A Phase 1 Study to Evaluate the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Immunogenicity of a Single Dose of ALXN1210 Administered Subcutaneously Compared to Intravenously in Healthy Subjects

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9. DOCUMENTATION OF STATISTICAL METHODS

ALXN1210-SC-101 Statistical Analysis Plan, Final Version 1.0, Dated 30 Jan 2017

Statistical Analysis Plan	RICHMOND Pharmacology

SPONSOR'S REFERENCE NUMBER: ALXN1210-SC-101

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STATISTICAL ANALYSIS PLAN

A PHASE 1 STUDY TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS, PHARMACODYNAMICS, AND IMMUNOGENICITY OF A SINGLE DOSE OF ALXN1210 ADMINISTERED SUBCUTANEOUSLY COMPARED TO INTRAVENOUSLY IN HEALTHY SUBJECTS

FINAL VERSION 1.0, 30 JAN 2017

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Explanation
λ_z	terminal elimination rate constant
ADA	antidrug antibody
AE	adverse event
AUC_∞	area under the serum concentration versus time curve from time 0 extrapolated to infinity
AUC_t	area under the serum concentration versus time curve from time 0 to the last quantifiable concentration
BLQ	below the level of quantification
CL	total body clearance
C_{max}	maximum observed serum concentration
CSR	clinical study report
cRBC	chicken red blood cell
CV	coefficient of variation
ECG	electrocardiogram
IMP	investigational medicinal product
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
QTcF	QT interval corrected using Fridericia's formula
RPL	Richmond Pharmacology Ltd
SAE	serious adverse event
SAP	statistical analysis plan
SAS	statistical analysis software
SC	Subcutaneous
SD	standard deviation
SOC	System Organ Class
TEAE	treatment-emergent adverse event
$Tt_{½}$	terminal elimination half-life
t_{max}	time to maximum observed serum concentration
V_d	volume of distribution

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1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to define the variables and analysis methodology to address the study objectives.

The protocol dated 28 Jul 2016, Amendment 2, was used in the preparation of this SAP.

Pharmacokinetic parameters will be calculated by Alexion or its designee. Richmond Pharmacology Ltd (RPL) is responsible for performing statistical analyses. Tables, figures, and listings will be produced using Statistical Analysis Software (SAS), Version 9.3 or higher.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Study objectives

The objectives of this study are:

Primary

- To evaluate the safety and tolerability of a single dose of ALXN1210 subcutaneous (SC) compared to ALXN1210 intravenous (IV) in healthy subjects, as assessed by physical examination findings, vital sign measurements, immunogenicity, laboratory analysis, and assessments of adverse events (AEs).
- To determine the absolute bioavailability of ALXN1210 SC.

Secondary

- To evaluate the pharmacodynamic (PD) effects of ALXN1210 SC compared to ALXN1210 IV, as assessed by the level of free C5 and chicken red blood cell (cRBC) hemolysis.

2.2 ENDPOINTS

The safety, immunogenicity, pharmacokinetics (PK), and PD endpoints are described in the sections to follow. The timing of assessments is displayed in the Schedule of Assessments (see protocol).

Safety Endpoints

Safety endpoints include the following:

- Change from baseline in physical examination findings
- Change from baseline in vital sign measurements
- Incidence of antidrug antibodies (ADA) measured via immunogenicity testing
- Change from baseline in electrocardiogram (ECG) parameters
- Change from baseline in laboratory measurements
- Incidence of AEs and serious AEs (SAEs)

Pharmacokinetic Endpoints

The following PK parameters will be evaluated:

- Maximum observed serum concentration (C_{max})
- Time to maximum observed serum concentration (t_{max})
- Area under the serum concentration versus time curve from time zero to the last quantifiable concentration (AUC_t)
- Area under the serum concentration versus time curve from time zero to infinity (AUC_∞)
- Terminal elimination rate constant (λ_z)
- Terminal elimination half-life ($t_{½}$)
- Total body clearance of drug from the serum (CL or CL/F)
- Apparent volume of distribution during terminal phase (V_d or V_d/F)

Pharmacodynamic Endpoints

The following PD effects will be evaluated:

- Change in serum total and free C5 concentrations over time
- Change in cRBC hemolysis over time

3. NUMBER OF SUBJECTS AND RANDOMIZATION

3.2 Number and Source of Subjects

Forty-two subjects are planned for evaluation of the primary and secondary objectives in this study: 6 (4 ALXN1210 SC, 2 placebo SC) subjects in Cohort 1a; 24 (20 ALXN1210 SC, 4 placebo SC) subjects in Cohort 1b, and 12 (ALXN1210 IV) subjects in Cohort 2. At the sponsor's discretion, up to 8 additional subjects (2 subjects each in Cohorts 1a and 1b and 4 subjects in Cohort 2) may be enrolled into the study if 2 or more subjects discontinue within 3 months for reasons other than drug-related AEs, and after consultation with the Safety Review Committee (SRC).

