CLINICAL STUDY PROTOCOL

Protocol Title: Effectiveness of Intra-articular Oxygen-ozone Injections and Splinting for the Treatment of Thumb Osteoarthritis: a Randomized Controlled Clinical Trial

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ABBREVIATIONS AND TERMS

ADE Adverse Device Effect
ADL Activities of daily life
ADP Adenosine DiPhosphate
AE Adverse Event
ANCOVA Analysis of Covariance
ASADE Anticipated Serious Adverse Device Effect
BMI Body Mass Index
CMCJ Carpo-Metacarpal joint
CRF Case Report Form
DASH The Disabilities of the Arm, Shoulder and Hand questionnaire
EC Ethics Committee
EL Eaton-Littler
GPIIb-IIIa Glycoprotein IIb and IIIa
HIV Human Immunodeficiency Virus
ICF Informed Consent Form
IEC Independent Ethics Committee
IGF-1 Insulin-like Growth Factor 1
IL-1 Interleukin-1
IL-6 Interleukin-6
IL-8 Interleukin-8
IL-10 Interleukin-10
IL-13 Interleukin-13
IL-17 Interleukin-17
IFN-γ Interferon- γ
IRB Institutional Review Board
JSN/JSW Joint Space Narrowing/Joint Space Width
MMP Matrix Metalloproteinase
MRI Magnetic Resonance Imaging
NSAID Non-Steroidal Anti-Inflammatory Drug
NRS Numeric Rating Scale
OA Osteoarthritis
OOT Oxygen-ozone therapy
PA Posterior-Anterior
pH potential Hydrogen
QL Quality of life
RCT Randomized Controlled Trial
SAE Serious Adverse Event
SADE Serious Adverse Device Effect
SAP Statistical Analysis Plan
TGF-β1 Transforming Growth Factor beta 1
TMJ trapezio-metacarpal joint
TMJO trapezio-metacarpal joint osteoarthritis
TNFα Tumor Necrosis Factor alpha
UADE Unanticipated Adverse Device Effect
USADE Unanticipated Serious Adverse Device Effect
VAS Visual Analog Scale

1- Introduction

Osteoarthritis is a degenerative condition that causes pain, impaired function and affects daily activities (1). OA of trapezio-metacarpal joint (TMJ) most commonly occurs in women over 50 years old, often bilateral and it is a disabling condition presenting with pain at base of the thumb, swelling, instability, deformity and impairment of hand function with limitation in gripping and pinching objects (2). Several factors have been associated with the development of degenerative changes in the carpometacarpal joint (CMCJ), including increased thumb base joint laxity and greater grip strength. In osteoarthritis, there is the destruction of cartilage and subchondral bone, with the consequent narrowing of articular space. Besides the biomechanical factors, trauma and adiposities, it is believed that inflammation plays an important role (3).

The inflammatory process is supported by several pro-inflammatory cytokines released by chondrocytes. Among these, the most important cytokines are IL1, IL6, IL8, IL17, LIF, TNF-α and IFN-γ, which cooperate in destroying the articular cartilage (4). Furthermore, IL1, which affects the production of ROS (reactive oxygen species), is implicated in the damage to chondrocytes DNA. ROS accelerate the disintegration of the cartilage matrix, and narrowing of the joint space, inhibiting the synthesis of collagen and proteoglycans (3). Another mechanism of joint destruction is proteolytic degradation (4). Based on this, future therapy in OA should inhibit proteolytic enzymes such as metalloproteinases (MMPs), nitric oxide synthesis (NOs), proinflammatory cytokines (IL1, IL6, TNF-α) and apoptosis. On the other hand, OA treatment should stimulate the synthesis of anti-inflammatory cytokines (IL4, IL10, IL13) and growth factors (TGF-β, IGF-1(5). The severity of trapezio-metacarpal osteoarthritis (TMJO) is classified radiologically by Eaton and Litter (6). There are several surgical treatment options for TMJO as well as many conservative treatments such as splinting, thumb strengthening exercises, anti-inflammatory drugs and intra-articular injections. There are no currently approved TMJO treatments capable of slowing OA-related structural progression, (7) so the main goals of the conservative treatment are to provide symptomatic relief, improve joint function, and delay surgical intervention. The patient with symptomatic TMJO in its initial stages are usually treated conservatively before surgical intervention: one of the main actions of intra-articular treatments, ranging from corticosteroids to hyaluronic acid and biologic products is to reduce inflammatory distress within the joint; injection treatments are usually used alone or in combination with thumb splint in a multimodal rehabilitation approach (8\9\10). In recent years, there has been a growing

interest in the effects of ozone (11), which can be safely delivered intra-articularly and whose use is in constant increase in an outpatient setting due to the ease of preparation methods.

