NOVARTIS

Clinical Development

SEG101/Crizanlizumab

CSEG101A2202 / NCT03264989

A phase 2, Multicenter, Open-Label Study to Assess PK/PD of SEG101 (crizanlizumab), with or without Hydroxyurea/Hydroxycarbamide, in Sickle Cell Patients with Vaso-Occlusive Crisis

Statistical Analysis Plan (SAP)

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25-Oct-2018	Prior to DBL	Amendment 1	Patient population has been changed from adults age 18-70 to age 16-70.	Title, Section 1
			It has been clarified that the maximum number of patients enrolled is 45 for 5.0 mg/kg treatment group.	Sections 1.1, 1.2, 2.1
			SEG101 has been changed to crizanlizumab.	Sections 2.1.1, 2.5.1, 2.8.1.1
			The definition of PAS1 and PDS1 have been updated.	Section 2.2
			The definition of dose interruption has been added.	Section 2.4.1
			The individual plots of concentration-time profile and P-selectin inhibition-time profile have been removed.	Section 2.5.2
			Geometric mean has been replaced by median in the PK and PD plots.	Sections 2.13.3, 2.13.4
			Analyses about liver have been updated.	Section 2.8.3
			[redacted].	Section 2.8.4.1
			CTCAE version has been updated.	Section 5.2, 5.3

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List of abbreviations

ACS	Acute Chest Syndrome
AE	Adverse event
AESI	Adverse events of special interest
ATC	Anatomical Therapeutic Classification
AUC	Area Under the Curve
BMI	Body Mass Index
CSR	Clinical Study report
CTC	Common Toxicity Criteria
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of Variation
DMC	Data Monitoring Committee
FAS	Full Analysis Set
ECG	Electrocardiogram
ER	Emergency Room
CRF	Case Report Form
FAS	Full Analysis Set
HbS	Human hemoglobin S (sickle cell hemoglobin)
HbSβ	Heterozygotes with one copy of the HbS gene and β-thalassemia variants (β^0 or β^+)
HbSC	Heterozygous sickle Hemoglobin C disease (hemoglobin SC disease)
HbSS	Homozygous sickle cell disease (hemoglobin SS disease)
HGLT	High level group terms
HLT	High level terms
HU/HC	Hydroxyurea/Hydroxycarbamide
MedDRA	Medical Dictionary for Drug Regulatory Affairs
NCI	National Cancer Institute
NMQ	Novartis MedDRA Queries
PAS	Pharmacokinetics Analysis Set
PD	Pharmacodynamics
PDS	Pharmacodynamics Analysis Set
PFS	Progression-Free Survival
PK	Pharmacokinetics
popPK	Population PK
PPS	Per-Protocol Set
PRO	Patient-reported Outcomes
PT	Preferred Term
QoL	Quality of Life
RAP	Report and Analysis Process
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCD	Sickle Cell Disease
SCPC	Sickle Cell Pain Crisis
SMQ	Standardized MedDRA Queries

SOC	System Organ Class
TFLs	Tables, Figures, Listings
VOC	Vaso-Occlusive Crisis
WHO	World Health Organization

1 Introduction

This statistical analysis plan (SAP) describes all planned analyses for the Clinical Study Report (CSR) of study CSEG101A2202, a phase II, open label PK/PD study in sickle cell disease (SCD) subjects with vaso-occulsive crisis (VOC).

The content of this SAP is based on protocol CSEG101A2202 v00, except an update of the definitions of PAS and PDS to be implemented in the next protocol amendment.

1.1 Study design

This is a multicenter, single-arm, open-label study to assess PK/PD of crizanlizumab in subjects with SCD and VOC.

Approximately 55 subjects will be enrolled in the trial. Up to 45 eligible subjects will be treated with crizanlizumab at a dose of 5.0 mg/kg and up to 10 additional eligible subjects will be treated with crizanlizumab at a dose of 7.5 mg/kg.

The study will include subjects, aged 16-70 years, with confirmed diagnosis of SCD (genotypes HbSS, HbSC, HbS β^0-thalassemia, HbS β^+-thalassemia, and others) who have experienced at least 1 VOC within the preceding 12 months. Subjects may not plan to initiate hydroxyurea/hydroxycarbamide (HU/HC) during the study. For subjects who are already treated with HU/HC, they should be taking HU/HC for at least 6 months and should accept to take the same dose at the same schedule during the trial.

The first 45 patients will receive crizanlizumab 5.0 mg/kg by IV infusion over 30 min on week 1 day 1, week 3 day 1, and then day 1 of every 4-week cycle. Once 27 patients have evaluable PK/PD data (or a total of up to 45 patients are enrolled) at the 5.0 mg/kg dose, an exploratory cohort of 10 patients will be enrolled at a dose of 7.5 mg/kg. Patients will receive

crizanlizumab 7.5 mg/kg by IV infusion over 30 min on week 1 day 1, week 3 day 1, and then day 1 of every 4-week cycle. All enrolled patients will have PK/PD sampling performed in order to characterize the PK/PD of crizanlizumab.

