Clinical Study Protocol IA/PAAG-SI/OA/2019
HBISA Endoprosthesis of synovial fluid NOLTREX™

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

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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

Synopsis

Study title	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
Protocol number	IA/PAAG-SI/OA/2019
Study MD	HBISA Endoprosthesis of synovial fluid NOLTREX^{TM} (4.0 ml for one injection with one-week interval between injections. Course - 2 injections).
Placebo	Sodium chloride solution 0.9% BUFUS®, in ampoules 10 ml (to be used with syringes 2.5 ml). (4.0 ml for one injection with one-week interval between injections. Course - 2 injections.)
Study period, number of study sites and patients	Proposed total study period – 12 months. Participation of each patient in the study – not more than 179 days: • Screening – days -7…0; • Treatment period: weeks 1-2; • Follow-up period: weeks 3-25. Number of study sites – 4. Number of patients: - 170 screened patients; - 144 randomizations (72 patients in each group); - 130 patients that should complete the study (65 patients in each group).
Study aim	The aim of this double-blinded controlled study is to assess clinical efficacy and safety of intra-articular polyacrylamide hydrogel with ions of silver in comparison with placebo (0.9% sodium chloride solution) in Kellgren Lawrence radiological grade II-III knee osteoarthritis
Study goals	• Assessment of **a change in knee joint functionality, stifnes and joint pain intensity estimated per the corresponding subscales and total arthritis index score** developed by Western Ontario and McMaster Universities **(WOMAC)** during administration of intra-articular MD NOLTREX™ in comparison with placebo

	(0.9% sodium chloride solution) in Kellgren Lawrence radiological grade II-III knee osteoarthritis; • Assessment of the **need in paracetamol and NSAID (in case of ineffective therapy with paracetamol) intake to relieve the knee pain** during administration of intra-articular MD NOLTREX™ in comparison with placebo (0.9% sodium chloride solution) in Kellgren Lawrence radiological grade II-III knee osteoarthritis; • Assessment of efficacy of MD NOLTREX™ in comparison with placebo (0.9% sodium chloride solution) in Kellgren Lawrence radiological grade II-III knee osteoarthritis **as subjectively assessed by a patient**; • Assessment of efficacy of MD NOLTREX™ in comparison with placebo (0.9% sodium chloride solution) in Kellgren Lawrence radiological grade II-III knee osteoarthritis **as subjectively assessed by the investigator**; • Assessment of **safety** of intra-articular MD NOLTREX™ in comparison with placebo (0.9% sodium chloride solution) in Kellgren Lawrence radiological grade II-III knee osteoarthritis.
Study methodology	The study MD – synovial fluid endoprosthesis NOLTREX™ – is intended for a symptomatic treatment leading to the decrease of joint pain intensity and improvement of functional characteristics of joint. Therefore the study MD is intended for symptom-modifying therapy of joint osteoarthritis (osteoarthrosis). The study design including the selection of the study population, endpoints, is developed in accordance with the recommendations laid down in the Guidelines on clinical investigations of medicinal 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. With regards to a slow but stable onset of the effect assumed in accordance with the mode of action of the medical device, the assessment of the primary endpoint (change of total WOMAC score – WOMAC total in the affected joint) is planned on Week 25, in addition, the effect onset in 3 months after the product administration, will be assessed on Week 13. Patients meeting the inclusion criteria and not having the exclusion criteria, will be randomized on Visit 1 to 2 groups: • MD NOLTREX™;

