STATISTICAL ANALYSIS PLAN

Study Title:	A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTICENTER STUDY TO ASSESS THE SAFETY AND EFFICACY OF INCLACUMAB IN PARTICIPANTS WITH SICKLE CELL DISEASE EXPERIENCING VASO-OCCLUSIVE CRISES
Development Phase:	Phase 3
Protocol Number:	GBT2104-131 (C5361001)
Protocol Date and Version:	13 July 2023 (Amendment 4), Version 5.0
Investigational Product:	Inclacumab (GBT2104 / PF-07940370)
Sponsor:	Global Blood Therapeutics, Inc., a wholly owned subsidiary of Pfizer Inc. 181 Oyster Point Boulevard South San Francisco, CA 94080 United States of America
Analysis Plan Version:	Version 3.0
Analysis Plan Date:	6 June 2025

STATISTICAL ANALYSIS PLAN REVIEW AND APPROVAL

Prepared by:

PPD

06 Jun 2025 11:32:019-0400

PPD , PhD
PPD
Global Blood Therapeutics, Inc., a wholly owned subsidiary of Pfizer Inc.

Date

Approved by:

PPD

06 Jun 2025 11:28:017-0400

PPD , PhD
PPD
Global Blood Therapeutics, Inc., a wholly owned subsidiary of Pfizer Inc.

Date

PPD

06 Jun 2025 11:27:032-0400

PPD , PhD
PPD
Pfizer Inc.

Date

PPD

06 Jun 2025 11:31:014-0400

PPD , MD
PPD
Global Blood Therapeutics, Inc., a wholly owned subsidiary of Pfizer Inc.

Date

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SUMMARY OF SAP CHANGES

Version	Version Date	Summary of Changes
1.0	26 JUL 2023	N/A (original document)
2.0	18 APR 2024	- Section 5.3: Intercurrent events and data handling strategies added based on feedback from regulatory agency. - Section 5.3.4: Pre-specified order for the fixed sequence hierarchical test procedure updated based on feedback from regulatory agency with regard to relative power for secondary endpoints.
3.0	4 JUN 2025	- Section 4.2: Added a modified Intent-to-Treat (mITT) analysis population to enable additional efficacy sensitivity analyses to account for potential uncertainties at Site 17-005. - Section 5.3: Added reference to the sensitivity analyses. - Section 5.3.6: Added sensitivity analyses based on mITT analysis population to asses robustness of results from the primary analyses.

1. GLOSSARY OF ABBREVIATIONS

ACS	acute chest syndrome
ADA	anti-drug antibodies
AESI	adverse event of special interest
ANOVA	analysis of variance
ASCQ-Me	Adult Sickle Cell Quality of Life Measurement
CGI-C	Clinician's Global Impression of Change
CI	confidence interval
CMH	Cochran-Mantel-Haenszel
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
EOS	end of study
HU	hydroxyurea
ITT	intent-to-treat
IRT	interactive response technology
MAR	missing at random
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
MNAR	missing not at random
NSAID	nonsteroidal anti-inflammatory drug
OLE	open-label extension
PD	pharmacodynamic(s)
PGI-C	Patient's Global Impression of Change
PK	pharmacokinetic(s)
RBC	red blood cell
RR	rate ratio
SAE	serious adverse event
SAP	statistical analysis plan
SCD	sickle cell disease
SOC	System Organ Class
TEAE	treatment-emergent adverse event
VOC	vaso-occlusive crisis

WHO	World Health Organization

2. INTRODUCTION

The primary objective of Study GBT2104-131 (C5361001) is to evaluate the safety and efficacy of treatment every 12 weeks with inclacumab to reduce the incidence of vaso-occlusive crises (VOCs) in participants with sickle cell disease (SCD).

This statistical analysis plan (SAP) provides details of the planned analyses and statistical methods for Study GBT2104-131.

Where this document differs from the high-level analysis plan described in the study protocol, the methodology described in this SAP is considered the latest and supersedes the corresponding section(s) in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

Population pharmacokinetic (PK), exploratory pharmacodynamic (PD), and exploratory biomarker analyses will be described in a separate document.

2.1. Study Design

Study GBT2104-131 is a Phase 3, randomized, placebo-controlled, double-blind, multicenter, parallel-group study to assess the safety and efficacy of inclacumab in reducing the frequency of VOCs in approximately 240 adult and adolescent participants (≥ 12 years of age) with SCD globally.

Initial enrollment will include participants ≥ 16 years of age until the independent Data Monitoring Committee (DMC) recommends to the Sponsor that adequate safety and PK data support the enrollment of participants 12 to 15 years of age.

Eligible participants will be randomized with a 1:1 ratio into one of two treatment arms as follows:

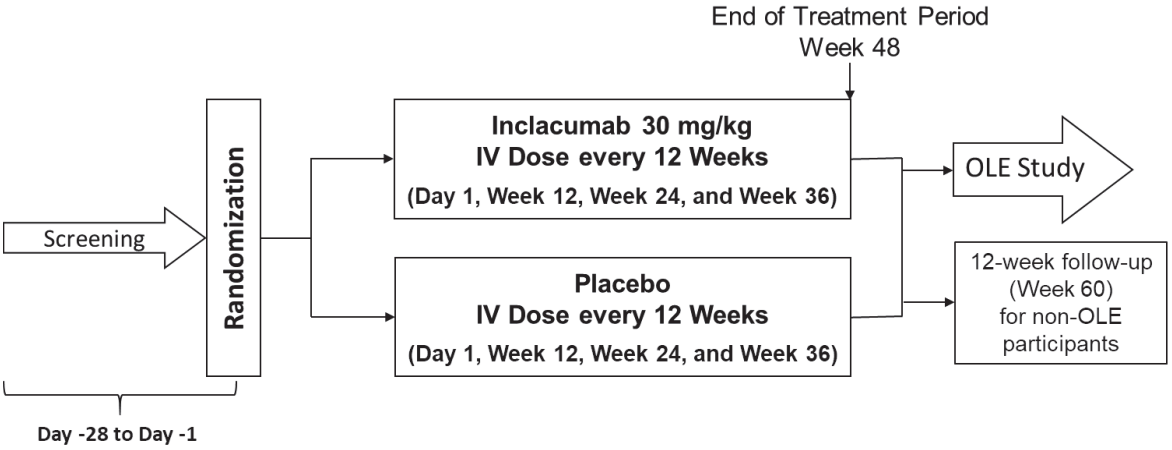
- Inclacumab 30 mg/kg administered IV every 12 weeks (Day 1, Week 12, Week 24, and Week 36); or
- Placebo administered IV every 12 weeks (Day 1, Week 12, Week 24, and Week 36).

All participants will undergo safety, efficacy, PK, and PD assessments at Baseline and through Week 48, as detailed in the Schedules of Assessments (see study protocol, Appendices 1 and 2).

Following completion of the Week 48 Visit, eligible participants will be given the option to enroll in an open-label extension (OLE) study (under a separate protocol). For participants enrolling in the OLE study, the Week 48 Visit will be the end of study (EOS) visit. For participants not enrolling in the OLE study, an additional required Follow-Up Visit at Week 60 will be the EOS visit.

A diagram of the study design is provided in Figure 1. Further study design details are provided in the study protocol.

Figure 1 GBT2104-131 Study Design



Abbreviations: IV=intravenous, OLE=open-label extension.