Subjects will receive treatment until study treatment is permanently discontinued due to unacceptable toxicity, death, are lost to follow-up or discontinued from the study treatment for any other reasons. Subjects will be followed in the mandatory safety follow-up period until 105 days after the last dosing. Subjects continuing to receive benefit, defined as no evidence of increased frequency of VOCs and no severe adverse events attributed to drug as per Investigator's assessment, will remain on study until crizanlizumab is commercially available or until subjects are rolled over in a different crizanlizumab study or access program.

Exploration of early access (EA) PK data may occur for preliminary PK analysis prior to clinical data base lock. For the derivation of PK parameters nominal time instead of actual elapsed time may be used.

In addition, an interim analysis will be performed when there are at least 27 subjects with single dose evaluable PK profiles and 5 subjects with both single dose and multiple dose evaluable PK profiles in the 5.0 mg/kg treatment group in the Pharmacokinetic Analysis Set 1

(PAS1).This interim analysis will inform the PKPD part of the pediatric study CSEG101B2201.

The primary analysis will be conducted after the primary PK parameters and PD-AUC are available for at least 27 subjects in the 5.0 mg/kg treatment group in PAS1 and Pharmacodynamics Analysis Set 1 (PDS1) respectively, or a total of 45 patients have been enrolled in the 5.0 mg/kg treatment group, whichever occurs first. The primary analysis data will be summarized in the primary CSR.

The final analysis will occur at the end of the study. All available data from all subjects up to the trial end will be analyzed and summarized in a final CSR.

1.2 Study objectives and endpoints

Objective	Endpoint	Analysis
Primary		Refer to Section 2.5
Characterize PK of crizanlizumab at 5.0 mg/kg in SCD subjects	-PK parameters after the starting dose and after multiple doses. -Trough concentrations prior to each study drug dose.	
Characterize PD (P-selectin inhibition) of crizanlizumab at 5.0 mg/kg in SCD subjects	-P-selectin inhibition and PD-AUC after the starting dose, after multiple dosing, and prior to each study drug dose.	
Secondary		
Assess efficacy of crizanlizumab	-Number of VOC events leading to healthcare visit in clinic/ER/hospital over time -Number of VOC events treated at home (based on documentation by health care provider following phone contact with subject) over time -Number of hospitalizations and ER visits (both total and VOC-related) over time -Days of ER/hospitalization (both total and VOC-related) over time -Number of each subcategory of VOC event (uncomplicated pain crisis, acute chest syndrome, hepatic sequestration, splenic sequestration, priapism) over time -Number of VOC events (including both healthcare visit and home treatment) over time	Refer to Section 2.7
Assess safety and tolerability of crizanlizumab	-Number, seriousness, severity, and causality assessments of treatment emergent adverse events and other safety data as considered appropriate -Absolute change from baseline in hemoglobin -Immunogenicity: measurement of anti-drug antibodies (ADA) to crizanlizumab	Refer to Section 2.8

2 Statistical methods

2.1 Data analysis general information

The analysis of study data for the primary CSR will be based on all subjects' data up to a data cut-off date when the primary PK parameters and PD-AUC are available for at least 27 subjects for 5.0 mg/kg treatment group in the PAS1 and PDS1 respectively, or a total of 45 patients have been enrolled in the 5.0 mg/kg treatment group, whichever occurs first.

The additional data for any new subjects or subjects continuing to receive study drug past this time, as allowed by the protocol, will be further summarized in a final study report once these subjects have completed or discontinued the study.

All analyses will be performed by Novartis and/or a designated CRO. SAS version 9.4 or later software will be used to perform all data analyses and to generate tables, figures and listings.

General analysis conventions

Unless otherwise specified, data from all study centers will be pooled for the analysis. No center effect will be assessed. Categorical data will be presented as frequencies and

percentages; continuous data will be presented as n, mean, standard deviation (SD), median, minimum, and maximum.

For PK concentration and PK parameters, coefficient of variation (CV) (%), geometric mean, and geometric CV% will be presented in addition to the previously mentioned summary statistics.

CV (%) is calculated as follows:

100*(SD/arithmetic mean).

Geometric CV (%) is calculated as follows:

sqrt (exp (variance for log transformed data)-1)*100.

Unscheduled assessments

The following points summarize the rules for unscheduled assessments:

- Baseline: All unscheduled assessments before the first dose should be included for consideration when calculating the baseline value.
- In summary tables by visit, unscheduled assessments should not be included unless they qualify as baseline.
- In shift and abnormality tables, all unscheduled assessments are included.

Unscheduled assessments will be reported with the scheduled assessments in the listings.

Data included in the analysis

The analysis cut-off date for the primary analysis of study data will be established after the primary PK parameters and PD-AUC are available for at least 27 subjects in the 5.0 mg/kg treatment group in the PAS1 and PDS1, respectively, or a total of 45 patients have been enrolled in the 5.0 mg/kg treatment group, whichever occurs first. All statistical analyses will be performed using all data collected in the database up to the data cut-off date. All data with an assessment date or event start date (e.g. vital sign assessment date or start date of an adverse event) prior to or on the cut-off date will be included in the analysis. Any data collected beyond the cut-off date will not be included in the analysis and will not be used for any derivations at the time of the primary analysis.