	• Placebo. On the Screening visit, it is determined whether a patient meets the inclusion/non-inclusion criteria, as well, the target knee joint is determined. The target knee joint is selected by the investigator using the following algorithm. If bilateral knee osteoarthritis is present, the target knee joint is selected in accordance with the greater pain intensity per WOMAC A scale, with the same tenderness of both knee joints – per radiologic changes (greater degree per Kellgren–Lawrence classification and/or lower value of the joint space width – JSW). If the same tenderness occurs in both knee joints, and the same picture of radiographic changes is observed, target knee joint is determined by the investigator at his/her discretion. The study MD or placebo will be injected to the target knee joint in the corresponding volume using the standard method: a patient being supine on his back, to the external superior recess, beginning from Visit 1 (randomization, start of treatment). Intra-articular injections will be made only if there are no signs of active joint inflammation (hyperemia, edema, joint effusion) and signs of skin inflammation or some other damage in the injection site for intra-articular administration of MD or placebo. The product will be administered in accordance with the instruction for the medical use of medical device NOLTREX™: 4.0 ml for one injection (2.5 + 1.5 ml, or 2.0 + 2.0 ml from two syringes through one needle [one puncture]) with one week interval. Course – 2 injections. The number of injections is determined by the physician depending on the stage of knee osteoarthritis and clinical response. To avoid the joint overfilling with the dense, slowly resorbing material, with a good clinical result (over 40% pain decrease per 100-mm VAS scale in comparison with baseline [2]), the course of injections will be stopped. Placebo will be administered per the analogous scheme.. Primary and secondary efficacy endpoints, and safety parameters will be assessed on Visits on Week 6, 13 and 25 (primary endpoint).
Patient visits	**Visit 0 (day -7…0): Screening** The following procedures will be done: • Obtaining the informed consent form for the study participation; • Collection of demographic data (date of birth, age); • Measurement of body weight and height; • Collection of complaints and medical history; • Recording of concomitant therapy;

	• Assessment of vital signs (blood pressure [BP], heart rate [HR], respiratory rate [RR], body temperature); • Physical joint examination; • Radiography of knee joints (or assessment of study X-rays made within 3 months prior the study inclusion). Screening examinations are continued if a patient meets the inclusion criteria. • Complete blood count; • Blood biochemistry: glucose, alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], gamma-glutamyl transpeptidase [GGTP], total bilirubin, creatinine, uric acid, rheumatoid factor; • Coagulogram (activated partial thromboplastin time [APTT], prothrombin time [PT], prothrombin index, fibrinogen); • Urine analysis; • Express urine pregnancy test for women with the preserved reproductive potential; • Test for human immunodeficiency virus (HIV), HBs-Ag, anti-HCV, RW; • Assessment of the inclusion/non-inclusion criteria; • Assessment of serious adverse events (SAE) related to study procedures. ***Treatment period (Week 1 – Week 2)*** **Visit 1 (Week 1, Day 1), randomization, start of treatment** The following procedures will be made: • Collection of complaints and medical history; • Recording of concomitant therapy; • Measurement of body weight; • Assessment of vital signs (BP, HR, RR, body temperature); • Physical joint examination; • Completion of algofunctional questionnaire WOMAC with the assessment of pain (WOMAC-A), stiffness (WOMAC-B), functionality (WOMAC-C) subscales and total score (WOMAC-T) (baseline values – WOMAC-X-w_1)

	• Assessment of compliance with the inclusion/non-inclusion criteria; • Selection of the target knee joint; • Assessment of pain intensity in the target knee joint per 100-mm visual analogue scale (100-mm VAS); • Randomization; • Injection № 1 of the study MD or placebo into the target joint; • Issuing of the patient diary; • Assessment of adverse events (AE); • Assessment of the subject exclusion criteria. **Visit 2 (Week 2, Day 8)** The following procedures will be made: • Collection of complaints and medical history; • Recording of concomitant therapy; • Measurement of body weight; • Assessment of vital signs (BP, HR, RR, body temperature); • Physical joint examination; • Assessment of the patient diary; • Return of the diary to the patient; • Express urine pregnancy test for women with the preserved reproductive potential; • Assessment of the subject exclusion criteria (the decision is made whether the intra-articular injection can be done); • Assessment of pain intensity in the target knee joint per 100-mm VAS; • By the decision of the research investigator, with regards to the rules for administration of the study MD determined by the protocol and the instruction for use of the medical device - injection № 2 of the study MD or placebo into the target joint; • AE assessment; • Repeat assessment of the subject exclusion criteria (after intra-articular injection).