2.2. Study Endpoints

2.2.1. Primary Efficacy Endpoint

The primary efficacy endpoint for the study is the rate of VOCs during the 48-week treatment period. A VOC is defined as an acute episode of pain that:

- Has no medically determined cause other than a vaso-occlusive event, and
- Results in a visit to a medical facility (hospitalization, emergency department, urgent care center, outpatient clinic, or infusion center), or results in a remote contact with a healthcare provider; and
- Requires parenteral narcotic agents, parenteral nonsteroidal anti-inflammatory drugs (NSAIDs), or an increase in treatment with oral narcotics.

Complicated VOCs of acute chest syndrome (ACS), hepatic sequestration, splenic sequestration, and priapism that meet the requirements listed above will be included in the primary endpoint.

To ensure consistency across study sites, all VOCs identified will be adjudicated by an independent, blinded VOC Adjudication Committee comprised of experts in SCD. Unless otherwise noted, VOC efficacy analyses will be performed on adjudicated data.

2.2.2. Secondary Efficacy Endpoints

The secondary efficacy endpoints for the study are the following:

- Time to first VOC during the 48-week treatment period.
- Time to second VOC during the 48-week treatment period.
- Proportion of participants with no VOCs during the 48-week treatment period.

- Rate of VOCs that required admission to a healthcare facility and treatment with parenteral pain medication during the 48-week treatment period where admission includes:
    - A hospital admission, or
    - An admission to an emergency room, observation unit, or infusion center for ≥ 12 hours, or
    - 2 visits to an emergency room, observation unit, or infusion center over a 72-hour period.
- Number of days of inpatient hospitalization for a VOC during the 48-week treatment period.

2.2.3. Exploratory Endpoints

The exploratory endpoints for the study are the following:

- Rate of all SCD-related urgent care visits to the clinic, emergency room, and hospital during the 48-week treatment period.
- Proportion of total days missed from school or work due to SCD during the 48-week treatment period.
- Rate of complicated VOCs (defined in protocol Section 5.1) during the 48-week treatment period.
- Rate of red blood cell (RBC) transfusions during the 48-week treatment period.
- Rate of inpatient hospital admissions for any reason during the 48-week treatment period.
- Number of days of inpatient hospitalization for any reason during the 48-week treatment period.
- Proportion of participants rated as “very much improved” or “much improved” based on the Patient’s Global Impression of Change (PGI-C) at Weeks 12, 24, 36, and 48.
- Proportion of participants rated as “very much improved” or “much improved” based on the Clinician’s Global Impression of Change (CGI-C) at Weeks 12, 24, 36, and 48.
- Change from Baseline in the cumulative score for the Adult Sickle Cell Quality of Life Measurement (ASCQ-Me) Pain Impact – Short Form over time to Week 48.

2.2.4. Safety Endpoints

The safety endpoints for the study are the following:

- Incidence of treatment-emergent adverse events (TEAEs).

- Change from Baseline in laboratory assessments (complete blood count, chemistry, and coagulation).

2.2.5. Exploratory Pharmacology Endpoints

2.2.5.1. Pharmacokinetic (PK) Endpoints

The following PK endpoint will be assessed:

- The PK of inclacumab by population PK analysis using nonlinear mixed-effects modeling.

2.2.5.2. Anti-drug Antibodies (ADA)

The following endpoint will be assessed:

- Incidence of ADA to inclacumab.

2.2.5.3. Pharmacodynamic (PD) Endpoints

The following exploratory PD endpoints will be assessed:

- Changes in non-activated and thrombin receptor activating peptide (TRAP)-activated platelet leukocyte aggregates (PLAs), PLT P-selectin expression, serum P-selectin inhibition measured by surface plasmon resonance (SPR), and plasma total and free soluble P-selectin (sP-selectin) over time.

2.2.5.4. Biomarker Endpoints

The following exploratory biomarker endpoints will be assessed:

- Changes in RBC adhesion (selected sites), genomic markers (optional), protein markers in the blood, urine markers of kidney function, and voxelotor plasma and whole blood concentrations (as applicable).

2.3. Determination of Sample Size

The following statistical hypothesis will be tested to address the primary objective:

H_0: RR =1 vs. H_1: RR ≠1

where RR is rate ratio for VOC for inclacumab arm vs. placebo arm.

Sample size determination was based on statistical power considerations for the primary efficacy endpoint, rate of VOCs during the 48-week treatment period.

A sample size of approximately 240 participants (120 participants per treatment arm [inclacumab and placebo]) provides approximately 90% power to detect a targeted 45% reduction in the rate of VOCs, from an average rate of 3.0 VOCs per year on placebo to 1.65 VOCs per year on inclacumab, using a 2-sided test at an overall $\alpha = 0.05$ level.

The number of VOCs per year was assumed to follow a negative binomial distribution with a dispersion parameter of 1.04. For the purposes of sample size calculation, a drop-out rate of 25% by Week 48 was assumed.

Calculations were based on the methodology of Zhu and Lakkis (2014).

2.3.1. Basis for Sample Size Assumptions

The assumption of an average rate of 3.0 VOCs per year for the placebo group is based on published clinical trial data for the placebo-controlled SUSTAIN study with a similar patient population (Ataga, 2017). Similarly, the targeted treatment effect of a 45% reduction in the rate of VOCs is based on the effect size reported in the SUSTAIN study with crizanlizumab (Ataga, 2017).

The assumption of 25% drop-out rate by Week 48 and a dispersion parameter of 1.04 is based on data from the Phase 3 clinical trial GBT440-031 in patients with SCD (data on file).

2.4. Randomization

Eligible participants will be randomized on Day 1 through a central interactive response technology (IRT) system.

Participants will be randomized with a 1:1 ratio to receive treatment with inclacumab or placebo. A stratified permuted block design will be used, with randomization stratified by baseline hydroxyurea (HU) use (yes; no), number of VOCs in the preceding 12 months (2 to 4 episodes; 5 to 10 episodes), and geographic region (North America; sub-Saharan Africa; Europe/rest of world).

The first dose of study drug is to be administered on the same day as randomization (Day 1).

2.5. Analysis Timing

2.5.1. Interim Futility Analysis

One interim analysis for futility will be performed at an information fraction of approximately 48%. The futility analysis will include participants who, by the data cutoff date, have the potential for at least 24 weeks of treatment.

For the futility analysis, the primary efficacy endpoint will be evaluated by the independent DMC. The study team will remain blinded. A Gamma family (-1) β-spending function will be used to determine the futility boundary. The futility boundary is considered non-binding. See Section 5.3.7 for additional details.

2.5.2. Final Analysis

The final analysis is intended to be based on complete data from the study, and performed after all randomized participants have completed the study (see Section 3.1) or discontinued early (i.e., last participant's last visit has occurred), and all corresponding data have been entered into the database, reviewed, and verified and the database is locked.

3. GENERAL CONSIDERATIONS

3.1. Definitions and Terminology

Study Drug

The term study drug refers to either inclacumab or placebo.

Baseline Value

Baseline is defined as the last available pre-treatment value taken on or before the day of randomization, and will be used for summary of baseline characteristics and change-from-baseline analyses, as appropriate. For ASCQ-Me, baseline is defined as the assessment taken on the day of randomization.

Day 1

Day 1 is the date of randomization.

Study Day

Study Day is defined relative to the date of randomization.

For study assessments or events that occur on or after the date of randomization, study day is calculated as:

Study Day = Event Date – Randomization Date + 1.

