# STATISTICAL ANALYSIS PLAN

**Version 1.0 final: December 03, 2020**

**Study IA/PAAG-SI/OA/2019**

**Multicenter, Double-blind, Randomized, Placebo-controlled Comparative Study of Efficacy and Safety of Intra-articular Polyacrylamide Hydrogel with Silver Ions (NOLTREX#) in patients with Knee Osteoarthritis K-L grades II-III**

**ClinicalTrials.gov Identifier: NCT03897686**

**Study sponsor**
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# List of abbreviations

| Abbreviation | Interpretation |
|---|---|
| 100-mm VAS | 100-millimeter visual analogue scale |
| BP | Blood pressure |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| APTT | Activated partial thromboplastin time |
| HIV | Human immunodeficiency virus |
| KOA | Knee osteoarthrosis |
| GGTP | Gamma-glutamyl transpeptidase |
| HA | Hyaluronic acid |
| CRO | Contract Research Organization |
| MD | Medical device |
| GCP | Good Clinical Practice |
| NSAID | 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 |
| JSW | Joint space width |
| WOMAC | Index of Western Ontario and McMaster Universities Osteoarthritis |

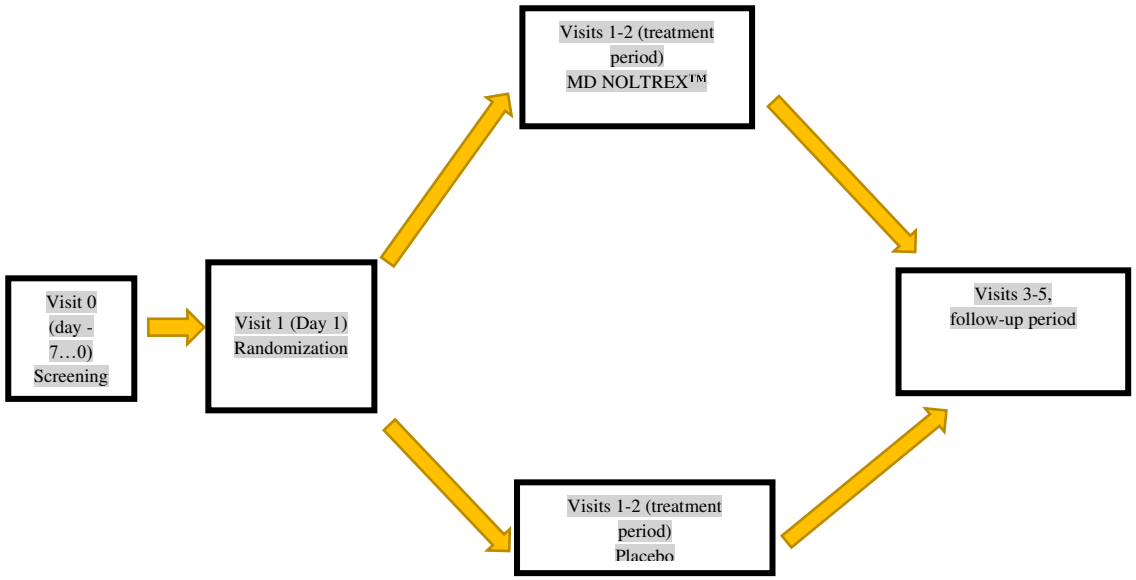
# 1.1 General provision

## 1.1.1 Justification of selection of the clinical study type

The study design including the selection of the study population, endpoints, is developed in accordance with the recommendations laid down in the Guidelines of EMA on clinical investigations of products intended for osteoarthritis treatment [1], on products intended for symptom-modifying therapy of osteoarthritis (osteoarthrosis): multicenter randomized double-blind placebo-controlled, in parallel groups study.

The study is double-blind – a patient, study investigator and Sponsor representative (monitoring specialist) will not know to which group a patient is included.