1.1. Mechanism of Oxygen-Ozone therapy

Ozone (O3), a gas discovered in the mid-19th century, is a molecule consisting of 3 atoms of oxygen in a dynamically unstable structure due to the presence of mesomeric states (12). Intra-articular administration of an adequate mixture of oxygen-ozone is supposed to reduce pain, to have protective immunomodulatory effects on cartilage, and to reduce oxidative stress, thus potentially representing an alternative to other injective methods (13). Several researchers worldwide and many years of clinical experience have indicated that O3 has the capacity to modulate inflammation (3). O3 is highly reactive and, when injected into a joint capsule, it is able to stimulate fibroblastic joint repair, reduce inflammation, and may promote new cartilage growth (3). O3 produces acute oxidative stress with a paradoxical antioxidant effect: it has been shown that the controlled administration of O3 may promote an oxidative preconditioning or adaptation to oxidative stress that in turn stimulates the antioxidant endogenous system, finally resulting in a protective state against tissue damage (14). When injected into the trapeziometacarpal joint, O3 is mixed with oxygen (O2) and dissolves into the synovial fluid, which contains antioxidants and proteins, and generates reactive oxygen species and lipid oxidation products. These molecules inactivate and inhibit proteolytic enzymes, decrease the release of proinflammatory cytokines, induce the proliferation of chondrocytes and fibroblasts, and promote the synthesis of antioxidant enzymes and immunosuppressive cytokines (15). All these processes counteract the proinflammatory and pro-oxidative circuit that arises in TMJO, resulting also in an increase in tissue oxygen supply through a hemorheologic action based on vasodilatation and angiogenesis stimulation (12).

1.2. Clinical Experience

From the literature, it is widely accepted that ozone has the biological properties of inducing analgesia, anti-inflammatory, and antioxidant effects mediated by activating the cellular metabolism and inhibiting prostaglandin synthesis (16), reduce edema and inflammation (17\18\19\20), and therefore reduce pain and improves function in OA (11\17\21\22\23\24\25\26). Intra-articular oxygen-ozone has been used in the medical domain for several decades. Although some articles have reported promising results on the effectiveness of oxygen-ozone for example in knee or temporomandibular OA, the evidence is low (17).

Many authors claim ozone is a good treatment with high success rate, minor complications and easy execution, reproducibility and sustainable outcome (17); however is still unclear what best therapeutic protocol is in terms of ozone concentration, volume to be injected, number of injections and time between injections. Patients could recovery the hand functions, pinch and grip movements.

1.3. OOT machine Description

Certified equipment "Medical Device Directive 93/42/CEE and S.M.I. in IIA class" and compliant with S.I.O.O.T. protocols. MEDICAL 99 IR is a portable unit for oxygen-ozone therapy, made for exclusively medical and/or research use.

MEDICAL 99 IR ensures consistent and repeatable conditions of use and ensures maximum reliability. The oxygen-ozone mix generator is operated by a microprocessor, which controls and displays the ozone concentrations produced, available according to ten custom selections. The equipment allows the withdrawal of the gas mixture in a state of total safety, carefully monitoring the chosen concentration. MEDICAL 99 IR guarantees the absolute purity of the gas delivered.

1.3.1. Indications for Use

The oxygen-ozone is injected inside the TMJ for the treatment of osteoarthritis and associated symptoms.

1.3.2. Contraindications

Contraindications: favism, pregnancy, severe cardiovascular diseases, hematological and respiratory failure, cancer and thyreopaties.

1.4. Thumb Splint

It is a functional thermoplastic splint for the stabilizing of the TMJ, maintaining the pulp of the distal phalange of the index finger free for gripping with the other fingers and leaving the thumb in a functional position.

1.4.1. Indications and Usage

Splints for TMJO provide external support to the joint, thereby stabilizing the adjacent joints capable of compensatory movements and maintaining the first joint space.

1.4.2. Contraindications

Contraindications: discomfort regarding the use of the splint, venous or lymphatic stasis present in the relevant hand.

1.5. Use and Training

Appropriate investigative site personnel will be trained on OOT syringe processing and preparation of the splint. The investigator or other personnel who will be administering treatment should have the appropriate medical training to give intra-articular trapezio-metacarpal injections.

1.6. Current Study Rationale

The aim of this study is to compare the effectiveness of combined treatment with intra-articular ozone injections and joint splinting in comparison to only thumb splint therapy in patients with TMJO by evaluating the following aspects:

- Clinical improvement of TMJO
- Reduction of pain
- Improvement in functional aspects of the thumb
- Improvement in patient's quality of life

1.7. Study Objectives

The primary study objective is to determine whether two injections of OO and thumb splint are superior to the use of only the joint splinting in reducing pain.

1) The primary study endpoint is to establish the improvement in DASH score from baseline.

2) Secondary objectives of this study are:

- Improvement in VAS Pain,
- Improvement in Grip Strenght Function,
- Improvement in Tip Pinch Function.
- Improvement in the Kapandji Score.
- Improvement in the Nine-Hole Peg Test.

2. Investigational Plan

2.1. Study Design

This is a randomized, controlled, prospective evaluation comparing combined treatment with intra-articular ozone injections plus thumb splint with only the splint therapy. The maximum study duration for each subject will be 6 months. A total of 40 patients will be enrolled. These patients will meet specific inclusion and exclusion criteria, but can be generally characterized as patients with painful unilateral thumb OA who have been unable to achieve satisfactory pain relief with previous conservative OA treatment.

2.2. Eligibility Criteria

Inclusion criteria

1- Male or female at least 18 years of age at time of screening.
2- Ability to comply with study procedures and visit schedules and able to follow oral and written instructions.
3- Patients with symptomatic OA in one thumb joint from 3 months. If bilateral, the side with major symptoms will be considered.
4- A thumb joint radiograph showing a E-L grade of 1 to 3 and an absence of severe osteoarthritis (defined as advanced stage osteoarthritis, including large osteophytes, chronic fractures or bone remodeling, severe deformity or bone attrition, and/or bone-on-bone contact indicative of severe osteoarthritis/full thickness cartilage loss).
5- Has undergone at least one prior conservative OA treatment (e.g. physical therapy, simple analgesics).
6- Signed an ethics committee-reviewed approved informed consent form.