	Every week between Visits 2 and 3 (weeks 3, 4 and 5), the study personnel contacts a patient by the phone to learn about complaints, AE and need of paracetamol and NSAID intake for the previous 2 weeks. If any AE or need of paracetamol and NSAID intake for over 4 days a week during each of the successive 2 weeks is found, the patient is invited to the clinic for the Unscheduled visit or Premature study discontinuation visit (depending on the visit reason). ***Follow-up period (Weeks 3–25)*** **Visit 3 (Week 6, Day 36 ± 2)** The following procedures will be done: • Collection of complaints and medical history; • Recording of concomitant therapy; • Measurement of body weight; • Assessment of vital signs (BP, HR, RR, body temperature); • Physical joint examination; • Assessment of the patient diary; • Dispensing of the new patient diary to the patient; • Completion of algofunctional questionnaire WOMAC with the assessment of pain (WOMAC-A-w_6), stiffness (WOMAC-B-w_6), functionality (WOMAC-C-w_6) subscales and total score (WOMAC-T-w_6) ("Week 6" values); • Patient's assessment of the treatment efficacy, OEP-w_6 value (per the scale from 1 – evident aggravation to 6 – significant improvement); • Investigator's assessment of the treatment efficacy, OEI-w_6 value (per the scale from 1 – evident aggravation to 6 – significant improvement); • Assessment of the total number of paracetamol tablets taken per the patient diary (one tablet = 500 mg), value PARACETAMOL-w_6; • Assessment of the total number of NSAID tablets taken, value NSAID-w_6 • Complete blood count;

	• Blood biochemistry: glucose, ALT, AST, ALP, GGTP, total bilirubin, creatinine, uric acid; • Coagulogram (APTT, PT, prothrombin index, fibrinogen); • Urine analysis; • Express urine pregnancy test for women with the preserved reproductive potential; • AE assessment; • Assessment of the subject exclusion criteria. ***On Weeks 8, 10 and 12, the study personnel contacts the patient by the phone to learn about complaints, adverse events (AE) and need of paracetamol and NSAID intake for the previous 2 weeks. If any AE or need of paracetamol intake for over 4 days a week during each of the successive 2 weeks is revealed, the patient is invited to the clinic for the unscheduled visit or premature study discontinuation visit (depending on the visit reason).*** **Visit 4 (Week 13, Day 84 ± 2)** The following procedures will be done: • Collection of complaints and medical history; • Recording of concomitant therapy; • Measurement of body weight; • Assessment of vital signs (BP, HR, RR, body temperature); • Physical joint examination; • Assessment of the patient diary; • Dispensing of the new patient diary to the patient; • Completion of algofunctional questionnaire WOMAC with the assessment of pain (WOMAC-A-w_{13}), stiffness (WOMAC-B-w_{13}), functionality (WOMAC-C-w_{13}) subscales and total score (WOMAC-T-w_{13}) (“Week 13” values); • Patient’s assessment of the treatment efficacy, OEP-w_6 value (per the scale from 1 – evident aggravation to 6 – significant improvement); • Investigator’s assessment of the treatment efficacy, OEI-w_{13} value (per the scale from 1 – evident aggravation to 6 – significant improvement);

	• Assessment of the total number of paracetamol tablets taken per the patient diary (one tablet = 500 mg) beginning from day 1, value PARACETAMOL-w_{13}; • Assessment of the total number of NSAID tablets taken, value NSAID-w_{13} • Complete blood count; • Blood biochemistry: glucose, ALT, AST, ALP, GGTP, total bilirubin, creatinine, uric acid; • Coagulogram (APTT, PT, prothrombin index, fibrinogen); • Urine analysis; • Express urine pregnancy test for women with the preserved reproductive potential; • AE assessment; • Assessment of the subject exclusion criteria. ***On Weeks 15, 17, 19, 21 and 23, the study personnel contacts the patient by the phone to learn about complaints, adverse events (AE) and need of paracetamol and NSAID intake for the previous 2 weeks. If any AE or need of paracetamol or NSAID intake for over 4 days a week during each of the successive 2 weeks is revealed, the patient is invited to the clinic for an unscheduled visit or premature study discontinuation visit (depending on the visit reason).*** **Visit 5 (Week 25, Day 169 ± 2)** The following procedures will be made: • Collection of complaints and medical history; • Recording of concomitant therapy; • Measurement of body weight; • Assessment of vital signs (BP, HR, RR, body temperature); • Physical joint examination; • Assessment of the patient diary; • Completion of algofunctional questionnaire WOMAC with the assessment of pain (WOMAC-A-w_{25}), stiffness (WOMAC-B-w_{25}), functionality (WOMAC-C-w_{25}) subscales and total score (WOMAC-T-w_{25}) (“Week 25” values);