### *1.1.1.1 Study flow chart*




## 1.1.2 Measures aimed to minimize subjectivity

Due to various viscosity (which leads to different exertion in intra-articular injection) and different appearance of the study MD an placebo, to preserve blinding of any patient and study team, an independent unblended physician is to be engaged, he/she will receive the product per randomization code and perform the procedure of intra-articular injection of the study MD and placebo.

Randomization – a measure aimed to minimize subjectivity of patient random allocation. Randomization will exclude subjective, as well non-informed, selection of patient population to one of the group which will differ significantly from the population enrolled to the second one.

Placebo-controlled study – investigator and patient blinding will be used, via administration of the MD and comparator intervention depending on randomization results.

Patients will be randomized to groups using the simple block randomization method.

# 2 Description of statistical methods

## 2.1 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.

### 2.1.1 Demographic data, baseline data and follow-up data

Demographic, baseline and follow-up characteristics, such as medical history (MedDRA classified) and co-medication will be described by treatment groups for the population depending on the initial treatment (intention-to-treat, ITT) and then safety, if they differ.

### 2.1.2 Analysis of the primary efficacy parameter

In this study, the primary endpoint of effectiveness is selected to **total index score WOMAC** (WOMAC-T) against the use of medical device NOLTREX ™ in comparison with placebo (0.9% sodium chloride solution).

***The null hypothesis ($H_0$)*** in this study is that the average change in the total WOMAC score (WOMAC-T) at visit 5 (week 25) compared to the basal value at visit 1 (week 1) will be equal in all groups.

***An alternative hypothesis ($H_A$)*** in this study is that the average change in the total WOMAC score (WOMAC-T) at visit 5 (week 25) compared to the basal value at visit 1 (week 1) will differ in at least one of the groups.

The analysis of the primary efficacy parameter will be carried out using covariance analysis (ANCOVA) (adjusted for the original WOMAC-T score). The factors will be the fixed factors of the treatment group and the radiological stage.

For pairwise comparison of groups of active medical device with placebo groups (for each stage of OA), after the covariance analysis, Tukey's HSD (honest significant difference) significance level

correction will be used, while 95% confidence intervals for corrected average changes will also be calculated (least square means).

The analysis of the primary efficacy parameter will be carried out both on the ITT population (main analysis) and on the PP population (additional analysis).

### 2.1.3 Analysis of secondary efficacy parameters

An analysis of the following secondary efficacy parameters is planned:

- Changing the overall score on the WOMAC scale (WOMAC-T) on Visit 4 (week 13) compared to the basal value on Visit 1 (week 1);
- The change in score on the subscale of pain (WOMAC-A) on Visit 3 (week 6), Visit 4 (week 13) and Visit 5 (week 25) and compared with the basal value on Visit 1 (week 1);
- The change in the score for the stiffness (WOMAC-B) and functionality (WOMAC-C) on Visit 3 (week 6), Visit 4 (week 13) and Visit 5 (week 25) compared to the basal value on Visit 1 (week 1);
- Evaluation of the treatment efficacy by the patient, the value OEP (on a scale of 1 - a clear deterioration to 6 - a significant improvement) at visits 3, 4 and 5 (OEP-$w_6$, OEP-$w_{13}$, OEP-$w_{25}$);
- Evaluation of the effectiveness of treatment by the investigator, the value OEI (on a scale of 1 - a clear deterioration to 6 - a significant improvement) on visits 3, 4 and 5 (indicators OEI-$w_6$, OEI-$w_{13}$, OEI-$w_{25}$);
- Assessment as per patient diary of the total number of paracetamol tablets taken (one tablet = 500 mg) starting on day 1, on visits 3, 4 and 5 (PARACETAMOL-$w_6$, PARACETAMOL-$w_{13}$ and PARACETAMOL-$w_{25}$, respectively);
- Assessment per patient diary of the total number of NSAID tablets, starting on day 1, at visits 3, 4, 5 (values NSAID-$w_6$, NSAID-$w_{13}$ and NSAID-$w_{25}$, respectively)
- Drop-out rate due to safety (exclusion criteria 2, 3, 6, 7);
- Drop-out rate due to low patient adherence to treatment (criteria 4 and 5).

