TITLE PAGE

STATISTICAL ANALYSIS PLAN

Version Number: 1.0

Protocol Title: A Phase 2a, Randomized, Open-Label Study to Evaluate Multiple Dosing Regimens of Subcutaneous ALXN1820 in Adult Patients With Sickle Cell Disease

Protocol Number: ALXN1820-SCD-201

Protocol Amendment Number: 1.0

Compound: ALXN1820

Brief Title: Safety, Efficacy, Pharmacokinetic, and Pharmacodynamic Study of ALXN1820 in Adult Patients With Sickle Cell Disease

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VERSION HISTORY

This statistical analysis plan (SAP) for Study ALXN1820-SCD-201 is based on Protocol Amendment 1.0, dated 22 Aug 2022.

SAP Version	Version Date	Change	Rationale
1.0	24 OCT 2023	Not applicable	Original version

APPROVAL SIGNATURES

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADA	antidrug antibody
AE	adverse event
CAP	complement alternative pathway
DBP	diastolic blood pressure
ECG	electrocardiogram
HR	heart rate
ICF	informed consent form
MedDRA	Medical Dictionary for Regulatory Activities
NCI	National Cancer Institute
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PT	Preferred Term
QTcF	corrected QT interval by Fridericia's formula
RR	respiratory rate
SAP	statistical analysis plan
SBP	systolic blood pressure
SC	subcutaneous
SCD	sickle cell disease
SOC	System Organ Class
TEAE	treatment-emergent adverse event
VOC	vaso-occlusive crisis
WHO-Drug	World Health Organization Drug Dictionary

1. INTRODUCTION

This statistical analysis plan describes the plan for presenting the data for final analysis for Protocol ALXN1820-SCD-201. Due to the early termination of the study, data will only be listed.

Safety, pharmacokinetic (PK), pharmacodynamic (PD), immunogenicity, biomarker, demographics and baseline characteristics, medical history, prior and concomitant medications and efficacy data are included. Standard data presentation instructions and listing specifications are contained in the Data Presentation Plan in a separate document.

Changes to the protocol-planned analyses are described in Section 4.5.

Due to the early termination of the study, Cohort 3 will not be initiated, the planned interim analysis will not be conducted, and the output of all participants will be listed without inclusion of tables and figures.

1.1. Objectives, Endpoints, and Estimands

The objectives and endpoints of ALXN 1820-SCD-201 are summarized in Table 1

Table 1: Objectives and Endpoints

Objectives	Endpoints
Primary	
To assess the safety and tolerability of ALXN1820 SC in participants with SCD	Safety assessed by incidence of TEAEs and SAEs, physical examination, vital sign measurements, clinical laboratory, and electrocardiogram results through Day 211 (Cohorts 1 and 2)
Secondary	
To assess the multiple-dose PK of ALXN1820 SC	Serum ALXN1820 multiple-dose PK profiles through Day 211 (Cohorts 1 and 2)
To assess the PD effects of ALXN1820 SC	Change in serum concentrations of total and free properdin over time through Day 211 (Cohorts 1 and 2) Change in CAP activity using the Wieslab AP assay through Day 211 (Cohorts 1 and 2)
To assess the effect of ALXN1820 on complement biomarkers	Change from Baseline in complement biomarkers through Week 12 (Cohorts 1 and 2)
To assess the effect of ALXN1820 on hemolysis biomarkers	Change from Baseline in hemoglobin levels at Week 12 (Cohorts 1 and 2) Change from Baseline in hemolysis markers (e.g., lactate dehydrogenase, reticulocytes, and bilirubin and hemopexin) at Week 12 (Cohorts 1 and 2)
To assess the immunogenicity of ALXN1820 SC	Incidence of ADAs to ALXN1820 through Day 211 (Cohorts 1 and 2)
Exploratory	
To explore the effect of ALXN1820 on VOC markers	Change from Baseline in VOC-related biomarkers through Week 12 (Cohorts 1 and 2)

