STATISTICAL ANALYSIS PLAN

Version 2.0 final: October 06, 2021

Study IA/PAAG-SI/OA/2019

Open-label Multicenter Postmarketing Extension Study of Efficacy and Safety of Intra-articular HBISA Endoprosthesis of Synovial Fluid NOLTREX™ per TU 9398-00152820385-2015 in Knee Osteoarthritis

ClinicalTrials.gov Identifier: NCT06429319

Study sponsor
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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	Osteoarthrosis
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

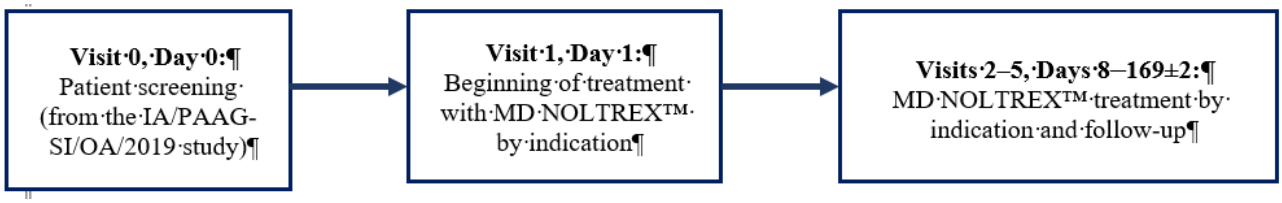
1.1 General provision

1.1.1 Justification of selection of the clinical study type

An open-label intervention study in one group with OA patients who received a single course of injections of the NOLTREX™ medical device as part of the IA/PAAG-SI/OA/2019 study.

The open nature of the study in one group is determined by the main purpose of the study - to assess the safety of MD NOLTREX™ with a single or repeated course of use. It is assumed that continued monitoring of patients in the placebo group will not lead to the identification of new AEs, the frequency of which should be compared with the frequency in the active therapy group, and is connected to unethical restriction of patients in the placebo group from the possibility to use potentially effective synovial fluid endoprostheses.

1.1.1.1 Graphical diagram of the study



1.1.2 Measures aimed to minimize subjectivity

The open nature of the study in one group is determined by the main purpose of the study - to assess the safety of MD NOLTREX™ with a single or repeated course of use. Placebo safety data at the 6-month point (Visit 5) in the IA/PAAG-SI/OA/2019 study will be used as a control. It is assumed that continued monitoring of patients in the placebo group will not lead to the identification of new AEs, the frequency of which should be compared with the frequency in the active therapy group, and is connected to unethical restriction of patients in the placebo group from the possibility to use potentially effective synovial fluid endoprostheses.

At the same time, many of the recorded performance parameters are objective (an assessment of the total number of paracetamol tablets or other NSAIDs taken from the patient's diary) or do not depend on the subjectivity of the investigator's assessment (parameters of evaluation on the scales WOMAC, VAS, OEP). The measurement of the JSW parameter is objective with strict adherence to the measurement methodology.

When evaluating the safety parameters, objective data is also recorded (for subjective assessment of the patient's tolerability of therapy, which does not depend on the subjectivity of the investigator's

assessment). Overall assessment of the treatment tolerability by the research physician will also be based on a comparison of objective data with basal parameters, which also minimizes subjectivity.

2. Basic and additional parameters under study

2.1. Basic safety parameters:

1. The frequency of AE and/or SAE;
2. Overall assessment of the treatment tolerability by the investigator and the patient;
3. The main parameters of vital signs (HR, BP, RR, body temperature)
4. Results of physical examination;
5. Results of laboratory and instrumental examination.

Separately it is planned to evaluate the cumulative (in the framework of a double-blind and open study) frequency of the most likely complications associated with periprocedural or temporary postprocedural (no more than 72 hours) pain or burning. These NSAIDs can be stopped with paracetamol or non-steroidal anti-inflammatory drugs permitted by the Protocol.

Other possible side effects and complications of special interest related to the method of administration by intra-articular injection:

- pain or swelling at the injection site, feeling of bursting, burning, arthralgia, effusion in the joint, synovitis, aseptic acute arthritis;
- infections (pyogenic arthritis, direct infection of the joint at infectious diseases, osteomyelitis, sepsis, etc.);
- subcutaneous neuropathies, drug-induced vascular embolism.

It is assumed that the AEs are divided into groups: somatic AEs and AEs associated with the pathology of the target joint, as well as the frequency of both these groups, as well as each AE according to the following classification:

- Very common ≥10%;
- Common (frequent) < 10%, but ≥ 1%;
- Uncommon < 1%, but ≥ 0.1%;
- Rare < 0.1%, but ≥ 0.01%;
- Very rare < 0.01%.