For study assessments or events that occur before the date of randomization, study day is calculated as:

Study Day = Event Date – Randomization Date.

Study Visit

Study Visit is the protocol visit as shown in Table 1.

Treatment Day

Treatment Day is defined relative to the date of first dose of study drug.

For study assessments or events that occur on or after the date of first dose, treatment day is calculated as:

Treatment Day = Event Date – First Dose Date + 1.

For study assessments or events that occur before the date of first dose, treatment day is calculated as:

Treatment Day = Event Date – First Dose Date.

Treatment-Emergent Adverse Event

Treatment-emergent adverse events (TEAEs) are defined as adverse events that occur on or after Day 1 of study treatment or the worsening of a preexisting condition on or after Day 1 of study treatment. Note: Given the long half-life of inclacumab, events occurring through study completion will be considered treatment emergent.

Study Completion

Completion of study is specified on the End of Study (EOS) CRF. Participants are considered to have completed the study if (1) the subject completed the study through the Week 48 visit and elected to enroll in the open-label extension study, or (2) the subject did not enroll in the open-label extension study and completed the study through Week 60.

Treatment Completion

Completion of treatment is specified on the End of Treatment (EOT) CRF and includes all participants who did not permanently discontinue study drug early.

3.2. Visit Windows

For summaries by timepoint (e.g., CGI-C, PGI-C, vital signs, weight, laboratory values), analysis visit windows will be used to classify assessments based on the actual study day of the measurement regardless of the original nominal visit label. This includes assessments collected at Unscheduled, Early Termination, or EOS visits. Target study days, the protocol-specified study day windows, and the analysis visit windows are shown in Table 1.

Table 1: Analysis Windows for Assessments Other than ASCQ-Me

Study Visit	Target Study Day per Protocol	Visit Window per Protocol (study days)	Window for Statistical Analysis (study days)
Screening	-	[-28, -1]	[-28, -1]
Day 1	1	1	1
Week 6	43	[36, 50]	[29, 57]
Week 12	85	[78, 92]	[64, 106]
Week 24	169	[162, 176]	[148, 190]
Week 36	253	[246, 260]	[232, 274]
Week 48	337	[330, 344]	[316, 358]
Week 60	421	[414, 428]	[400, 442]

ASCQ-Me=Adult Sickle Cell Quality of Life Measurement.
Note: Baseline is defined as the last available pre-treatment value taken on or before the day of randomization. If multiple measurements fall within the statistical analysis window, the measurement closest to the target study day will be used. If two measurements are equally close, the earlier value will be used.

For the ASCQ-Me questionnaire (completed weekly at home), the analysis windows shown in Table 2 will apply for data summary.

Table 2: Analysis Windows for Assessment of ASCQ-Me

Analysis Timepoint	Target Study Day	Window for Statistical Analysis (study days)
Baseline	1	1
Week 1	8	[2, 11]
Week 2	15	[12, 18]
Week 3	22	[19, 25]
Week 4	29	[26, 32]
Week 5	36	[33, 39]
Week 6	43	[40, 46]
Week 7	50	[47, 53]
Week 8	57	[54, 60]
Week 9	64	[61, 67]
Week 10	71	[68, 74]
Week 11	78	[75, 81]
Week 12	85	[82, 88]
Week 13	92	[89, 95]
Week 14	99	[96, 102]
Week 15	106	[103, 109]
Week 16	113	[110, 116]
Week 17	120	[117, 123]
Week 18	127	[124, 130]
Week 19	134	[131, 137]
Week 20	141	[138, 144]
Week 21	148	[145, 151]
Week 22	155	[152, 158]
Week 23	162	[159, 165]
Week 24	169	[166, 172]
Week 25	176	[173, 179]
Week 26	183	[180, 186]
Week 27	190	[187, 193]

Analysis Timepoint	Target Study Day	Window for Statistical Analysis (study days)
Week 28	197	[194, 200]
Week 29	204	[201, 207]
Week 30	211	[208, 214]
Week 31	218	[215, 221]
Week 32	225	[222, 228]
Week 33	232	[229, 235]
Week 34	239	[236, 242]
Week 35	246	[243, 249]
Week 36	253	[250, 256]
Week 37	260	[257, 263]
Week 38	267	[264, 270]
Week 39	274	[271, 277]
Week 40	282	[278, 284]
Week 41	288	[285, 291]
Week 42	295	[292, 298]
Week 43	302	[299, 305]
Week 44	309	[306, 312]
Week 45	316	[313, 319]
Week 46	323	[320, 326]
Week 47	330	[327, 333]
Week 48	337	[334, 340]

ASCQ-Me=Adult Sickle Cell Quality of Life Measurement.
Note: Baseline is defined as the assessment taken on the day of randomization. If multiple measurements fall within the statistical analysis window, the measurement closest to the target study day will be used. If two measurements are equally close, the earlier value will be used.

4. ANALYSIS POPULATIONS

Two main analysis populations are defined for this study: the intent-to-treat (ITT) population and the safety population. A per-protocol population is also defined.

4.1. Intent-to-Treat Population

The ITT population includes all randomized participants. For analyses based on this population, participants will be grouped according to treatment assigned at randomization.

The ITT population will be the main analysis population for efficacy analyses and summaries of demographic and baseline characteristics.

4.2. Modified Intent-to-Treat Population

The modified intent-to-treat (mITT) population includes all randomized participants in the ITT population except participants from Site 17-005 (n=18). The mITT population is a subset of the ITT population.

The mITT population will serve as the basis for additional sensitivity analyses for efficacy, as detailed in Section 5.3.6. For analyses based on this population, participants will be grouped according to treatment assigned at randomization. Findings will be used to assess robustness of results from the primary analyses.

4.3. Safety Population

The safety population includes randomized participants who received treatment with study drug. For analyses based on this population, participants will be grouped according to the actual study treatment received. Any participant who receives one or more doses with inclacumab will be classified in the inclacumab treatment arm. Participants who receive treatment with placebo only will be classified in the placebo treatment arm.

The safety population will be the primary analysis population for safety and exposure data.

4.4. Per-Protocol Population

The per-protocol population includes randomized participants who met all study eligibility criteria, received all 4 doses of assigned study drug during the 48-week treatment period, and have no significant protocol deviations with the potential to impact efficacy assessments (e.g. did not receive any prohibited concomitant medication). For analyses based on this population, participants will be grouped according to treatment assigned at randomization. The per-protocol population will be identified based on blinded data and documented prior to the primary analysis. If the number of participants in the per-protocol population is > 90% of that in the ITT population, then analyses based on the per-protocol population will not be performed.

The per-protocol population may be used for sensitivity analysis of the primary efficacy endpoint, as appropriate.

5. STATISTICAL METHODS

5.1. Summaries of Study Conduct

The number of participants randomized will be tabulated by region, country, study site, and treatment group. Participant disposition (the number of participants randomized, treated, completing study treatment, and completing the study) will be tabulated by treatment group. Reasons for early study drug discontinuation and study discontinuation will be summarized.

Any eligibility criteria deviations, dosing errors, and other significant protocol deviations will also be tabulated by treatment group and evaluated for potential impact on the interpretation of study results.

5.2. Summaries of Demographic, Baseline Characteristics, and Concomitant Medications

Demographic and baseline characteristics (such as age, sex, race, body weight in kg, estimated glomerular filtration rate (eGFR), sickle cell genotype, number of VOCs in the 12 months prior to the screening visit, baseline HU use, baseline voxelotor use, prior crizanlizumab use, and geographic region) will be summarized for the ITT population by treatment group.

Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA) and summarized for each treatment group. Prior and concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary and summarized.

Time on study, defined as time from randomization (Day 1) to the participant's end of study date, will also be tabulated.

5.3. Efficacy Analyses

The primary efficacy analyses will be based on the ITT patient population (Section 4.1), with participants grouped according to the treatment assigned at randomization. In addition, sensitivity analyses based on the mITT population will be performed for the primary efficacy and all secondary efficacy endpoints. For the primary efficacy endpoint, sensitivity analysis based on the per-protocol population will also be performed (see Section 5.3.6).

For the primary efficacy analyses, data from all randomized participants, regardless of adherence to study drug or to the protocol will be included in the efficacy analyses. This includes data from participants who discontinued study drug early but continued with study assessments. One exception is the initiation of prohibited therapies (ie, crizanlizumab, chronic transfusions, stem cell transplant, gene therapy for SCD), for which only data up to the time of initiation of the prohibited therapy would be used in efficacy analyses.

Table 3 summarizes intercurrent events and corresponding data handling strategies.

Table 3: Strategies for Handling Intercurrent Events in Efficacy Analyses

Intercurrent Event	Data Handling Strategies
Discontinuation of study treatment due to any reason but stay in study	Analyze data as collected (ie, "treatment policy" strategy: the values of the variable of interest are used regardless of whether the intercurrent event occurs).
Study discontinuation due to any reason (including death)	Analyze data as collected (ie, all data collected while on study will be used in the analysis and missing data that arise due to study withdrawal will be handled as specified in this analysis plan).
Change in standard of care treatment, including hydroxyurea, ESAs, voxelotor, L-glutamine, and episodic transfusions	Analyze data as collected (ie, "treatment policy" strategy: the values of the variable of interest are used regardless of whether the intercurrent event occurs.)
Initiation of the listed prohibited therapies (ie, crizanlizumab, chronic transfusions, stem cell transplant, gene therapy for SCD)	Data up to the time of occurrence of the intercurrent event will be used in the analysis (ie, "while-on-treatment policy" strategy).

Additional analyses of the primary efficacy endpoint based on the per-protocol population are described in Section 5.3.6.

Covariate Adjustment
Unless otherwise noted, analyses of primary and secondary efficacy endpoints will be adjusted for the following randomization stratification variables:

- Baseline hydroxyurea use (yes; no)
- Number of VOCs in the 12 months prior to study entry (2 to 4 episodes; 5 to 10 episodes)
- Geographic region (North America; sub-Saharan Africa; Europe/rest of world)

For stratification variables, the value recorded in the clinical database (i.e., per case report form [baseline HU use and number of VOCs in the 12 months prior to study entry] or as provided in the site list [geographic region]) will be compared to value captured in the IRT. If differences are observed, the values from the clinical database will be used in the analysis.

Time Period at Risk
For each participant, the time period at risk for evaluation of VOCs is from date of randomization (Day 1) to the participant's end of study date or Study Day 358 (end of Week 48 analysis window), whichever is earlier.

Statistical Tests
All statistical tests will be conducted at a two-sided alpha level of 0.05. Adjustment for multiple comparisons is described in Section 5.3.4.

5.3.1. Primary Efficacy Endpoint

For the primary efficacy endpoint, all VOCs that meet the protocol-specified definition as assessed by the VOC Adjudication Committee with an onset date during the time period at risk will be included in the analysis. For each treatment group, unadjusted rates of VOCs will be estimated by dividing the total number of protocol-defined VOCs observed by the total time at risk for the treatment group.

The rate of VOCs during the 48-week treatment period will be compared between the inclacumab and placebo arms with the use of negative binomial regression model.

The regression model will include covariates for treatment group (inclacumab, placebo) and the randomization stratification factors. The logarithm of observed patient-time at risk will be used as an offset term in the model to account for different lengths of follow-up across participants. The rate of VOCs adjusted for the specified baseline covariates will be estimated for each treatment group based on the regression model. Similarly, the ratio of the VOC rate (inclacumab versus placebo) along with the associated 95% confidence interval (CI) and p-value will be estimated from the regression model.

The regression model will be fit based on all observed data from randomized participants, regardless of adherence to study drug or to the protocol, and without imputation for premature study discontinuation. Sensitivity analyses will be performed to assess the robustness of the primary analysis results, including missing data assumptions (see Section 5.3.6).

SAS code similar to the following will be used for the primary analysis (where trtgrp is the randomized treatment group, numvoc is the number of protocol-defined VOCs during the 48-week treatment period, and logtime is the natural log of patient-time at risk). Patient-time at risk is expressed in years and the VOC rate per 48 weeks will be calculated by multiplying the estimated annual rate by 0.92.

```
PROC GENMOD;
 CLASS trtgrp <categorical_variables>;
 MODEL numvoc = trtgrp <categorical_variables> / LINK=log
   DIST=negbin OFFSET=logtime;
 LSMEANS trtgrp / EXP DIFF CL OM;
RUN;
```

A plot of the mean cumulative function of VOC episodes will be presented using recurrent events analysis methods.

For the primary endpoint, subgroup analyses will be performed to evaluate the consistency of results across pre-specified subgroups (see Section 5.3.5).

5.3.2. Secondary Efficacy Endpoints

5.3.2.1. Time to First VOC and Time to Second VOC

Time to first VOC and time to second VOC will be measured from randomization (Day 1) to onset date of the first or second VOC event, respectively. Participants who do not experience a protocol-defined VOC (per primary endpoint definition [Section 2.2.1]) will be censored at the end of their time period at risk.

Summary statistics will include number of participants with event, number of participants censored, median time to event along with 95% CI for the median and quartiles. The CIs for the median will be calculated according to Brookmeyer and Crowley (1982) and the CIs for the survival function estimates at fixed time points will be derived using the log-log transformation according to Kalbfleisch and Prentice (2002) (conftype=loglog default option in SAS Proc LIFETEST) with back transformation to a CI on the untransformed scale. The estimate of the standard error will be computed using Greenwood's formula.

For each time to event endpoint, treatment comparison between inclacumab and placebo will be performed based on log-rank test stratified by the randomization stratification factors. Kaplan-Meier plots will be generated. For each endpoint, a Cox regression model will be used to estimate the hazard ratio between the inclacumab and placebo groups, as appropriate.

5.3.2.2. Participant Status with no VOCs

For the proportion of participants with no VOCs during the 48-week treatment period, a Cochran-Mantel-Haenszel (CMH) test, stratified by the randomization stratification factors will be used for the treatment comparison between inclacumab and placebo.

For early study discontinuation, participants without an observed protocol-defined VOC (per primary endpoint definition [Section 2.2.1]) who discontinue the study prior to the end of the 48-week treatment period will be assumed to have experienced at least one VOC by Week 48 (i.e., counted as 'failure'). An analysis based on observed data only (i.e., without imputation for early study discontinuation) will be performed as a sensitivity analysis (Section 5.3.6).

5.3.2.3. VOCs that Required Healthcare Facility Admission and Parenteral Pain Medication

For the rate of VOCs that required admission to a healthcare facility and treatment with parenteral pain medication during the 48-week treatment period, the same statistical method for the primary endpoint will be used (Section 5.3.1).