Exclusion criteria

1- Presence of clinically observed active infection or severe inflammation in the index trapezio-metacarpal joint or skin disease/breakdown or infection in the area of the planned injection site of the index thumb joint.
2- Diagnosed with rheumatoid arthritis, Reiter's syndrome, psoriatic arthritis, gout, ankylosing spondylitis, or arthritis secondary to other inflammatory diseases; Human Immunodeficiency Virus (HIV), viral hepatitis; chondrocalcinosis, Paget's disease, or villonodular synovitis.
3- Diagnosed with leukemia, known presence of metastatic malignant cells, or ongoing or planned chemotherapeutic treatment.
4- Disease of cervical spine, shoulder or other upper extremity joints judged by the investigator to be contributing to the pain in the index thumb joint (e.g. radiculopathy, De Quervain S., ecc).
5- Untreated symptomatic injury of the index hand (e.g., acute traumatic injury, tendinopathy, nerve lesion).
6- Presence of surgical hardware or other foreign body intended to treat arthritis or cartilage-related pathology in the index thumb joint.
7- Presence of venous or lymphatic stasis in the index arm.
8- Orally administered systemic steroid use within 2 weeks prior to screening
9- Planned/anticipated surgery of the index hand during the study period.
10- Major surgery of the index hand within 12 months prior to screening.
11- Minor surgery (e.g. arthroscopy) of the index hand within 6 months prior to screening.

12- Any documented clinically significant degree of cognitive impairment or other condition, finding, or psychiatric illness at screening, which, in the opinion of the investigator, could compromise patient safety or interfere with the assessment of the safety and treatment effects of the study injection.
13- Pregnant or nursing mothers or women planning to become pregnant during the time they will be participating in the study.
14- Previously documented failed treatment with OOT or splint
15- Known drug or alcohol dependence currently or within the last year.
16- Use of any investigational drug or device within 30 days prior to screening.
17- Use of any investigational biologics within 60 days prior to screening.

2.3. Restricted Medications/Nonpharmacological Therapies

Patients will be advised that participation in the study will require them to abstain from certain medications and therapies for the entire 1 month period following the procedure. **In case of pain, it's possible to take Paracetamol 1000 mg 1 tablet (maximum dose 2 tablets per day).** In contrast, the following drugs and non-drug therapies should not be taken or used immediately upon enrollment (after signing informed consent) for the full 1 month period:
- Oral NSAIDs
- Topical NSAIDs (including plasters and patches) applied to the index hand
- Other topical pain therapies applied to the index hand (e.g., capsaicin, lidocaine, heat patches)
- Orally administered systemic corticosteroids
- Narcotics
- Centrally acting medications for analgesia

After completion of Month 1 visit, patients are allowed to take any medication they need to use.
The following therapies should not be used beginning immediately upon enrollment (after signing informed consent) throughout the entire study follow-up:
- Intra-articular corticosteroids administered to the index hand
- Intra-articular HA administered to the index hand
- Other intra-articular therapy administered to the index hand
- Surgical procedure performed on the index hand

Subjects may utilize any medication they would like to use for concomitant OA treatment, unless listed above. General frequency and reason for use will be reported at each follow- up.

2.4. Study Assessments and Procedures

The table in Appendix A summarizes clinical study assessments, procedures, and information collected on case report forms (CRFs).

2.4.1. Screening Visit and Baseline Recording

Subject procedure flow is outlined in Appendix B.

2.4.1.1. General Pre-Screening

Patients with symptomatic OA in one trapezio-metacarpal joint, who have been unable to get satisfactory pain relief with prior treatment, will be pre-screened for eligibility. The investigator will keep a log listing all patients who are pre-screened for the study. Patients potentially meeting the inclusion and exclusion criteria will be asked to sign an informed consent form.

"Non-standard of care" test or procedure assessments specific to this Clinical Investigation may not be done before an informed consent has been signed.

2.4.1.2. Informed Consent

The patient must complete the informed consent process and sign and date the informed consent form prior to participation in this study, including completion of any non-standard-of-care procedures required for this Clinical Investigation. Designated center staff will explain the study purpose, data to be collected, how data will be used, time and travel commitments and other expectations of a study subject. The patient will be given the opportunity to discuss the study with the investigator, including any medical aspect of their disease and the study treatment. If the patient decides to participate, then he/she will sign and date the informed consent form along with the Investigator and other staff participating in the consent process; and the treatment visit will be scheduled.

A copy of the informed consent form will be given to the subject.

2.4.1.3. Confirm Eligibility

Screening assessments include:

- DASH questionnaire for index thumb joint
- X-Ray

2.4.1.4. Baseline Recording

During the screening visit, the following information will be obtained and recorded in the subject study record and CRFs for all subjects.

- Signed and dated original ICF

- Inclusion and exclusion criteria
- Demographics
- Relevant Medical history, baseline conditions and medication use

The following baseline assessments will be completed prior to treatment and after final eligibility confirmation (during injection visit and prior to the injection procedure itself).
- VAS
- Grip strength test
- Tip pinch strength test
- Kapandji Score
- Nine-Hole Peg Test

All preoperative procedures are to be completed within 28 days prior to the injection procedure.

2.4.2. Randomization

Randomization will occur through a module in the CRF. Once randomization is complete and a treatment arm is assigned.