The main parameters of the effectiveness of the studied MD:

1. Change in the overall score on the WOMAC scale (WOMAC-T) at Visit 3 (week 13), Visit 5 (week 25) compared to the basal value at Visit 0 (screening) of the open study and compared to the basal value at Visit 1 (week 1) of the IA/PAAG-SI/OA/2019 study;

2. Change in the pain subscale score (WOMAC-A) on Visit 3 (week 13), Visit 5 (week 25) compared to the basal value on Visit 0 (screening) of the open study and compared to the basal value on Visit 1 (week 1) of the IA/PAAG-SI/OA/2019 study;
3. Change in the score for stiffness subscale (WOMAC-B) and functional performance (WOMAC-C) at Visit 3 (week 13), Visit 5 (week 25) compared to the basal value at Visit 0 (screening) of open study and compared to the basal value at Visit 1 (week 1) of the study IA/PAAG-SI/OA/2019;
4. The change in the severity of pain in the target knee on a 100-mm visual analogue scale (100 mm VAS) at Visit 2 (week 1), Visit 3 (week 13), Visit 5 (week 25) compared to the basal value at Visit 0 (screening) open safety study and compared to the basal value at Visit 1 (week 1) study IA/PAAG-SI/OA/2019;
5. Evaluation of the effectiveness of treatment by the patient, parameter (on a scale from 1-clear deterioration to 6 - significant improvement) on Visits 3 and 5 (parameters OEP-w_{13} and OEP-w_{25}, respectively);
6. Evaluation of the effectiveness of treatment by the investigator, parameter (on a scale from 1-clear deterioration to 6-significant improvement) on Visits 3 and 5 (parameters OEI-w_{13}, OEI-w_{25}, respectively);
7. Assessment of the total number of paracetamol tablets taken (one tablet = 500 mg) starting from Day 1 at Visit 3 and 5 (parameters PARACETAMOL-w_{13} and PARACETAMOL-w_{25}, respectively);
8. Assessment of the total number of NSAID tablets taken starting from Day 1 at Visits 3 and 5 (parameters NSAID-w_{13} and NSAID-w_{25}, respectively);
9. The parameter JSN of the target knee joint at Visit 5 of the open study compared to the basal value on Visit 0 of the IA/PAAG-SI/OA/2019 study retrospectively.

All performance parameters will be analyzed among all patients included in open study no. IA/PAAG-SI/OA/2020, as well as in subgroups of patients:

- who received only one course of injections in the placebo-controlled study IA/PAAG-SI/OA/2019;
- those who received two courses of therapy – both in the placebo-controlled and at Visit 1 (and according to their indications at Visit 2) of the open study
- those who received two courses of therapy – both in the placebo-controlled and at Visit 3 (and according to their indications at Visit 4) of the open study

3. Description of statistical methods

3.1. Description of statistical methods to be used

Statistical analysis will be carried out using specialized software, the selection of which will be carried out during the preparation of the statistical analysis plan.

Continuous (quantitative) data will be presented using the number of observations, arithmetic mean, 95% confidence interval (CI) for mean, standard (mean-square) deviation, median, interquartile range (25th and 75th centile), minimum and maximum.

Qualitative data (ordinal, nominal) will be presented using absolute frequencies (number of observations), relative frequencies (percent) and 95% CI.

Unless otherwise specified in the statistical analysis plan, statistical tests will be two-sided with a 5% confidence level.

The medical history and AEs will be encoded using the MedDRA classifier in the current version. Concomitant and prior therapy will be encoded using the ATX classifier.

This section briefly describes the planned analysis. The full analysis will be described in the statistical analysis plan.

3.1.1. Demographic data, baseline data, and follow-up data.

As part of this open continuation study, an initial (on Visit 0) assessment of parameters similar to those for the double – blind IA/PAAG-SI/OA/2019 study will be performed, and data from the group originally included in the IA/PAAG-SI/OA/2019 study that received the medical device under study and the group that was included in the current open study will be compared. This comparison will be performed using descriptive statistics.

In addition, the initial characteristics will be compared depending on the actual distribution into the following subgroups:

- who received only one course of injections in the placebo-controlled study IA/PAAG-SI/OA/2019;
- those who received two courses of therapy – both in the placebo-controlled and at Visit 1 (and as per the indications at Visit 2) of the open study;
- those who received two courses of therapy – both in the placebo-controlled and at Visit 3 (and as per the indications at Visit 4) of the open study.

