptively the effect of crizanlizumab + standard of care and standard of care alone on protein to creatinine ratio (PCR) at 12 months	Proportion of patients with PCR improvement and stable PCR (improvement: ≥ 20% decrease in PCR from baseline; stable: within ± 20% change from baseline) at 12 months from baseline
To evaluate descriptively the effect of crizanlizumab + standard of care and standard of care alone on the percentage change in eGFR	Percentage change in eGFR from baseline to 3, 6, 9, and 12 months of treatment
To evaluate descriptively the effect of crizanlizumab + standard of care and standard of care alone on ACR decline rate	Slope of ACR decline from baseline to 12 months of treatment based on ACR values at baseline and at 3, 6, 9, and 12 months
To evaluate descriptively the effect of crizanlizumab + standard of care and standard of care alone on eGFR decline rate	Slope of eGFR decline from baseline to 12 months of treatment based on eGFR values at baseline and at 3, 6, 9, and 12 months
To evaluate descriptively the effect of crizanlizumab + standard of care and standard of care alone on the progression of CKD at 12 months	Proportion of patients with progression of CKD from baseline to 12 months
To evaluate descriptively overall safety and, tolerability of crizanlizumab + standard of care and standard of care alone	Safety will be assessed by the frequency and severity of adverse events (AEs), deaths, measurement of vital signs, ECG assessments, SAEs, and laboratory abnormalities
To assess the immunogenicity of crizanlizumab over the study period (treatment of 1 year + 105 days of follow-up)	Immunogenicity: measurement of anti-drug antibodies (ADA) to crizanlizumab at select time points Crizanlizumab PK measurements will accompany immunogenicity measurements
To evaluate descriptively healthcare resource utilization (visits to emergency room [ER] and hospitalizations) in crizanlizumab + standard of care arm and standard of care alone	Annualized rate of visits to ER and hospitalizations due to AKI events, VOCs, or other SCD complications

1.2.1 Primary estimands

The estimand is the precise description of the treatment effect and reflects strategies to address events occurring during trial conduct which could impact the interpretation of the trial results (e.g. premature discontinuation of treatment).

The primary clinical question of interest is: What is the effect of crizanlizumab + standard of care and standard of care alone on renal function in SCD patients with SCD-related CKD.

The detailed information regarding primary estimands can be found in section 2.5.3.

2 Statistical methods

Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, 25th and 75th percentiles, minimum, and maximum will be presented.

The data from all centers that participate will be combined in the analyses.

This study will be considered for Article 46 regulation. If at least 5 pediatric patients (<18 years of age) in each treatment are included in the study, all analysis outputs will be performed for pediatric patients separately. Otherwise, separate analyses will not be provided.

2.1 Data analysis general information

Data will be analyzed by Novartis GMA Oncology Biostatistics and Statistical Programming personnel according to the data analysis plan in this SAP [redacted]. Important information is given in the following sections and details are provided, as applicable, in Section 5 [redacted]

SAS® version 9.4 (or later version if available at time of database lock) will be used in all analyses.

Data from all patients with signed informed consent will be used in the analysis. Data collected after withdrawal of informed consent will not be reported. Due to expected small size of enrollment at individual centers, data from all centers will be pooled together for analysis.

General analysis conventions

Pooling of centers: Unless specified otherwise, data from all study centers will be pooled for the analysis. Due to expected small size of enrollment at individual centers, no center effect will be assessed.

Qualitative data (e.g., gender, race, etc.) will be summarized by means of contingency tables; a "missing" category will be included as applicable. Percentages will be calculated using the number of patients in the relevant population as the denominator.

Quantitative data (e.g., age, body weight, etc.) will be summarized by appropriate descriptive statistics (i.e. mean, standard deviation, median, 25th and 75th percentiles, minimum, and maximum).

2.1.1 General definitions

Investigational drug and study treatment

Investigational drug refers to crizanlizumab only. Whereas study treatment refers to both treatment arms. The term investigational treatment may also be referred to as study treatment which is used throughout this document.

Treatment and group

For presentation in the outputs, **treatment** refers to crizanlizumab 5.0 mg/kg plus SOC; and SOC alone.

Date of first administration of investigational drug

The date of first administration of investigational drug is defined as the first date when a non-zero dose of investigational drug is administered and recorded on the Study Treatment –

Infusion case report form (CRF). The date of first administration of investigational drug will also be referred as start of investigational drug.

Date of last administration of investigational drug

The date of last administration of investigational drug is defined as the last date when a non-zero dose of investigational drug is administered and recorded on Study Treatment CRF. The date of last administration of investigational drug will also be referred as end of investigational drug.

Date of first administration of study treatment

The date of first administration of study treatment is the same as the date of first administration of non-zero dose of study treatment as per the Dosage Administration CRF. The date of first administration of study treatment will also be referred as start of study treatment.

For SOC arm, the date of first administration of study treatment is the first administration of SOC after randomization.

For crizanlizumab plus SOC arm, the date of first administration of study treatment is the first administration of crizanlizumab or SOC after randomization.

Date of last administration of study treatment

The date of last administration of study treatment is the same as the date of last administration of non-zero dose of study treatment as per the Dosage Administration CRF.

For SOC arm, the date of last administration of study treatment is the last administration of SOC after randomization.

For Crizanlizumab plus SOC arm, the date of last administration of study treatment is the last administration of Crizanlizumab or SOC after randomization.

Study day

The study day describes the day of the event or assessment date, relative to the reference start date.

The study day is calculated as:

- The date of the event (visit date, onset date of an event, assessment date etc.) − reference start date + 1 if event is on or after the reference start date;
- The date of the event (visit date, onset date of an event, assessment date etc.) − reference start date if event precedes the reference start date.

The reference start date for safety assessments (e.g. AE onset, laboratory abnormality occurrence, vital sign measurement, dose interruption, PK, electrocardiogram [ECG], etc.) is the start of study treatment.

The reference start date for all other assessments, i.e. vaso-occlusive crisis (VOC), healthcare resource utilization, SCD-related renal and other organ function, other acute pain crisis not requiring a healthcare visit and managed at home, [redacted] is the date of randomization.

The study day will be displayed in the data listings. If an event starts before the reference start date, the study day displayed on the listing will be negative.

Time unit

A year length is defined as 365.25 days. A month length is 30.4375 days (365.25/12). If duration is reported in months, duration in days will be divided by 30.4375. If duration is reported in years, duration in days will be divided by 365.25.

Baseline

For efficacy evaluations, the last non-missing assessment, including unscheduled assessments on or before the date of randomization is taken as the "baseline" value or "baseline" assessment. [redacted]

For safety evaluations, the last available assessment on or before the date of start of study treatment is taken as the "baseline" assessment.

In case the time of assessment and time of treatment start are captured (e.g. pre-dose ECG), the last available assessment before the treatment start date/time is used for baseline.

For safety parameters (e.g. ECGs), where the study requires multiple replicates per time point, the average of these measurements will be calculated for baseline (if not already available in the database).

In cases where multiple measurements meet the baseline definition, with no further flag or label that can identify the chronological order, then the following rule should be applied: If values are from central and local laboratories, the value from central assessment should be considered as baseline. If multiple values are from the same laboratory (local or central) or collected for ECGs or vital signs, then the last entry should be considered as baseline. Further details to cover case by case situations may be described in the programming data specifications.

If patients have no value as defined above, the baseline result will be missing.

For safety parameters other than ECG, scheduled pre-dose collections as well as unscheduled collections on Day 1 for which no time is available will be considered as pre-dose.

For ECG, study Day 1 scheduled pre-dose ECGs will be considered to have been obtained prior to start of study treatment if dosing time or ECG time is missing and used in the calculation of the baseline value. If a scheduled pre-dose measurement actually occurred post-dose, then the corresponding measurement will be treated and analyzed similar to an unscheduled post-dose measurement.

On-treatment assessment/event

For AE reporting the overall observation period will be divided into three mutually exclusive segments:

1. **Pre-treatment period**: from day of patient's informed consent to Day -1 (i.e., the day before first dose of crizanlizumab/day before the Week 1 Day 1 visit)

2. **On-treatment period**: from Day 1 (i.e., day of first dose of crizanlizumab/day of Week 1 Day 1 visit) to 105 days after last dose of discontinued study treatment or the end of the safety follow-up period (i.e., 30 or 105 days after the Week 51 Day 1 visit)
3. **Post-treatment period**: starting at Day 106 after last dose of discontinued study treatment or Day 31 or 106 after the Week 51 Day 1 visit.