5.3.2.4. Inpatient Hospitalization Days for VOC

For each VOC event requiring inpatient hospitalization (regardless of treatment received) during the 48-week treatment period, the number of days hospitalized will be determined based on the hospital admission and discharge dates. The total number of days of inpatient hospitalization for VOCs during the 48-week treatment period will be analyzed using the same statistical method used for the primary endpoint.

5.3.3. Exploratory Efficacy Endpoints

Exploratory endpoints will be summarized by descriptive statistics. For comparison between inclacumab and placebo groups, point estimates, CIs, and p-values may be presented as appropriate, without adjustment for multiplicity.

The rate of all SCD-related urgent care visits to the clinic, emergency room, and hospital during the 48-week treatment period will be summarized descriptively using similar patient-time methodology as the primary endpoint. A similar analysis will be performed for the rate of

complicated VOCs, the rate of RBC transfusions during the 48-week treatment period, and the rate of inpatient hospital admissions for any reason during the 48-week treatment period.

The number of days of inpatient hospitalization for any reason during the 48-week treatment period will be analyzed using the same methods as for the secondary endpoint of number of days of inpatient hospitalization for VOCs during the 48-week treatment period.

For each participant working outside of the home or attending school, the proportion of total days missed from school or work due to SCD out of the total expected days will be calculated for the 48-week treatment period. Results will be summarized descriptively by treatment group and compared between treatment groups based on an analysis of variance (ANOVA) model including treatment group and the randomization stratification factors. Only participants for whom the number of days of work and/or school that should have been attended is > 0 will be included in the analysis.

The proportion of participants rated as "very much improved" or "much improved" based on the PGI-C at Weeks 12, 24, 36, and 48 will be summarized descriptively by timepoint and treatment group. A CMH test, stratified by the randomization stratification factors, will be used for the treatment comparison between inclacumab and placebo. A similar analysis will be performed for the proportion of participants rated as "very much improved" or "much improved" based on the CGI-C.

A cumulative score for the ASCQ-Me Pain Impact (Short Form) will be calculated for each participant and assessment timepoint. For each assessment a cumulative score will only be calculated if all five questions on the form were answered. The cumulative score is the sum of the individual raw scores according to the response mapping in Table 4.

Table 4: ASCQ-Me Pain Impact (Short Form) Response Mapping

Response to Each Question	Raw Score
Always	1
Often	2
Sometimes	3
Rarely	4
Never	5

Changes from baseline over time to Week 48 will be calculated and summarized descriptively by timepoint (weekly) and treatment group. An ANOVA model including treatment group and the randomization stratification factors will be used to compare results by treatment group at selected timepoints (i.e., Week 6, Week 12, Week 24, Week 36, and Week 48). The same analysis will be repeated for (1) adult subjects only (≥ 18 years) and (2) subjects with high baseline VOC burden (number of VOCs in the 12 months prior to screening = 5 to 10 events).

5.3.4. Adjustment for Multiple Comparisons (Primary and Secondary Efficacy Endpoints)

A fixed sequence hierarchical test procedure will be used to control Type I error when evaluating the treatment effect of inclacumab compared with placebo for the primary and secondary

efficacy endpoints. The endpoints will be tested sequentially based on the following pre-specified order:

1. Rate of VOCs during the 48-week treatment period (primary endpoint)
2. Time to first VOC during the 48-week treatment period
3. Rate of VOCs that required admission to a healthcare facility and treatment with parenteral pain medication during the 48-week treatment period
4. Number of days of inpatient hospitalization for a VOC during the 48-week treatment period
5. Time to second VOC during the 48-week treatment period
6. Proportion of participants with no VOCs during the 48-week treatment period

Each endpoint will be tested at a two-sided alpha level of 0.05. Formal testing of endpoints will continue until the first non-significant result. Testing of endpoints subsequent to a non-significant result will be considered exploratory in nature.

5.3.5. Subgroup Analyses

Subgroup analyses will be performed to evaluate the consistency of analysis results across pre-specified subgroups defined by demographic and baseline characteristics.

The following subgroups will be analyzed with respect to the primary efficacy endpoint (rate of VOCs during the 48-week treatment period), pending sufficient participant counts within the subgroups.

- Age (< 18 years; ≥ 18 years)
- Sex (male; female)
- Race (African, Black or African American, or North African; Arab or Middle Eastern; other)
- Baseline HU use (yes; no)
- Baseline voxelotor use (yes; no)
- Number of VOCs in the 12 months prior to study entry (2 to 4 episodes; 5 to 10 episodes)
- SCD genotype (HbSS, HbSC, HbSβ0 thalassemia, or HbSβ+ thalassemia)
- Baseline weight (< 60 kg; ≥ 60 kg)
- Geographic region (North America; sub-Saharan Africa; Europe/rest of world)

For the primary efficacy endpoint, a negative binomial regression model similar to that specified for the primary analysis (Section 5.3.1) will be used for each subgroup analysis based on data subset for the patient subgroup of interest. Baseline covariates included in the primary analysis but no longer relevant given the subgroup of interest will be excluded from the model.

In the case of a low number of participants within a category (< 10 participants in a given treatment arm), the category may not be analyzed, or be simplified by combining some of the subgroups.

The estimated treatment effects (inclacumab vs. placebo) and corresponding 95% CIs will be displayed graphically for each level of the subgroups specified.

5.3.6. Sensitivity Analyses

The following sensitivity analyses will be performed with respect to the primary efficacy endpoint (rate of VOCs during the 48-week treatment period):

- Per-protocol population: The primary endpoint will be re-analyzed based on the per-protocol analysis population (Section 4.3) using the same analysis methodology as for the primary ITT analysis. If the number of participants in the per-protocol populations is > 90% of that in the ITT population, then analyses based on the per-protocol population will not be performed.
- Additional sensitivity analyses involving missing data imputation detailed in Section 5.3.6.1:
    - Missing at random (MAR) multiple imputation
    - MAR tipping point analysis
    - Missing not at random (MNAR) "Jump to reference" (J2R) multiple imputation
    - MNAR dropout reason-based (DR) multiple imputation

The following sensitivity analysis will be performed with respect to the secondary efficacy endpoint proportion of participants with no VOCs during the 48-week treatment period:

- The secondary endpoint will be re-analyzed using the same analysis methodology as specified in Section 5.3.2.2, however, the sensitivity analysis will be based on observed data only (i.e., without imputation of VOC events for early study discontinuation). Participants who discontinue the study prior to the end of the 48-week treatment period without an observed protocol-defined VOC by the time of early study discontinuation will be assumed not to have experienced at least one VOC by Week 48.

In addition, the primary and all secondary efficacy endpoints will be re-analyzed based on the mITT analysis population (Section 4.2) using the same methodology as specified for the ITT population.

5.3.6.1 Accounting for Missing VOC Data

To minimize the amount of missing data in the study, participants are encouraged to remain in the study after premature discontinuation of treatment and complete visits according to the protocol. However, participants dropping out of the study will potentially lead to unobserved VOCs.

This section summarizes the plan to account for missing data in VOCs, including sensitivity analyses using multiple imputation to assess the robustness of the treatment effect under different underlying assumptions.

Sensitivity analyses under MAR and MNAR assumptions

To assess the robustness of inference about treatment effects to various missing data assumptions, additional sensitivity analyses that capture departures from the underlying assumptions will be performed using the controlled multiple imputation method introduced in

Keene et al. (2014). As with the primary analysis, the sensitivity analyses include all data until participants withdraw from the study regardless of if they discontinue from randomized treatment.