3.3 Randomization and Blinding

Eligible subjects who meet the inclusion and exclusion criteria will be assigned unique numbers for enrollment and randomization. The randomization list will be generated using SAS PROC PLAN by a statistician not involved in the development of this SAP and analyses.

This is a partially blinded study such that:

- ***Cohort 1a.*** Dosing (a single 400-mg dose of ALXN1210 SC or placebo SC) is double-blind. Subjects in Cohort 1a will be randomly assigned in a 2:1 ratio (4 ALXN1210 SC, 2 placebo SC; N = 6).

  Subjects will be randomized in consecutive subject number order as per the above list. Randomization will occur at the point of study drug dosing.

Within Cohort 1a, the treatment will be allocated with permuted block randomization (blocksize = 3).

- Thirty-six subjects will be randomly assigned, in a 2:1 ratio, to either **Cohort 1b** (N=24) or **Cohort 2** (N=12) (blocksize = 6).
    - ***Cohort 1b.*** Dosing (a single 400-mg dose of ALXN1210 SC or placebo SC) is double-blind. Subjects in Cohort 1b will be randomly assigned in a 5:1 ratio (20 ALXN1210 SC, 4 placebo SC; N = 24). Within Cohort 1b, the treatment will be allocated with permuted block randomization (blocksize = 12).
    - ***Cohort 2***. Dosing (a single 400-mg dose of ALXN1210 IV) is open-label (N = 12); both subjects and onsite medical/nursing staff will know the study drug/dose being administered.

  Subjects will be randomized in consecutive subject number order as per the above list. Randomization will occur at the point of study drug dosing.

For the blinded part of the randomization schedule, (Cohort 1b) only pharmacy staff preparing the SC injections will be aware of treatment allocation. The finished doses will be labelled in a double blind fashion, thus protecting the study blind for all other staff and study subjects but the unblinded members of the pharmacy team.

Details of the subject enrollment and assignment of randomization can be found in the Study Operations Manual, Section 6.

4. STATISTICAL ANALYSES

In general, descriptive statistics for continuous variables will include number of observations, mean, standard deviation (SD), median, minimum, and maximum, by treatment group.

Descriptive statistics for PK parameters will include number of observations, arithmetic mean, SD, arithmetic coefficient of variation (CV), geometric mean, geometric CV, median, minimum and maximum, by treatment group.

Descriptive statistics for PD parameters will include number of observations, arithmetic mean, SD, median, minimum, and maximum, by treatment group.

Categorical variables will be summarized using frequency counts and percentages by treatment group.

The analyses will be presented by treatment group. Treatment group refers to ALXN1210 SC-, ALXN1210 IV-, and ALXN1210 SC placebo- treated subjects.

Baseline will be defined as the last value of the assessment recorded prior to first administration of study drug (ALXN1210 IV, ALXN1210 SC, or placebo SC). For post-dose assessments, the first scheduled value will be used for summary analysis if repeated measurements are made at a time point.

Deviations from the planned analyses will be described in the final integrated clinical study report (CSR).

4.1. Analysis Populations

The safety population will consist of all subjects who receive the protocol-defined, single dose of study drug (ALXN1210 IV, ALXN1210 SC, or placebo SC). Subjects in this population will be used for the safety analysis.

The PK population will consist of all subjects from the safety population who received either ALXN1210 SC or ALXN1210 IV and who have sufficient serum concentration data to enable the calculation of PK parameters. The PK population will be used for PK summaries.

The PD population will consist of all subjects from the safety population who have sufficient total and free C5 concentration data and cRBC hemolysis data. The PD population will be used for PD summaries.

The immunogenicity analysis population will consist of all subjects from the safety population who have a pre-dose and post-dose ADA sample collected.