Eligible subjects will be randomized in a 1:1 ratio to one of following two treatment arms:
- Subject device: OOT plus thumb splint
- Comparator device: thumb splint

Details regarding randomization can be found in Section 4.4.1.

2.4.3. OOT Preparation

Take a disposable syringe with a needle of adequate size for the area to be treated. The syringe must have a capacity of 3 cc. The syringe is placed on the ozone-oxygen mix generator through a sterile filter, is loaded and is ready for use.

2.4.4. Injection Procedure

Administer the contents of the syringe as a single injection into the joint. The procedure for the injection involves the following steps:

1. Clean the injection area with an antiseptic solution.
2. Apply a local anesthetic on the injection site (optional). If local anesthetic is used, then a topical anesthetic, such as ethyl chloride, is recommended. Under no circumstances may the anesthetic be injected intra-articularly.
3. Position needle into the intra-articular space and confirm needle placement with an ultrasound image (if ultrasound guidance is being used).

4. Attach an empty syringe to the needle, aspirate all available joint fluid (aspiration volume must be recorded).

5. Transfer the syringe that contains the injection solution to the needle positioned at the injection site.

6. Inject all contents of the syringe into the synovial space of the joint. (Take care not to inject extra-articularly or into the synovial tissues and capsule).

A clear ultrasound image is to be taken to document needle placement in the synovial space. Any AEs associated with joint aspiration and injection procedure will be recorded. Additionally, any device processing issues will be recorded. Before discharge subjects will be instructed not to exceed their pre-injection physical activity level for 14 days post-injection.

2.4.5. Splinting procedure

An expert physiotherapist will create two personalized thermoplastic splint for every patients for the stabilizing of the TMJ (one for the day that has a functional aim and one for the night with the objective of TMJ rest). The splint maintains the pulp of the distal phalange of the index finger free for gripping with the other fingers and leaving the thumb in a functional position. Splints for TMJO provide external support to the joint, thereby stabilizing the adjacent joints capable of compensatory movements and maintaining the first joint space. The two splint will be used for 2 months.

2.4.6. Follow-up Visits

Follow-up assessment visits will be at the following intervals following the injection:

1 Month (± 7 days)

3 Month (± 7 days)

6 Month (± 7 days)

The following assessments and information will be collected at follow-up visits:

- DASH (all follow-up visits)
- VAS (all follow-up visits)
- Grip strength test (all follow-up visits)
- Tip pinch strength (all follow-up visits)
- Kapandji score (all follow-up visits)
- Nine-hole Peg test (all follow-up visits)
- Medication use (During the first 6-Month follow-up period, information on all medication usage will be collected)
- AEs (During the first 6-Month follow-up period, all AEs have to be reported.)

The study follow-up visits consist of a structured telephone interview and electronic self-report. The investigative center will contact the subject to complete the Follow-up, Adjunct Therapies and AE forms remotely.

Use of certain pain medication must be discontinued 48 hours prior to assessments (with the exception of aspirin taken for cardio protection). Aspirin in low dose would not be enough to affect any pain sensations. In addition, the low dose aspirin is supposed to be taken every day when taken for cardio-protection, so they should remain consistent on their regimen throughout the study. **In case of pain, it's possible to take Paracetamol 1000 mg 1 tablet (maximum dose 2 tablets per day).**

2.5. Subject Discontinuation and Withdrawal

2.5.1. Subject Deregistration

Subjects assigned a clinical investigation subject ID, but not having a study treatment administered, are considered deregistered and will not be considered as part of the intent-to- treat population and may be replaced (In case the subject is de-registered, a study exit form should be completed).

2.5.2. Subject Discontinuation

Subjects will participate in the study until they complete the 6-months post treatment assessments. Subjects who do not complete the study per protocol may be exited from the study in the following manner:

<u>Subject Voluntary Withdrawal</u>

Subjects may withdraw their consent and discontinue participation in the study at any time for any reason or no reason, without jeopardizing their medical care. If a subject withdraws consent then it will be documented on the study exit form, and the reason for withdrawal will be requested.

Investigators must also report this to their respective Ethics Committee (EC) as defined by their institution's procedure and local EC requirements. No additional follow–up will be required and no additional data will be recorded from subjects once a subject has withdrawn from the trial. Subjects will not be replaced.

<u>Subject Lost-to-Follow-up</u>

In the event a subject cannot be contacted for post-treatment assessments, at least three attempts should be made to locate the subject and these efforts will be documented. Subjects will be

discontinued from the study if they are lost to follow-up. It should be understood that more than three attempts can be made, but three is the minimum required.

Investigator Withdrawal

Subjects may be withdrawn by the investigator prior to receiving an injection if, on the day of injection, it is determined by the investigator that the subject is no longer an appropriate candidate for injection (e.g., due to development of infection of the index joint) then the subject will be withdrawn from the study and will not receive the injection.

Subjects who are withdrawn from the study prior to receiving the index injection will be replaced. The investigator may decide to withdraw the patient from the study at any time based on medical judgment. In all instances of withdrawal, data collected up to the time of patient withdrawal may be used in the study. The site should complete a study exit form.

Endpoint Reached

Subjects who are not satisfied with the improvements in pain, function or stiffness following OOT or splinting treatment in the index thumb joint within 6 months and choose to seek additional treatment for their index trapezio-metacarpal OA will be discontinued from the study.

