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Biostatistics & Statistical Programming /
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LNA043

CLNA043X2201

A randomized, placebo-controlled, patient and investigator blinded, single dose, Proof of Concept study investigating the safety, tolerability and preliminary efficacy of intra-articular LNA043 in regenerating the articular cartilage of the knee at donor sites in patients undergoing autologous chondrocyte implantation

Statistical Analysis Plan (SAP)

Personal Protected Data

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Document History – Changes compared to previous final version of SAP

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
22-Jun-17	Prior to DB lock	Creation of initial version	Finalized Document	
18-Dec-17	Prior to DB lock	Protocol amendment	Increased the number of days between randomization and treatment (updated Figure 1-1) and add a clarification for the 7T MRI as an exploratory medical device	Section 1.4
09-Jan-18	Prior to DB lock	Reviewed SAP	**Corporate Confidential Information**	
			Specified that descriptive statistics will be done for raw, percentage change from baseline and change from baseline for each primary variable	Section 6.1.2
23-Feb-18	Prior to DB lock	Specification of parameters	**Corporate Confidential Information**	
		Add a graphical method for correlation	Investigation of gagCEST and Sodium MRI relathionship through scatterplot.	Section 6.1.2
		Add a supportive analysis model	**Corporate Confidential Information**	
		Add formula for the calculation of parameter	We specify that data for the statistical analysis of the %Refilling, will be available only for donor and defect regions. Formula for the calculation of the %Refilling has been also added. The formula is given in percentage change from baseline and so redundant wording for the statistical model was removed.	Section 6.2.3
8-Mar-18	Prior to DB lock	Change on the descriptive and statistical analyses	**Corporate Confidential Information**	

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
4-Jun-18	Prior to DB lock	Protocol amendment	**Corporate Confidential Information**	
11-Oct-18	Prior to DB lock	Discussion with the team on the timepoints	Removed Defect region from the %refilling endpoint. Refer to donor as 'Surgically created region' (SCD), defect 'Defect to be treated' (DTBT), healthy non-weight bearing (HNWB) and healthy weight bearing (HWB)	Section 6.2.3
15-Apr-2019	Prior to DB lock	Based on IA findings	**Corporate Confidential Information**	
		Early termination	LNA043 40mg is removed from the SAP since there will be no subjects receiving 40mg due to early termination of the study	Section 2, 6.1.2, 6.2.2, 6.2.3, 7.2
		Based on IA findings	**Corporate Confidential Information**	
12-Aug-2019	Prior to DB lock	Protocol Deviations and clarifications on secondary analysis	Filled Table 4-1, protocol deviation codes and analysis sets	Section 4
			Clarify the baseline assessments and statistical analysis	Section 6.2.1, 6.2.3
18-Feb-2020	After DB lock	ICRS scoring	ICRS scoring analyses updated since the team would like to distinguish analyses between different biopsy types	Section 6.2.1, 6.2.3

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1 Introduction

1.1 Scope of document

The RAP documents contain detailed information to aid the production of Statistics & Programming input into the Clinical Study Report (CSR) for trial ***"CLNA043X2201".***

The Statistical analysis plan (SAP) describes the implementation of the statistical analysis planned in the protocol.

1.2 Study reference documentation

Study protocol (v03) is available at the time of finalization of Statistical Analysis Plan.

1.3 Study objectives

1.3.1. Primary objective(s)

Primary objective(s)	***Endpoints related to primary objectives***
• To assess the efficacy of a single LNA043 i.a. injection in regenerating hyaline cartilage tissue at the Surgically Created Donor (SCD) regions of patients undergoing autologous chondrocyte implantation (ACI)	• Cartilage glycosaminoglycan (GAG) content and bi-layer collagen organization based on 7T MRI at Week 4
• To assess safety and tolerability of a single LNA043 i.a. injection in patients undergoing ACI	• Adverse Events • ECGs • Vital signs • Hematology, blood chemistry, urinalysis • Physical examination

1.3.2. Secondary objective(s)

Secondary objective(s)	***Endpoints related to secondary objective(s)***
• To assess extent and quality of the repair tissue at the SCD region before surgery	• International Cartilage Repair Society (ICRS) II histology scoring system at Week 4 • Percentage (%) of SCD region filling based on 7T MRI at Week 4