Safety summaries (tables, figures) include only data from the on-treatment period with the exception of baseline data, which will also be summarized where appropriate (e.g. change from baseline summaries). In addition, a separate summary for death including on treatment and post treatment deaths will be provided. In particular, summary tables for AEs will summarize only on-treatment events, with a start date during the on-treatment period (**treatment-emergent** AEs).

However, all safety data (including those from the post-treatment period) will be listed and those collected during the post-treatment period will be flagged.

Windows for efficacy analysis

In order to summarize assessments over time (including unscheduled visits) by time point, the assessments will be time slotted. The following general rule will be applied in creating the assessment windows (for baseline refers to definition above): If more than one assessment is done within the same time window, the assessment performed closest to the target date will be used. If 2 assessments within a time window are equidistant from the target date, then the earlier of the 2 assessments will be used. If multiple assessments are on the same date then the worst case will be used (minimum or maximum depending of the parameter direction). Data from all assessments (scheduled and unscheduled), including multiple assessments, will be listed.

Statistical approaches to handle multiple assessments in a given visit window are specified below.

- For ACR/eGFR and PCR assessments ±7 days time window was considered for the visits before week 53 and ±14 days time window was used for the visit time point at week 53.
- Following rules are used to determine the visit windows post baseline for other Lab assessments:
- “Lower limit” = “upper limit of prior applicable visit” + 1.
- “Upper limit” = “target day of current visit” + integer part of (“target day of next applicable visit” – “target day of current visit”)/2 with the exception of Day 365. For Day 365 the upper limit is calculated as 365+14.

The mapped visits will be used in the by visit analyses. However, the listings will show all the collected data regardless of used in the by visit analyses.

Table 2-1 Analysis visit windows based on study day and time for ACR/eGFR, other Lab and PCR.

Analysis Visit	Target day (days)	ACR/eGFR	Clinical chemistry/ Hematology	PCR
Baseline (Week 1 Day 1)	1	≤ Study Day 1	≤ Study Day 1	≤ Study Day 1
Week 3 Day 1	15	-	> 1 to 43	-
Week 7 Day 1	43	-	-	-
Week11 Day 1	71	-	> 43 to 85	-
Week 15 Day1	99	>91 to 106	> 85 to 141	-
Week 19 Day 1	127	-	-	-
Week 23 Day 1	155	-	-	-
Week 27 Day 1	183	>175 to 190	> 141 to 225	>175 to 190
Week 31 Day 1	211	-	-	-
Week 35 Day 1	239	-	-	-
Week 39 Day 1	267	>259 to 274	> 225 to 316	-
Week 43 Day 1	295	-	-	-
Week 47 Day 1	323	-	-	-
Week 51 Day 1	351	-	-	-
Week 53 Day 1	365	>350 to 379	>316 to 379	>350 to 379
End of treatment (EOT)		Assessment taken at EOT visit	Assessment taken at EOT visit	Assessment taken at EOT visit
105 days follow-up	105 days after last infusion	From 29 days within last infusion	-	From 29 days within last infusion

2.2 Analysis sets

A patient is considered to be enrolled into the study if signed informed consent has been obtained. Only patients with signed informed consent will be included in the analysis data sets.

2.2.1 Full Analysis Set

The Full Analysis Set (FAS) comprises all patients to whom study treatment has been assigned by randomization. According to the intent to treat principle, patients will be analyzed according to the treatment they have been assigned to during the randomization procedure.

2.2.2 Safety Set

The Safety Set includes all patients who received at least one dose of study treatment. Patients will be analyzed according to the study treatment received, where treatment received is defined as the randomized treatment if the patient took at least one dose of that treatment or the first treatment received if the randomized treatment was never received.

2.3 Patient disposition, demographics, and other baseline characteristics

The FAS will be used for all baseline and demographic summaries and listings unless specified otherwise. Summaries will be reported by treatment group and for all patients, and listings will be reported by treatment group to assess baseline comparability. No inferential statistics will be provided.

Basic demographic and background data

All demographic and baseline disease characteristics data will be summarized and listed by treatment group. Categorical data (e.g. age, gender, race, ethnicity, performance status) will be summarized by frequency counts and percentages; the number and percentage of patients with missing data will be provided. Continuous data (e.g. age, weight, height, body mass index (BMI)) will be summarized by descriptive statistics (N, mean, median, standard deviation, minimum and maximum). For selected parameters, 25^{th} and 75^{th} percentiles will also be presented. BMI (kg/m^2) will be calculated as weight[kg] / (height[m]^2) using weight and height at screening.

Summary statistics will also be tabulated for other baseline characteristics and assessments, including Hb, ACR, PCR, eGFR, CKD history and CKD progression category.

Baseline stratification factors

The number (%) of patients in each stratum (HU/HC usage: yes, no; CKD risk category: moderate risk, high/very high risk) based on data obtained from the IRT system and the CRF will be summarized overall and by treatment group for the FAS.

Sickle cell and VOC history

Summary statistics will be tabulated for sickle cell and VOC history (e.g., AKI event at baseline, Other acute pain crisis [APC] event at baseline, VOC history including genotype and HU/HC or L-glutamine use, frequency of VOCs at baseline).

Medical history

Medical history and ongoing conditions entered on eCRF will be summarized and listed by treatment group. Separate summaries will be presented for ongoing and historical medical conditions. The summaries will be presented by primary SOC, PT and treatment group. Medical history and current medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) terminology. The MedDRA version used for reporting will be specified in the CSR and as a footnote in the applicable tables/listings.

Patient demographic data and other baseline characteristics (e.g., medical history, disease history etc.) will be listed.

Patient disposition

Enrollment will be summarized for all screened patients and also by treatment group using the FAS. The number (%) of randomized patients included in the FAS will be presented overall and by treatment group. The number (%) of screened and not-randomized patients and the reasons for screening failure will also be displayed. The number (%) of patients in the FAS who are still on treatment, who discontinued the study phases and the reason for discontinuation will be presented overall and by treatment group.

The following summaries will be provided (with % based on the total number of FAS patients):

- Number (%) of patients who were randomized (based on data from IRT system)

- Number (%) of patients who were randomized but not treated (based on 'Study Treatment – Infusion' eCRF page not completed for any study treatment component)
- Primary reason for not being treated (based on 'disposition' eCRF page)
- Number (%) of patients who were treated (based on 'Study Treatment – Infusion' eCRF pages with non-zero dose administered)
- Number (%) of patients who are still on-treatment (based on the 'disposition' page not completed);
- Number (%) of patients who discontinued the study treatment phase (based on the 'disposition' page)
- Primary reason for study treatment phase discontinuation (based on the 'disposition' page)
- Number (%) of patients who have entered the follow-up
- Number (%) of patients who have discontinued from the post-treatment follow-up (30 days or 105 days as applicable)
- Reasons for discontinuation from the post-treatment follow-up

Protocol deviations

The number (%) of patients in the FAS with any protocol deviation will be tabulated by deviation category (as specified in the study edit checks specifications) overall and by treatment group for the FAS. All protocol deviations will be listed. The protocol deviations are defined in the protocol deviations specifications, which will be finalized before final database lock.

In addition, COVID-19 related protocol deviations and issues with potential impact on quality will be summarized.

Analysis sets

The number (%) of patients in each analysis set will be summarized by treatment group and stratum.

2.4 Treatments (study treatment, concomitant therapies, compliance)

2.4.1 Study treatment / compliance

The duration of exposure in weeks to crizanlizumab, actual cumulative dose, dose intensity (computed as the ratio of total dose received and actual duration) and the relative dose intensity (computed as the ratio of dose intensity and planned dose intensity), will be summarized. The duration of exposure in weeks will also be summarized for the standard of care medications for both arms by standard of care categories (HU, ACE, ARB, etc.). Duration of exposure will be categorized into time intervals; frequency counts and percentages will be presented for the number (%) of patients in each interval.

The number of patients with dose adjustments (interruption, or permanent discontinuation) and the reasons will be summarized and all dosing data will be listed for crizanlizumab. The number of patients with dose modifications (dose interruption, dose change or permanent

discontinuation) and the reasons will be summarized and all dosing data will be listed for standard of care medications.

The Safety Set will be used for all summaries and listings of study treatment. Categorical data will be summarized as frequencies and percentages. For continuous data, mean, standard deviation, median, 25th and 75th percentiles, minimum, and maximum will be presented.

Duration of exposure to study treatment

Duration of exposure to study treatment is considered by taking into account the duration of exposure to the investigational drug:

Duration of exposure to study treatment (weeks) = ((last date of exposure to study treatment) – (date of first administration of study treatment) + 1)/7.