2.5.3. Trial Completion

A study exit form must be completed when:

- The subject withdraws from the clinical trial
- The subject is considered lost-to-follow-up per the above definition
- The subject did not received the index treatment due to disqualifying factors
- The investigator withdraws the subject
- Subject receives additional treatment for index thumb OA within 6 months.
- More than one cross-over of study treatment in index hand
- Subject receives invasive treatment for thumb OA other than one of the study treatments in index hand
- Upon trial follow-up completion (6-months follow-up time point has been reached)
- The subject has died

2.6. Description of Assessments and CRFs

All data to be collected is described below.

2.6.1. Demographics

Demographic information collected will include gender, age, height and weight.

2.6.2. Medical History

Information regarding systemic inflammatory conditions, currently active malignancy, pregnancy, lactation status, leukemia and current chemotherapeutic drug use will be obtained.

2.6.3. Medication Use

Information regarding the subject's medication use for treatment of thumb OA will be collected.

2.6.4. Patient Reported Outcome Measures

- Disabilities of the Arm, Shoulder and Hand (DASH):

The DASH questionnaire is a tool already validated and used for the assessment of the patient's disability related to the pathology of the upper extremity (in this case of the hand) in the control of normal actions of daily life. The questionnaire consists of 38 questions, for a possible score ranging from the value 0 to the value 100. A higher score indicates the greater severity of the degree of disability felt by the patient in relation to the pathology.

- Visual Analogic Scale: The VAS is a validated measure of knee pain. The NRS is an 11-point Likert type scale anchored by 0 "no pain" and 10 "worst possible pain". Subjects rate their average pain over the last 48 hours.

- Grip strength test e tip pinch strength test:

The Grip Strength Test and the Tip Pinch Strength Test are designed to measure the maximum isometric force exerted by the muscles responsible for flexion of the metatarsals and phalanges, flexion of the fingers and adduction of the thumb. The tests are carried out by means of a dynamometer to record the changes in force in the gripping and gripper movements.

- Kapandji Score:

The Kapandji score assesses the patient's ability to oppose the thumb, assigning a score based on the point of the hand they can touch.

- Nine-Hole Peg Test:

The Nine-Hole Peg Test is a test that uses 9 pegs and a board with 9 holes: the patient is asked to place the pegs one by one in the holes and then remove them and put them back in the container. The score is given by the time in seconds that the patient takes to perform the maneuver.

2.6.5. Imaging Assessments

2.6.5.1. Trapezio-metacarpal joint Radiograph

A standing posterior-anterior (PA) and lateral fixed radiographs of the index hand are acquired and

classified using the E-L grade by an expert radiologist.

2.6.5.2. Magnetic Resonance Imaging

MRI will be used to assess baseline comorbidities and will not be used to determine eligibility. Key comorbidities, including but not limited to subchondral fractures, subchondral bone cysts, micro fractures, ligament tears, and bone marrow lesions will be recorded.

2.6.6. Additional Case Report Forms

2.6.6.1. Adverse Events

AE forms will document a description of the AE, onset date and duration, severity, seriousness, treatment, and relatedness. The form will also document whether the event was anticipated or unanticipated.

2.6.6.2. Study Exit Form

This form will document termination of each subject's study participation and capture the reason for study exit.

2.6.6.3. Protocol Deviation Form

This form will document deviations from the study protocol.

3. Adverse Event reporting and management

All AEs reported to or identified by investigative center personnel occurring during the study period and after the treatment procedure will be documented. AEs will have the onset and resolution date(s) listed (when known), will have their management and outcome documented (where feasible), and will be assessed for severity, relatedness, and whether they were anticipated. SAEs will be described in a narrative in the final study report.

Anticipated AEs associated with the injection procedure include worsening of hand pain and/or function, effusion, and infection.

3.1. Definitions

<u>Adverse Event</u>

An AE is any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons whether or not related to the investigational medical device.

NOTE 1: This definition includes events related to the investigational device or the comparator.

NOTE 2: This definition includes events related to the procedures involved.

NOTE 3: For users or other persons, this definition is restricted to events related to investigational

medical devices.

Exceptions include events that are pre-existing (prior to injection) and have not changed in severity or intensity. If a pre-existing complication or event changes in severity or intensity then it should be reported as an AE.

Serious Adverse Event

A SAE is an AE that:

a) leads to a death,

b) leads to a serious deterioration in health of the subject, that either resulted in:

- Life-threatening illness or injury, or
- Permanent impairment of a body structure or a body function, or
- In-patient hospitalization[2] or prolongation of existing hospitalization, or
- Medical or surgical intervention to prevent life threatening illness or injury or permanent impairment to a body structure or a body function,

NOTE 1: Planned hospitalization for a pre-existing condition, or a procedure required by the clinical study protocol (without a serious deterioration in health) is not considered a SAE

Adverse Device Effect

AE related to the use of an investigational medical device.

NOTE 1: This includes any AE resulting from insufficiencies or inadequacies in the instructions for use, the deployment, the implantation, the installation, the operation, or any malfunction of the investigational medical device.

NOTE 2: This includes any event that is a result of a use error or intentional abnormal use of the investigational medical device.