For comparison between these subgroups, variance analysis (for quantitative parameters) or the "Chi-square" criterion (for qualitative parameters) will be applied, followed by the application of multiple comparison criteria (if necessary).

3.1.2. Analysis of safety parameters

In this study, the main parameters are safety parameters:

1. The frequency of AE and/or SAE;
2. Overall assessment of the tolerability of therapy by the investigator and the patient;
3. The main parameters of vital activity (heart rate, blood PRESSURE, body temperature, BDD);
4. Results of physical examination of the knee joint;
5. Results of laboratory and instrumental examination.

In addition, it is planned to evaluate the cumulative (in the framework of a double-blind and open study) frequency of the most likely complications associated with periprocedural or temporary postprocedural (no more than 72 hours) pain or burning.

Other possible side effects and complications of special interest related to the method of intra-articular injection will also be presented separately:

- pain or swelling at the injection site, feeling of bursting, burning, arthralgia, effusion in the joint, synovitis, aseptic acute arthritis;
- infections (pyogenic arthritis, direct infection of the joint at infectious diseases, osteomyelitis, sepsis, etc.);
- subcutaneous neuropathies, drug-induced vascular embolism.

It is planned to analyze the safety parameters for all included patients (safety population or FAS) using descriptive statistics, as well as in the following subgroups:

- those who received only one course of injections in the placebo-controlled study IA/PAAG-SI/OA/2019;
- those who received two courses of therapy – both in the placebo-controlled and at Visit 1 (and as per the indications at Visit 2) of the open study;
- those who received two courses of therapy – both in the placebo-controlled and at Visit 3 (and as per the indications at Visit 4) of the open study.

For comparison between these subgroups, variance analysis (for quantitative parameters) or the "Chi-square" criterion (for qualitative parameters) will be applied, followed by the application of multiple comparison criteria (if necessary).

3.1.3. Analysis of efficacy parameters

Main efficacy parameters of the studied MD:

1. Change in the overall score on the WOMAC scale (WOMAC-T) on Visit 3 (week 13), Visit 5 (week 25) compared to the basal value on Visit 0 (screening) of the open study and compared to the basal value on Visit 1 (week 1) of the IA/PAAG-SI/OA/2019 study;

2. Change in the pain subscale score (WOMAC-A) on Visit 3 (week 13), Visit 5 (week 25) compared to the basal value on Visit 0 (screening) of the open study and compared to the basal value on Visit 1 (week 1) of the IA/PAAG-SI/OA/2019 study;

3. Change in the score for stiffness subscale (WOMAC-B) and functional performance (WOMAC-C) at Visit 3 (week 13), Visit 5 (week 25) compared to the basal value at Visit 0 (screening) of open study and compared to the basal value at Visit 1 (week 1) of the study IA/PAAG-SI/OA/2019;

4. The change in the severity of pain in the target knee on a 100-mm VAS at Visit 2 (week 1), Visit 3 (week 13), Visit 5 (week 25) compared to the basal value at Visit 0 (screening) of open safety study and compared to the basal value at Visit 1 (week 1) study IA/PAAG-SI/OA/2019;

5. Evaluation of the effectiveness of treatment by the patient, parameter OEP (on a scale from 1-clear deterioration to 6-significant improvement) on Visits 3 and 5 (parameters OEP-w13 and OEP-w25, respectively);

6. Evaluation of the effectiveness of treatment by the investigator, parameter OEI (on a scale from 1-clear deterioration to 6-significant improvement) on Visits 3 and 5 (parameters OEI-w13, OEI-w25, respectively);

7. Assessment of the total number of paracetamol tablets taken (one tablet = 500 mg) starting from day 1 on Visits 3 and 5 (PARACETAMOL -w13 and PARACETAMOL-w25, respectively);

8. Assessment of the total number of NSAID tablets taken starting from day 1 on Visits 3 and 5 (NSAID-w13 and NSAID-w25, respectively);

9. The parameter JSN of the target knee joint at Visit 5 of the open study compared to the basal value on Visit 0 of the IA/PAAG-SI/OA/2019 study retrospectively.

All performance parameters will be analyzed both among all patients included in open study no. IA/PAAG-SI/OA/2020 (using descriptive statistics) and in subgroups of patients:

- those who received only one course of injections in the placebo-controlled study IA/PAAG-SI/OA/2019;
- those who received two courses of therapy – both in the placebo-controlled and at Visit 1 (and as per the indications at Visit 2) of the open study;
- those who received two courses of therapy – both in the placebo-controlled and at Visit 3 (and as per the indications at Visit 4) of the open study.

