

STATISTICAL ANALYSIS PLAN

IA/PAAG-SI/OA/2021

Version 2.0 final: October 06, 2021

Survey Data Analysis to Assess the Long-term Efficacy and Safety of Intra-articular HBISA Endoprosthesis of Synovial Fluid (NOLTREX™) in Knee Osteoarthritis

ClinicalTrials.gov Identifier: NCT06523491

Study sponsor
"RC "BIOFORM" LLC
Block G, 5th Floor, Bldg. 2, Property 4, 22nd km,
Kievskoye Shosse (Moskovskiy Settlement)
Moscow, 108811, Russian Federation

«APPROVED»	
Director General «RC «BIOFORM» LLC	_____ Dar'evich E.N.
	«____»_____ 2021

List of abbreviations

Abbreviation	Meaning
BP	Blood pressure
ALT	Alanine aminotransferase
anti-HCV	Total antibodies to Hepatitis C Virus
AST	Aspartate aminotransferase
ATC	Anatomical-therapeutic-chemical classification
APTT	Activated partial thromboplastin time
VAS	Visual-analog scale
HIV	Human immunodeficiency virus
GGTP	Gamma-glutamyl transpeptidase
DEE	Confidence interval
CS	Clinical study
CRO	Contract research organization
MD	Medical device
GCP	Good clinical practice
NSAIDs	Non-steroidal anti-inflammatory drugs
IEC	Independent ethics committee
AE	Adverse event
OA	Osteoarthritis
PT	Prothrombin time
SAE	Serious adverse event
SOP	Standard operating procedure
ESR	Erythrocyte sedimentation rate
CRD	Chronic renal disease
RR	Respiration rate
HR	Heart rate
ALP	Alkaline phosphatase
eCRF	electronic Case Report Form
ACR	American College of Rheumatology
HBs-Ag	hepatitis B surface antigen

ICH	International council for harmonization of technical requirements for pharmaceuticals for human use
JSN	Joint space narrowing
JSW	Joint space width
MedDRA	Medical Dictionary for Regulatory Activities
OEI	Evaluation of the treatment effectiveness by the investigator
OEP	Evaluation of the treatment effectiveness by the patient
WOMAC	Western Ontario and McMaster Universities Osteoarthritis
WOMAC-A	Subscale of pain according to the osteoarthritis index developed by researchers at the Western Ontario and McMaster Universities Osteoarthritis
WOMAC-B	Subscale of stiffness according to the osteoarthritis index developed by researchers at the Western Ontario and McMaster Universities Osteoarthritis
WOMAC-C	Subscale of function according to the osteoarthritis index developed by researchers at the Western Ontario and McMaster Universities Osteoarthritis
WOMAC-T	Change in the total score on the scale of the osteoarthritis index, developed by the researchers at the Western Ontario and McMaster Universities Osteoarthritis

Name of the study

1-year follow-up of a randomized controlled trial IA/PAAG-SI/OA/2019 with open-label extension IA/PAAG-SI/OA/2020 to assess the long-term efficacy and safety of Intra-articular HBISA Endoprostheses of Synovial Fluid NOLTREX™ in patients with symptomatic Kellgren and Lawrence (KL) grade 2 and 3 knee OA.

The duration of the study, the number of research centers and patients

This study was 1-year follow-up involving patients with knee OA who received IA injections of NOLTREX™ in the RCT IA/PAAG-SI/OA/2019 (CS1) and OLE IA/PAAG-SI/OA/2020 (CS2). The follow-up data were collected through telephone interview (using questionnaire) conducted in April-July 2022, 12 months after the completion of OLE IA/PAAG-SI/OA/2020. Analysis included data collected at Visit 1 (week 1) of the parent study IA/PAAG-SI/OA/2019, visit 0 (screening) and Visit 5 (week 23) of OLE.

Number of patients:

In the current study (CS3) including patients who met inclusion criteria and had no exclusion criteria who received MD NOLTREX™ in the CS1 (IA/PAAG-SI/OA/2019) and/or CS2 (IA/ PAAG-SI/OA/2020) study and also participated in CS3. Therefore, a formal sample size calculation was not performed.