The last date of exposure to study treatment is the earliest date between:

- last date of treatment + 27 days (Infusion of crizanlizumab every 4 weeks)
- date of death (if the patient died)

Summary of duration of exposure of study treatment in months will include categorical summaries (less than 3 months, at least 3 months, at least 6 months, at least 9 months, at least 12 months) and continuous summaries (i.e. mean, SD etc.).

Cumulative dose

Cumulative dose of a study treatment is defined as the total dose given during the study treatment exposure and will be summarized.

The **planned cumulative dose** for a study treatment component refers to the total planned dose as per the protocol up to the last date of investigational drug administration.

The **actual cumulative dose** refers to the total actual dose administered, over the duration for which the patient is on the study treatment as documented in the Study Treatment – Infusion eCRF. In order to determine the actual cumulative dose, the dose administered as reported in the eCRF in mg will be divided by the last weight of a given patient at time of the dosing.

Dose intensity and relative dose intensity

Dose intensity (DI) for patients with non-zero duration of exposure is defined as follows:

DI (mg/kg/28days) = (Actual cumulative dose (mg/kg) / Duration of exposure to study treatment (weeks)) x 4.

Planned dose intensity (PDI) is defined as follows:

PDI (mg/kg/28days) = (Planned cumulative dose (mg/kg) / Duration of exposure (weeks)) x 4.

Relative dose intensity (RDI) is defined as follows:

RDI = DI (mg/kg/28days) / PDI (mg/kg/28days).

Dose interruptions or permanent discontinuations

The number of patients who have permanent discontinuations or interruptions, and the reasons, will be summarized separately by treatment group.

Interruptions are considered as infusion completely skipped or delayed.

Interruption: A dose cannot be administered within 21 days of the scheduled day of infusion as per protocol. If a dose was temporarily stopped during infusion, it should not be considered as a dose interruption. Duration of a dose interruption is calculated as the time between the scheduled date of infusion and the actual date of infusion after the interruption.

'Dose permanently discontinued' fields from the Study Treatment CRF pages will be used to determine the dose permanent discontinuations.

The corresponding fields 'Reason for dose interrupted' and 'Reason for permanent discontinuation' will be used to summarize the reasons, respectively.

2.4.2 Prior, concomitant and post therapies

Concomitant medications and significant non-drug therapies/procedures prior to and after the start of the study treatment, as well as the incidence of transfusion, will be summarized and listed for all patients by treatment arm based on the Safety Set.

Concomitant medications

Concomitant therapy is defined as all interventions (therapeutic treatments and procedures) other than the study treatment administered to a patient coinciding with the study treatment period. Concomitant therapy include medications (other than study drugs) starting on or after the start date of study treatment or medications starting prior to the start date of study treatment and continuing after the start date of study treatment.

Concomitant medications will be coded using the World Health Organization (WHO) Drug Reference Listing (DRL) dictionary that employs the WHO Anatomical Therapeutic Chemical (ATC) classification system and summarized by lowest ATC class and PT using frequency counts and percentages. Surgical and medical procedures will be coded using MedDRA and summarized by SOC and PT. These summaries will include:

1. Medications starting on or after the start of study treatment but no later than 30 or 105 days after start of last dose of study treatment and
2. Medications starting prior to start of study treatment and continuing after the start of study treatment.

All concomitant therapies will be listed by treatment arm. Separate listings will be provided for L-Glutamine, Analgesics, Anticoagulants, NSAIDS and opioids. Any concomitant therapies starting and ending prior to the start of study treatment will be flagged in the listing. Unless otherwise specified, the Safety Set will be used for the concomitant medication tables and listings. Non-drug therapies and medical procedures will be coded using MedDRA and summarized by SOC and PT.

L-Glutamine

The number and percentage of patients who were treated with L-Glutamine (as recorded in the "Sickle cell – VOC History" eCRF page) prior to study start and the number of patients who started or stopped these medications during the on-treatment period (as recorded on the corresponding Prior and Concomitant Medications L-Glutamine eCRF pages) will be summarized by treatment group using FAS.

L-Glutamine starting on or after the start date of study treatment will be listed using FAS.

Analgesics

Separate summaries will be provided to present the number and percentage of patients who received analgesics (as entered on "Prior and Concomitant Medications Analgesics" eCRF page) on or after the start of study treatment but no later than 105 days after start of last dose of study treatment.

- **NSAIDS**
  The number and percentage of patients who were treated with NSAIDS on or after the start of study treatment will be summarized by treatment group using FAS.
- **Opioids**
  The number and percentage of patients who were treated with Opioids on or after the start of study treatment will be summarized by treatment group using FAS.

Transfusions

The proportion of patients who received transfusion and the number of transfusions during the on-treatment period will be summarized descriptively. All transfusion will be listed.

2.5 Analysis supporting primary objectives

The primary objective of this study is to evaluate the effect of crizanlizumab + standard of care and standard of care alone on albuminuria (ACR) decrease at 12 months, as assessed by the proportion of patients with ≥ 30% decrease in ACR at 12 months from baseline.

2.5.1 Primary endpoint

The primary endpoint of this study is the proportion of patients with ≥ 30% decrease in ACR at 12 months from baseline.

2.5.2 Statistical hypothesis, model, and method of analysis

The primary endpoint will be analyzed descriptively based on the data from the FAS. A logistic regression model that includes effects for treatment and randomization stratification factors will be utilized. The treatment effect based on the log-odds ratio will be estimated by the model.

The odds ratio for the relative difference between treatments in the primary endpoint, and its corresponding 95% confidence interval, will be presented.

In addition, the proportions of patients in each group with at least 30% decrease in ACR will be presented, along with a 95% two-sided confidence interval for the difference in proportions.

2.5.3 Handling of intercurrent events of primary estimand

The primary estimand is described by the following attributes:

1. The target population comprises patients with kidney dysfunction due to SCD.
2. The primary variable is proportion of patients with ≥ 30% decrease in ACR at 12 months.
3. The treatment of interest is the effect of crizanlizumab 5.0 mg/kg + standard of care on the ACR value taken for the entire study duration with or without the intercurrent events
4. The intercurrent events are the events occurring after randomization that may impact the treatment effect. The intercurrent events of interest are:
   a. Treatment discontinuation
   b. Initiation or discontinuation of HU/HC, HA approved form(s) of L-glutamine (Endari™ in USA), ACE, and/or ARB
   c. Intake of NSAIDs within 48 hours prior to ACR measurements
   d. Intake of Voxelotor
   e. Blood transfusion or VOC occurred within 7 days of urine and blood sample collection
   f. Renal replacement therapy (i.e. hemodialysis, peritoneal dialysis, hemofiltration and kidney transplantation)
5. The summary measure is the odds ratio of treatment effect for patients with ≥ 30% decrease in ACR between crizanlizumab + standard of care and standard of care alone.

Handling of the intercurrent events

The approach of accounting for intercurrent events is as follows:

- **For the intercurrent events 4.a:** The composite strategy will be applied for treatment discontinuation due to death of any cause, drug-related AEs, and progressive disease. Define treatment discontinuation due to death of any cause, drug-related AEs, and progressive disease as non-responders. The hypothetical strategy will be applied for treatment discontinuation due to other reasons. The interest focuses on the treatment effect if patients stay on treatment for 12 months, if possible.
- **For the intercurrent events 4.b:** The hypothetical strategy. The interest focuses on the treatment effect if patients had not initiated or discontinued HU/HC, HA approved form(s) of L-glutamine (Endari™ in USA), ACE and/or ARB.
- **For the intercurrent events 4.c:** The hypothetical strategy. The interest focuses on the treatment effect if patients had not received NSAIDs within 48 hours prior to ACR measurement.
- **For the intercurrent events 4.d:** The hypothetical strategy. The interest focuses on the treatment effect if patients had not received Voxelotor
- **For the intercurrent events 4.e:** The hypothetical strategy. The interest focuses on the treatment effect if patients had not had blood transfusion or VOC occurred.
- **For the intercurrent events 4.f:** The composite strategy will be applied for treatment discontinuation due to renal replacement therapy, define treatment discontinuation due to renal replacement therapy as non-responders. The hypothetical strategy will be applied if the renal replacement therapy occurred and patient did not discontinue. The interest focuses

on the treatment effect if patients had not had renal replacement therapy. The reason of using hypothetical strategy is because for patients who had renal replacement therapy and continued the treatment with their renal function return to within 10% of their pre-AKI level should not be considered as treatment failure.