The following are identified as possible examples of ADEs:

- Injection site reaction – swelling, redness, burning, itching at the injection site – to a degree that is atypical of an intra-articular aspiration and injection procedure
- Hand arthralgia – severe pain in the thumb joint
- Thumb joint ache – to suffer dull, continued pain
- Thumb joint inflammation – a localized protective reaction of tissue to irritation, injury, or infection—characterized by pain, redness, swelling, and sometimes loss of function

[2]Any admission over 24 hours will be considered as an in-patient hospitalization

- Thumb joint effusion – the escape of fluid from the blood vessels or lymphatics into the trapezio-metacarpal joint
- Trapezio-metacarpal arthrosis – degenerative disease of the thumb joint – beyond what is considered normal OA progression

Serious Adverse Device Effect

A Serious Adverse Device Effect (SADE) is an ADE that has resulted in any of the consequences characteristic of a SAE.

Unanticipated Serious Adverse Device Effect

An Unanticipated Serious Adverse Device Effect (USADE) is a SADE which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report.

NOTE: Anticipated SADE (ASADE): an effect which by its nature, incidence, severity or outcome has been previously identified in the risk analysis report

Device Deficiency

Inadequacy of an investigational medical device related to its identity, quality, durability, reliability, safety or performance. This may include malfunctions, use error, or inadequacy in the information supplied by the manufacturer.

3.2. Adverse Event Assessments

All AEs reported to or identified by investigative center personnel will be assessed by the investigator and recorded in the patient's study chart and on the AE form, including but not limited to the following:

- Observed or volunteered problems
- Physical signs and symptoms
- Medical condition that occurs during the study, having been absent at baseline
- Medical condition present at baseline that appears to worsen during the study

All AEs will be documented on the AE Form regardless of whether the medical/clinical event is associated with the use of the investigational device.

Each AE record must include a description of the event, date of onset, date of resolution (when known), severity, action taken, relationship to study device and seriousness criteria. Each AE must be recorded separately.

An outcome which may be expected to occur following any joint aspiration and injection procedure (e.g. transient pain at the injection site, mild swelling of the joint) should not be classified as an AE unless it is considered to be more severe or of longer duration or otherwise more pronounced than is typical.

Negative responses on follow-up questionnaires, which are intended to evaluate clinical efficacy, will not be recorded as AE. A worsening of index thumb OA (i.e. joint space narrowing observed on X-ray) which is considered by the investigator to be part of the normal progression of the disease will not be recorded as an AE.

The investigator using the following definitions will assess **severity**:

- Mild: aware of sign or symptom, but easily tolerated
- Moderate: discomfort enough to cause interference with usual activity
- Severe: incapacitating, with inability to work or do usual activity

The investigator will assess **relationship to the study device** for each AE using the following definitions:

1. Not related: the relationship to the device or procedures can be excluded when:

- the event is not a known side effect of the product category the device belongs to or of similar devices and procedures;
- the event has no temporal relationship with the use of the investigational device or the procedures;
- the event does not follow a known response pattern to the medical device (if the response pattern is previously known) and is biologically implausible;
- the discontinuation of medical device application or the reduction of the level of activation/exposure - when clinically feasible - and reintroduction of its use (or increase of the level of activation/exposure), do not impact on the event;
- the event involves a body-site or an organ not expected to be affected by the device or procedure;
- the event can be attributed to another cause (e.g. an underlying or concurrent illness/clinical condition, an effect of another device, drug, treatment or other risk factors);
- the event does not depend on a false result given by the investigational device used for diagnosis, when applicable;
- harms to the subject are not clearly due to use error;

In order to establish the non-relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the event.

2. Unlikely: the relationship with the use of the device seems not relevant and/or the event can be reasonably explained by another cause, but additional information may need to be obtained.
3. Possible: the relationship with the use of the investigational device is weak but cannot be ruled out completely. Alternative causes are also possible (e.g. an underlying or concurrent illness/clinical condition or/and an effect of another device, drug or treatment). Cases where relatedness cannot be assessed or no information has been obtained should also be classified as 'possible'.
4. Probable: the relationship with the use of the investigational device seems relevant and/or the event cannot be reasonably explained by another cause, but additional information may need to be obtained.
5. Related: the event is associated with the investigational device beyond reasonable doubt when:
the event is a known side effect of the product category the device belongs to or of similar devices and procedures;
- the event has a temporal relationship with investigational device use/application or procedures;
- the event involves a body-site or organ that
- the investigational device or procedures are applied to;
- the investigational device or procedures have an effect on;
- the event follows a known response pattern to the medical device (if the response pattern is previously known);
- the discontinuation of medical device application (or reduction of the level of activation/exposure) and reintroduction of its use (or increase of the level of activation/exposure), impact on the serious event (when clinically feasible);
- other possible causes (e.g. an underlying or concurrent illness/clinical condition or/and an effect of another device, drug or treatment) have been adequately ruled out;
- harm to the subject is due to error in use;
- the event depends on a false result given by the investigational device used for diagnosis, when applicable;
- In order to establish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the event.

Seriousness: If the AE meets any of the SAE criteria mentioned in section 4.1 it is regarded as serious.

3.3. Reporting Procedures

3.3.1. Adverse Event Reporting

Index Procedure and during 6-month Follow-up

All AEs that occur either during the injection procedure or during the 6-month follow-up phase of the study will be documented as described above as soon as possible upon knowledge of the event.

3.3.2. Investigator responsibilities

Independent Ethics Committees Reporting

Report to the IEC by The principal investigator(s) must report to the IEC all reportable events, as required by the clinical study protocol or by the IEC.