All events with start date before or on the cut-off date and end date after the cut-off date will be reported as ‘ongoing’. The same rule will be applied to events starting before or on the cut-off date and not having documented end date. This approach applies, in particular, to adverse event and concomitant medication reports. For these events, the end date will not be imputed and therefore will not appear in the listings.

For the final analysis, all data collected will be used, without notion of cut-off date.

2.1.1 General definitions

Investigational drug and study treatment

Both investigational drug and study treatment refer to crizanlizumab. The term investigational treatment may also be referred to as study treatment which is used throughout this document.

Treatment

For presentation in the outputs, treatment refers to crizanlizumab 5.0 mg/kg and 7.5 mg/kg.

Date of first administration of investigational drug

The date of first administration of investigational drug is defined as the first date when a nonzero dose of investigational drug is administered and recorded on the Dosage Administration Record 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 nonzero dose of investigational drug is administered and recorded on dose administration 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 derived as the first date when a nonzero dose of study treatment was administered as per the Dosage Administration CRF. The date of first administration of study treatment will also be referred as start of study treatment.

The date of first administration of study treatment is the same as the date of first administration of investigational drug.

Date of last administration of study treatment

The date of last administration of study treatment is defined as the last date when a nonzero dose of study treatment was administered as per Dose Administration CRF.

The date of last administration of study treatment is the same as the date of last administration of investigational drug.

Study day

The study day, describes the day of the event or assessment date, relative to the start of study treatment. Study day 1 for all assessments is taken to be the start of study treatment.

The study day is defined as:

- The date of the event (visit date, onset date of an event, assessment date etc.) – Start of study treatement + 1, if event is on or after the start of study treatment;
- The date of the event (visit date, onset date of an event, assessment date etc.) – Start of study treatment, if event precedes the start of study treatment.

The study day will be displayed in the data listings. If an event starts before start of study treatment, the study day displayed in 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 safety evaluations, the last available assessment on or before the date of start of study treatment is taken as 'baseline' assessment. In case time of assessment and time of treatment start is captured, 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 would be calculated (if not already available in the database) before determining baseline.

In rare 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 subjects 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

The overall observation period will be divided into three mutually exclusive segments:

1. Pre-treatment period: from day of subject's informed consent to before date of first administration of study treatment
2. On-treatment period: from date of first administration of study treatment to 105 days after date of last administration of study treatment (including start and stop date)
3. Post-treatment period: starting at day 106 after last administration of study treatment.

Note: If dates are incomplete in a way that clear assignment to pre-, on-, post-treatment period cannot be made, then the respective data will be assigned to the on-treatment period.

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 adverse events

(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.

2.2 Analysis sets

Full analysis set

The Full Analysis Set (FAS) comprises all subjects to whom study treatment has been assigned and who received at least one dose of study treatment. Subjects will be analyzed according to the crizanlizumab dose (5.0 mg/kg or 7.5 mg/kg) they have been assigned to.

Safety set

The Safety Set includes all subjects who received at least one dose of study treatment. Subjects will be analyzed according to the dose level received (5.0 mg/kg or 7.5 mg/kg), where dose level received is defined as the assigned dose level if the subject took at least one dose of that treatment or the first dose received if the assigned dose level was never received.

Pharmacokinetic analysis set

The Pharmacokinetic analysis set 1 (PAS1) includes all subjects who provide at least one evaluable PK profile. A profile is considered evaluable if all of the following conditions are satisfied:

- Subject receives the planned treatment of 5.0 mg/kg or 7.5 mg/kg before single dose PK profile or 3 consecutive doses of the planned treatment before the multiple dose PK profile
- Subject provides at least one primary PK parameter
- Subject does not have any transfusion of blood product in the last 4 weeks before the first PK sample of the full PK profile, or during the full PK profile

The Pharmacokinetic analysis set 2 (PAS2) includes all subjects who receive at least one planned treatment of 5.0 mg/kg or 7.5 mg/kg and provide at least one corresponding evaluable PK concentration.

A usbject is considered to receive the planned treatment if the actual dose received divided by the last weight measured before the dose is within a ±5% window of the planned dose.

Pharmacodynamics analysis set

The pharmacodynamics analysis set 1 (PDS1) includes all subjects who provide at least one evaluable PD profile. A profile is considered evaluable if all of the following conditions are satisfied:

- Subject receives the planned treatment of 5.0 mg/kg or 7.5 mg/kg before single dose PD profile or 3 consecutive doses of the planned treatment before the multiple dose PD profile
- Subjects provides at least one PD-AUC (single dose or multiple dose) parameter

- Subject does not have any transfusion of blood product in the last 4 weeks before the first PD sample of the full PD profile, or during the full PD profile

The pharmacodynamics analysis set 2 (PDS2) includes all subjects who receive at least one planned treatment of 5.0 mg/kg or 7.5 mg/kg and provide at least one corresponding evaluable PD assessment.

Details on pharmacokinetic analysis

If a profile is not evaluable per PAS definition, all scheduled concentrations for that profile as well as corresponding parameters will be flagged for exclusion from summaries and figures. Additionally, if Rsq_adj < 0.75 or AUC%Extrap > 20%, then Lambda_z, T1/2, AUCinf, Vz/F (VZ), CL/F (CL) will be flagged for exclusion from summaries and figures.