The primary estimand is described in Table 2-2 below, together with its key attributes. The supplementary estimands are described in Table 2-3.

Table 2-2 Primary estimand

Estimand	Target population	Summary Measure	Handling of the intercurrent events	Rationale
Primary estimand	Patients with kidney dysfunction due to SCD	Odds ratio of treatment effect on ACR response	Exclude ACR data collected after treatment discontinuation. Treatment discontinuation due to death of any cause, drug-related AEs, progressive disease will be considered as non-responders; other reasons will use multiple imputation (MI). Exclude ACR data after initiation or discontinuation of HU/HC, HA approved form(s) of L-glutamine (Endari™ in USA), ACE and/or ARB. Will use MI. Exclude ACR data collected after intake of NSAIDs within 48 hours prior to ACR measurement. Will use MI. Exclude ACR data collected after intake of Voxelotor. Will use MI. Exclude ACR data collected after blood transfusion or VOC occurred (if within 7 days before the ACR measure). Will use MI. Exclude ACR data collected after renal replacement therapy. Treatment discontinuation due to renal	See Section 2.5.3 Handling of the intercurrent events

Estimand	Target population	Summary Measure	Handling of the intercurrent events	Rationale
			replacement therapy will be considered as non-responders; If the patient required renal replacement therapy and did not discontinue study treatment, will use MI.	

2.5.4 Sensitivity analyses

No sensitivity analysis will be conducted.

2.6 Analysis of the key secondary objective

Not applicable.

2.7 Analysis supporting secondary objective(s)

The secondary objectives in this study are to describe the two treatment groups with respect to albuminuria/proteinuria, including change in ACR, ACR decline rate, and PCR improvement, and with respect to eGFR, percentage change in eGFR, eGFR decline rate, and the progression of CKD, also safety, and healthcare resource utilization will be evaluated. Another secondary objective is to assess the immunogenicity and accompanying PK of crizanlizumab.

2.7.1 Secondary endpoints

The secondary endpoints are the mean change in ACR from baseline to 3, 6, 9, and 12 months of treatment; the slope of ACR decline from baseline to 12 months of treatment based on ACR values at baseline and at 3, 6, 9, and 12 months, the percentage change in eGFR from baseline to 3, 6, 9, and 12 months of treatment, the slope of eGFR decline from baseline to 12 months of treatment based on eGFR values at baseline and at 3, 6, 9, and 12 months, the proportion of patients with ≥ 30% decrease in ACR at 6 months from baseline, the proportion of patients with PCR improvement and stable PCR (improvement: ≥ 20% decrease in PCR from baseline; stable: within ± 20% change from baseline) at 12 months from baseline and the proportion of patients with progression of CKD from baseline to 12 months.

2.7.2 Statistical hypothesis, model, and method of analysis

The secondary efficacy endpoints will be summarized descriptively using the FAS.

The mean change in ACR from baseline to 3, 6, 9, and 12 months of treatment

The mean change in ACR from baseline will be summarized and plotted over time by treatment arm. An Analysis of Covariance (ANCOVA) model that includes effects for treatment, randomization stratification factors, and baseline ACR, will be carried out for analysis.

The slope of ACR decline from baseline to 12 months of treatment based on ACR values at baseline and at 3, 6, 9, and 12 months

The slope of ACR decline will be estimated as a random coefficient in a linear mixed effect model: the model will be fitted to ACR data collected at baseline and at Months 3, 6, 9, and 12. It includes treatment, stratification factors, time and the interaction of treatment by time as the fixed effects, and intercept as a random effect. The mean slopes within each treatment and the difference in mean slopes between two treatments will be presented.

The percentage change in eGFR from baseline to 3, 6, 9, and 12 months of treatment

The percentage change in eGFR is calculated as the post-baseline eGFR value minus the baseline eGFR divided by the eGFR at baseline. The calculation of eGFR is based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (for patients ≥ 18 at screening) and Creatinine-based "Bedside Schwartz" (for patients < 18 at screening) equations. Percentage change in eGFR from baseline will be summarized and plotted over time by treatment arm. An ANCOVA model that includes effects for treatment, HU/HC use (Yes vs. No), and baseline eGFR, will be carried out for analysis using a logarithmic scale.

The slope of eGFR decline from baseline to 12 months of treatment based on eGFR values at baseline and at 3, 6, 9, and 12 months

The slope of eGFR decline will be estimated as a random coefficient in a linear mixed effect model: the model will be fitted to eGFR data collected at baseline and at Months 3, 6, 9, and 12. It includes treatment, stratification factors, time and the interaction of treatment by time as the fixed effects, and intercept as a random effect. The mean slopes within each treatment and the difference in mean slopes between two treatments will be presented.

Proportion of patients with progression of CKD from baseline to 12 months

The progression of CKD, which is based on both eGFR and ACR, will be assessed according to the classification presented based on KDIGO 2013 (See amended protocol v03 Figure 8-1).

The following analysis will be performed on CKD progression.

- For the proportion of patients with progression of CKD from baseline to 12 months (including patients with increase in CKD progression category defined by Figure 8-1 in protocol v03, patients with a 25% or greater drop in eGFR from baseline and patients with at least 50% increase in ACR for patients with severe (A3) albuminuria and a doubling of albumin levels in patients with moderate (A2) albuminuria), a logistic regression model that includes effects for treatment, HU/HC use, will be carried out.
- For the proportion of patients with a 25% or greater drop in eGFR from baseline to 12 months, a logistic regression model that includes effects for treatment, HU/HC use, will be carried out.
- For the proportion of patients with at least 50% increase in ACR for patients with severe (A3) albuminuria and a doubling of albumin levels in patients with moderate (A2) albuminuria, a logistic regression model that includes effects for treatment, HU/HC use, will be carried out.
- A shift table will be provided for patients with increase in CKD progression category for each treatment based on Figure 8-1 in Protocol v03.

Proportion of patients with ≥ 30% decrease in ACR at 6 months from baseline

For the proportion of patients with ≥ 30% decrease in ACR at 6 months from baseline, a logistic regression model that includes effects for treatment, stratification factors, will be carried out.

Proportion of patients with PCR improvement and stable PCR (improvement: ≥ 20% decrease in PCR from baseline; stable: within ± 20% change from baseline) at 12 months from baseline

For the proportion of patients with PCR improvement and stable PCR (improvement: ≥ 20% decrease in PCR from baseline; stable: within ± 20% change from baseline) at 12 months from baseline, a logistic regression model that includes effects for treatment, stratification factors, will be carried out.

2.8 Safety analyses

For all safety analyses, the Safety Set will be used. Safety summaries will include only on-treatment assessments; safety listings include all assessments with those that occur more than 30-days or 105-days after last study treatment flagged. All listings and tables will be presented by treatment group.

For all safety analyses, the Safety Set will be used.

2.8.1 Adverse events (AEs)

AE summaries will include all AEs occurring during the on-treatment period. All AEs collected in the AE eCRF page will be listed along with the information collected on those AEs e.g. AE relationship to study drug, AE outcome etc. AEs with start date outside of on-treatment period will be flagged in the listings.

AEs will be summarized by number and percentage of patients having at least one AE, having at least one AE in each primary SOC and for each PT using MedDRA coding. A patient with multiple occurrences of an AE will be counted only once in the respective AE category. A patient with multiple CTCAE grades for the same PT will be summarized under the maximum CTCAE grade recorded for the event. AE with missing CTCAE grade will be included in the 'All grades' column of the summary tables.

In AE summaries, the primary SOC will be presented alphabetically, and the PTs will be sorted within primary SOC in descending frequency. The sort order for the PT will be based on their frequency in the crizanlizumab + SOC group.

The following AE summaries will be produced by treatment group; overview of AEs and deaths, AEs by SOC and PT, summarized by relationship, seriousness, leading to treatment discontinuation, leading to dose interruption, requiring additional therapy and leading to fatal outcome. In addition, for EudraCT requirements a summary of (1) Serious AEs and deaths, with number of occurrences and (2) Non-serious AEs, with number of occurrences will be produced (an occurrence is defined as >1 day between start and prior end date of record of same PT).

All deaths (on-treatment and post-treatment) will be summarized.

All AEs, deaths, and SAEs (including those from the pre- and post-treatment periods) will be listed and those collected during the pre-treatment and post-treatment period will be flagged.

AEs will be assessed according to the CTCAE version 5.0.

Adverse events identified in the Novartis MedDRA Query (NMQ) topic of "COVID-19 diagnosis, manifestations, risks and complications including death" will be summarized and

listed. Summaries will be provided overall for this COVID-19 topic and by different classification levels defined within the topic.