For secondary efficacy parameters, the following statistical analysis methods are planned:

- For quantitative indicators, an analysis similar to the analysis of the primary endpoint (covariance analysis) followed by the identification of groups with significant differences using Tukey's HSD.
- For frequency indicators (segments) - comparison of groups using the $\chi^2$ criterion ("chi-square").

Analysis of secondary efficacy parameters will be conducted on the ITT population (main analysis) and on the PP population (additional analysis).

### 2.1.4 Analysis of safety parameters

The descriptive part of the safety analysis will include an assessment of frequencies and bilateral 95% CIs for the following indicators in each treatment group:

- AE: the overall frequency and frequency of individual categories in general within the study and in groups;
- SAE: the overall frequency and frequency of individual categories in general within the study and in groups;
- the frequency of clinically significant changes in the results of physical examination by visits and groups;
- the frequency of clinically significant changes in laboratory parameters by visits and groups;
- the frequency of clinically significant changes in the indices of the main parameters of vital activity (body temperature, blood pressure, heart rate, respiration rate) by visits and groups;
- Comprehensive assessment of the tolerability of treatment by the researching doctor and the patient (assessment of the frequency for each response category);

Comparison of frequencies between treatment groups will be carried out using the $\chi^2$ criterion; the relative risk index will additionally be calculated. Evaluation of the dynamics in the course of the study ("before-after") for all re-studied frequencies will be conducted within each group using the McNemar criterion or Madansky criterion.

In addition to frequency indicators, the tabular form will show the values of all quantitative indicators of safety (laboratory data, vital signs) by visits and groups. The decision to conduct a statistical analysis of the specified data on visits and groups will be made at the stage of finalizing the plan for statistical data analysis.

The analysis of safety parameters will be carried out in the safety population (safety).

## 2.2 Intermediate statistical analysis

The study does not plan to conduct an intermediate statistical analysis.

## 2.3 Planned number of participants in a clinical trial with justification of sample size

The calculation of the sample size is based on the study results of the medical device NOLTREX ™ for OA (osteoarthritis) of the knee joint (N. V. Zagorodny et al. The use of new biopolymer material Noltrex in the complex treatment of patients with gonarthrosis. 2012, t. 17, No. 6, p. 49-52)[3]. Application in this study during the first 10 days of NSAID Movalis does not affect the assessment of results after 13 and 25 weeks.

As the primary endpoint in the study the change in total score on the WOMAC scale in the affected joint (WOMAC total) was chosen, at Visit 7 (week 25) compared to the basal value at Visit 1 (week 1). The choice of "Week 25" assessment point is related to the fact that in the above study the effect of medical device NOLTREX ™ was maximal after 6 months from the injection, and then the effect of the treatment was decreasing. An additional endpoint - a change in the total score on the WOMAC

scale (WOMAC total) at Visit 6 (week 13) compared to the basal value at Visit 1 (week 1) will be used to assess the onset of the effect of medical device after 3 months from the injection.

For the calculations the following assumptions were used:

1) Two groups (fixed factor A) - medical device NOLTREX™ and placebo
2) Baseline of WOMAC level and the total dose of NSAIDs received in the duration of study as covariates
3) Effect size - 0.25: calculated on the basis of data from the above publication, in which the magnitude of differences between groups, taking into account the variability, was up to 100 points, and the variability (standard deviation) - up to 400 points
4) Level of significance = 5% (type I error - $\alpha = 0.05$)
5) Power - 80% (type II error - $\beta = 0.20$)
6) Statistical model - covariance analysis (adjusted for the initial value) with fixed factors of the group
7) Drop-outs during the study will not exceed 10%.
8) Drop-out during inclusion period (screening) will not exceed 15%.