Only PK parameters and concentrations which are not flagged for exclusion programmatically or by the pharmacokineticist (e.g. due to conmeds, high concentration despite dose modification, etc.) will be used for figures and summaries. All values will be listed with excluded values flagged in the listings.

Subject classification

Subjects may be excluded from the analysis populations defined above based on the protocol deviations entered in the database and/or on specific subject classification rules defined in Table 2-1.

Table 2-1 Subject classification based on protocol deviations and non-PD criteria

Analysis set	Protocol deviations leading to exclusion	Non protocol deviation leading to exclusion
FAS	No written inform consent	No dose of study treatment
Safety set	No written inform consent	No dose of study treatment
PAS1	No written inform consent	No dose of study treatment No evaluable PK profile
PAS2	No written inform consent	No dose of study treatment No evaluable PK concentration
PDS1	No written inform consent	No dose of study treatment No evaluable PD profile
PDS2	No written inform consent	No dose of study treatment No evaluable assessment

Withdrawal of Informed Consent

Any data collected in the clinical database after a subject withdraws informed consent from all further participation in the trial, will not be included in the analysis. The date on which a subject withdraws full consent is recorded in the CRF.

[redacted]

2.3 Subject disposition, demographics and other baseline characteristics

The FAS will be used for all baseline and demographic summaries and listings unless otherwise specified.

Demographics and baseline data

Demographic and other baseline data (e.g. age, sex, race, ethnicity, height, weight, and Body Mass Index, ECOG, etc.) will be summarized descriptively by treatment group.

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

Relevant medical histories will be summarized by system organ class and preferred term.

BMI will be calculated based on height and weight at screening:

$$\text{BMI } (kg/m^2) = \text{weight } [kg] / (\text{height } [m])^2$$

All demographic data and other baseline characteristics (e.g., medical history, vaso-occlusive crisis history including genotype and HU/HC use, alcohol history, smoking history, hepatitis and HIV screen, drug screen and pregnancy test etc.) will be listed.

2.3.1 Subject disposition

Treated subjects included in the FAS will be presented. The number (%) of subjects 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 subjects):

- Number (%) of subjects who are still on-treatment (based on the 'Treatment Disposition' page not completed);
- Number (%) of subjects who discontinued the study treatment phase (based on the 'Treatment Disposition' page)
- Primary reason for study treatment phase discontinuation (based on the 'Treatment Disposition' page)
- Number (%) of subjects who have entered the post-treatment follow-up (based on the 'Treatment Disposition' page);
- Number (%) of subjects who have discontinued from the post-treatment follow-up (based on the Post-treatment follow-up Disposition page);
- Reasons for discontinuation from the post-treatment follow-up (based on Post-treatment follow-up Disposition page);

Subject disposition data will be listed.

Screened subjects not treated will be listed.

Protocol deviations

All protocol deviations will be listed.

Analysis sets

The number (%) of subjects in each analysis set (defined in Section 2.2) will be summarized by treatment group. Subjects included in each analysis set will be listed.

2.4 Treatments (study treatment, rescue medication, concomitant therapies, compliance)

2.4.1 Study treatment / compliance

The Safety set will be used for the analyses below. Categorical data will be summarized as frequencies and percentages. For continuous data, mean, standard deviation, median, minimum, and maximum will be presented.

The duration of exposure in weeks to study drug as well as the dose intensity (computed as the ratio of actual cumulative dose received and actual duration of exposure) and the relative dose intensity (computed as the ratio of dose intensity and planned dose intensity) will be summarized by means of descriptive statistics using the safety set.

The number of subjects with dose adjustments (interruption or permanent discontinuation or reduction for 7.5 mg/kg group only) and the reasons will be summarized for all subjects by treatment group and all dosing data will be listed.

Duration of exposure to study treatment

Duration of exposure to study treatment (week) = ((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 of the last date of treatment + 27 days, the date of death (if the subject died), and the date of data cutoff.

Summary of duration of exposure of study treatment in appropriate time units will include categorical summaries (less than 6 weeks, at least 6 weeks, at least 54 weeks, at least 106 weeks) and continuous summaries (i.e. mean, standard deviation etc.).

Cumulative dose

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

The planned cumulative dose 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 subject is on the study treatment as documented in the Dose Administration CRF.

Dose intensity and relative dose intensity

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

DI (mg/kg/28 days) = Actual Cumulative dose (mg/kg) / Duration of exposure to study treatment (week) × 4.

Planned dose intensity (PDI) is defined as follows:

PDI (mg/kg/28 days) = Planned Cumulative dose (mg/kg) / Duration of exposure (week) × 4.

Relative dose intensity (RDI) is defined as follows:

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

DI and RDI will be summarized.

Dose reductions, interruptions or permanent discontinuations

The number of subjects who have dose reductions (for 7.5 mg/kg treatment group only), permanent discontinuations or interruptions, and the reasons, will be summarized by treatment group.

'Dose change', 'Dose interrupted', and 'Dose permanently discontinued' fields from the Dosage Administration CRF pages (DAR) will be used to determine the dose reductions, dose interruptions, and permanent discontinuations, respectively.

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

A dose change is either 'change in prescribed dose level' or 'dosing error' where actual dose administered/total daily dose is different from the prescribed dose.

