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Statistical Analysis Plan Cingal 20-01, Cingal 20-02, and Cingal 20-03

A Prospective Study of a Single Injection Cross-linked Sodium Hyaluronate with Triamcinolone Hexacetonide (CINGAL®) to Provide Symptomatic Relief of Osteoarthritis of Hip Joint (Cingal 20-01), Shoulder (Cingal 20-02), and Ankle (Cingal 20-03)

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1.0 Introduction

This document presents the statistical analysis plan (SAP) for the Anika Therapeutics, Inc. protocols CINGAL 20-01 (Hip), CINGAL 20-02 (Shoulder), and CINGAL 20-03 (Ankle). This SAP incorporates the data listings, summary tables, and statistical analyses. This document is based on the original final protocols and case report forms (CRFs) for each study.

The three studies are the same except for the anatomical area of interest and so all of the analyses will be consolidated into a single SAP.

2.0 Study Design

This is a prospective, expanded indications multi-center, open-label study to evaluate safety and performance of injection of Cingal for relief of pain in patients with a diagnosis of an osteoarthritic hip joint (CINGAL 20-01), shoulder joint (CINGAL 20-02), and ankle joint (CINGAL 20-03).

The subjects in each of these studies will be patients with a diagnosis of osteoarthritic (OA) joint who the investigator determines are appropriate candidates for treatment with an injection of CINGAL.

In each study, up to 25 subjects will be enrolled and treated at up to 10 investigational sites in the EU. Subject participation will last approximately 6 Months, with visits scheduled at Screening, Baseline, 1 month, 3 month and 6 months.

3.0 Objectives

The goal of these studies is to demonstrate the clinical improvement and safety in patients treated with CINGAL for hip osteoarthritis, shoulder osteoarthritis, and ankle osteoarthritis. Specifically, this study will provide confirmation to the effectiveness and safety of CINGAL at relieving the specified joint pain to 6 months post-treatment.

PRIMARY ENDPOINT:

- Reduction of index joint Numerical Rating Scale (NRS) pain on walking from baseline to 6 Months post injection (CINGAL 20-01), joint pain from baseline to 6 Months post injection (CINGAL 20-02), and pain on walking from baseline to 6 Months post injection (CINGAL 20-01).

SECONDARY ENDPOINTS:

- Improvement in Lequesne Hip index joint from baseline to 6 months post injection. (CINGAL 20-01 only)
- Improvement in DASH score for index joint from baseline to 6 months post injection (CINGAL 20-02 only)

- Improvement in AOFAS index joint from baseline to 6 months post injection (CINGAL 20-03 only).
- Improvement in Patient Global Assessment (PGA) from baseline to 6 months post injection.
- OMERACT-OARSI responder rate in the index joint at 6 months post injection.
- Time to treatment failure
- Reduction in Medication usage from baseline to 6 months post injection.

EXPLORATORY ENDPOINTS

Any comparisons across timepoints (baseline to 6 months) not described in the primary or secondary endpoints, including but not limited to:

- Demographics
- Medical History
- History of joint osteoarthritis
- Rescue Medication Use
- Treatment Failure
- Injection procedure
- Concomitant medications
- Non-drug therapy

SAFETY ENDPOINT:

The incidence, severity, and relationship to treatment of all Adverse Events (AE) will be collected from the treatment injection to the 6 month assessment.

4.0 Statistical Methods in Protocol

4.1 STATISTICAL METHODS

This Statistical Analysis Plan provides the details of the statistical analysis of the study data that is described in the protocol.

4.1.1Sample Size

The primary analysis will be the mean change in index joint pain from baseline to 6 months as measured by the Numerical Rating Scale.

The hypothesis to be tested is:

H_0: $\mu_{DMON} = 0$ versus H_A: $\mu_{DMON} > 0$.

In this hypothesis, μ_{DMON} is the mean change in pain on walking from baseline at 6 Months in CINGAL treated patients.

The data will be analyzed via a one sample t-test.

It is assumed that a mean change in pain from baseline of 2 points represents a clinically significant improvement with a standard deviation of 2 - 2.5 points, for 80% power and alpha of 5% then a total of 15 subjects are required. To ensure sufficient subjects are available at the 6 month follow up, it is proposed to enroll and treat at least 25 subjects which should be more than adequate to demonstrate that treatment of the study population with CINGAL would reduce joint pain at 6 months.