	• Patient's assessment of the treatment efficacy, OEP-w_{25} value (per the scale from 1 – evident aggravation to 6 – significant improvement); • Investigator's assessment of the treatment efficacy, OEI-w_{25} value (per the scale from 1 – evident aggravation to 6 – significant improvement); • Assessment of the total number of paracetamol tablets taken per the patient diary (one tablet = 500 mg) starting from day 1, value PARACETAMOL-w_{25}; • Assessment of the total number of NSAID tablets taken per the patient diary starting from day 1, value NSAID-w_{25}, • Complete blood count; • Blood biochemistry: glucose, ALT, AST, ALP, GGTP, total bilirubin, creatinine, uric acid; • Coagulogram (APTT, PT, prothrombin index, fibrinogen); • Urine analysis; • Express urine pregnancy test for women with the preserved reproductive potential; • AE assessment. • In case of planned completion of the study and completion of all procedures provided in the clinical study protocol, the patient will be provided with studied medical device NOLTREX regardless of the treatment group to which the patient was assigned. In this case the patient will receive 4 packages of NOLTREX which corresponds to one treatment course. **Unscheduled visit** Unscheduled visits will be made as necessary, for example, in the event of aggravation of the studied medical condition, AE or intolerability of the study MD or placebo therapy. When an unscheduled visit is made, regardless of its reason, the investigator makes the following procedures and completes corresponding eCRF pages (Unscheduled visit): • Collection of complaints and medical history; • Recording of concomitant therapy; • Measurement of body weight; • Assessment of vital signs (BP, HR, RR, body temperature);

	• Physical joint examination; • Assessment of the patient diary; • AE assessment. If clinically indicated and meeting the criteria, any of the study procedures can be additionally made on the unscheduled visit by the decision of the Principal Investigator. **Premature study discontinuation visit** • On the premature study discontinuation visit, the same procedures are made as on Visit 5
Main parameters of efficacy and safety assessment	**Primary endpoint** – change of the total WOMAC score (WOMAC-T) on Visit 5 (Week 25) in comparison with baseline on visit 1 (Week 1). **Secondary endpoints:** • Change of the total WOMAC score (WOMAC-T) on Visit 4 (Week 13) in comparison with baseline on Visit 1 (Week 1); • Change of the pain subscale score (WOMAC-A) on Visit 3 (Week 6), Visit 4 (Week 13) and Visit 5 (Week 25) and in comparison with baseline on Visit 1 (Week 1); • Change of stiffness (WOMAC-B) and functionality (WOMAC-C) subscale scores on Visit 3 (Week 6), Visit 4 (Week 13) and Visit 5 (Week 25) in comparison with baseline on Visit 1 (Week 1); • Patient's assessment of the treatment efficacy, value OEP (per scale from 1 – evident aggravation to 6 – significant improvement) on Visits 5, 6 and 7 (values OEP-w_6, OEP-w_{13}, OEP-w_{25}); • Investigator's assessment of the treatment efficacy, value OEI (per scale from 1 – evident aggravation to 6 – significant improvement) on Visits 3, 4 and 5 (values OEI-w_6, OEI-w_{13}, OEI-w_{25}); • Assessment of the total number of paracetamol tablets taken (one tablet = 500 mg) beginning from day 1, on Visits 3, 4 and 5 (values PARACETAMOL-w_6, PARACETAMOL-w_{13} and PARACETAMOL-w_{25}, correspondingly);