Reduction: A dose change where the prescribed dose level is lower than the previous prescribed dose level or where the actual dose administered is lower than the calculated dose amount based on the prescribed dose. Therefore any dose change to correct a dosing error will not be considered a dose reduction. Only dose change is collected in the CRF, and number of reductions will be derived programmatically based on the change and the direction of the change.

Interruption: A dose cannot be administered within 7 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.

2.4.2 Prior, concomitant and post therapies

Concomitant medications

Concomitant therapy is defined as all interventions (therapeutic treatments and procedures) other than the study treatment administered to a subject 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 using the latest version available prior to clinical database lock and summarized by lowest ATC class and preferred term. These summaries will include:

1. Medications starting on or after the start of study treatment but no later than 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. Any concomitant therapies starting and ending prior to the start of study treatment or starting more than 105 days after the last date of study treatment will be flagged in the listing. The safety set will be used for all concomitant medication tables and listings.

2.5 Analysis of the primary objective

The primary objective is to characterize PK and PD of crizanlizumab at 5.0 mg/kg in SCD subjects.

2.5.1 Primary endpoint

The primary variables of the study are the PK and PD parameters after single dose and after multiple doses.

Primary PK parameters:

- AUCd15, AUCtau, Cmax

All other PK parameters in Table 2-2 are considered to be secondary.

PD parameters of crizanlizumab

- PD-AUCd15 after single dose
- PD-AUCd29 after multiple doses

Table 2-2 Non-compartmental PK parameters for crizanlizumab

AUCd15	The AUC from time zero to the last measurable concentration sampling time (tlast) (mass x time x volume-1) after single dose
AUCtau	The AUC calculated to the end of a dosing interval (tau) at steady-state (amount x time x volume-1)
Cmax	The maximum (peak) observed serum drug concentration after dose administration (mass x volume-1)
Tmax	The time to reach maximum (peak) serum drug concentration after dose administration (time)
Lambda_z	Smallest (slowest) disposition (hybrid) rate constant (time-1)
T1/2	The elimination half-life associated with the terminal slope (λz) of a semi logarithmic concentration-time curve (time)

The PK parameters are derived based on the non-compartmental methods using WinNonlin® software with the most recent version available at the time of analysis. Refer to the Novartis Pharmacokinetic/Pharmacodynamic Analysis Manual (2015).

2.5.2 Statistical hypothesis, model, and method of analysis

The inter-subject variations and CV% will be presented for the primary PK parameters. The point estimate and the corresponding two-sided 90% confidence interval (CI) for the mean of the log-transformed primary PK parameters after single dose and multiple doses will be derived respectively. The point estimate and CI will be anti-log transformed to obtain the point estimate and the 90% confidence interval for the geometric mean on the original scale.

The descriptive statistics (n, mean, CV%, standard deviation (SD), median, geometric mean, geometric CV%, minimum and maximum) will be presented for all PK parameters defined in Table 2-2 except Tmax, where only n, median, minimum and maximum will be presented.

The PAS1 for 5.0 mg/kg treatment group will be used for all PK analyses described above.

Descriptive statistics for crizanlizumab concentration will be presented at each scheduled timepoint.

In addition, the mean (+/- SD) and median concentration-time profiles over time will be displayed graphically on the linear and semi-log view.

All individual PK parameters and PK concentration data for crizanlizumab will be listed.

All individual pre-dose concentrations for crizanlizumab with median will be displayed graphically on semi-log view over time. In addition, the mean (+/- SD) and median pre-dose concentrations over time will be displayed graphically on the linear and semi-log view.

The PAS2 for 5.0 mg/kg treatment group will be used for these PK analyses.

PD-AUCd15 and PD-AUCd29 will be derived from the P-selectin inhibition data of week 1 and week 15, respectively. The point estimate and the corresponding two-sided 90% CI for the mean of the log-transformed PD-AUC after single dose and multiple doses will also be provided. The point estimate and CI will be anti-log transformed to obtain the point estimate and the 90% confidence interval for the geometric mean on the original scale.

The PDS1 for 5.0 mg/kg treatment group will be used for all PD analyses described above.

Descriptive statistics for PD inhibition will be presented at each scheduled timepoint.

In addition, the mean (+/- SD) and median PD-time profiles over time will be displayed graphically on the linear and semi-log view.

All individual PD parameters and PD data will be listed.

Median pre-dose inhibition will be displayed graphically on semi-log view over time. In addition, the mean (+/- SD) and median pre-dose inhibition over time will be displayed graphically on the linear and semi-log view.

The PDS2 for 5.0 mg/kg treatment group will be used for these PD analyses.

2.5.3 Handling of missing values/censoring/discontinuations

Missing values for any PK parameters or concentrations will not be imputed and will be treated as missing.

Below the limit of quantitation (BLQ) values will be set to zero by the Bioanalyst, and will be displayed in the listings as zero and flagged. BLQ values will be treated as missing for the calculation of the geometric means and geometric CV%.

Missing values for the PD parameter or data will not be imputed and will be treated as missing.

2.5.4 Supportive analyses

No supportive analyses are planned.

2.6 Analysis of the key secondary objective

There is no key secondary objective.