Table 1: Objectives and Endpoints

Objectives	Endpoints
To explore the clinical efficacy of ALXN1820 SC in SCD	VOC events (rate of VOC, time to first VOC) through Week 12 (Cohorts 1 and 2)

Abbreviations: ADA = antidrug antibody; AP = alternative pathway; CAP = complement alternative pathway; PD = pharmacodynamic; PK = pharmacokinetic(s); SAE = serious adverse event; SC = subcutaneous; SCD = sickle cell disease; TEAE = treatment-emergent adverse event; VOC = vaso-occlusive crisis.
Note: Estimand is not applicable.

1.2. Study Design

This is a Phase 2a study, with up to 3 multiple-dose cohorts of open-label ALXN1820 subcutaneous (SC) in adult participants with sickle cell disease (SCD; HbSS and HbSß^0-thalassemia).

The study is planned to be conducted in up to 30 adults participants with SCD enrolled in up to 3 open-label cohorts (Cohorts 1, 2, and 3 [optional]) to receive multiple SC doses of open-label ALXN1820. At the time of the early termination of the study, 2 participants are enrolled in the study. ALXN1820 is administered as described in Table 2.

Table 2: ALXN1820-SCD-201 Dosing Cohorts

Cohort	N	Study Intervention	Route of Administration	Planned Dose	Number of Doses/Dose Interval
1	Up to 12 Maximum of 6 on a stable dose of hydroxyurea	ALXN1820	SC	300 mg	QW × 13
2	Up to 12 Maximum of 6 on a stable dose of hydroxyurea	ALXN1820	SC	600 mg	Q4W × 4

Abbreviations: N = number of participants; QW = once weekly; Q4W = once every 4 weeks; SC = subcutaneous; SCD = sickle cell disease

The dose and dosing intervals for Cohorts 1 and 2 were determined using cumulative safety data, an interim PK/PD analysis from participants enrolled in Study ALXN1820-HV-101, and data from the 6-month Good Laboratory Practice toxicology study in monkeys.

Cohorts 1 and 2 run in parallel, and participants are randomized 1:1 to either of the cohorts upon determining eligibility. Each cohort is to be stratified to ensure participants with SCD who are treated with a stable dose of hydroxyurea and participants with SCD who are not currently treated with hydroxyurea are included. The Treatment Period is 12 weeks for Cohorts 1 and 2.

For participants who were previously treated with hydroxyurea but are not currently on hydroxyurea, treatment is to be stopped at least 30 days prior to providing informed consent.

This study uses an independent Data Monitoring Committee to monitor safety and to perform the planned interim analyses of the study. At Alexion's discretion and after consultation with the Data Monitoring Committee, additional participants with SCD may be enrolled as replacement participants if a participant discontinues during the Dosing Period for reasons other than drug-related adverse events (AEs).

2. STATISTICAL HYPOTHESES

Not applicable.

3. ANALYSIS SETS

Due to the early termination of the study, no analysis set will be defined except Enrolled Set, which includes all participants who signed the informed consent form, excluding screen failures. All the listing outputs will be based on the Enrolled Set.

4. STATISTICAL ANALYSES

4.1. General Considerations

All analyses will be performed using Statistical Analysis Software® (SAS®) Version 9.4 or higher (SAS Institute Inc., Cary, NC, USA) or other validated statistical software.

Baseline is defined as the last nonmissing observation before the first dose of the study intervention.

Treatment Period is 12 weeks for Cohorts 1 and 2. Follow-up Period is 18 weeks after the last dose or may be longer if CAP activity is below the normal range or 80% of the baseline value, as defined in the protocol.

Study and participant characteristics (e.g., disposition, demographics and baseline characteristics, medical history, and protocol deviations) are described in Section 6.2.