2.8.1.1 Adverse events of special interest / grouping of AEs

Data analysis of AESIs

An adverse event of special interest (AESI) is a grouping of AEs that are of scientific and medical concern specific to compound crizanlizumab. These groupings are defined using MedDRA terms, SMQs (standardized MedDRA queries), HGLTs (high level group terms), HLT (high level terms) and PTs. Customized SMQs (Novartis MedDRA queries, NMQ) may also be used. A NMQ is a customized group of search terms which defines a medical concept for which there is no official SMQ available or the available SMQ does not completely fit the need. It may include a combination of single terms and/or an existing SMQ, narrow or broad. For each specified AESI, number and percentage of patients with at least one event of the AESI occurring during the on-treatment period will be summarized. The list of AESI to be taken into account for crizanlizumab trials is documented in electronic case retrieval sheet (eCRS) for the project. The most up to date version of the eCRS available at the time of a database lock for an analysis will be used to define on which AESIs the analysis will be conducted.

Summaries of these AESIs will be provided by treatment group, (specifying grade, serious AE, relationship, leading to treatment discontinuation, leading to dose interruption).

A listing of all grouping levels down to the MedDRA PTs used to define each AESI will be generated.

In addition, all AEs and serious AEs will be listed.

2.8.2 Deaths

Separate summaries for on-treatment and all deaths will be produced by treatment group, SOC and PT.

All deaths will be listed, post treatment deaths will be flagged. A separate listing of deaths prior to starting treatment will be provided for all screened patients.

2.8.3 Laboratory data

Data handling

For urine sample replicates, the average of the parameters at that assessment will be used in the analyses.

Data analysis

On analyzing laboratory, data from all sources (central and local laboratories) will be combined, except ACR/PCR and eGFR parameters, these kidney-related parameters are from central lab. The summaries will include all assessments available for the lab parameter collected no later than 105 days (if applicable) after the last study treatment administration date.

The following summaries will be produced for hematology (including coagulation), biochemistry, and urinalysis (macroscopic only) laboratory data (by laboratory parameter and treatment group):

- Worst post-baseline CTCAE grade (regardless of the baseline status). Each patient will be counted only for the worst grade observed post-baseline.
- Shift tables using CTCAE grades to compare baseline to the worst on-treatment value
- For laboratory tests where CTCAE grades are not defined, shift tables using the pre-specified thresholds to compare baseline to the worst on-treatment value.

The following listings will be produced for the laboratory data:

- Listings of all laboratory data with values flagged to show the corresponding CTCAE v5.0 grades and classification relative to the laboratory normal range. Lab data collected during the post-treatment period will be flagged.
- Listing of all CTCAE Grade 3 or 4 laboratory toxicities

For hemoglobin and leukocytes parameters, a summary and box plot of the absolute change from baseline over time based on time windows will be provided.

Liver function parameters

Liver function parameters of interest are total bilirubin (TBL), direct bilirubin (DBILI), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP). The number (%) of patients with worst post-baseline values as per Novartis Liver Toxicity guidelines will be summarized in addition to the baseline values.

Shift tables using ALT, AST, and TBL will be generated, respectively. Evaluation of drug-induced serious hepatotoxicity (eDISH) plot will be produced and a similar plot of DBILI vs. ALT will also be generated. Individual patient plots will be produced for patients with potential severe drug-induced liver injury. Plot of prothrombin time / international normalized ratio (PT-INR) and albumin over time will also be produced for the same patients. A corresponding listing of patients with potential severe drug induced liver injury will be generated.

The following summaries will be produced:

- ALT or AST > 3 x upper limit of normal (ULN)
- ALT or AST > 5 x ULN
- ALT or AST > 8 x ULN
- ALT or AST > 10 x ULN
- ALT or AST > 20 x ULN
- TBL > 2 x ULN
- TBL > 3 x ULN
- ALT or AST > 3 x ULN & TBL > 2 x ULN
- ALT or AST > 3 x ULN & TBL > 2 x ULN & ALP < 2 x ULN (potential Hy's law)
- ALT or AST > 3 x ULN & TBL > 2 x ULN & ALP ≥ 2 x ULN
- ALT > 3 x ULN & DBILI > 2 x ULN & ALP < 2 x ULN

Potential Hy's Law events are defined as those events with concurrent occurrence of AST or ALT > 3 x ULN and TBL > 2 x ULN and ALP < 2 x ULN in the same assessment sample during the on-treatment period. Patients with potential severe drug-induced liver injury are defined as those patients with concurrent occurrence of ALT > 3 x ULN and DBILI > 2 x ULN and ALP < 2 x ULN in the same assessment sample during the on-treatment period.

Other laboratory parameters

Hepatitis markers and additional tests only performed at screening will be listed only.

2.8.4 Other safety data

2.8.4.1 ECG and cardiac imaging data

Data handling

For ECG replicates, the average of the ECG parameters at that assessment will be used in the analyses.

Data analysis

12-lead ECGs including PR, QRS, QT, QTcF and HR intervals will be obtained for each patient during the study. ECG data will be read and interpreted centrally.

The number and percentage of patients with notable ECG values will be presented by treatment group.

- QT, QTcF
  - New value of > 450 and ≤ 480 ms
  - New value of > 480 and ≤ 500 ms
  - New value of > 500 ms
  - Increase from Baseline of > 30 ms to ≤ 60ms
  - Increase from Baseline of > 60 ms
- HR
  - Increase from baseline > 25% and to a value > 100 bpm
  - Decrease from baseline > 25% and to a value < 50 bpm
- PR
  - Increase from baseline >25% and to a value > 200 ms
  - New value of > 200 ms
- QRS
  - Increase from baseline >25% and to a value > 120 ms
  - New values of QRS > 120 ms

A listing of notable ECG values will be produced by treatment group. In the listing, the assessments collected during the post-treatment period will be flagged.

[redacted]

2.8.4.2 Vital signs

Vital sign assessments are performed in order to characterize basic body function. The following parameters were collected: height (cm), weight (kg), body temperature (°C), pulse rate (beats per minute), respiratory rate (breaths per minute), systolic and diastolic blood pressure (mmHg).

Data handling

Vital signs collected on treatment will be summarized. Values measured outside of the on-treatment period will be flagged in the listings.

Data analysis

For analysis of vital signs the clinically notable criteria are provided in Table 2-4 below.

Table 2-4 Clinically notable changes in vital signs

Vital sign (unit)	Criteria	< 18 years at baseline and < 18 years at time of assessment	< 18 years at baseline and ≥ 18 years at time of assessment	≥ 18 years at baseline
Systolic blood pressure (mmHg)	High	≥ 95th percentile of the age and height group[1]	≥ 180 with increase from updated baseline[5] of ≥20	≥180 with increase from baseline of ≥20
	Low	≤ 5th percentile of the age and height group[1]	≤ 90 with decrease from updated baseline[5] of ≥20	≤90 with decrease from baseline of ≥20
Diastolic blood pressure (mmHg)	High	≥ 95th percentile of the age and height group[1]	≥ 105 with increase from updated baseline[5] of ≥15	≥105 with increase from baseline of ≥15
	Low	≤ 5th percentile of the age and height group[1]	≤ 50 with decrease from updated baseline[5] of ≥15	≤50 with decrease from baseline of ≥15
Pulse rate (bpm)	High	≥16years: >92	≥120 with increase from updated baseline[5] of ≥15	≥100 with increase from baseline of >25%
	Low	≥16 years: <58	≤50 with decrease from updated baseline[5] of ≥15	≤50 with decrease from baseline of >25%
Weight (kg)	High	increase from baseline[3] of ≥2 BMI-for-age percentile categories[4]	increase from updated baseline[5] of ≥10%	increase >10% from baseline
	Low	decrease from baseline[3] of ≥2 BMI-for-age percentile categories[4]	decrease from updated baseline[5] of ≥10%	decrease >10% from baseline
Respiratory rate (breath per minute)[2,6,7]	High	≥16years: ≥20	≥20	≥20

Vital sign (unit)	Criteria	< 18 years at baseline and < 18 years at time of assessment	< 18 years at baseline and ≥ 18 years at time of assessment	≥ 18 years at baseline
	Low	≥16 years: <12	<12	<12
Oral body temperature (°C)	High	≥38.4	≥39.1	≥39.1
	Low	≤35.0	≤35.0	≤35.0

bpm=beats per minute; NHLBI= National Heart, Lung, and Blood Institute;

[1] Blood pressure percentiles are calculated for each blood pressure record using the method described in Appendix B of the following reference: The Fourth Report on Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents. Pediatrics 2004; 114; 555.