Let Y_{i1} denote the observed pre-withdrawal counts from participant *i,* which follow a negative binomial distribution with parameter k and p_{i1}.

$$P[Y_{i1} = y_1] = \frac{\Gamma(y_1 + k)}{\Gamma(y_1 + 1)\Gamma(k)}(1 - p_{i1})^k p^{y_1}$$

Note that the inverse of k is also denoted by k in SAS PROC GENMOD procedure as pointed out by Keene et al (2014). The mean $\mu_{i1} = \frac{kp_{i1}}{1-p_{i1}}$ and variance $\frac{kp_{i1}}{(1-p_{i1})^2}$.

For this method, post study withdrawal counts from participant *i* denoted by Y_{i2} will be imputed conditional upon the observed number of events prior to the withdrawal, a post-withdrawal model assumption, the baseline covariates included in the primary model and the time remaining after discontinuation to end-of study (48 weeks) denoted by T_{i2}.

The method involves first fitting the primary analysis, i.e. negative binomial regression Model 1 to the observed data Y_{i1},

$$\log(\mu_{i1}) = \log(T_{i1}) + X_{i1}\beta$$

where T_{i1} is the pre-withdrawal follow-up time from participant *i*, the design matrix X_{i1} includes both the baseline covariates and the treatment group, β is the parameter vector.

After that, independent samples are drawn from the joint distribution of the model parameters, creating a number of parameter sets that consist of the linear regression terms and the log of the dispersion parameter.

Imputed post-withdrawal counts Y_{i2} are then generated for each discontinued participant by, for each generated set of model parameters, drawing a random number from the probability function for post-withdrawal counts, conditioned on the observed number of events prior to withdrawal for that participant. According to Keene et al. (2014), the conditional distribution of post-withdrawal counts Y_{i2} given the pre-withdrawal counts Y_{i1}=y_1 from participant *i* is also a negative binomial distribution with parameter p_i^* and dispersion parameter k^*=$k + y_1$, i.e.,

$$P[Y_{i2} = y_2 | Y_{i1} = y_1] = \frac{\Gamma(k^* + y_2)(\psi_{i2}/(1 + \psi_{i1}))^{y_2}}{\Gamma(y_2 + 1)\Gamma(k^*)(1 + \psi_{i2}/(1 + \psi_{i1})^{(k^* + y_2)})}$$

with k^*=$k + y_1$ and $p_i^* = \frac{\psi_{i2}}{1+\psi_{i1}+\psi_{i2}}$. The link between ψ_{ij} and p_{ij} is $\psi_{ij} = \frac{p_{ij}}{1-p_{ij}}$.

y_1 is the number of counts before withdrawal from the study, *k* is the dispersion parameter which can be estimated from observed data.

Furthermore, from fitted Model 1 estimate of dispersion parameter k can be obtained and denoted by $\hat{k}$; similarly estimate of p_i^* can be obtained as

$\hat{p}_i^* = Pred_{i,\ post} / (\hat{k} + Pred_{i,\ pre} + Pred_{i,\ post})$

where $Pred_{i,\ pre}$ and $Pred_{i,\ post}$ are the predicted event rates of participant *i* from negative binomial regression model before and after withdrawal, respectively. The predicted rates depend on

various assumptions and the baseline covariates included in the primary analysis model. Note that for implementation in SAS, parameters $\left(p = 1 - \hat{p}_i^*, k = \hat{k}^*\right)$ need to be supplied as input of RAND('NEGBINOMIAL', p, k) function to generate randomly a negative binomial distribution due to different parameterization (see documentation for the SAS RAND FUNCTION).

The imputed number of VOCs is then combined with the observed VOCs and then the combined data is analyzed using the primary analysis methodology. This analysis is repeated multiple times and the results combined using Rubin's formulae (Fleming [2011] and Ratitch et al. [2013]).

The following assumptions that will be used to determine $\hat{p}_i^*$ and impute the missing data who withdraw early from the study (see Table 5):

(a) MAR multiple imputation:

Missing number of VOCs for a participant is imputed using the observed VOC rate within the treatment group of that participant ($Pred_{i,\ pre} = Pred_{i,\ post}$).

(b) MNAR "jump to reference" (J2R) multiple imputation:

It is assumed that the expected postwithdrawn event rate of participant on inclacumab arm is shifted to that of the placebo arm. Specifically, the participant's mean event rate profile follows that observed for a control group. The covariance matrix matches that from the randomized arm for the pre-withdrawal event counts and the placebo arm for the conditional components for the post-withdrawal given the pre-withdrawal measurements. Post-withdrawal events in the placebo arm are imputed under the placebo arm just as under MAR. (Keene et al. [2014]). This approach is mimics the scenario when the post-withdrawn participants on inclacumab arm cease their treatment and started treatment similar to placebo.

(c) MNAR dropout reason-based (DR) multiple imputation:

Missing counts will be imputed differently depending on the reason for dropout; counts for subjects in the inclacumab arm who dropped out for a potentially treatment related reasons are imputed based on the expected rate in the placebo arm, whereas the remaining subjects who have dropped out are imputed assuming MAR. Potentially treatment related reasons include AE, physician decision, protocol deviation, withdraw by participant, and withdraw by parent/guardian.

(d) MAR tipping point analysis:

Missing counts for a participant on the placebo arm will be imputed using the observed event rate. Missing counts for a participant on inclacumab arm will be imputed according to the rate of inclacumab arm multiplied by a factor delta. A series of analyses will be performed with a range of increasing deltas for inclacumab arm so that one could assess at which point the study conclusions would change from favorable to unfavorable; i.e., to identify a tipping point.

Table 5: Overview of Imputation Methods for Sensitivity Analyses

Method	Treatment Group	Pre-withdrawal Rate	Post-withdrawal Rate
MAR MI	Inclacumab	Inclacumab	Inclacumab
	Placebo	Placebo	Placebo
MNAR Jump to reference MI	Inclacumab	Inclacumab	Placebo
	Placebo	Placebo	Placebo
MNAR Dropout Reason-based MI	Inclacumab	Inclacumab	Dropout reason specific (see Table 6)
	Placebo	Placebo	Placebo
MAR Tipping Point	Inclacumab	Inclacumab	Inclacumab*delta
	Placebo	Placebo	Placebo

MAR=missing at random; MI=multiple imputation; MNAR=missing not at random.

Table 6 specifies the treatment arm used to calculate imputation for participants on the inclacumab arm. Participants on the placebo arm will be imputed using the rate of placebo arm.

Table 6: Treatment Arms Used to Calculate Imputation Rate by Reason for Withdrawal

Reason for Withdrawal	Post-withdrawal Rate
Adverse Event	Placebo
Lost To Follow-Up	Inclacumab
Physician Decision	Placebo
Pregnancy	Inclacumab
Protocol Deviation	Placebo
Study Terminated by Sponsor	Inclacumab
Withdrawal by Participant	Placebo
Withdrawal by Parent/Guardian	Placebo
Other	Based on review prior to study unblinding

Sensitivity analyses will be performed based on the assumptions stated above. 5000 imputations will be carried out, and a seed of 2104131 will be used.

5.3.7. Interim Futility Analysis Details

A non-binding futility interim analysis for the primary efficacy endpoint (i.e., the rate of VOCs during the 48-week treatment period) is planned to allow for potential of early stopping of the

study. The futility analysis will be performed on data adjudicated by an independent blinded VOC Adjudication Committee. To enable robust decision making at interim, two analyses will be performed. The *main analysis* will be based on subjects randomized with the potential for at least 24 weeks of treatment (2 doses of study drug) while a *supportive analysis* will be based on all randomized subjects.