4.2. Primary Endpoints Analysis

The primary endpoint for the study is safety, which will be assessed by the following parameters:

- AEs
- Clinical laboratory assessments
- Vital signs
- Physical examination
- Electrocardiogram (ECG) parameters, including heart rate (HR), PR, RR, QRS, and QT intervals and corrected QT interval by Fridericia's formula (QTcF)

4.2.1. Analysis of AEs

All the AEs will be listed for each participant.

The adverse event will be presented using primary Medical Dictionary for Regulatory Activities (MedDRA) System Organ Classes (SOCs) and Preferred Terms (PTs) according to the version of the MedDRA coding in effect dictionary at the time of database lock. The SOCs will be presented in alphabetical order, and the PTs will be presented in descending order of frequency overall within each SOC.

Treatment-emergent adverse events (TEAEs) (refer to Section 6.1 for the definition of TEAE) will be indicated. The following information of AEs will also be listed:

- Relationship as assessed by the investigator
- Severity by severity grades according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE, v5.0) (Grade 1, Grade 2, Grade 3, Grade 4, Grade 5)
- Seriousness criteria
- VOC exacerbated
- Leading to discontinuation from study intervention

- Leading to study withdrawal
- Leading to death
- Injection site reaction (ISR) according to the Investigator.

Clinically significant ISR will be reported as AE and will be indicated on the AE listing.

4.2.2. Analysis of Laboratory Assessments

Laboratory results based on standardized units will be used.

Absolute values and changes from Baseline in clinical chemistry, hematology, coagulation, and urinalysis will be listed at each visit.

Clinical chemistry, hematology, coagulation, and urinalysis values will be graded according to NCI CTCAE, v5.0, where applicable, and will be listed.

4.2.3. Analysis of Vital Signs

Vital sign measurements include body weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), HR, temperature and respiratory rate (RR). Actual values and change from Baseline in vital sign measurements will be listed at each visit for each participant.

Abnormal vital signs will be listed for each participant, criteria for abnormal vital signs are defined as follows:

- Body weight ≥ 7% decrease from Baseline; Body weight ≥ 7% increase from Baseline
- SBP < 90 mmHg; SBP > 140 mmHg; SBP > 160 mmHg
- DBP < 50 mmHg; DBP > 90 mmHg; DBP > 100 mmHg
- HR < 60 beats per minute; HR > 100 beats per minute
- Temperature < 36 degrees Celsius; temperature > 38 degrees Celsius
- RR < 12 breaths per minute; RR > 20 breaths per minute

4.2.4. Analysis of Physical Examination

All physical examination data will be provided in data listings. Abnormal physical examination finding during the study will be listed.

4.2.5. Analysis of ECG Results

The ECG parameters include HR, PR, RR, QRS, and QT intervals and QTcF. The mean of the triplicate ECG readings at the timepoints collected will be calculated. Absolute value and change from Baseline values will be listed at each visit for each participant. Change from Baseline values will be based on the calculated mean at each timepoint.

Outlier ECG results will be listed for each reading per visit, criteria for outlier ECG results are defined as follows:

- QT interval and QTcF > 450 msec

- QT interval and QTcF > 480 msec
- QT interval and QTcF > 500 msec
- QT interval and QTcF increases from Baseline > 30 msec
- QT interval and QTcF increases from Baseline > 60 msec

All ECG individual measurements as well as the calculated mean of triplicate ECG readings, will be provided in data listings.

4.3. Secondary Endpoints Analysis

4.3.1. Analysis of PK/PD

Serum concentration will be listed over time for each participant. All serum concentrations below the lowest quantifiable sample concentration of the assay (BQL) or missing data will be labeled as such in the concentration data listings.

Due to the early termination of the study and the limited sample size of enrolled participants, there will be no PK parameters outputs.

Absolute values, changes from Baseline, and percent change from Baseline of PD parameters (serum total and free properdin concentrations and CAP activity) will be listed over time for each participant.

4.3.2. Analysis of SCD Biomarkers

Absolute value, change from Baseline, and percent change from Baseline in biomarkers, including Ba, C3a, C4a, C5a, sC5B9, LDH, bilirubin (total, direct and indirect), reticulocytes (absolute and percent), haptoglobin and hemopexin will be listed over time for each participant.