The survey was conducted in a population that included 57 patients, divided into 3 subgroups depending on the treatment they received: 30 patients (52.63%) received 2 courses of therapy; 17 patients (29.82%) received 1 course of therapy; 10 patients (17.54%) patients received placebo. The placebo group was very small for statistical assessment for most parameters and was not mainly analyzed in main part of them. That is why it is single arm study in comparation of 1 or 2 course of Noltrex (47 patients).

Purpose of research

The aim of 1-year follow-up is to evaluate the long-term safety and efficacy of a single or repeated course of IA NOLTREX™ in patients with symptomatic Kellgren and Lawrence (KL) grade 2 and 3 knee OA.

Study design and methodology:

The 1-year follow-up study involved patients with knee OA who had received a course of IA injections of NOLTREX™ or placebo in the RCT IA/PAAG-SI/OA/2019 and OLE IA/PAAG-SI/OA/2020. The follow-up data were collected during a telephone interview/questionnaire conducted in April–July 2022 – 12 months after the completion of OLE. Analysis included data collected at Visit 1 (week 1) of the parent study IA/PAAG-SI/OA/2019, visit 0 (screening) and Visit 5 (week 23) of OLE.

The multicenter, double-blind, randomized, comparative, placebo-controlled study of the efficacy and safety of NOLTREX™ with intra-articular administration in the treatment of gonarthrosis IA/PAAG-SI/OA/2019 (CS1) involved 72 patients over 50 years of age with a verified diagnosis of gonarthrosis in accordance with the criteria of the American College of Rheumatology (ACR) II-III radiographic stage according to the Kellgren-Lawrence classification. These patients received 1 to 2 injections (at an interval of 1 week) of MD NOLTREX™ into the most affected (target) knee joint.

The open-label interventional study IA/PAAG-SI/OA/2020 (CS2) included patients who received at least one intra-articular injection of MD NOLTREX™ as part of the CS1 study, underwent procedures at Visit 5 (25 weeks - 6 months after the injection) and signed an informed consent form to participate in the open-label clinical study IA/PAAG-SI/OA/2020 (CS2).

This study was conducted 12 months after the completion of CS2 in order to obtain an expert assessment of the long-term efficacy and safety of MD NOLTREX™ based on data from CS1, CS2 and the results of a telephone interview/questionnaire for patients conducted in April-July 2022. The survey/questionnaire was conducted according to the following scheme:

1. Patient survey - current pain status on a visual analogue scale (VAS) and 3 questions about treatment after completion of the study, which is filled in from the patient's words.
2. Questionnaire for the physician about treatment after completion of the study, which is filled in by collecting the history of the disease and the methods of treatment used after exiting the study(s), from medical records, a medical information storage system (if applicable).
3. The Osteoarthritis Index questionnaire, developed by employees of the University of Western Ontario and McMaster Universities Osteoarthritis (WOMAC) for completion from the patient's words - identical to that previously used MD NOLTREX™ under two study protocols: CS1 and CS2.

Since the WOMAC osteoarthritis index and VAS pain scores are similar to those used in CS1 and CS2, the aim of this study was to jointly analyze the data obtained in CS1, CS2, and current CS3.

Diagnosis and main inclusion criteria:

Current study included patients who had previously participated in the IA/PAAG-SI/OA/2020 and/or IA/PAAG-SI/OA/2019 studies. Patients were included in these studies in accordance with the following criteria:

Inclusion criteria for the IA/PAAG-SI/OA/2019 (CS1) study

1. Men and women over 50 years old;
2. Verified gonarthrosis according to the ACR criteria (knee pain in combination with one of the following signs: age over 50 years, crepitus in the joint or morning stiffness in the joint lasting less than 30 minutes in combination with radiographic signs of gonarthrosis);

3. II-III radiographic stage of gonarthrosis according to the Kellgren-Lawrence classification with predominant damage to the medial tibiofemoral region of the knee joint;
4. Radiographic joint space width (JSW) of the target knee joint of at least 2.5 mm.