The estimated *information fraction* associated with the primary endpoint based on subjects with the potential for at least 24 weeks of treatment is expected to be approximately 48% (see Table 8). The methodology including calculation of information fraction is described in this section.

Unblinded efficacy results from the interim analysis for VOC will be reviewed by the DMC. Unblinded results will not be communicated to Sponsor's study team or to any party directly involved in the study conduct until the DMC has recommended and the Sponsor determined that the study needs to be terminated due to any cause, including futility or safety reasons.

5.3.7.1. Methodology

At the interim analysis timepoint, the critical boundary for the group sequential test will be derived from the predefined error spending function. The calculation of boundaries is performed using statistical software R and associated packages including RPACT.

For comparison of treatment effect, a non-binding futility boundary is constructed using the type II error spending function methodology by Hwang-Shih-DeCani (Jennison, 2000). Specifically, a type II error spending function with parameter γ = -1 will be used to determine the non-binding futility boundary.

A Hwang-Shih-Decani (HSD) error spending function is a function of information fraction *w*. When the parameter $\gamma \neq 0$ it takes the following form

$$HSD(w;\beta,\gamma) = \beta(1 - e^{-\gamma w})/(1 - e^{-\gamma})$$

where β denotes the target type II error rate, i.e., the target power is *1*- β.

According to Mütze (2019), for testing the null hypothesis H_0 at kth look (e.g., $k = 1$ for interim analysis) is based on the difference of log-rates i.e., $\hat{\beta}_{1k} - \hat{\beta}_{2k}$.

Let the information level at interim analysis ($k = 1$) be

$$\mathbb{I}_k = \frac{1}{\frac{1}{I_{\beta_1}^{(k)}} + \frac{1}{I_{\beta_2}^{(k)}}} = \frac{I_{\beta_1}^{(k)} I_{\beta_2}^{(k)}}{I_{\beta_1}^{(k)} + I_{\beta_2}^{(k)}}$$

where the expected Fisher information of the parameter β_i at *k*th look is given by

$$I_{\beta_i}^{(k)} = \sum_{j=1}^{n_i} \frac{t_{ijk} e^{\beta_i}}{1 + \phi t_{ijk} e^{\beta_i}} = \sum_{j=1}^{n_i} \frac{t_{ijk} \mu_i}{1 + \phi t_{ijk} \mu_i}$$

where ϕ is the negative binomial dispersion parameter, t_{ijk} is the follow-up time from *j*th subject from *i*th treatment group at *k*th look ($i = 0,1; j = 0,1,\ldots,n_i; k = 1,2$).

The actual (unknown) information fraction of *k*th look w_k is defined by

$$w_k = \frac{I_k}{I_{max}}$$

where I_k and I_{max} denotes the information level at kth look and at final (without early discontinue) respectively. The actual information fraction at the interim analyses can be estimated the information fraction by plugging in maximum likelihood estimates $(\hat{\beta}_0, \hat{\beta}_1, \hat{\phi})$

from a negative binomial regression model with subject follow-up duration at log-scale as offset and treatment group.

When the interim analysis is performed, the estimated information fraction w_1 can be obtained. To determine the non-binding HSD Gamma(-1) futility boundary, the type II error rate h_1 allocated to the interim analysis can be calculated from β-spending function

$$h_1 = HSD(w_1; \beta = 0.1, \gamma = -1)$$

Then the non-binding futility boundary d_1 is determined by solving the following equation numerically:

$$h_1 = \mathrm{P}_{H_1}(T_1 < d_1)$$

where T_1 is the Wald-test statistic observed at interim. In the following sections, calculation of the non-binding futility boundary d1 is implemented using function *getDesignGroupSequential* function from RPACT package.

5.3.7.2. Operating Characteristics of Futility Boundary

Table 7 displays the operating characteristics of the futility boundary. The futility boundary is non-binding. If the futility boundary is crossed, the DMC may recommend to the Sponsor that the study be stopped for futility.

Table 7: Operating Characteristics of Futility Boundary

Parameter	Inclacumab vs. Placebo	
Analysis	Interim[a]	Final
Analysis cut-off	Approximately 130 participants with potential for at least 24 weeks of treatment (i.e., 2 doses of study drug)	Last participant completes last visit
Futility boundary[b]		
Z-value scale	Z>-0.509	Z>-1.96
One-sided p-value scale	p>0.305	p>0.025

Note: The non-binding HSD Gamma(-1) futility boundary is calculated based on assumptions including target type II error rate 0.1; annual VOC rate in placebo is 3; dispersion parameter is 1.04; subjects randomized on or before 30DEC2022 were included in this calculation.

[a] Information fraction 0.48 used for calculation of main interim analysis futility boundary.

[b] Futility boundary is non-binding. In this calculation, placebo is set as reference group.

The actual information fraction at the interim analysis (IA) may not match the planned one. The futility boundary will be updated using specified β-spending function based on the actual information fraction from unblinded observed data (sample size, individual follow-up times, VOC event rates for each treatment group, and dispersion parameter). Therefore, the observed Z-test statistic at the interim analysis will be compared with the updated futility boundary.

Based on the stopping boundary defined above and the timing of the interim and final analyses as described above, the design has the following operating characteristics.

Table 8: Simulated Cumulative Probabilities of Stopping for Futility at Interim Analysis

Scenario	Look	Information Fraction[a]	Probability of Stop for Futility[b]
Main IA			
Null hypothesis is true (RR=1)	IA	48.2%	69.6%
Alternative hypothesis is true (RR=0.55)	IA	47.0%	1.8%

IA=interim analysis; RR=rate ratio.

Note: Simulation performed in R with number of iterations=10000 and seed=20230428.

[a] Information fraction calculation includes subjects with at least 24 weeks of follow up.

[b] Probability of stop for futility is defined by probability of accept null hypothesis.

5.3.7.3. Impact of Futility Interim Analyses to Study Power

In Section 2.3, the sample size calculation is based on a fixed design without interim analyses. This section evaluates any potential impact from adding a non-binding futility interim analysis to study power by comparing the current study design with futility IA vs. original fixed study design (i.e., without futility IA).

By law of total probability, it can be shown that the following equation holds,

P_{H_1}(Final test is significant)

= P_{H_1}(Test statistic does not hit futility boundary at IA, Final test is significant) +

P_{H_1}(Test statistic hits futility boundary at IA, Final test is significant)

Note that

- Under the alternative hypothesis, P_{H_1}(Final Test is significant) is the power of original fixed design, which is approximately 90% per Section 2.3;
- P_{H_1}(Test statistic does not hit futility boundary at IA, Final Test is significant) is the power of current design with a non-binding-Gamma(-1) futility boundary;
- P_{H_1}(Test statistic hits futility boundary at IA, Final Test is significant)

  =P_{H_1}(Test statistic hits futility boundary at IA) * P_{H_1}(Final Test is significant | Test statistic hits futility boundary at IA)

  < P_{H_1}(Test statistic hits futility boundary at IA) = 1.8% (per Table 8) under the alternative hypothesis.

Hence the power of current design is at least approximately 88%. The impact on power from adding a non-binding futility interim analysis is minimal.