Single sites should not enroll more than 75% of the total enrollment.

4.1.2 Primary Efficacy Endpoint

4.1.2.1 The mean change in index joint Pain from baseline to 6 Months as measured with NRS comparing the CINGAL treated group to baseline measurements .

The primary analysis will be the mean change in index joint pain from baseline to 6 months as measured by the Numerical Rating Scale.

The hypothesis to be tested is:

H_0: $\mu_{DMON} = 0$ versus H_A: $\mu_{DMON} > 0$.

In this hypothesis, μ_{DMON} is the mean change in pain on walking from baseline at 6 Months in CINGAL treated patients.

The data will be analyzed via a one sample t-test.

This will be analyzed using SAS PROC UNIVARIATE. ODS statements will be used to output the descriptive statistics and p-values into data sets that can be easily displayed using PROC REPORT.

The tables for this analysis will be displayed as follows showing all time points for reference:

This analysis will be performed for each of the protocols

Summary of Differences in Mean Responses over time -

Time Perios	**Descriptive Statistic**	**Baseline Result**	**Visit Result**	**Change from Baseline**
1 Week	N			
	Mean			
	Std.Drv			
	Median			
	(Min,Max)			
	p-value diff=0			
1 Month	N			
	Mean			
	Std.Drv			
	Median			
	(Min,Max)			
	p-value diff=0			
. . .				
6 Months	N			
	Mean			
	Std.Drv			
	Median			
	(Min,Max)			
	p-value diff=0			

4.1.3 Secondary Efficacy Endpoints

4.1.3.1 **Improvement in Lequesne Hip index joint from baseline to 6 months post injection. (CINGAL 20-01 only)**

Since this is a secondary endpoint the data will be analyzed for each time point individually with no adjustments for multiplicity Data will be analyzed via a one sample t-test on the difference between the month elbow pain score and the baseline elbow pain score. Formally, the hypothesis to be tested is:

H_0: $\mu_{DMON} = 0$ versus H_A: $\mu_{DMON} > 0$.

In this hypothesis, μ_{DMON} is the mean change from baseline at a time point for CINGAL treated patients.
This will be analyzed using SAS PROC UNIVARIATE. ODS statements will be used to output the descriptive statistics and p-values into data sets that can be easily displayed using PROC REPORT.

This will be analyzed and tabled like the primary endpoint in section 4.1.2.1.

4.1.3.2 **Improvement in AOFAS index joint from baseline to 6 months post injection (CINGAL 20-03 only)**.

Since this is a secondary endpoint the data will be analyzed for each time point individually with no adjustments for multiplicity Data will be analyzed via a one sample t-test on the difference between the month elbow pain score and the baseline elbow pain score. Formally, the hypothesis to be tested is:

H_0: $\mu_{DMON} = 0$ versus H_A: $\mu_{DMON} > 0$.

In this hypothesis, μ_{DMON} is the mean change from baseline at the time points for CINGAL treated patients.
This will be analyzed using SAS PROC UNIVARIATE. ODS statements will be used to output the descriptive statistics and p-values into data sets that can be easily displayed using PROC REPORT.

This will be analyzed and tabled like the primary endpoint in section 4.1.2.1.

4.1.3.3 **The mean and percent change in Patient Global Assessment from Baseline to 1 Months, 3 Months and 6 Months.**

Since this is a secondary endpoint the data will be analyzed for each time point individually with no adjustments for multiplicity Data will be analyzed via a one sample t-test on the difference

between the month elbow pain score and the baseline elbow pain score. Formally, the hypothesis to be tested is:

H_0: $\mu_{DMON} = 0$ versus H_A: $\mu_{DMON} > 0$.

In this hypothesis, μ_{DMON} is the mean change from baseline the time points for CINGAL treated patients.
This will be analyzed using SAS PROC UNIVARIATE. ODS statements will be used to output the descriptive statistics and p-values into data sets that can be easily displayed using PROC REPORT.

This will be analyzed for each of the protocols and tabled like the primary endpoint in section 4.1.2.1.

4.1.3.4 **Responder index based on OMERACT- OARSI responder index at 1 Month, 3 Months and 6 Months**.

The response rate for each time point will be tabulated and exact 95% confidence intervals will be calculated.

This will be estimated and tested using PROC FREQ with the BINOMIAL option. The results will be displayed similar to:

Summary of the OMERACT-OARSI Responder Rates at each visit and each protocol.