Inclusion criteria for the IA/PAAG-SI/OA/2020 (CS2) study

1. Men and women over 50 years of age;
2. Signed informed consent form by the study participant;
3. Verified gonarthrosis according to the ACR criteria (knee pain in combination with one of the following: age over 50 years, crepitus in the joint or morning stiffness in the joint lasting less than 30 minutes in combination with radiographic signs of gonarthrosis);
4. II-III radiographic stage of gonarthrosis according to the Kellgren-Lawrence classification with predominant damage to the medial tibiofemoral region of the knee joint

Completion of participation in the clinical study IA/PAAG-SI/OA/2019 in the NOLTREX™ medical device group with the completion of visit 5 procedures (25 weeks).

Evaluation criteria:

Efficacy:

The integral assessment of efficacy was based on the statistical analysis of the main efficacy parameters.

The efficacy of the study MD was assessed by the following endpoints.

Primary Outcome Measure(s):

1. Change in the total WOMAC score (WOMAC-T) 12 months after completion of CS2 compared with the baseline value at Visit 0 (screening) of the IA/PAAG-SI/OA/2020 study, compared with the baseline value at Visit 1 (week 1) of the IA/PAAG-SI/OA/2019 study and the last value at Visit 5 (week 23) of the IA/PAAG-SI/OA/2020 study;

Secondary Outcome Measure(s):

2. Change in the WOMAC Pain (WOMAC-A) Score 12 months after completion of CS2 compared to the baseline value at Visit 0 (screening) of the CS2 study, compared to the baseline value at Visit 1 (week 1) of the IA/PAAG-SI/OA/2019 study and the last value at Visit 5 (week 23) of the CS2 study;
3. Change in the WOMAC Stiffness (WOMAC-B) score 12 months after completion of CS2, after completion of CS2 compared with the baseline value at Visit 0 (screening) of the CS2 study, compared with the baseline value at Visit 1 (week 1) of the CS1 study and the last value at Visit 5 (week 23) of the IA/PAAG-SI/OA/2020 study;
4. Change in the WOMAC Physical Function (WOMAC-C) Score 12 months after completion of CS2 compared with the baseline value at Visit 0 (screening) of the CS 2 study, compared with the baseline value at Visit 1 (week 1) of the CS1 study and the last value at Visit 5 (week 23) of the CS2 study;

5. Change in the 100-mm VAS Pain Score 12 months after completion of CS2 compared with the baseline value at Visit 0 (screening) of the IA/PAAG-SI/OA/2020 study, compared with the baseline value at Visit 1 (week 1) of the CS1 study and the latest value at Visit 5 (week 23) of the CS2 study;

Safety:

This study did not specifically collect or analyze adverse event data.

The current CS 3 assessed safety using medical absolute and relative contraindications for repeated intra-articular treatment, as well as physician-reported data, including information on pain medication use.

Statistical methods:

Statistical analysis was performed using the R statistical software package (R: A language and environment for statistical computing. R Core Team. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>) version 4.x.x, using package version control adapted and validated by Microsoft (Microsoft R Open. Microsoft and R Core Team. Microsoft, Redmond, Washington. URL <https://mran.microsoft.com/>) using "A Guidance Document for the Use of R in Regulated Clinical Trial Environments" (<https://www.r-project.org/doc/R-FDA.pdf>).

Statistical analysis was performed for data on all patients who participated in the study in accordance with the definitions of the populations for the analysis.

All available patient data were used for the analysis; due to the lack of a statistical hypothesis and the planned statistical analysis of any parameters, only the results of the descriptive analysis are presented.

Demographic, efficacy, and safety data were presented using descriptive statistics:

Continuous (quantitative) data were presented using the following parameters:

- Number of observations (n);
- Arithmetic mean (M);
- Standard deviation (SD);
- 95% confidence interval for the mean (95% CI);
- Minimum value (min);
- Maximum value (max);
- Median (Me);
- Interquartile range (ICR).

For variables presented as qualitative and ordinal indicators, absolute (n) and relative (—) frequencies for each category, as well as 95% CI, were calculated.