Inclusion and exclusion from each analysis set will be decided at the Blind Data Review Meeting prior to database lock.

4.2. Sample Size and Power

A total evaluable sample size of 36 subjects, (24 ALXN1210 SC subjects [receiving active treatment] from Cohort 1 and 12 ALXN1210 IV subjects from Cohort 2), will provide > 80% power to infer that the lower bound of a 90% confidence interval (CI) for the ratio of the bioavailability of ALXN1210 SC to IV is > 0.4, assuming an absolute bioavailability of 0.6 and a coefficient of variation (CV) of 0.35. Additionally, 6 subjects will receive placebo treatment via the SC route, including 2 in Cohort 1a and 4 in Cohort 1b. Randomization within Cohort 1a (6 subjects) will be conducted in a 2:1 ratio. After Cohort 1a, 36 subjects will be randomized in a 2:1 ratio to Cohort 1b (ALXN1210 SC or placebo SC) or Cohort 2 (ALXN1210 IV), and within Cohort 1b, subjects will be randomized in a 5:1 ratio, to receive either ALXN1210 SC or ALXN1210 SC placebo. This brings the total planned number of subjects to N = 42.

4.3. Demographics, Baseline Characteristics, and Subject Disposition

All subjects will be included in the summary of subject disposition. This will present the overall number of subjects screened and by treatment group and overall the frequency and percentage of subjects randomized and treated, and who completed or discontinued from the study, along with reason for discontinuation. Furthermore, the number and percentage of subjects in each study population will be tabulated. Non-eligible and discontinued subjects will be listed. Subject assignment to study populations will be listed. Demographics and baseline characteristics (including height, body weight and body mass index) will be listed and summarized by each treatment group and overall for the safety population. If the remaining populations are different from the safety population, separate demographic tables will be produced.

4.4. Safety Analysis

Safety analyses will be performed on the safety population, and will be reported by each treatment group and overall. Safety analyses will include an analysis of all

AEs, ECGs, clinical laboratory data, vital sign measurements, and physical examination results and will be presented using descriptive statistics. No inferential statistical analyses are planned on the safety parameters of this study.

Adverse Events

The incidence of treatment-emergent (after dosing) AEs (TEAEs) will be summarized using the safety population. The Medical Dictionary for Regulatory Activities (MedDRA) dictionary Version 19.0 (or higher) will be used to classify all AEs reported during the study by System Organ Class (SOC) and Preferred Term. A summary of TEAEs including the incidence of subjects who experienced TEAEs (number and percentage of subjects) and incidence of TEAEs (number of events) will be presented for each treatment group and overall, by severity and by relationship to study drug. TEAEs and serious TEAEs will be summarized by SOC and Preferred Term for each treatment group and overall, by severity, and by relationship to study drug. Treatment group will be categorized by the treatment that was actually received at the date of AE onset (i.e., ALXN1210 SC, ALXN1210 IV or ALXN1210 placebo SC). Subjects having multiple AEs within a category (e.g., overall, SOC and Preferred Term) will be counted once in that category. For severity tables, a subject's most severe event within a category will be counted. For relationship tables, a subject's event with greatest relationship to study drug within a category will be counted. In each table, SOC and Preferred Term will be presented in descending order of overall incidence rate (alphabetical order will be used in case of equal rates). All TEAEs, serious TEAEs, TEAEs resulting in withdrawal from the study, TEAEs occuring during study drug administration and AEs that occurred before first dose administration will be presented in by-subject listings.

Medical History

Medical history data will be coded using MedDRA dictionary Version 19.0 (or higher) and listed individually.

Vaccination Status

A listing of MCV4 and Serogroup B vaccination status and dates will be presented individually.

Study Drug Administration

Study drug administration data will be listed individually.

Vital Signs

Vital signs data (systolic and diastolic blood pressure, heart rate, temperature, and respiration rate) will be listed for individual subjects. In addition, height and body weight will be listed for individual subjects. Summary statistics of absolute (observed) values and changes from Baseline will be calculated for each parameter by each treatment group.

Laboratory Data

Clinical laboratory parameters (blood chemistry, hematology, coagulation, urine chemistry, urinalysis and other laboratory results) will be listed and abnormal parameters will be flagged as high (H) or low (L) according to reference ranges. Absolute (observed) values and changes from Baseline (continuous variables) will

be summarized by each treatment group based on scheduled values. The first scheduled lab value will be used for summary analysis if repeated measurements are made at any time point.