For comparison between these subgroups, variance analysis will be used (for quantitative parameters other than those based on WOMAC), covariance analysis (for quantitative parameters based on WOMAC, in this case the initial value of WOMAC in the double-blind phase of the study will be used as a covariate, and the results will be presented as the average, calculated by the least squares method), or the "Chi-square" criterion (for qualitative parameters), followed by the application of multiple comparison criteria (if necessary).

3.2. Interim statistical analysis

In this study, no interim data analysis is planned.

3.3. Planned number of participants in a clinical trial study based on the sample size

This open-label MD NOLTREX™ safety assessment study includes eligible and non-eligible patients who received MD NOLTREX™ as part of the IA/PAAG-SI/OA/2019 study. Therefore, no formal calculation of the sample size was performed. The maximum expected number of study participants is the number of patients who were randomized to the MD NOLTREX™ group in the IA/PAAG-SI/OA/2019 study, i.e. 72 patients.

3.4. Applicable level of significance

Statistical analysis of all the presented efficacy and safety parameters will be carried out at a 5% significance level, using two-way versions of statistical criteria.

3.5. Criteria for termination of the study

The study can be stopped for the following reasons:

1. at the initiative of the sponsor:

a. obtaining new toxicological or pharmacological data, or data on SAE, which force to revise the previously conducted assessment of the benefits/risks of participation in the study;

b. the frequency of AE and/or their severity does not allow to continue the study;

c. other reasons, including administrative.

2. at the initiative of the investigator: the frequency of AE and/or their severity unacceptably increases the risk for patients participating in the study

3. by decision of regulatory authorities.

If the study is stopped early, the Sponsor is obliged to notify the personnel of the research centers, as well as the regulatory bodies, indicating the reason for the early termination of the study.

The rules for terminating the research for each research participant are listed in Section **Ошибка! Источник ссылки не найден.**

3.6. Procedures to count the missing, non-analyzable, and questionable data

During the monitoring visits to the clinical center, the monitoring specialists authorized by the sponsor will conduct an analysis of patient's eCRF to identify any lack of necessary data. In the absence of data in the eCRF and the availability of relevant information in the primary documentation, the questions to the investigators and instructions for eliminating the inconsistencies will be formulated.

The statistician, authorized by the sponsor, and the principal investigator, when checking the database of research results, will analyze for the presence of dubious, missing and non-analyzable data, and will also formulate questions for investigators.

Researchers, if possible, will eliminate the identified errors in eCRF and inform the main researcher and authorized representatives of the sponsor about this. If the detected errors in the data cannot be eliminated after the completion of patient participation in the study, the statistical analysis of the data will be used to analyze the sensitivity of the resulting parameters to the doubtful data found. Information about the missing, doubtful and non-analyzable data will be presented in the final clinical trial report.

For all parameters of efficacy, safety and tolerability, data recovery is not provided due to the fact that the distribution of patients into subgroups for analysis is based on the actual need for treatment, and is not based on any procedures for distribution into groups, so the missing values cannot be considered as missing accidentally or completely accidentally.

3.7. Procedures for reporting any deviations from the original statistical plan

The decision to change the statistical plan reflected in this Protocol is made by the Sponsor.

All changes in the original statistical plan and their justification are reflected in the final report on the clinical study.

3.8. Selection of study participants for analysis

Due to the fact that the main purpose of this study is to analyze safety parameters, the main population for the analysis of all studied parameters of efficacy and safety will be the safety population, which is defined as the population of all patients included in the study (due to the fact that all included patients were exposed to the studied medical device), which most corresponds to the full data set for analysis (Full Analysis Set, FAS).

Additionally, performance data will also be analyzed in a population that strictly conforms to the study Protocol, known as the Per Protocol – PP data set.

4. Data management

4.1. Retention of randomization codes and their disclosure procedures

Randomization is not provided in this study.

4.2. Description of data management and maintaining records

All study site records and documents related to the clinical site, as well those in the investigator's file (including informed consent forms, logs, subject accountability sheets, etc.), as well subject source medical documents should be kept for 15 years after the study completion. The study sponsor should control integrity and availability of all clinical study materials for the entire life cycle of the study MD. Archived data can be kept as xerocopies, as well on optic and electronic information media. The principal investigator should immediately inform the sponsor about the facts of unintended damage/destruction, as well change of the storage location of clinical study materials. The targeted destruction of archived materials is possible only with written permission of the Study sponsor.

All obtained information including AE/SAE information will be recorded to source documents and then transferred to eCRF. eCRF will not contain data not presented in source documents.