Calculations of the sample size (the number of completed cases) were performed using the G*Power 3.1.9.2 software package with the above assumptions:

**F tests –** ANCOVA: Fixed effects, main effects and interactions

| | | | |
|---|---|---|---|
| **Analysis:** | A priori: Compute required sample size | | |
| **Input:** | Effect size f | = | 0.25 |
| | α err prob | = | 0.05 |
| | Power (1−β err prob) | = | 0.80 |
| | Numerator df | = | 1 |
| | Number of groups | = | 2 |
| | Number of covariates | = | 2 |
| **Output:** | Noncentrality parameter λ | = | 8.0000000 |
| | Critical F | = | 3.9175498 |
| | Denominator df | = | 124 |
| | Total sample size | = | 130 |
| | Actual power | = | 0.8013621 |

In this case, taking into account rounding, the study should complete 130 patients (65 in each group), for which it is necessary to randomize 144 patients (72 in each group).

Accordingly, to achieve the required number of randomized patients, it is necessary to screen up to 170 patients.

## 2.4 Applicable level of significance

For all parameters of efficacy, safety and tolerability the bilateral statistical criteria will be used at a 95% significance level (the threshold value p for confirming statistical significance is less than 0.05).

## 2.5 Study termination criteria

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 preliminary ended, 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 study for each study participant are listed in section 6.3.

## 2.6 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 to investigators.

Investigators, if possible, will eliminate the identified errors in eCRF and inform the main investigator 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.

The missing data for the primary efficacy parameter will be replenished by the last observation carried forward (LOCF) method (transfer of data from the last available observation point to the point for the main analysis).

For the remaining parameters of efficacy, safety and tolerability, data replenishment is not provided.

## 2.7 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 with their justification are reflected in the final report of the clinical study.

## 2.8 Selection of study participants for analysis

### 2.8.1 Population selected on the basis of prescribed treatment (ITT)

For the ITT data set, also known as the full analysis set (FAS), all randomized participants are included who were treated with the medical device under the study, regardless of the extent to which the protocol was followed during the study. This data set is essential for analyzing primary and secondary efficacy parameters.

### 2.8.2 Population selected based on compliance with protocol (PP)

Primary and secondary efficacy parameters will additionally be analyzed using a set of data from study participants selected by the protocol compliance principle (PP). The participant will be excluded from the PP data set in the following cases:

- Significant violation of the inclusion and non-inclusion criteria.
- The use of prohibited concomitant therapy.
- Any other significant violation of the protocol, recognized as significantly violating the basic assessment of the efficacy of particular study participant.

### 2.8.3 Population for safety assessment

The data set analyzed for safety assessment is identical to the ITT data set. However, unlike the population, depending on the treatment prescribed, participants are analyzed depending on the actual medical device received (if it is different from the medical device that was assigned by randomization). All types of safety analysis will be based on the use of a data set for safety assessment.

All decisions regarding the choosing of the databases being analyzed will be made before the research database is closed.

# 3 Data management

## 3.1 Retention of randomization codes and their disclosure procedures

Patient randomization code is stated in documents intended for the use beyond a study site (eCRF, SAE reports, etc.). The assigned patient randomization code is entered to eCRF and corresponding forms. Patient randomization code is not changed during the study.

Randomization plan is kept for control access. The study personnel is responsible for randomization compliance with the established guidelines.

The access to the list of randomization codes will be limited to unblinded Sponsor personnel involved in preparation of randomization table, its implementation to IxRS (if applicable), and responsible for emergency code break. Unblinded personnel will not take part in the study and will not contact with other members of project team and blinded study site representatives.

## 3.2 Description of data handling and recording

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 as 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 intended 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 the 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 at the stage of 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 answer is deemed 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 4 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 the principal investigators of the clinical sites who confirm study results and conclusions, sealing the report with a stamp of the institution.

# 4 Protocol updates/amendments

Investigator signatures on the protocol signature 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.

# 5 Deviations from 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 exclusion from the study or non-inclusion 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/CRO 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.