For summary statistics, a lab value with "<" will be replaced with a numeric value by removing the "<" sign. In the listings, the values will be displayed as originally reported by the laboratory.

Laboratory parameter values will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE). Parameters that are not present in this document will be omitted. Shift tables by treatment group will be produced for these laboratory parameters by grade. These tables will summarize the number of subjects with each baseline grade relative to the normal ranges and changes to the worst highest grade assessed post-dose during the study.

Electrocardiograms

Electrocardiogram parameters will be measured at the specified time points and will include heart rate, PR, RR, QRS, QT, and corrected QT interval corrected using Fridericia's formula (QTcF)(QTcF=QT/RR^1/3) intervals and will be listed individually. The average of the ECG readings at the time points collected will be calculated, and absolute (observed) values and changes from Baseline values will be summarized by each treatment group.

An outlier analysis will be performed that will summarize the frequency and percentage of subjects who meet any of the following outlier criteria at each visit by treatment group:

- QT, QTcF interval > 450 msec
- QT, QTcF interval > 480 msec
- QT, QTcF interval > 500 msec
- QT, QTcF interval increases from baseline > 30 msec
- QT, QTcF interval increases from baseline > 60 msec

Prior and Concomitant Medications

All prior and concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary version June 1, 2016 and will be listed individually. The frequency and percentage of prior and concomitant medications will be summarized by Anatomical Therapeutic Chemical (ATC) and Preferred Name. Separate tables will be given for prior and concomitant medications. Prior medications are defined as those for which the end date is prior to the date of first study drug administration. Concomitant medications are defined as those with start date on or after the date of first study drug administration and those with start date prior to the first study drug administration but with end date on or after the date of first study drug administration. Non-pharmacologic therapies and procedures and prophylactic antibiotic treatment will be listed.

Physical Examination

Physical examination data will be listed.

Infusion/Injection Site Evaluation

Data from the injection/infusion site evaluations will be listed individually.

Visual Assessment Scale for Pain at Infusion/Injection Site

Data from the pain at infusion/injection site will be listed individually.

4.5 Pharmacokinetic Analysis

All serum concentration data will be listed for each individual subject and summarized at each time point by treatment group for ALXN1210-treated subjects.

If a Below the Level of Quantitation (BLQ) value occurs in a profile before the first measurable concentration, it will be assigned a value of zero concentration. If a BLQ value occurs after a measurable concentration in a profile and is followed by a value above the Lower Level of Quantitation (LLQ), then the BLQ will be omitted following visual inspection of the serum concentration versus time profile to assess the appropriateness of this assignment. If a BLQ value occurs at the end of a collection profile (after the last quantifiable concentration), the value will be treated as missing data. If 2 BLQ values occur in succession, the profile will be deemed to have terminated at the first BLQ value and any subsequent concentrations will be omitted from PK calculations.

Samples with no reportable value due to a bioanalytical issue or missing samples will be set to missing, and will not be included in the PK calculations.

When calculating the mean or median value for a concentration at a given time point, all BLQ values will be set to zero except when an individual BLQ falls between 2 quantifiable values, in which case it will be omitted.

For tabulation, graphical representation, and calculation purposes, all samples with no reportable value (or missing samples) observed after dosing will be set to missing.

Individual and mean concentrations versus nominal time on linear and semi-log scales will be presented graphically.

Individual serum concentration data for ALXN1210-treated subjects, with actual sampling dates and times calculated relative to the start of the infusion/injection, will be used to derive the PK parameters by non-compartmental analyses using Phoenix WinNonlin Version 6.3 or higher.

The following PK parameters will be derived:

Maximum observed serum concentration (C_{max})

- Time to maximum observed serum concentration (T_{max})
- Area under the serum concentration versus time curve from time zero to the last quantifiable concentration (AUC_t)
- Area under the curve from time zero to infinity (AUC_∞)
- Terminal elimination rate constant (λ_z)
    - Only those data points that are judged to describe the terminal log-linear decline will be used in the regression.

- A minimum number of 3 data points in the terminal phase will be used in calculating λ_z, with the line of regression starting at any post-C_{max} data point (C_{max} should not be part of the regression slope)

- Terminal elimination half-life ($t_{½}$)
- Total clearance (CL or CL/F), calculated as dose/AUC_∞
- Apparent volume of distribution during terminal phase (V_d or V_d/F), calculated as dose/(λ_z*AUC_∞)

All pharmacokinetic parameters will be listed for each individual subject and will be summarized by treatment group. In addition, 95% CI of the mean and geometric mean parameters will be calculated.