After completion of scheduled visits by subjects and eCRF filling by the investigator, eCRF will be verified against source documents by the authorized sponsor monitors. If eCRF is completed correctly and precisely in accordance with source document data, the monitor confirms the verification of source documents and eCRF data in eCRF entering the verification flag. If on the stage on data assessment in eCRF, the quality control manager and/or biostatistician have any data questions, all clarifications and changes in eCRF data will be documented via generation of electronic queries for data clarification in eCRF. Responses on such queries are checked by the monitor, as well for compliance of corrected data to query text (if applicable), and, if the question is acknowledged sufficient, the query will be closed. Otherwise, the query will be re-opened with additional clarifying text for the investigator.

The investigator should provide information confirming possibility of timely subject enrollment following the criteria provided by the protocol.

The study should be carried out in accordance with the protocol and applicable sponsor standard operating procedures. If it is necessary to introduce changes to the protocol, the procedure stated in Section 0 of the present protocol should be followed.

Investigators should complete source medical documents and eCRF of all subjects included to the study.

The investigator is responsible for complete and accurate eCRF completion. All data recorded in eCRF should be presented in subject's source medical documents in printed form or as records made by the investigator or another authorized person in the clinical site.

In eCRF, in accordance with source documents, all significant details of subject participation in the study are recorded. eCRF should contain data on completion of subject participation in the study. eCRF should be completed within 7 days after subject visit to the study site.

eCRF should be completed in accordance with the instruction on eCRF completion. The errors made should be corrected entering a new value to eCRF, and the old value will be saved in the history of changes (audit trail). All missing data should be explained in eCRF which will be implemented with a special marker field (check mark) confirming missing data. If necessary, the investigator can enter a comment to a corresponding field clarifying reasons for missing data. eCRF should be certified with the electronic signature of the study investigator. The signatures certify that information contained in eCRF is reliable.

All study information and collected data are strictly confidential. The Investigator has right to report the study information to persons directly not taking part in the study, only with the Sponsor permission.

The final report consisting of statistical and clinical report is formed after database lock and completion of statistical processing of the study results.

The final report is signed by principal investigators of the clinical sites who confirm study results and conclusions sealing the report with a stamp of the institution.

5. Additions/amendments to the Protocol

Investigator signatures on the protocol signatures page mean the written confirmation of the consent to carry out the study in accordance with the protocol. During the clinical study, study materials can be changed and updated. Such changes and updates are considered as amendments.

Protocol amendment – a written description of changes or formal clarification of the clinical study protocol text. Amendments can be major and minor. Any protocol amendment, prior being implemented, should be duly approved in accordance with internal SOPs of the sponsor company and then approved by regulatory bodies, local IEC and signed by the investigator.

In the Decision of the Council of the Eurasian Economic Commission dated 12 February 2016 № 29 “On the rules for clinical and clinical, and laboratory tests (studies) of medical devices”, the definition of clinical study protocol amendment is given, whereby an amendment of test (study) program – a written description of changes or formal clarification of program text which affect or can influence reliability of obtained results and outcome of the clinical trial (study).

Amendments to clinical study materials are considered minor if they do not influence aims, organization forms, conduct methodology, statistical methods for clinical study processing and measures taken to provide safety of patients participating in the study. Protocol amendments should be kept together with initial protocol version. Amendment number and date should be stated on the title protocol.

6. Deviations from the clinical study plan

Protocol deviation – unintended divergence from the approved Study protocol.

Serious Protocol deviation – the deviation which can, by the judgment of the investigator or responsible person appointed by the investigator, can lead to subject withdrawal from the study or exclusion of his data from clinical and/or statistical study part. Deviations not classified as serious are considered minor deviations from the Study protocol.

Clinical site personnel and/CRP and monitor (if he is present in the site) should report a serious Protocol deviation to the sponsor as soon as possible. The Sponsor can offer to re-classify the Protocol deviation (minor to serious, or vice versa) based on the assessment. In such case, the classification made by the sponsor prevails and should be reported to CRO with a written justification.

The Sponsor should be informed about minor Protocol deviations within 10 working days, but prior the next study period/stage or prior the clinical phase/statistical phase.

Notifications and reports about the Protocol deviations are submitted to corresponding local ethical committees.

Procedure for documenting of Protocol deviations

The Investigator or responsible person appointed by the investigator should document and explain any deviation from the approved Study protocol. The Sponsor notification about the protocol deviation can be submitted in exceptional cases in verbal form (if immediate action/notification is required) which should be followed by the written notification (for example by email; in the study progress report). All protocol deviations should be described in the final study report.