2.7 Analysis of secondary efficacy objective(s)

2.7.1 Secondary endpoints

The secondary efficacy endpoints include the following.

- Annualized rate of VOC events leading to healthcare visit in clinic/ER/hospital
- Annualized rate of VOC events treated at home (based on documentation by health care provider following phone contact with subject)
- Annualized rate of all VOC events (including both healthcare visit and home treatment)
- Annualized rate of each subcategory of VOC event (uncomplicated pain crisis, acute chest syndrome, hepatic sequestration, splenic sequestration, priapism)
- Annualized rate of hospitalizations and ER visits (both total and VOC-related)
- Annualized days of ER/hospitalization (both total and VOC-related)

Annualized rate of a VOC event = Total number of VOC events × 365.25/(end date – treatment start date + 1), and annualized days of ER/hospitalization = Total number of days × 365.25/(end date – treatment start date + 1), where end date is the date of the last treatment date + 27.

2.7.2 Statistical hypothesis, model, and method of analysis

All the above mentioned efficacy endpoints will be summarized descriptively based on FAS by treatment group in the final CSR only and not at the time of the primary analysis CSR, due to the expected short follow-up that will not allow valid efficacy assessment.

2.7.3 Handling of missing values/censoring/discontinuations

Missing values or data will not be imputed and will be treated as missing.

2.8 Safety analyses

All safety analyses will be based on the Safety Set unless otherwise specified. Safety summaries include only on-treatment assessments (refer to Section 2.1.1); safety listings include all assessments with those more than 105 days after last study treatment flagged.

2.8.1 Adverse events (AEs)

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

AEs will be summarized by number and percentage of subjects having at least one AE, having at least one AE in each primary system organ class (SOC) and for each preferred term (PT) using MedDRA coding. A subject with multiple occurrences of an AE will be counted only once in the respective AE category. A subject with multiple CTCAE grades for the same preferred term 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 system organ class will be presented alphabetically and the preferred terms will be sorted within primary SOC in descending frequency. The sort order for the preferred term will be based on their frequency in all subjects.

The following adverse event summaries will be produced by treatment group:

- AEs by SOC and PT (Post-text)
- AEs by PT (In-text only)
- Treatment-related AEs by SOC and PT (Post-text)

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 preferred term).

2.8.1.1 Adverse events of special interest / grouping of AEs

Data analysis of AESIs

An adverse event of special interest is a grouping of adverse events 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 (preferred terms). 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 subjects with at least one event of the AESI occurring during on treatment period will be summarized.

Summaries of these AESIs will be provided by treatment group, (specifying grade, SAE, relationship, leading to treatment discontinuation, leading to dose adjustment/interruption, hospitalization, death etc.). If sufficient number of events occurred, analysis of time to first occurrence will be applied.

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

2.8.2 Deaths

Separate summaries for on-treatment and all deaths (including post-treatment death) will be produced by treatment group, system organ class and preferred term.

All deaths will be listed using Safety set and post treatment deaths will be flagged. A separate listing of deaths prior to starting treatment will be provided for all screened subjects.

2.8.3 Laboratory data

On analyzing laboratory values, data from all sources (central and local laboratories) will be combined. The summaries will include all assessments available for the lab parameter collected no later than 105 days after the last study treatment administration date (see Section 2.1.1).

The following summaries will be produced for hematology and biochemistry laboratory data by laboratory parameter and treatment group:

- Worst post-baseline CTCAE grade (regardless of the baseline status). Each subject will be counted only once for the worst grade observed post-baseline.
- Shift tables using CTC grades to compare baseline to the worst on-treatment value; for laboratory tests where CTC grades are not defined, shift tables using the low/normal/high/(low and high) classification 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 CTC grades and classification relative to the laboratory normal range. Lab data collected during the post-treatment period will be flagged.
- Listing of all CTC grade 2, 3, or 4 laboratory toxicities

Liver function parameters

Liver function parameters of interest are total bilirubin (TBL), ALT, AST 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 total bilirubin will be generated, respectively. eDISH plot will be produced. A similar plot of direct bilirubin (DBILI) vs. ALT will also be generated. Individual subject plots will be produced for subjects matching liver function criteria ALT > 3xULN, DBILI > 2xULN and ALP < 2xULN. Individual subject reticulocyte count plots, and a plot of PT-INR and albumin over time will also be produced for the same patients.

The following summaries will be produced:

- ALT or AST > 3xULN
- ALT or AST > 5xULN
- ALT or AST > 8xULN
- ALT or AST > 10xULN
- ALT or AST > 20xULN
- TBL > 2xULN
- TBL > 3xULN
- ALT or AST > 3xULN & TBL > 2xULN
- ALT or AST > 3xULN & TBL > 2xULN & ALP < 2xULN (potential Hy's law)
- ALT or AST > 3xULN & TBL > 2xULN & ALP ≥ 2xULN
- ALT > 3xULN & DBILI >2xULN & ALP <2xULN

Potential Hy's Law events are defined as those subjects with concurrent occurrence of AST or ALT > 3xULN and TBL > 2xULN and ALP < 2xULN in the same assessment sample during the on-treatment period. Further medical review has to be conducted to assess potential confounding factor such as, liver metastates, liver function at baseline etc.