	• Assessment of the total number of NSAID tablets taken starting from day1, on Visits 3, 5 and 5 (values NSAID-w_6, NSAID-w_{13}, NSAID-w_{25} correspondingly); • Patient exclusion rates due to safety (subject exclusion criteria 2, 3, 6, 7); • Patient exclusion rates due to poor patient's treatment compliance (criteria 4 and 5). **Safety assessment parameters:** • Frequency of AE and/or SAE reporting in treatment groups; • Global study physician and patient assessment of the therapy intolerability; • Vital signs (HR, BP, body temperature, RR); • Laboratory test results. AE description will be presented per the following scheme: • AE description; • Severity of manifestation; • Duration; • Relationship with the study product; • Outcome. AE will be encoded in accordance with the Medical Dictionary for Regulatory Activities MedDRA.
Inclusion criteria	1) Men and women above 50 years; 2) Verified knee osteoarthritis in accordance with the ACR criteria (knee pain combined with one of the following signs: age above 50 years, knee crepitus or morning joint stiffness lasting for less than 30 minutes combined with radiologic signs of knee osteoarthritis); 3) Kellgren Lawrence radiological grade II-III knee osteoarthritis with the predominant involvement of the medial tibiofemoral region of the knee joint; 4) Joint space width (JSW) of the target knee joint at least 2.5 mm.
Non-inclusion criteria	1) History of any injury or surgical intervention on the target knee joint (except for diagnostic arthroscopy made more than 60 days before study entry);

	2) Severe degenerative changes in the target knee joint determined as the joint space narrowing less than 2 mm; 3) Varus or valgus deformation of the target knee joint; 4) Instability of the target knee joint; 5) Active inflammation of the target knee joint (edema, hyperemia, present effusion) at the study entry; 6) Microcrystalline arthropathies; 7) Systemic inflammatory disease (rheumatoid arthritis, systemic lupus erythematosus, etc.); 8) Seronegative spondiloarthritis and reactive arthritis; 9) Inflammatory diseases of the skin and soft tissues in the proposed injection site for the study MD or placebo in the target joint; 10) History of venous thrombosis and thromboembolias; 11) Coagulogram abnormalities (APTT, prothrombin time, prothrombin index, fibrinogen); 12) Inflammatory changes in the complete blood count (leukocytosis, increase of erythrocyte sedimentation rate [ESR]); 13) Platelet count abnormality (in accordance with the reference ranges of the local laboratory; 14) Increase of rheumatoid factor level; 15) Increase of uric acid level > 360 μmol/l; 16) Diabetes mellitus; 17) Hemophilia and other hemorrhagic diatheses, as well administration of anticoagulants and disaggregants; 18) Positive results of HIV, HBs-Ag, anti-HCV, RW tests; 19) Intra-articular injection to the target knee joint: • MD NOLTREX™ – within 24 months prior patient's inclusion to the study; • hyaluronates – within 6 months prior patient's inclusion to the study; • glucocorticosteroids – within 1 month prior the study inclusion; • non-steroidal anti-inflammatory drugs (NSAID) – within 3 weeks prior patient's inclusion to the study.

	20) Oral administration of non-steroidal anti-inflammatory drugs (NSAID) within 2 weeks prior the study inclusion; 21) Necessity of systemic glucocorticosteroids intake in any dosage form; 22) Paracetamol administration within 48 hours prior the study inclusion; 23) Pregnancy and lactation; 24) Hypersensitivity to components of the study MD or placebo; 25) Severe liver disorder determined as the increase of one of the values: ALT, AST, ALP, total bilirubin, GGTP more than 3 times the upper limit of normal; 26) Renal diseases with the glomerular filtration rate estimated per Cockraft-Gault formula less than 60 ml/min/1.73 m^2 (III-V stage chronic renal disease [CRD]); 27) Clinically manifested hip osteoarthritis; 28) History of knee and coxofemoral endoprosthesis; 29) Acute infectious diseases or infectious aggravations of chronic diseases (respiratory infections) within one month prior the study inclusion; 30) Severe decompensated chronic or acute diseases and other conditions which, by the opinion of the study physician, may preclude the patient's participation in the study or affect the study results.
Subject exclusion criteria	1) Ethics Committee, regulatory bodies or the Sponsor terminate the study or participation of the clinical site in the study for any reasons; 2) Need in paracetamol or NSAID intake due to knee osteoarthritis 4 days a week or more during 2 successive weeks; 3) The investigator makes the decision that a patient should discontinue the study in the best interests of the patient; 4) Informed consent revocation (patient's unwillingness to continue the study); 5) Major deviation from the study protocol; 6) Individual intolerability or contraindications to the study MD, placebo or paracetamol; 7) AE/SAE which requires examination and/or treatment affecting significantly the study procedures (in particular, development of active arthritis or hemarthrosis of the target knee joint);