4. Risk evaluation

4.1. Potential Risks to Study Subjects

Subjects in this study are exposed to potential risks associated with aspiration of joint fluid, and solution injection into the thumb joint. These include pain, bleeding, bruising, infection, deep venous thrombosis, scar tissue formation, thrombotic complications and nerve damage. These risks are not unique to this study and may occur with any aspiration, or joint injection procedure. The use of joint splinting could create discomfort, venous or lymphatic stasis present in the relevant hand.

4.2. Investigation Suspension or Termination

The Investigator, the IEC, may suspend or terminate the investigation at any/all investigational sites at any time if they believe:

- There is unacceptable risk to the subjects enrolled in the study
- Failure of the investigator to enroll patients into the study at an acceptable rate
- Failure of the investigator to comply with the protocol or appropriate regulations, especially with respect to subject safety
- Site personnel knowingly submit false information from the investigative site to the study monitor, or IEC.

4.3. Potential Benefits of the Procedure

The potential benefit of OOT and joint splinting for the treatment of thumb OA includes symptomatic pain relief, hand function restoration, and anatomical improvement within the joint.

4.4. Bias Minimization

4.4.1. Randomization

The randomization plan will be produced using SAS v 9.4 or similar software. Balanced randomization with random block sizes (1:1, OOT plus splint:only splint) will be implemented. In the event that, post-randomization, no study treatment was given, randomization will not be reassigned and in this case will not count toward the overall sample size. Randomization will continue with the next case enrolled until the minimum sample size of 20 subjects is reached in both treatment groups. Subjects that have signed informed consent and are in screening process when the minimum study sample size is reached, will be randomized and treated per protocol provided that they found to be eligible. The enrollment activities will be monitored closely to limit number of these subjects. Randomization will be stratified by site, and each site will receive separate randomization plans using random predetermined block sizes that will remain undisclosed to the sites. The randomization file will be uploaded into the electronic data capture (EDC) system. Once the subject is enrolled into the EDC and has been identified as eligible for randomization, the randomization allocation will be visible within the EDC and viewable only by the un-blinded research associate. The subject will be treated according to the contents of the displayed randomization allocation.

5. Data collection, handling and retention

5.1. Source Documentation

Source documentation for this study will be maintained to capture the course of treatment and to substantiate trial data integrity. Source documentation will include, but is not limited to, worksheets, hospital and/or clinic or office records documenting subject visits including study procedures and other treatments or procedures, medical history and physical examination information, imaging results, device accountability records, medical consultations and laboratory results and reports.

5.2. Case Report Forms

Data for this clinical trial will be collected and documented on the subject's CRFs. CRFs provided may appear in paper or electronic form. Only authorized study site personnel or subjects will complete CRFs as appropriate to the specific CRF. CRFs must be reviewed and signed by the investigator or their documented designees. This may be done electronically within the electronic

data capture system. Because there is a potential for errors, inaccuracies, and misinterpretation in the process of transcribing data onto CRFs (whether paper or electronic), the following documents must be available at all times for inspection and comparison to the CRFs by the study monitor: data query forms, originals or photocopies/certified copies of all relevant records and reports, copies of test results and reports.

5.3. Records Retention

All CRF information, study records, reports and source documents that support the CRF must be retained in the files of the responsible investigator for a minimum 2 years following notification by designee that all investigations have been completed, and will further be retained in accordance with local and international guidelines as identified in the Investigator Site Agreement.

6. Reporting

6.1. End of Study Report

The investigator should notify the IEC in writing within three months after completion, termination, or discontinuation of the study at the site. The same procedure will be applied to regulatory authorities where required.

7. Statistical analysis

A complete Statistical Analysis Plan (SAP) will be finalized prior to database lock and un-blinding. All statistical analyses will be performed according to valid, prospectively defined methods. Briefly, key planned analyses are described below.

7.1. Sample Size Calculation

The sample size is calculated on the basis of the study: Heyworth BE, Lee JH, Kim PD, Lipton CB, Strauch RJ, Rosenwasser MP. Hylan versus corticosteroid versus placebo for treatment of basal joint arthritis: a prospective, randomized, double-blinded clinical trial. J Hand Surg Am. 2008;33(1):40-48. doi:10.1016/j.jhsa.2007.10.009. In designing this injective clinical trial, a power analysis was performed to determine the sample size required to detect differences the size of 1 SD in the DASH outcome measure. A calculation of 16 patients per group was shown to demonstrate 80% power and a 5% 2-tailed type I error rate to detect DASH differences seen in previous studies investigating treatment of basal joint arthritis. To safeguard against loss to follow-up and other

unexpected variances, we determined that approximately 20 patients per group (40 patients in total) were needed to observe these clinically relevant differences.

7.2. Efficacy Analysis

Data were described as number and percentage, if categorical, or mean and standard deviation, if continuous, unless otherwise specified. Differences were described as mean, standard deviation and 95% confidence interval. Baseline characteristics were presented for the whole sample, while test values over time were presented only for patients who weren't lost to follow up.

Differences between groups were explored with chi square test, with Fisher correction if necessary, for categorical variables, or with Student t-test for continuous variables approximately Gaussian, or with Mann Whitney test otherwise. Adherence to Gaussian distribution were verified with Shapiro Wilks test.

About primary outcome, DASH and NRS were also described as percentage of patients reaching the minimal importance difference of 10.83 and 2 points, respectively.

Significance threshold was set to 0.05, all analyses were made with Stata version 18.