Renal disorders will be evaluated using estimated Glomerular Filtration Rate (eGFR) shift table, and serum creatinine shift table.

Hematuria (defined as urinanalysis RBC of 6-8/HPF or more), glycosuria (defined as urine glucose of 1+ or more), and proteinuria (defined as urine protein of 1+ or more) will be evaluated using frequency table by time (baseline vs. post-baseline).

Shift table for leucocytosis (leucocytes: ≤ 100,000 mm^3; > 100,000 mm^3) will be produced.

2.8.4 Other safety data

2.8.4.1 ECG and cardiac imaging data

Data handling

When ECG triplicates are collected at any assessment, 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 centrally for each subject during the study. ECG data will be read and interpreted centrally.

The number and percentage of subjects with notable ECG values will be presented by treatment group. In addition, a listing of these subjects will be produced.

- 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 ≤ 60 ms
- 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 > 110 ms
  - New values of QRS > 110 ms

[redacted]

2.8.4.2 Vital signs

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

Data handling

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

Data analysis

The number and percentage of subjects with notable vital sign values (high/low) will be presented by treatment group. A listing of all vital sign assessments will be produced by treatment group with the notable values flagged. In the listing, the assessments collected outside of on-treatment period will be flagged.

For analysis of vital signs the clinically notable vital sign criteria are provided in Table 2-3 below.

Table 2-3 Clinically notable changes in vital signs

Vital sign (unit)	Clinically notable criteria	
	above normal value	**below normal value**
Weight (kg)	increase ≥ 10% from Baseline	decrease ≥ 10% from Baseline
Systolic blood pressure (mmHg)	≥ 180 and increase from baseline of ≥ 20	≤ 90 and decrease from baseline of ≥ 20
Diastolic blood	≥ 105 and	≤ 50 and

Vital sign (unit)	Clinically notable criteria	
	above normal value	**below normal value**
pressure (mmHg)	increase from baseline of ≥ 15	decrease from baseline of ≥ 15
Pulse rate (bpm)	≥ 100 and increase from baseline of > 25%	≤ 50 and decrease from baseline of > 25%
Body temperature (°C)	≥ 39.1	-

2.8.4.3 Immunogenicity

Immunogenicity will be characterized descriptively tabulating antidrug antibodies (ADA) prevalence at baseline and ADA incidence on-treatment.

2.9 Pharmacokinetic endpoints

Refer to Section 2.5 [redacted]