[2] Fleming S, 2011

[3] Baseline BMI-for-age weight status categories are underweight (less than the 5th percentile), healthy weight (5th percentile to less than the 85th percentile), overweight (85th to less than the 95th percentile) and obese (equal to or greater than the 95th percentile);

[4] BMI-for-age percentiles categories (P3, P5, P10, P25, P50, P75, P85, P90, P95, P97) are obtained from the WHO Growth Charts;

[5] Updated baseline is the last value collected before the 18th birthday.

[6] Eldridge L, 2015;

[7] Kou .R, 2009.

The number and percentage of patients with notable vital sign values (high/low) will be presented by treatment group.

A listing of notable vital sign values will be produced by treatment group. In the listing, the assessments collected outside of on-treatment period will be flagged.

2.8.4.3 Immunogenicity

Immunogenicity will be measured pre-dose on Week 1 Day 1, during treatment, at visit EOT, and at the 105-day follow-up visit (as applicable).

All immunogenicity results will be summarized for the crizanlizumab + standard of care treatment group by patient and visit/time.

Immunogenicity will be characterized descriptively tabulating ADA incidence on-treatment.

2.9 Pharmacokinetic endpoints

Crizanlizumab pre-dose/trough PK samples will be taken to accompany immunogenicity measurements to evaluate the impact of immunogenicity on exposure (PK) (see amended protocol v03 Section 12.5.3).

Crizanlizumab pre-dose serum concentration data will be summarized for the crizanlizumab + standard of care treatment arm by patient and visit/sampling time point. Descriptive summary statistics will be provided for the crizanlizumab + standard of care treatment arm by visit/sampling time point, including the frequency (n, %) of concentrations below the LLOQ and reported as zero.

Descriptive summary statistics will include mean (arithmetic and geometric), SD, coefficient of variance (CV; arithmetic and geometric), median, minimum, and maximum.

2.10 PD and PK/PD analyses

Not applicable.

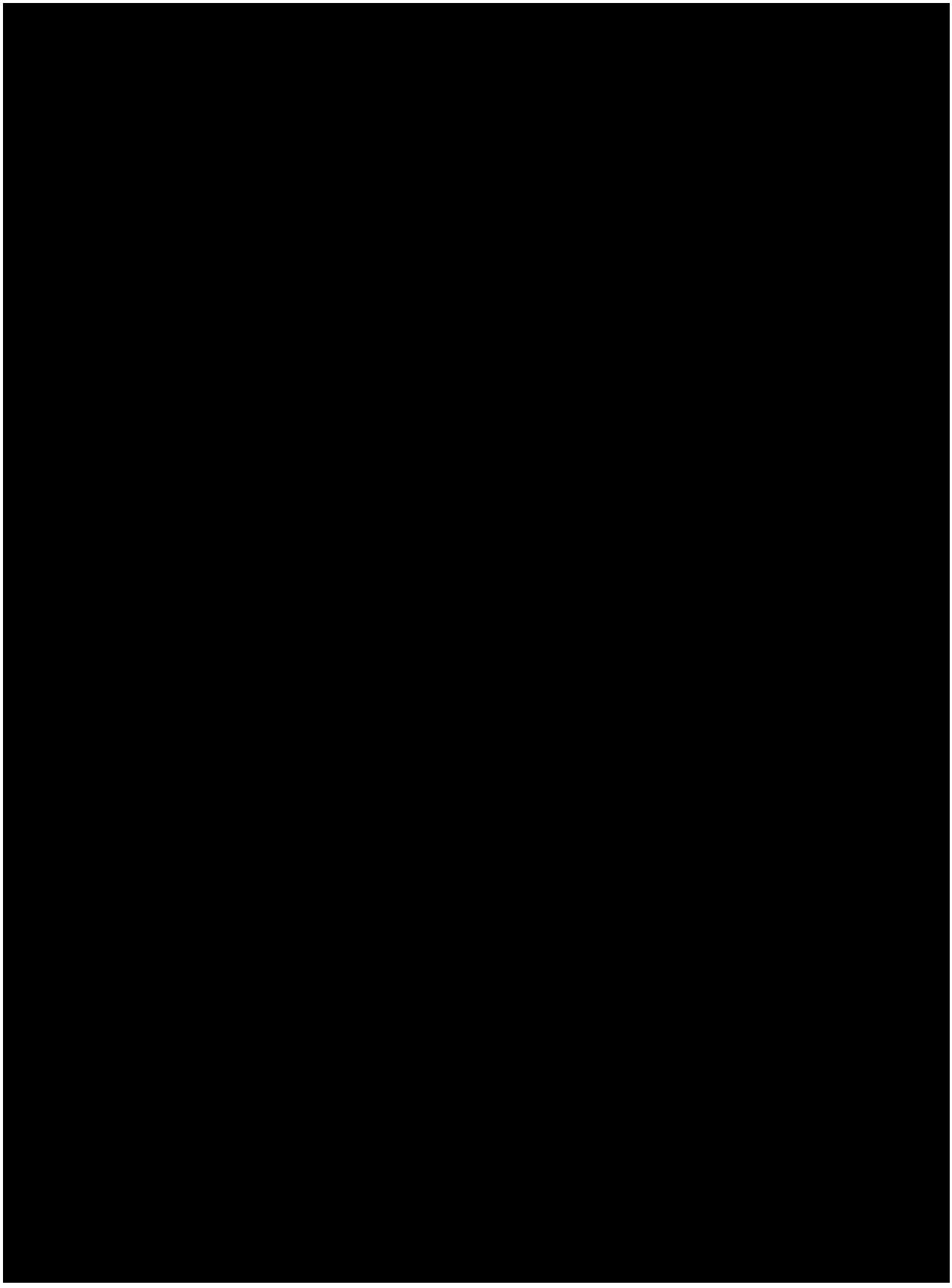
2.11 Resource utilization

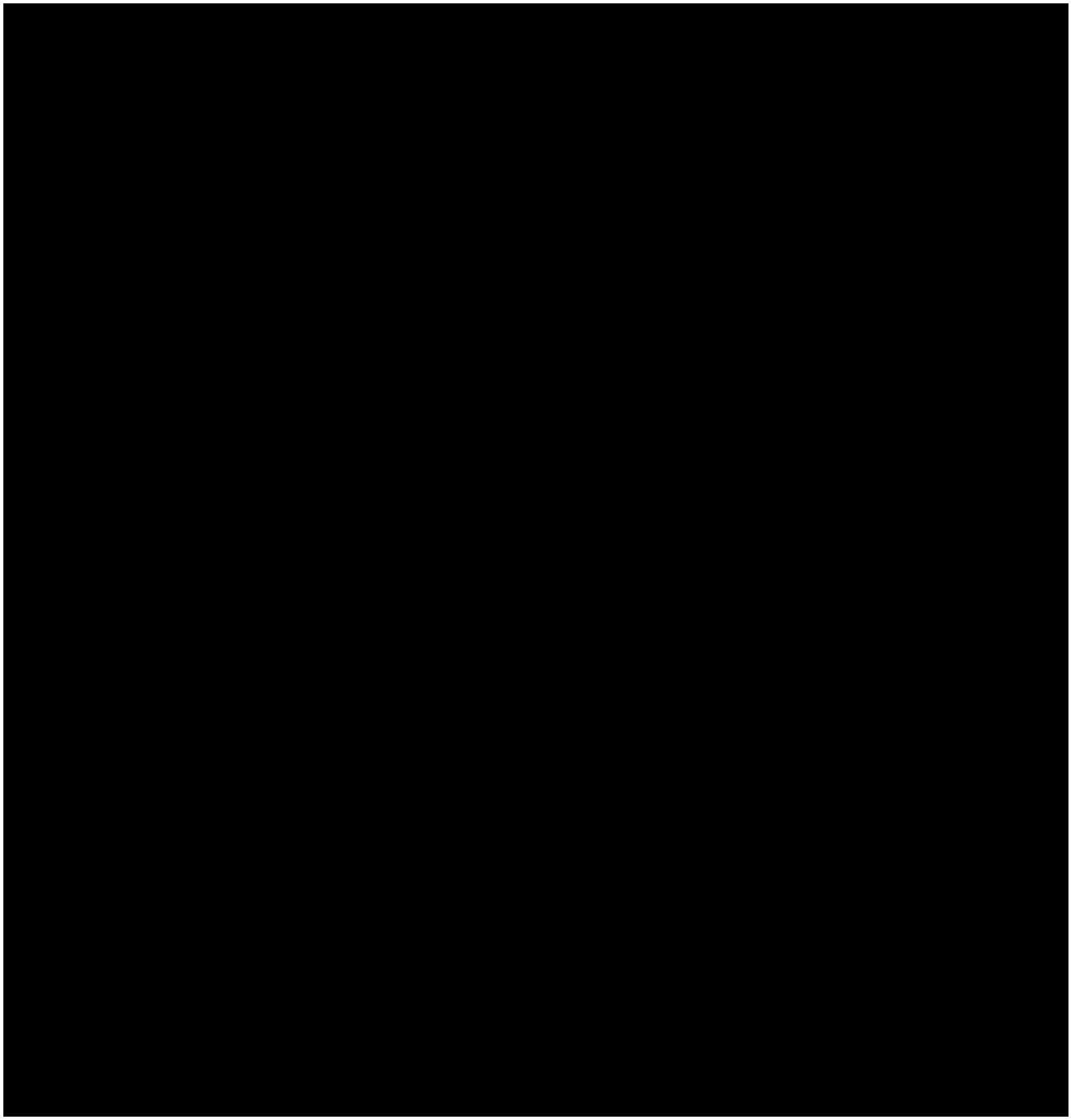
Annualized rate of visits to ER and hospitalizations due to AKI events, VOCs, or other SCD complications will be used to evaluate the healthcare resource utilization (visits to clinic, ER and hospitalizations) in crizanlizumab + standard of care arm and standard of care alone. In addition, the number/proportion of patients with VOC events managed at home (not requiring healthcare resource utilization) will also be summarized.