5.4. Safety Analyses

Safety analyses will be based on the safety patient population (Section 4.2), with participants grouped according to the actual study treatment received. Safety will be assessed through descriptive summaries of adverse events, clinical laboratory test results, and vital signs.

5.4.1. Exposure to Study Drug and Compliance

Exposure to study drug (number of study drug treatments received and duration of treatment) will be summarized by treatment group. Duration of treatment will be defined based on the difference between the dates of the first and the earlier of last dose of study drug and participant's end of study date plus 12 weeks (inclusive), i.e.,

$$Duration\ (weeks) = \frac{\min(LastDosingDate + 84,\ EndofStudyDate) - FirstDosingDate + 1}{7}$$

For each dosing visit, the actual dose administered (in mg) and the difference between the planned dose (30 mg/kg) and actual dose administered (in mg/kg) will be summarized descriptively by treatment group.

For each participant, the overall percent compliance with study drug will be calculated based on the ratio of the average dose administered (mg/kg) during the participant's time on study to the planned dose (i.e., 30 mg/kg):

$$Compliance\ (\%) = 100\ \text{x}\ \frac{Average\ (ActualDose\ [mg/kg])}{30\ mg/kg}$$

The overall percent compliance with study drug will be summarized descriptively by treatment group.

In addition, the number and percentage of participants with infusion interruptions will be summarized by dosing visit and treatment group. In addition, the number and percentage of participants with at least one infusion interruption during the study will be summarized by treatment group.

5.4.2. Adverse Events

Adverse events will be classified according to MedDRA; the most current version at the time of analysis will be used. Severity of adverse events will be graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0, when possible, or based on the protocol-specified grading for adverse events not covered in the NCI CTCAE.

Summaries of treatment-emergent adverse events (TEAEs), defined as adverse events that occur on or after Day 1 of study treatment or the worsening of a preexisting condition on or after Day 1 of study treatment, will be tabulated by system organ class (SOC) and preferred term, as appropriate.

Summaries of TEAEs by treatment group will be provided for the following categories:

- All TEAEs
- All TEAEs by maximum severity
- TEAEs assessed as related to study drug by the investigator
- TEAEs leading to study drug interruption
- TEAEs leading to study drug discontinuation
- Adverse events of special interest (AESIs) (i.e., infusion-related reaction TEAEs)
- Treatment-emergent serious adverse events (SAEs)

Listings for all adverse events, TEAEs leading to study drug discontinuation, AESIs, SAEs, and deaths will be provided.

VOC events will be collected and summarized separately (uncomplicated VOC, ACS, hepatic sequestration, splenic sequestration, and priapism).

5.4.3. Clinical Laboratory Assessments

Laboratory abnormalities assessed by the Investigator as clinically significant will be recorded as adverse events.

Descriptive summaries of laboratory parameters (hematology, serum chemistry, and coagulation) at baseline and each evaluation post baseline, as well as changes from baseline over time, will be provided by treatment group. If any of the results are below the limit of quantitation or above the limit of quantitation, then the numerical limit will be used in the descriptive summaries.

Laboratory abnormalities will be graded via the Common Terminology Criteria for Adverse Event (CTCAE). A treatment-emergent laboratory abnormality is any post-baseline laboratory value obtained on or after initiation of study drug which demonstrates an increase of 1 grade or more from the baseline toxicity value. If the baseline value is missing, any graded abnormality (grade 1 or higher) that occurs following initiation of study drug will be deemed treatment-emergent. The number and percentage of participants experiencing treatment-emergent laboratory abnormalities will be summarized by treatment group. Laboratory abnormality shifts from baseline through each evaluation post baseline will be summarized by treatment group.

5.4.4. Vital Signs and Weight

Descriptive summaries of vital signs (e.g., systolic and diastolic blood pressure, heart rate, and body temperature) and weight at baseline and each evaluation post baseline, as well as changes from baseline over time, will be generated by treatment group.

5.5. Exploratory PK, ADA, and PD Analyses

5.5.1. Pharmacokinetic Analyses

Population PK analysis will be performed on data from all participants who receive active study drug and have at least 1 measurable post-dose concentration. If any participants are found to have incomplete data, protocol deviations, or clinical events affecting PK, a decision will be made on a case-by-case basis as to their inclusion in the analysis. The influence of demographic covariates (such as body weight, sex, age, race, ethnicity, renal function) on inclacumab PK parameters will be investigated. Further details of the population PK analyses will be described in a separate document.

5.5.2. Anti-drug Antibody Analyses

Analyses for ADA will include data from all participants who receive active study drug and have pre-dose and at least 1 assessment for ADA after the start of dosing. Anti-drug antibody characterization will include, but is not limited to, incidence, timing, and persistence of ADA.

A multi-tiered ADA testing approach will be used for sample immunogenicity assessment. The screening assay will be performed on all collected samples to detect the presence of binding ADA to inclacumab. Samples deemed positive in the screening assay will then be tested in the confirmatory assay to confirm specificity to the response. Participants with samples that are confirmed positive will be reported as positive for the presence of anti-inclacumab antibody and these samples will be titered to determine the relative level of ADA.

The percentage of participants with confirmed ADA positivity during the study out of the total number tested will be reported. The ADA positive samples will be run in a neutralizing antibody (NAb) assay to assess their ability to neutralize drug activity and results reported.

If any participants are found to have incomplete data, protocol deviations, or clinical events affecting ADA, a decision will be made on a case-by-case basis as to their inclusion in the analysis. The relationship of ADA with PK, safety, and efficacy observations will be explored, as appropriate.

5.5.3. Pharmacodynamic Analyses

Exploratory pharmacodynamic analyses will be performed on data from all participants who have at least 1 measurable result. The relationship of PD results with PK, ADA, safety, other PD markers, and efficacy observations will be explored, as appropriate. Further details of the pharmacodynamic analyses will be described in a separate document.

6. TABLES, LISTINGS, AND FIGURES

A separate document will provide mockups of the tables, listings, and figures that support the analyses proposed in this SAP.

7. REFERENCES

1. Ataga K, et al. Crizanlizumab for the Prevention of Pain Crises in Sickle Cell Disease. N Engl J Med. 2017;376(5):429–439.
2. Brookmeyer R, and Crowley JJ. (1982). A Confidence Interval for the Median Survival Time. Biometrics, 38: 29–41.
3. Fleming TR. Addressing Missing Data in Clinical Trials. Ann. Intern. Med. 2011;154:113–117.
4. Jennison C, Turnbull BW. In: Group Sequential Methods with Applications to Clinical Trials. Chapman & Hall/CRC, Boca Raton, 2000.
5. Kalbfleisch JD, and Prentice RL. (2002). Statistical Analysis of Failure Time Data. 2nd Edition. Hoboken, Wiley Interscience.
6. Keene O, Roger J, Hartley B, and Kenward . Missing Data Sensitivity Analysis for Recurrent Event Data Using Controlled Imputation, Pharmaceutical Statistics. 2014;13(4):258–264.
7. Mütze T, Glimm E, Schmidli H, Friede T. Group sequential designs for negative binomial outcomes. Stat Methods Med Res. 2019 Aug; 28(8):2326–2347.
8. Ratitch B, O'Kelly M, Tosiello R. Missing data in clinical trials: from clinical assumptions to statistical analysis using pattern mixture models. Pharmaceutical Statistics 2013; 12: 337–347.
9. Zhu H and Lakkis H. Sample Size Calculation for Comparing Two Negative Binomial Rates. Statistics in Medicine, 2014;33(3):376–387.