Parameter	**1 Week**	**1 Month**	**3 Months**	**6 Months**
Estimate n/N (%)				
95% Confidence Interval				

4.1.3.5 **Time to Treatment Failure**.

The time to treatment failure will be assessed via the estimation of the Kaplan-Meier curve. This will be done via the PROC LIFEREG procedure in SAS. The estimate of the median survival time and the 95% confidence intervals will be reported. This will be done for each protocol.

For all studies, a patient is considered a treatment failure if any of the following occur:

Corticosteroid injections
Shock-wave therapy
Dry Needling
Nitroglycerin patches
Prolotherapy (PrT).
Platelet / PRP / BMAC injection
Hyaluronic Acid.
Surgical treatment – open or arthroscopic

4.1.3.6 **Reduction in Pain Medication usage at 1 Month, 3 Months and 6 Months**.

The reduction from baseline in the mean reduction of medication usage at each time point will be tabulated and 95% confidence intervals will be calculated.

This will be estimated using PROC MEANS. The results will tabled like the primary endpoint in section 4.1.2.1.

4.1.4 Exploratory Endpoints

Any comparisons across timepoints (baseline to 6 months) not described in the primary or secondary endpoints, including but not limited to:

- Demographics
- Medical History
- History of joint osteoarthritis
- Injection procedure
- Concomitant medications
- Non-drug therapy

For the exploratory analyses, the continuous variables will be summarized via descriptive statistics (n, mean, median, standard deviation, minimum, and maximum. The discrete variables will be summarized via counts and percents.

4.2 STATISTICAL METHODS

Tabulation of summary statistics, graphical presentations, and statistical analyses will be performed using SAS® software 9.4 or higher version. Where not otherwise specified, the last pre-treatment observation will be used as baseline for calculating post-treatment changes from baseline. The primary presentations and analyses will be based on data pooled across study centers.

Relevant summaries for individual centers, or combinations of centers, may be presented for primary data. All testing and confidence intervals will use a significance level of 5%.

4.3 DEMOGRAPHIC AND BASELINE CHARACTERISTICS

All demographic and baseline characteristics will be tabulated by treatment group and a test of homogeneity between the treatment groups will be conducted. For continuous variables (e.g. age, height, weight) a one way ANOVA will be used. For categorical variables (e.g. gender, race), a Fisher's exact test or chi-squared test will be used. Medical history findings, physical examinations and concomitant medications will be tabulated by treatment group.
The baseline, Vital Signs, and BMI data will be summarized via descriptive statistics and tested for homogeneity using a one way ANOVA.

4.4 ADVERSE EVENTS

All AEs will be coded according to MedDRA. Safety assessments will include Treatment-Emergent Adverse Events (TEAEs) which are defined as AEs with an Investigator assessment of definitely, probably, or possibly related to CTM. TEAEs will be summarized with frequencies and percentages by system organ class and preferred term, severity, and relationship to study CTM for each treatment group. In summaries of TEAEs by severity and relationship to CTM for subjects reporting multiple episodes, all reported events will be included, not only the worst reported case. Serious Adverse Events will also be presented by relationship to the CTM.
The number of subjects with at least one AE will be tabulated for each treatment group. Differences between the treatment groups will be tested using Fisher's exact test. Then the number of AEs for each treatment group will also be tabulated.

The number of subjects and the number of AEs will be tabulated by severity, relationship, and local injection site specific events versus non local events.

4.5 SUBJECT POPULATIONS

All safety analyses will be conducted on all subjects who undergo treatment in any group.

The Safety Population will be defined as all subjects who undergo at least one Study Treatment, and the safety analysis will be performed on this population.

The primary analysis on the primary endpoint will be performed on the Intent to Treat (ITT) population, defined as all patients who were treated in the study. All Primary and Secondary endpoints will be analyzed using the ITT population.

A secondary analysis will be conducted on the Per Protocol (PP) population. Since the primary endpoint is at 6 Months, this is all subjects who complete the 6 Month visit and who do not have a major deviation from the protocol. For all other assessments, this is defined as the subjects who complete those assessments according to the protocol.

4.6 ADDITIONAL ANALYSES

All of the analyses are performed on the data with the specified covariates or other factors assumed in the model. If it is determined that certain factors may influence the outcomes of the endpoints, then additional analyses will be performed