The frequency of hospitalization and ER visits over the treatment (overall and AKI events-related, VOCs, or other SCD complications) will be summarized by treatment group. This analysis will also include the baseline rate of VOCs leading to healthcare visit in 12 months prior to screening visit as per CRF.

- Sickle cell VOC history, including the number of:
    - VOCs at home (not requiring healthcare resource utilization) in the previous 12 months
    - VOCs requiring healthcare resource utilization in the previous 12 months, including the number of:
        - Hospitalizations in the 12 months
        - ER visits in the previous 12 months
    - SCD complications other than VOCs requiring healthcare resource utilization in the previous 12 months, including the number of:
        - Hospitalizations in the previous 12 months
        - ER visits in the previous 12 months

  Data will be collected for complicated and uncomplicated VOCs. Complicated VOCs are defined as Acute chest syndrome (ACS), hepatic sequestration, splenic sequestration, and priapism
- Number of missed school days or work days due to VOCs and/or other SCD complications in the previous 12 months. Data will be collected for complicated and uncomplicated VOCs
- Other complications of SCD, including, but not limited to, stroke, leg ulcers, asthma, avascular necrosis, pulmonary hypertension, retinopathy, other (investigator to specify)





2.15 Interim analysis

No interim analysis is planned. However, safety DMC analyses will be performed at specified time points, as defined in the DMC Charter. Additional DMC meetings may be scheduled if unexpected safety findings arise.

3 Sample size calculation

The sample size was adjusted to reflect current recruitment status.

When the estimation based approach for the 95% CI for the difference between treatment arms is used to calculate the precision estimates with sample size of 25 patients per treatment arm (total N=50 patients without drop-outs), then the width is 0.429 and the precision (half of the width) is around 0.215. See Table 12-3 for the 95% CI calculation of the difference (30% - 10%) varying the sample size per arm from 10 to 40.

Table 3-1 Confidence intervals for the difference between two proportions using proportions numeric results for two-sided confidence intervals for the difference in proportions confidence interval method: Chi – Square – Simple Asymptotic (Pearson)

Confidence Level	N1	N2	Allocation Ratio	Actual Width	P1	P2	P1-P2	Lower Limit	Upper Limit
0.95	10	10	1.000	0.679	0.300	0.100	0.200	-0.139	0.539
0.95	15	15	1.000	0.554	0.300	0.100	0.200	-0.077	0.477
0.95	20	20	1.000	0.480	0.300	0.100	0.200	-0.040	0.440
0.95	25	25	1.000	0.429	0.300	0.100	0.200	-0.015	0.415
0.95	30	30	1.000	0.392	0.300	0.100	0.200	0.004	0.396
0.95	35	35	1.000	0.363	0.300	0.100	0.200	0.019	0.381
0.95	40	40	1.000	0.339	0.300	0.100	0.200	0.030	0.370

4 Change to protocol specified analyses

No changes from the protocol-specified analysis were made.

5 Appendix

5.1 Imputation rules

5.1.1 Study drug

The following rule will be used for the imputation of the dose end date for a given study treatment component:

Scenario 1: If the dose end date is completely missing and there is no end of treatment (EOT) page and no death date, the patient is considered as on-going:

The patient should be treated as on-going and the cut-off date should be used as the dose end date.

Scenario 2: If the dose end date is completely or partially missing and the EOT page is available:

Case 1: The dose end date is completely missing, and the EOT completion date is complete, then this latter date should be used.

Case 2: Only Year(yyyy) of the dose end date is available and yyyy < the year of EOT date:

Use Dec31yyyy

Case 3: Only Year(yyyy) of the dose end date is available and yyyy = the year of EOT date:

Use EOT date

Case 4: Both Year(yyyy) and Month (mm) are available for dose end date, and yyyy = the year of EOT date and mm < the month of EOT date:

Use last day of the Month (mm)

All other cases should be considered as a data issue and the statistician should contact the data manager of the study.

After imputation, compare the imputed date with start date of treatment, if the imputed date is < start date of treatment:

Use the treatment start date

Patients with missing start dates will be considered missing for all study treatment component related calculations and no imputation will be made. If start date is missing then end-date will not be imputed.

5.1.2 AEs, concomitant medications, and safety assessment date imputation

Table 5-1 Imputation of start dates (AEs, concomitant medications) and assessments (laboratory, ECG, vital signs)

Missing Element	Rule
day, month, and year	• No imputation will be done for completely missing dates

day, month	• If available year = year of study treatment start date then
	  o If stop date contains a full date and stop date is earlier than study treatment start date then set start date = 01JanYYYY
	  o Else set start date = study treatment start date.
	• If available year > year of study treatment start date then 01JanYYYY
	• If available year < year of study treatment start date then 01JulYYYY

Missing Element	Rule
day	• If available month and year = month and year of study treatment start date then o If stop date contains a full date and stop date is earlier than study treatment start date then set start date= 01MONYYYY. o Else set start date = study treatment start date. • If available month and year > month and year of study treatment start date then 01MONYYYY • If available month and year < month year of study treatment start date then 15MONYYYY

Table 5-2 Imputation of end dates (AE, concomitant medications)

Missing Element	Rule (*=last treatment date plus 30 or 105 days not > (death date, withdrawal of consent date))
day, month, and year	• Completely missing end dates (incl. ongoing events) will be imputed by the end date of the on-treatment period*
day, month	• If partial end date contains year only, set end date = earliest of 31DecYYYY or end date of the on-treatment period *
day	• If partial end date contains month and year, set end date = earliest of last day of the month or end date of the on-treatment period*

Any AEs and concomitant medications with partial/missing dates will be displayed as such in the data listings.

Any AEs and ConMeds which are continuing as per data cut-off will be shown as 'ongoing' rather than the end date provided.

For AEs with start date before or on the cut-off date and end date after the cut-off date, the outcome will be reported as unknown.

5.2 AEs coding/grading

AEs are coded using the MedDRA terminology.

AEs will be assessed according to the CTCAE version 5.0.

The CTCAE represents a comprehensive grading system for reporting the acute and late effects of cancer treatments. CTCAE grading is by definition a 5-point scale generally corresponding to mild, moderate, severe, life threatening, and death. This grading system inherently places a value on the importance of an event, although there is not necessarily proportionality among grades (a grade 2 is not necessarily twice as bad as a grade 1).

5.3 Laboratory parameters derivations

Grade categorization of lab values will be assigned programmatically as per CTCAE version 5.0. The calculation of CTCAE grades will be based on the observed laboratory values only, clinical assessments will not be taken into account. The criteria to assign CTCAE grades are given in Novartis internal criteria for CTCAE grading of laboratory parameters. The latest available version of the document based on the underlying CTCAE version 5.0 at the time of analysis will be used (Guideline for implementation of CTCAE version 6). For laboratory tests where grades are not defined by CTCAE v5.0, results will be graded by the low/normal/high (or other project-specific ranges, if more suitable) classifications based on laboratory normal ranges.