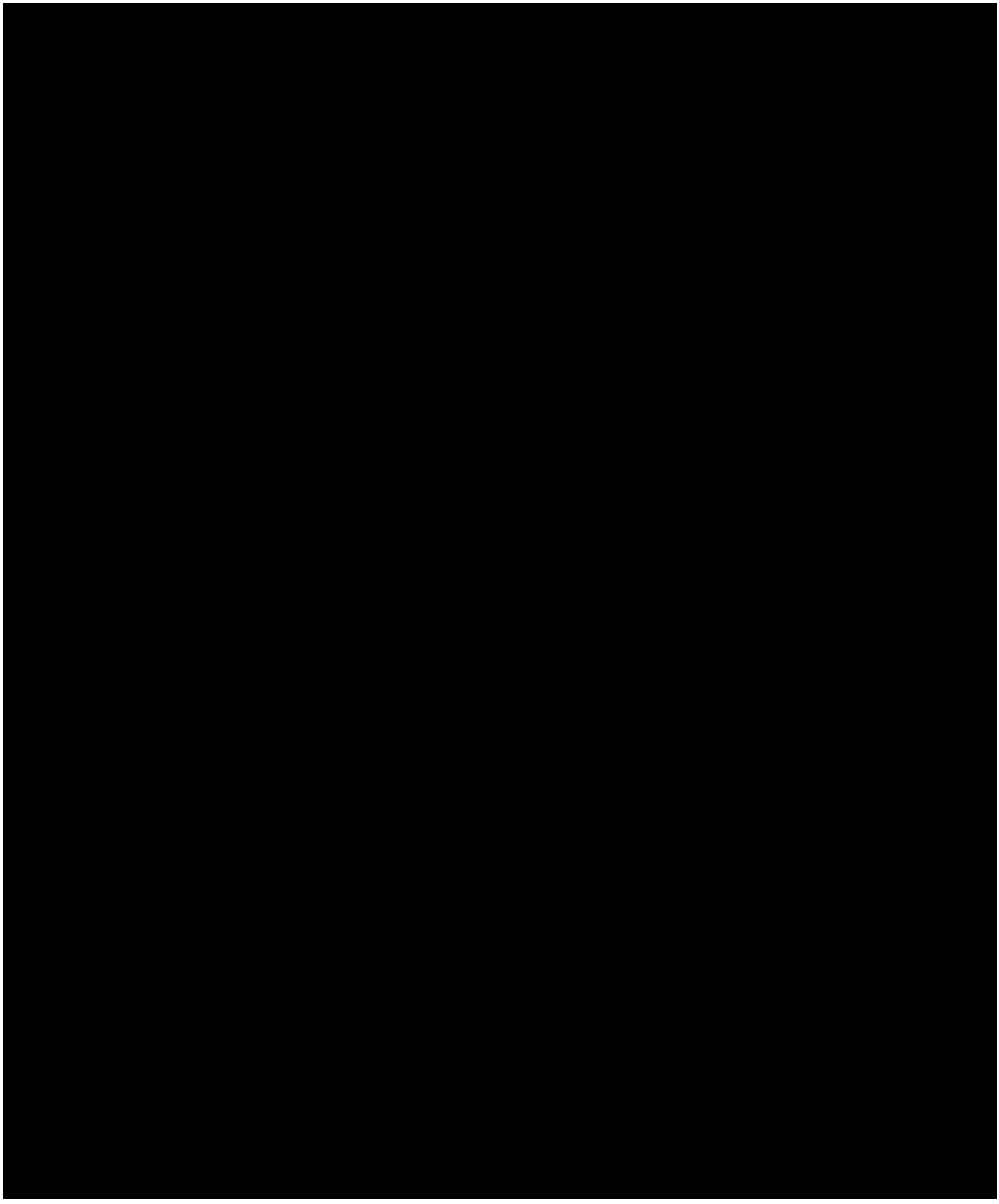
2.10 PD and PK/PD analyses

Refer to Section 2.5 [redacted]

2.11 Patient-reported outcomes

Not applicable.

[redacted]



3 Sample size calculation

5.0 mg/kg treatment group:

For sample size consideration, PK and PD data from study CSEG101A2101 and study CSEG101A2201 were used. Currently, the inter-subject variability of PK parameters, such as AUC and Cmax, of VOC subjects is not known and needs to be approximated from the Ctrough results of subject study A2201. In that study the inter-subject variability of Ctrough was found to be 50.8%.

Data of study A2101 has revealed a high correlation between PK concentrations 336h post-dose and AUCt. Assuming this correlation holds true for subjects together with the assumption of an inter-subject CV = 50.8% of pre-dose concentrations allows an approximation of expected inter-subject CV = 46.1% for AUCt.

Currently, the inter-subject variation of the P-Selectin inhibition at 5.0 mg/kg and the respective PDAUC672hr is not known. However, approximations utilizing results from lower

dose groups tested in study CSEG101A2101 suggest an inter-subject coefficient of variation of approximately CV = 34.1%.

When the sample size is 27 evaluable subjects, a two-sided 90% confidence interval for a single mean of log-transformed AUC will extend 0.140 from the observed mean, ensuring a precision of 15%, assuming that the standard deviation is known to be 0.439 and the confidence interval is based on the large sample z statistic.

Similarly, with the same sample size of 27 evaluable subjects, a two-sided 90% confidence interval for a single mean of log-transformed PD-AUC will extend 0.105 from the observed mean, representing a precision of about 11%, assuming that the standard deviation is known to be 0.332 and the confidence interval is based on the large sample z statistic.

Exploratory 7.5 mg/kg treatment group:

When the sample size for this dose group is 6, a two-sided 90% confidence interval for a single mean will extend 0.172 from the observed mean, assuming that the standard deviation is known to be 0.209 and the confidence interval is based on t-statistic.

Considering a drop-out rate of 35% from the CSEG101A2201 study, and to account for the heterogeneity of subjects in terms of genotype and renal function, the multiplication factor for enrollment versus evaluable subjects is 67%, and 45 subjects will need to be enrolled in the 5.0 mg/kg treatment group. The same holds for the exploratory cohort, requiring enrollment of 10 subjects to yield 6 evaluable subjects at the 7.5 mg/kg dose.

4 Change to protocol specified analyses

No change from protocol specified analyses was made.

5 Appendix

5.1 Imputation rules

5.1.1 Study drug

Missing dates for study drug administraton should be queried and will not be imputed.

5.1.2 AE, ConMeds and safety assessment date imputation

Table 5-1 Imputation of start dates (AE, CM) and assessments (LB, EG, VS)

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.

Missing Element	Rule
	• If available year > year of study treatment start date then 01JanYYYY • If available year < year of study treatment start date then 01JulYYYY
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, CM)

Missing Element	Rule (*=last treatment date plus 105 days not > (death date, cut-off 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 ConMeds 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.

5.1.2.1 Other imputations

5.2 AEs coding/grading

Adverse events are coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

Note: The latest available MedDRA version at the time of the analyses should be used. The MedDRA version used should be specified in the footnote of relevant tables.

AEs will be assessed according to the Common Terminology Criteria for Adverse Events (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 NCI Common Terminology Criteria for Adverse Events (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 the 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.

For laboratory tests where grades are not defined by CTCAE version 5.0, results will be graded by the low/normal/high 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

CTC grading for blood differentials is based on absolute values. However, this data may not be reported as absolute counts but rather as percentage of 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 CTC grades 0 and 1, the normal range for derived corrected calcium is set to the same limits (in mg/dL) as for calcium.

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

6 Reference

Pharmacokinetic / Pharmacodynamic Analysis Manual, *Novartis*, 17 Dec 2015.

Smith B, Vandenhende FR, DeSante KA, Farid NA, Welch PA, Callaghan JT and Forgue ST . Confidence Interval Criteria for Assessment of Dose Proportionality. *Pharmaceutical Research*, 17(10), 1278-83 (2000).