• To assess over a longer term the extent and quality of filling of the SCD region	• Percentage (%) of SCD region filling based on 7T MRI at Week 12 and 28 • Cartilage GAG content and bi-layer collagen organization estimated using 7T MRI at Week 12 and 28
• To evaluate local and systemic pharmacokinetics (PK) of LNA043 following a single i.a. administration	• Serum PK profile of LNA043 and AngPTL3, Cmax and AUC • Concentration of LNA043 and AngPTL3 in synovial fluid at Week 4
• To assess the potential immunogenicity of LNA043	• Presence and characterisation of anti-LNA043 antibodies in serum at Day 1 (predose), Day 8, 29, 85, 197

1.4 Study design and treatment

This is a non-confirmatory, patient and investigator blinded, randomized, placebo-controlled, parallel group, 2 cohorts, single dose study in patients with cartilage lesions undergoing autologous cartilage implantation (ACI) with the use of an exploratory 7T MRI for efficacy assessment. Approximately 22 patients will be enrolled in the study to allow 18 completers, divided as follows: 12 completers in Cohort 1 and 6 completers in Cohort 2.

Participants will be treated only on one occasion (Day 1) with a single i.a. injection that will be performed under arthroscopic visualization. The two cohorts differ only by the dose administered.

Patients will be assigned to one of the following Cohorts. Each cohort has “two (2)” treatment arms in a ratio of “2:1, (LNA043: placebo)”.

Cohort 1, study treatments are defined as:

- A: single dose of 20 mg LNA043
- B: single dose of matching placebo to 20 mg LNA043

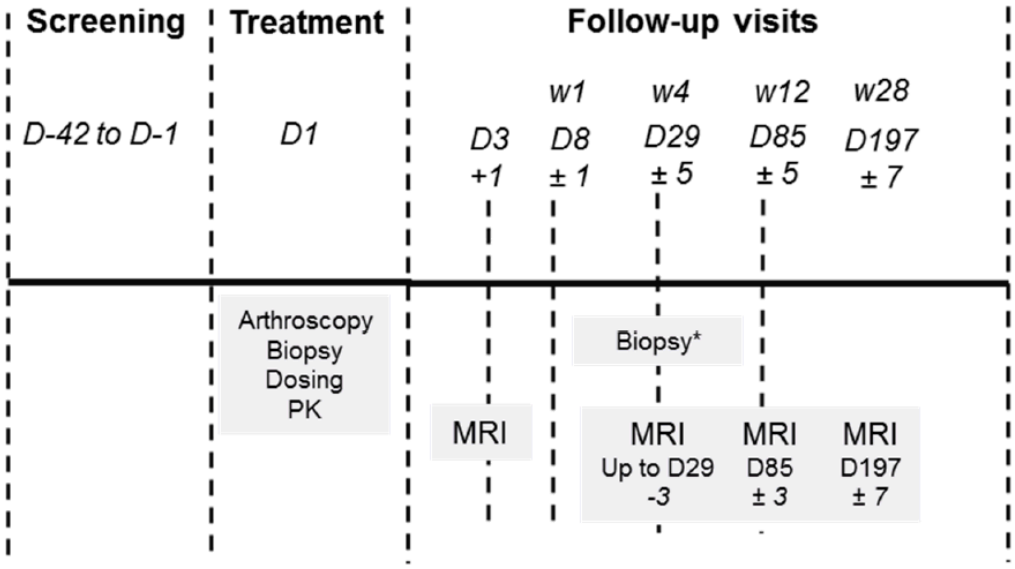
Cohort 2, study treatments are defined as:

- C: single dose of 40 mg LNA043
- D: single dose of matching placebo to 40 mg LNA043

However, due to the early termination of the study, LNA043 40mg will not be dosed.

Figure 1-1 depicts the steps that will be followed in the study for both cohorts, starting from a screening epoch, a Day 1 treatment epoch in which an arthroscopy for cartilage harvest will be done as part of the normal ACI procedure, and the patient will be dosed. Post-treatment follow-up epoch will be at Day 2, Day 3, Week 1, Week 4 (i.e., time of ACI graft implantation), Week 12 and Week 28 for the End of Study (EoS) assessments.