A severity grade of 0 will be assigned for all non-missing lab values not graded as 1 or higher. Grade 5 will not be used. For laboratory tests that are graded for both low and high values, summaries will be done separately and labelled by direction, e.g., sodium will be summarized as hyponatremia and hypernatremia.

Imputation Rules

CTCAE grading for blood differentials is based on absolute values. However, this data may not be reported as absolute counts but rather as percentage of white blood cells (WBC).

If laboratory values are provided as '<X' (i.e. below limit of detection) or '>X', prior to conversion of laboratory values to SI unit, these numeric values are set to X.

The following rules will be applied to derive the WBC differential counts when only percentages are available for a xxx differential

xxx count = (WBC count) * (xxx %value / 100)

Further derivation of laboratory parameters might be required for CTCAE grading. For instance, corrected calcium can be derived using the reported total calcium value and albumin at the same assessment using the following formula:

Corrected Calcium (mg/dL) = Calcium (mg/dL) – 0.8 [Albumin (g/dL)-4]

In order to apply the above formula, albumin values in g/L will be converted to g/dL by multiplying by 0.1), calcium values in mmol/L will be converted to mg/dL by dividing by 0.2495. For calculation of laboratory CTCAE grades 0 and 1, the normal range for derived corrected calcium is set to the same limits (in mg/dL) as for calcium.

CTCAE grades for the derived absolute WBC differential counts (neutrophils, lymphocytes) and corrected calcium will be assigned as described above for grading

5.4 Statistical models

5.4.1 Primary analysis

Logistic regression

The primary endpoint will be analyzed based on the data from the FAS. A logistic regression model that includes effects for treatment and randomization stratification factors will be utilized.

The test of the treatment effect (based on the log-odds ratio estimated by the model) will be carried out at the 1-sided significance level of 0.025. The null hypothesis is that the odds ratio of the treatment effect is equal to 1 (i.e., there is no difference in the proportion of patients with ≥ 30% decrease in ACR at 12 months between crizanlizumab + standard of care and standard of care alone). The alternative hypothesis is that the odds ratio is greater than 1 (i.e., the proportion of patients with ≥ 30% decrease in ACR at 12 months is higher in the crizanlizumab + standard of care group than in the standard of care alone group).

Odds ratios will be computed for comparisons of Crizanlizumab + SOC versus SOC utilizing the logistic regression model fitted.

The odds ratio will be calculated such that an odds ratio >1 is favorable for Crizanlizumab + SOC arm. Using PROC GENMOD to calculate the confidence interval for the odds ratios assumes asymptotic normality of the Wald estimate for the regression coefficient. The 95% confidence interval for the regression parameter of the active treatment effect relative to control will be calculated using an exponential transformation to create the confidence interval for the odds ratio.

The SAS procedure GENMOD will be used with the following code:

```
proc genmod data=.... DESCENDING;
  class trt stratum1 stratum2;
  model aval = trt stratum1 stratum2/
                  dist=bin link=logit;
  lsmeans trt /diff;
  estimate "Crizanlizumab +SOC vs SOC" trt 1 -1;  ods output
Estimates=imp_est;
 run;
```

where binary event AVAL = The patient with at least a 30% decrease ACR (=1 or "Yes")

trt = planned treatment

stratum1 = CKD risk category (moderate risk or high/very high risk)

stratum2 = HU/HC prescription (Yes/No)

In cases where logistic regression doesn't converge, the following steps will be performed:
1. Run the PROC GENMOD procedure with EXACT statement;
2. If convergence not reached, perform Fisher's exact test. In this case, no odds ratios or confidence intervals will be estimated, but p-values may be calculated.

```
Proc freq data=....;
Table TRT * AVAL / fisher;
Where TRT in ("crizanlizumab + SOC", "SOC");
Run;
```

Wald CI for absolute difference in response rates

The 100 × (1 – α) % Wald CI of the absolute difference between the proportion of

responders (binary outcome = 1 or "Yes"), for a given treatment arm **X** compared to that for treatment group **Y**, without applying a continuity correction, is obtained from the following:

```
proc freq data =
dataset;
      where treatment group = Crizanlizumab+SOC or SOC;
      table treatment group * binary event / riskdiff alpha = alpha
      level;
```

Multiple imputations

Multiple imputation (MI) is a simulation based approach where missing values are replaced by multiple Bayesian draws from the conditional distribution of missing data given the observed data and covariates, creating multiple completed data sets. These completed data sets can then be analyzed using standard methods. Rubin (1987) presented rules how to combine the multiple sets of estimates to produce overall estimates and confidence intervals that adequately incorporate missing data uncertainty. In the multiple imputation analysis, the response status will be imputed separately for each treatment group including CKD risk category and HU/HC use.

The number of imputations will be set to 100 (NIMPUTE), the seed for the random function will be set to 101<studycode> for this study. To generate the multiple imputed data sets, the SAS procedure MI can be used as follows:

```
PROC MI DATA=<ACR> OUT=<impdata> SEED=1012203<studycode> NIMPUTE=100;
 CLASS <AVAL> <stratum1> <stratum2> <trt>;
 BY <treatment group>;
 fcs logistic (<AVAL>=<trt> <stratum1> <stratum2>/details);
 VAR <AVAL>;
RUN;
```

The ACR response rate will be calculated for each imputation and then combined using Rubin's rules. In order to calculate the response rate for each imputation, PROC FREQ will be used as follows.

Calculate binomial proportion and standard error for each imputation.

```
proc freq data=<ACR>;
by treat visit _imputation_ ;
tables <response> / binomial (level=2 cl=wilson correct) ;
ods output BinomialProp=imp_bpr;
run;
```

Transpose the dataset for subsequent use with PROC MIANALYZE.

```
proc transpose data=imp_bpr out=imp_trs(drop=_name_) ;
by treat visit _imputation_ ;
var nvalue1; id name1; idlabel label1;
run;
```

Apply LOGIT transformation: y=log(p/(1-p)) and std. err. transformation: <new se> = se/(p*(1-p))

```
data logit;
set imp_trs(rename=(_bin_=p e_bin=se));
by treat visit _imputation_ ;
lmean=log(p/(1-p));
lse=se/(p*(1-p));
run;
```

The transformed binomial proportion estimates and standard errors are combined by applying Rubin's rules for multiple imputed data sets.

```
proc mianalyze data=logit;
by treat;
modeleffects lmean;
stderr lse;
ods output ParameterEstimates=logitres;
run;
```

The combined data should be transformed back using the following formula: p=1/(1+exp(-y))

```
data miexpres;
set logitres;
by treat visit ;
resti = 1/(1+exp(-estimate));
rlow = 1/(1+exp(-lclmean));
rupp = 1/(1+exp(-uclmean));
run;
```

Of note, sometimes all responses may be imputed to 0 or 1 at a given combination of response variable, treatment group and visit. Such cases should be considered separately. The combined final response rate would be the same as the original response but the 95% CI will be undefined.
The odds ratio will be derived using GENMOD for each imputation, then combined using Rubin's rules again.

```
proc genmod data = acr_mi descending;
by avisitn _imputation_;
class trt stratum1 stratum2;
model aval = trt stratum1 stratum2/ link=logit dist=bin;
lsmeans trt_ / diff;
estimate ' Crizanlizumab +SOC vs SOC' trt_ 1 -1;
ods output Estimates=imp_est;
run;
proc mianalyze data=imp_est;
by avisitn trt_ ;
modeleffects LBetaEstimate;
stderr StdErr;
ods output ParameterEstimates=_res;
run;
```

Details of Implementation

Steps in implementing MI and monotherapy response for binary variables

1. Set ACR data collected after patient discontinued study treatment due to other reasons (except death, drug-related AEs, and progressive disease) to missing.
2. Set ACR data collected after patient initiatiated or discontinuationed of HU/HC, HA approved form(s) of L glutamine, ACE and/or ARB to missing.
3. Set ACR data collected after patient intook of NSAIDs within 48 hours prior to ACR measurement to missing.
4. Set ACR data collected after patient intook of Voxelotor to missing.

5. Set ACR data collected after patient had blood transfusion or VOC occurred (if within 7 days before the ACR measure) to missing.

6. Apply multiple imputation to create multiple sets of complete dataset.

7. Set as non-responder if the patient discontinued study treatment due to death of any cause, drug-related AEs, progressive disease.

8. Proceed with the analysis.

5.4.2 Key secondary analysis

Not applicable.

6 Reference

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