Some PK parameters may not be calculated for all or some subjects if the concentration data are not deemed to be amenable to evaluation. Explanations for PK parameters that could not be estimated will be provided in the clinical study report (CSR).

Additional PK parameters may be calculated, as appropriate.

4.5.1 Absolute Bioavailability of ALXN1210 SC

The geometric means ratio (ALXN1210 SC/ALXN1210 IV) and its 90% and 95% CI will be computed for the PK-parameters C_{max}, AUC_t, and AUC_∞, and will be tabulated. To calculate CIs of geometric means ratios, parameters will be log-transformed and CIs will be constructed for the mean difference of log-transformed parameters, assuming that log-transformed parameters are normally distributed and have unequal variances. The CIs are then back-transformed to receive CIs for the geometric mean ratios.

4.6. Pharmacodynamic Analyses

The PD effects of ALXN1210 administered SC/IV will be evaluated as follows:

- Changes and percent changes in serum total and free C5 concentration over time.
- Change and percent change in cRBC hemolysis over time.

Pharmacodynamic data will be listed for each individual subject and absolute (observed values) as well as changes and percent changes from Baseline will be summarized at each time point by treatment group.

Individual and mean percent change from baseline versus nominal time profiles will be presented graphically for each PD parameter.

4.7. Pharmacokinetic/ Pharmacodynamic Analysis

An exploratory analysis may be performed to examine exposure-response relationships between ALXN1210 serum concentrations and the PD endpoints total C5, free C5, and/or cRBC hemolysis. If completed, the analysis will be the responsibility of Alexion and results will be described in the CSR and will be the responsibility of Alexion.

4.8. Immunogenicity Analyses

Immunogenicity, as measured by ADA, will be summarized in tabular form (number and percentage of subjects developing ADA) by treatment group. Titer result will be presented in frequency tables, descriptive statistics and by-subject listings.

4.9 Methods for Withdrawals, Missing Data, and Outliers

The individual serum concentration data and the actual timing of study drug administration and blood sampling will be used throughout the analyses. If there is any doubt about the actual time at which a sample was taken, then the scheduled time will be used. For PK data analysis, please see Section 4.5 regarding the handling of missing and BLQ values. For PD data analysis, there will be no imputation for missing values.

If medication dates are incomplete and it is not clear whether the medication was concomitant, it will be assumed to be concomitant.

AEs with unknown start date/time will be assumed to be treatment-emergent unless the end date/time is known to be before the first administration of study drug. Otherwise missing or partial dates will be listed as such.

There will be no further imputation of missing data (i.e., subjects who prematurely discontinue from the study will not be included in summary statistics or analyses beyond the time of discontinuation).

Depending on the extent of missing values, the appropriateness of the methods described for handling missing data may be reassessed prior to database lock (to examine the sensitivity of results to handling of missing data).

4.10 Protocol deviations

A protocol deviation is defined as a lack of compliance with the protocol which may interfere with the subject's safety or with the outcome of the trial. The protocol deviations will be identified and reviewed throughout the study, reviewed by the principal investigator and sponsor and graded as minor or major. The final review will be performed at the Blind Data Review Meeting prior to database lock. The protocol deviations will be listed.

5. CONVENTIONS

All listings will be ordered by treatment and subject number and will include all enrolled subjects. The listings will be presented by treatment group ALXN1210 SC, ALXN1210 IV and ALXN1210 placebo SC placebo.

For all tables, except PK parameter tables, descriptive statistics for minimum and maximum will be presented with the same decimal digits as the original data, and with 1 more decimal place than the original data for mean and median; SD will be reported with 2 or more decimal than the original data.

For PK data, the individual and summaries of PK parameter results will be reported in 3 significant digits in the listings and in the tables with the following exceptions:

- Tmax will be presented as follows: individual minimum, and maximum values will be reported exactly as the raw data are reported

- % CV will be presented to 1 decimal place.

Page layout of tables and listings will be in landscape mode and will be produced in Microsoft Word and as bookmarked PDF. Details of page layout will be provided in the tables, figures and listings (TFL) shell document.