4.3.3. Analysis of Immunogenicity

Baseline is defined as the last nonmissing antidrug antibody (ADA) signal obtained on or before the first study intervention administration.

For assessment of immunogenicity, the signal of ADAs will be categorized as positive or negative. Additionally, following confirmation of positive ADAs, samples will be further categorized into ADA response categories and will be assessed for ADA titer over time. ADA categories are defined as follows:

- **ADA negative:** An ADA-negative signal in the ADA assay at all timepoints collected for ADA analysis
- **ADA positive:** An ADA-positive signal in the ADA assay at any timepoint collected for ADA analysis

Participants who are ADA positive may be further categorized into ADA response categories as follows:

- **Pre-existing immunoreactivity:** An ADA-positive response with either of the following 2 conditions met:

  - ADA-positive response at Baseline with all post-first-dose ADA results negative

    OR

  - ADA-positive response at Baseline with all post-first-dose ADA responses < 4-fold over the baseline titer level

- **Treatment-emergent ADA responses:** An ADA-positive response post-first dose when baseline results are negative or missing
- **Treatment-boosted ADA responses:** An ADA-positive response post-first dose that is ≥ 4-fold over the baseline titer level when the baseline result is positive

4.4. Exploratory Endpoints Analysis

Absolute value, change from Baseline, and percent change from Baseline in the markers of VOC, including hemopexin and nitric oxide, will be listed over time for each participant.

SCD-related pain crises (VOC) are defined as acute episodes of pain with no medically determined cause other than a VOC event that results in a medical facility visit and treatment with either oral or parenteral narcotic agents or with a parenteral nonsteroidal anti-inflammatory drug. Uncomplicated VOCs are defined as no occurrence of any other SCD complication during the VOC episode. Complicated VOCs are defined as the presence of a diagnosis of other SCD complications during the VOC episode. Acute chest syndrome, hepatic sequestration, splenic sequestration, and priapism are considered VOC events in this study.

The following information of VOC assessments will be listed:

- VOCs leading to a healthcare visit
- Start and end date of VOC
- Complicated VOC or uncomplicated VOC
- If complicated VOC, the complicated VOC categories will be listed (acute chest syndrome, hepatic sequestration, splenic sequestration, priapsim or other)
- Location of pain
- Tiredness during pain crisis
- Intensity of pain
- Medication Taken
- VOC exacerbate (if yes, reporting to AE)

4.5. Changes to Protocol-Planned Analyses

Due to the early termination of the study and limited enrolled participants, data will only be listed. Applicable changes from Protocol Amendment 1.0 are as follows:

- The interim analyses have been removed

- The Cohort 3 will not be initiated and have been removed
- Analysis sets have been removed except for Enrolled Set, all analyses will be based on the Enrolled Set
- Analyses of PK parameters have been removed
- Summary descriptive statistics will not be provided
- Outlier analyses of ECG have been removed
- Inflammatory biomarkers and cell adhesion biomarkers have been removed from the analysis of VOC-related biomarkers
- Visit windows have been removed

5. SAMPLE SIZE DETERMINATION

Twelve participants were planned to be enrolled in each of Cohorts 1 and 2. In participants with stable SCD, hemoglobin level is unlikely to change. The sample size was determined based on a targeted hemoglobin change from Baseline to exclude 0 g/dL with a lower 2-sided 95% confidence bound. Assuming a standard deviation of 1 g/dL, a sample size of 12 participants can provide 88% power to detect a change from Baseline of 1 g/dL with a 2-sided significance level of 0.05. In Optional Cohort 3, 6 participants were planned to be enrolled to define the exposure/response relationship of ALXN1820 by combining Cohorts 1, 2, and 3 data using a PK/PD modeling approach. The Optional Cohort 3 sample size was not determined for power purposes.

After the decision of the study early termination, the Cohort 3 will not be enrolled.