7.3. Demographics and Medication Use

Subject demographics and medication use will be summarized and thoroughly characterized with descriptive statistics including error measures. Categorical variables will be summarized using frequency and percentage. Continuous variables will be summarized using mean, median standard deviation, minimum, and maximum. To assure that randomization was successful the following tests will be conducted; (1) age and baseline DASH comparisons of OOT and splint groups will be tested using two- tailed independent sample t-tests, (2) gender distribution will be tested using a Fisher's Exact test, and (3) Race distribution will be tested using a Likelihood-Ratio Chi-square test. Each of these three tests will be conducted using alpha = 0.05.

7.4. Subject Reported Outcomes

Subject reported outcome measures: DASH, VAS, Grip Strength Test and Tip Pinch Strenght test, Nine-Hole Peg Test, and Kapandji Score will be summarized and thoroughly characterized with appropriate descriptive statistics including error measures. Categorical variables will be summarized using frequency and percentage. Continuous variables will be summarized using mean, median standard deviation, minimum, and maximum.

These measures will also be analyzed using inferential statistics. Planned exploratory analyses, along with associated statistical tests, are described in the SAP.

7.5. Safety Analysis

The safety profile of OOT will be characterized by summarizing AEs, which will be standardized using the Medical Dictionary for Regulatory Activities. Characterization will include narratives of all SAEs and descriptive statistics including AE incidence overall and per subject, AE severity, AE device relatedness, and AE onset for each treatment group. AEs will also be used to characterize the safety of a repeat OOT injection. Results will be tabulated by treatment group.

8. Study monitoring and quality control

The investigational sites will be monitored to ensure compliance with the trial protocol, adherence to applicable regulations, and accuracy of trial data. Monitoring visits will be conducted primarily to ensure that the safety and wellbeing of the subjects is preserved. Monitoring visits will also be used to verify that trial data submitted on case report forms are complete and accurate with respect to the subject medical records and to verify device accountability. The principal investigator, his/her delegate(s) and the study coordinator(s) shall be accessible during monitoring. Accessibility is of particular importance for reviewing data in the CRF. Site personnel will complete CRFs following each subject visit. Trial data submitted will be reviewed against patient charts and other sources containing original patient records. As needed, intermittent monitoring visits will be conducted to compare data entered into the database to a selected sample of the subjects' source documents. Monitoring efforts will be expanded where the sample suggests irregularities.

Prior to the first site activation, a Monitoring Plan will be established outlining the activities above, as well as management of trial materials supplied to sites.

9. Ethical and legal considerations

9.1. Regulation Statement

This protocol has been developed and the study will be run in accordance with the ethical principles that have their origin in the Declaration of Helsinki (2013). Further, the study will be run in compliance with ISO 14155 (2nd ed.) 2011. Investigators will conduct this study in accordance with all applicable local or regional laws and regulations. Investigators will neither conduct procedures specific to the study nor collect subject data without prior approval or favorable opinion of any IEC under whose jurisdiction the conduct of this study falls. During the conduct of study activity, the investigators shall act in accordance with any further requirements as imposed by an IEC, IRB or other regulatory agency.

9.2. Informed Consent Procedure

Prior to the collection of any study specific data, the patient must complete the informed consent process and sign and date the informed consent form. The informed consent may be approved by an IEC, and is subject to local laws and regulations. Designated site staff will explain to the potential subject the study purpose, data to be collected and how it will be used, time and travel commitments and other expectations of a study subject. The patient will be given adequate time to consider participation in the study and review the informed consent form.

Any new information or protocol amendment that substantially alters the scientific validity of the study or affects the subjects' rights, safety, welfare, or their willingness to continue participation in the study, will result in re-consenting of currently active patients on an updated and approved ICF (as required by local law and regulation).

9.3. Protocol Amendments

If a protocol amendment substantially alters the scientific validity of the study or affects the subjects' rights, safety, welfare, or their willingness to continued participation in the study, then the subject should be re-consented on an updated and approved ICF(as required by local law and regulation). New procedures or processes which substantially alter the scientific validity of the study or affects the subjects' rights, safety, welfare, or their willingness to continued participation the study will not be implemented until an approval or favorable opinion has been granted by the reviewing IEC.

9.4. Subject Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Only the subject number will be recorded in the CRF. Study findings stored on a computer will be stored in accordance with local data protection laws. As part of the informed consent process, the subjects will be informed in writing that representatives of IEC or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws. If the results of the study are published, then the subjects' identity will remain confidential. The investigator will maintain a list to enable subjects to be identified.

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Appendix A – Study Schedule

Assessments			Post Treatment follow-up[1]			
	Screening	Injections[2] (1\week for 2 weeks) and/or Splint (2 months)	1 Month	3 Month	6 Month	Study Exit
Visit window			± 7 days	± 7 days	± 7 days	
Informed Consent	X					
Eligibility	X					
Demographics	X					
Radiographs	X					
Follow-up			X	X	X	
Medication Use	X	X	X	X	X	
DASH	X		X	X	X	
VAS		X[3]	X	X	X	
Grip strength and Tip pinch strength test	X		X	X	X	
Kapandji Score and Nine-Hole Peg Test	X		X	X	X	
Adverse Events		❑	❑	❑	❑	❑
Study Exit Form		☐	☐	☐	☐	X

1: Follow-up assessments may consist of a structured telephone interview, electronic self-report or, if standard of care, an office visit. 2: The first injection should take place within 28 days of completed screening. 3: Questionnaires should be obtained prior to injection procedure

Appendix B – Subject Procedure Flow