Figure 1-1: Study Design for Cohort 1 and Cohort 2



4 Statistical methods: Analysis sets

For all analysis sets, patients will be analyzed according to the study treatment(s) received.

The safety analysis set will include all patients who received any study drug.

The PK analysis set will include all subjects with at least one available valid (i.e. not flagged for exclusion) PK concentration measurement, who received any study drug and with no protocol deviations that impact on PK data.

The PD analysis set will include all subjects with any available PD data, who received any study drug and experienced no protocol deviations with relevant impact on PD data.

For subjects for which the actual treatment received does not match the randomized treatment, the treatment actually received will be used for the analysis. The analysis sets and protocol deviation codes are related as follows:

Table 4-1 Protocol deviation codes and analysis sets

Category Deviation code	Text description of deviation	Data exclusion
Subjects are excluded from PK analysis in case of these PDs:		Exclude subject from PK analysis set
Subjects are excluded from PD analysis in case of these PDs:		Exclude subject from PD analysis set
OTH03	*Critical assessment not carried out as expected per protocol*	Yes
Subjects are excluded from PK and PD analysis in case of these PDs:		Exclude subject from PK and PD analysis sets

If updates to this table are needed, an amendment to the SAP needs to be implemented prior to DBL.

5 Statistical methods for Pharmacokinetic (PK) parameters

All subjects within the PK analysis set will be included in the PK data analysis.

5.1 Variables

The following pharmacokinetic parameters will be determined using the actual recorded sampling times and non-compartmental method(s) with Phoenix WinNonlin (Version 6.2 or higher): Cmax, Tmax, AUClast from the serum concentration-time data.

5.2 Descriptive analyses

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Descriptive summary statistics will be provided by treatment and visit/sampling time point. including the frequency (n, %) of concentrations below the LLOQ and reported as zero. Pharmacokinetics parameters and will be listed by treatment and subject.

Summary statistics will include mean (arithmetic and geometric), SD, CV (arithmetic and geometric), median, minimum and maximum. An exception to this is Tmax where median, minimum and maximum will be presented. Concentrations below LLOQ will be treated as zero

in summary statistics and for PK parameter calculations. A geometric mean will not be reported if the dataset includes zero values.

6 Statistical methods for Pharmacodynamic (PD) parameters

All subjects within the PD analysis set will be included in the PD data analysis.

6.1 Primary objective

The primary aim of this study is to determine the change from baseline cartilage GAG content and change from baseline in bi-layer collagen organization based on 7T high-field MRI at week 4.

6.1.1 Variables

The primary variables are:

- GAG content (gagCEST and Sodium) by MRI: measurements will be available at baseline and Week 4 from both donor region and a nearby "healthy" cartilage region (as a reference tissue). Specifically, the ratio of normalized GAG content in the SCD to healthy non-weight bearing region (HNWB), i.e. SCD/HNWB and the DTBT to healthy weight bearing region (HWB), i.e. DTBT/HWB.

  The SCD/HNWB ratio is of primary interest.

  The quantity of proteoglycans in the knee articular cartilage is subject to variation depending on: i) the region (weight-bearing areas have larger proteoglycan quantity; ii) the age (proteoglycans are progressively lost over time); the time of the day (in the evening they are usually lower). In order to account for this intrinsic variability, in addition to the typical measures taken in MRI studies (e.g., patients have to lie down for 30’ before being scanned, age range is narrow), proteoglycan content in the index region is normalized to a reference healthy region, which represents the theoretical maximum proteoglycan content in that particular knee.

- The bi-layer collagen organization: The change from baseline of the difference in T2 relaxation time between the superficial layer and deep layer of cartilage will be determined similarly to the GAG content assessment (i.e., same cartilage regions and time-points), for each region (SCD, DTBT, HNWB, HWB) and layer (superficial and deep layer), as well as for the T2 ratio (superficial/deep).

  The SCD region is of primary interest.

Baseline is defined at the Day 3 assessment.

6.1.2 Descriptive analyses

The cartilage GAG content and bi-layer collagen organization will be listed by treatment group, region (DTBT ,SCD, HNWB and HWB), and the ratios (SCD/HNWB, DTBT/HWB) patient and visit/time, and descriptive statistics will be provided, for raw and change from baseline, by region, treatment group and visit/time.