	8) Erroneous study inclusion (for example, the patient was included with a violation of the protocol inclusion/non-inclusion criteria); 9) A patient has non-inclusion criteria during the study; 10) A patient takes/needs additional treatment which can affect the study result or patient's safety (see section "Disallowed concomitant therapy"); 11) Other conditions or events which require, by the opinion of the study physician, the patient's exclusion from the study. 12) Injury of the studied joint.
Disallowed concomitant therapy	1. Glucocorticosteroids in any dosage form; 2. Any intra-articular or peri-articular injections, physical therapy, as well other therapeutic or rehabilitation measures for knee osteoarthritis, except the study procedures; 3. NSAIDs and analgesics including the local ones (except for paracetamol and NSAIDs permitted by the protocol in cases when paracetamol is ineffective); 4. Paracetamol intake in dose over 4000 mg a day; 5. the use of one of the NSAIDs permitted by the Protocol in doses exceeding those permitted by the Protocol (permitted drugs and doses: diclofenac potassium 50 mg 2 times a day, diclofenac sodium tablets 75 mg 2 times a day, naproxen tablets 500 mg 2 times a day, naproxen sodium tablets 550 mg 2 times a day, meloxicam tablets 7.5 mg once a day, celecoxib tablets 200 mg 2 times a day); 6. use of NSAID in combination with paracetamol or combination of two or more NSAID; 7. Rehabilitation measures due to knee osteoarthritis; 8. Physical therapy in knee osteoarthritis; 9. Acupuncture; 10. Surgeries and arthroscopies of the target knee joint. 48 hours prior the visits where the assessment of pain intensity in the knee joint (Screening visit, Visits 1 – 7) is made paracetamol or NSAID intake is not allowed.
Justification of the sample size	The calculation of the sample size is based on the study results of product Noltex in knee osteoarthritis (osteoarthrosis) (N.V. Zagorodniy et al. Administration of new biopolymer material "Noltrex" in complex treatment of patients with knee osteoarthritis. 2012, v. 17, №6, p. 49-52)

[3]. The use of NSAID Movalis in the study during the first 10 days does not interfere with assessment of results in 13 and 25 weeks.

As the study primary endpoint, we selected the change of the total WOMAC score of the affected joint (WOMAC total), at Visit 7(Week 25) in comparison with baseline at Visit 1 (Week 1). The selection of assessment point "Week 25" is related to the fact that Noltrex effect was the greatest in the abovementioned study in 6 months after the administration, subsequently the product effect was reduced. The additional endpoint – change of total WOMAC score (WOMAC total) on Visit 6 (Week 13) in comparison with baseline on Visit 1 (Week 1) will be used for assessment of the onset of the MD effect in 3 months after the administration.

The following assumptions were used for calculations:

1) Two groups (fixed factor A) – Noltrex and placebo
2) The initial value of the WOMAC score and the total dose of NSAIDs obtained during the study as covariates
3) Effect size – 0.25: calculated based on the data from the publication presented above in which the value of intergroup differences with regards to variability was up to 100 scores, and variability (standard deviation) – up to 400 scores
4) Significance level = 5% (type I error – $\alpha = 0.05$)
5) Power – 80% (II type error – $\beta = 0.20$)
6) Statistical model – analysis of covariance (baseline-adjusted) with fixed factors of group
7) Study exclusion rate will not exceed 10%
8) Non-inclusion rate in selection (screening) period will not exceed 15%

The sample size (number of completed cases) was calculated using applied software package G*Power 3.1.9.2 with the abovementioned 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, with regards to approximation, 130 patients (65 in each group) should complete the study, for that, 144 patients should be randomized (72 in each group). Correspondingly, to achieve the necessary number of randomized patients, up to 170 patients should be screened.
Blinding, randomization	Due to various viscosity (which leads to different exertion efforts in intra-articular injection) and different appearance of the study MD and placebo, to preserve blinding of any patient and study team, an independent non-blinded 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. Patients will be randomized to groups using the simple block randomization method.