6. SUPPORTING DOCUMENTATION

6.1. Technical Specifications for Derived Variables

Adverse Events

The analysis of AE is described in detail in Section **Error! Reference source not found.**.

TEAEs are events with start dates and start times on or after the date and time of the first dose of study intervention and up to 100 days after the last dose of the study intervention. If the start date of an AE is partially or completely missing and the end (stop) date and time of the AE do not indicate that it occurred prior to the first dose, then the determination of treatment-emergent status will be based on the following:

- If the start year is after the year of the first study intervention dose, then the AE is treatment emergent; else,
- If the start year is the same as the year of the first study intervention dose and
  - the start month is missing, then the AE is treatment emergent; else if
  - the start month is present and is the same or after the month of the first study intervention dose, then the AE is treatment emergent; else,
- If the start date is completely missing, then the AE is treatment emergent.

All other AEs are considered pretreatment AEs.

If the relationship to study intervention is missing for TEAEs, then the relationship will be counted as related to study intervention for the listing tables. Missing severity for TEAEs will be counted as Grade 3.

6.2. Study and Participant Characteristics

6.2.1. Participant Disposition

Listings of the screen failures and reasons for screen failure will be generated.

Listings of participants' disposition will be based on the Enrolled Set, including treatment disposition, study duration, completion, or discontinuation of the study with a reason for study discontinuation (including COVID-19-related reasons), and completion or discontinuation of the treatment with a reason for treatment discontinuation (including COVID-19-related reasons)**Baseline Characteristics and Demographics**

Baseline characteristics and demographics will be listed for each participant using the Enrolled Set. The following variables will be listed:

- Age (years)
- Sex (female, male, undifferentiated or unknown)
- Ethnicity (Hispanic or Latino, not Hispanic or Latino, nor reported or unknown)

- Race (White, Black or African American, American Indian or Alaska Native, Asian, Native Hawaiian or other Pacific Islander, not reported, unknown, multiple or other)
- Baseline body weight (kilogram)
- Baseline height (centimeter)
- Baseline body mass index (kilogram/meters^2)
- Geographical region (European Union, North America or Rest of world)
- Number of VOC in the past 12 months
- Number of VOC in the past 3 months
- VOC categories in the past 12 months (< 3 crisis or ≥ 3 crisis)
- Genotype (HbSS, HbSC, HbSß$^+$ thalassemia, HbSß^0-thalassemia or Other)
- Baseline hemoglobin (g/dL)
- Stable dose of hydroxyurea (yes or no)
- Prior opioid use (yes or no)

6.2.3. Medical History

Medical history will be coded using MedDRA version 23.1 or above and listed for the Enrolled Set, presenting the start and end date within each PT grouped by the SOC.

6.2.4. Prior and Concomitant Medications

Prior and concomitant medications/nonpharmacologic therapies and procedures will be listed using the Enrolled Set.

Prior medications or procedures are defined as medications or procedure taken prior to the first dose of study intervention. Concomitant medications or procedures are defined as medications or procedures taken on or after the first dose of study intervention, including those started before the first dose of study intervention and continued after the first dose of the study intervention.

Medications will be coded using the World Health Organization (WHO) Drug Dictionary version in use by Alexion at the time of the analysis, while nonpharmacologic therapies and procedures will be coded using MedDRA.

Medications will be coded and listed by WHO-Drug Anatomical Therapeutic Chemical Level 3 or the next lower level if level 3 is unavailable and by WHO-Drug generic name. Nonpharmacologic therapies and procedures will be coded and listed by MedDRA SOC and PT.

The medications for Meningococcal Vaccination will be listed for each participant.

6.2.5. Protocol Deviations

The protocol deviations will be listed for each participant in the Enrolled Set.

6.3. Instrument Scoring Details

Not applicable.

6.4. Additional Details on Statistical Methods

Not applicable.

7. REFERENCES

Not applicable.

ALXN1820-SCD-201 SAP V1.0_24Oct2023_final

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