Summary statistics will be provided for each region and their difference, and the ratios by treatment group (LNA043 20 mg vs. placebo) and visit/time. Summary statistics will include mean (arithmetic), SD, CV, median, minimum, maximum.

Graphical methods will be employed to show group and individual summary plots over time by treatment.

Graphical methods such as scatterplots may also be used to explore the relationship between the gagCEST and Sodium.

6.1.3.1 Handling of missing values/censoring/discontinuations

The primary analysis model described above is valid under the assumption of data missing at random.

6.2 Secondary objectives

6.2.1 Variables

The secondary variables are the:

1. SCD region filling (absolute volume and % refilling) at Weeks 4, 12 and 28
2. ICRS scoring at Week 4

For SCD region filling the baseline is defined as the Day 3 assessment; for biopsy type "curette scraping" the baseline for ICRS scoring is defined as the Day 1 assessment.

6.2.2 Descriptive analyses

The variables will be listed by treatment group, patient and visit/time. Summary statistics will be provided by treatment group (LNA043 20 mg, vs.placebo) and visit/time. Summary statistics will include mean (arithmetic), SD, CV, median, minimum, maximum.

Graphical methods will be employed to show group and individual summary plots over time by treatment. Other secondary efficacy variables will be summarized graphically and in summary tables.

6.2.3 Statistical model, assumptions and hypotheses

SCD region filling

The change from baseline in SCD volume (mm3) of cartilage defect will be analyzed using similar MMRM analysis of variance

A single

MMRM model will be used, since there are no layers for this secondary endpoint.

% Refilling for SCD region is derived from the following formula:
$\%\text{Refilling}_{\text{SCD region}} = [(\text{SCD volume}_{\text{baseline}} - \text{SCD volume}_{\text{week4}})/ \text{SCD volume}_{\text{baseline}}]*100$

ICRS scoring at Week 4

ICRS scoring will be analyzed using an ANOVA model, by biopsy type, for treatment comparison, with scoring at Week 4 the dependent variable and treatment as fixed effect. In addition, the ICRS scoring for "curette scraping" biopsy type will be analyzed using an ANCOVA change from baseline model with treatment as fixed effect and baseline as covariate.

For both statistical analyses, model estimated means and their differences of each LNA043 dose vs. placebo, will be obtained along with the 95% CI for the difference.

7 Statistical methods for safety and tolerability data

All subjects within the safety analysis set will be included in the safety data analysis.

7.1 Variables

Adverse events, vital signs (blood pressure, heart rate, body temperature), ECG intervals, clinical laboratory measurements, immunogenicity, hematology, blood chemistry as well as subject demographics, baseline characteristics, and treatment information.

7.2 Descriptive analyses

Subject demographics and other baseline characteristics

All data for background and demographic variables will be listed by treatment group and subject. Summary statistics will be provided by treatment group (LNA043 20 mg vs. placebo).

Relevant medical history, current medical conditions, results of laboratory screens, drug tests and any other relevant information will be listed by treatment group and subject.

Treatment

Data for study drug administration (rescue medication) and concomitant therapies will be listed by treatment group and subject.

Vital signs

All vital signs data will be listed by treatment group, subject, and visit/time and if ranges are available abnormalities (and relevant orthostatic changes) will be flagged. Summary statistics will be provided by treatment (LNA043 20 mg vs. placebo) and visit/time.

ECG evaluations

All ECG data will be listed by treatment group, subject and visit/time, abnormalities will be flagged. Summary statistics will be provided by treatment (LNA043 20 mg vs. placebo) and visit/time.

Clinical laboratory evaluations

All laboratory data will be listed by treatment group, subject, and visit/time and if normal ranges are available abnormalities will be flagged. A separate listing is provided presenting all parameters in a subject with any abnormal values. Summary statistics will be provided by treatment (LNA043 20 mg vs. placebo) and visit/time.

Adverse events

All information obtained on adverse events will be displayed by treatment group (LNA043 20 mg vs. placebo) and subject.

The number and percentage of subjects with adverse events will be tabulated by body system and preferred term with a breakdown by treatment. A subject with multiple adverse events within a body system is only counted once towards the total of this body system and treatment.

Immunogenicity

All immunogenicity results will be listed by subject and visit/time.