CLINICAL INVESTIGATION PLAN
ABOUT MEDICAL DEVICE
"MEDANTRO PILOT STUDY"

TITLE: Evaluation of the efficacy of the use of a Type I Collagen medical device (MD Tissue *Collagen Medical Device*) in the infiltrative treatment of the Grand Trochanter Pain Syndrome. "MEDANTRO PILOT STUDY".

IDENTIFICATION CODE: MDG2020711

EUDRACT: NA

CLINICAL INVESTIGATION PLAN VERSION AND DATE: n° 2.5 del 08 January 2021

NUMBER OF PAGES n° 25

This document contains confidential information belonging to GUNA S.p.a. Milan, Italy and the Gaetano Pini Orthopaedic Institute of Milan.
The information contained in this document may not be made available to or used by third parties without the written permission of GUNA S.p.a. and the Gaetano Pini Orthopaedic Institute of Milan, Italy.
Only investigators participating in this Clinical Investigation are excluded from these restrictions.

INDEX

1 SYNOPSIS

2 LIST OF ABBREVIATIONS

3 INTRODUCTION

3.1 Tendon Tissue

3.2 The Great Trochanter's Pain Syndrome

2.3 Preclinical Studies

4 SCIENTIFIC REQUIREMENTS

4.1 Research Title

4.2 Purpose of the research

4.3 Design of the Investigation

4.4 Duration of the investigation

4.5 Participating population

4.6 Enrolment

4.7 Inclusion criteria

4.8 Exclusion criteria

4.9 Coding of subjects

4.10 Procedures

4.10.1 Evaluation Times

4.10.2 Visit Plan

4.11 Materials needed to conduct the Clinical Investigation

4.12 Study groups

4.13 Infiltrative technique

4.13.1 Primary End Point

4.13.2 Secondary End Points

4.14 Data collection

4.15 Early Termination of the Investigation

5 STATISTICS

5.1 Sample size

5.2 Statistical Analysis Plan

5.2.1 Descriptive statistics

5.2.2 Primary endpoint

5.2.3 Secondary endpoints

6 ETHICAL REQUIREMENTS

6.1 Ethical authorisations

6.2 Amendments to the Investigation Plan

6.3 Informing the Subject

6.4 Adverse Events

6.5 Protection of Privacy

6.6 Data Access

6.7 Data Ownership

6.8 Data Processing and Publication

6.9 Financing

6.10 Code of Ethics

6.11 Subject Insurance

7 PREMATURE TERMINATION OF THE CLINICAL INVESTIGATION

8 GLOSSARY

9 ANNEXES TO THE CLINICAL INVESTIGATION PLAN

10 BIBLIOGRAFY

1. SYNOPSIS

TITLE	Evaluation of the efficacy of the use of a Type I *Collagen medical device* (MD-Tissue *Collagen Medical Device*) in the infiltrative treatment of Great Trochanter Pain Syndrome. "MEDANTRO PILOT STUDY''.
STUDY DESCRIPTION	Single-centre pilot clinical investigation based on a one-sample design. Variables will be assessed at 6 different times: at baseline (day 0), after week 1, weeks 2, weeks 6, weeks 10 and after weeks 24, i.e. 6 months after the start of the Clinical Investigation.
OBJECTIVES	The objective of this study is to evaluate the efficacy of the experimental treatment in terms of reduction of referred pain in the peritrochanteric area of the hip affected by GTPS.
PRIMARY ENDPOINT	The primary endpoint will be assessed at week 10 and will include: • Assessment of the NRS score at weeks 10 compared to time day 0. It can be considered clinically significant if at least 50% of the treated subjects show a reduction of at least 3 points on the NRS scale.
POPULATION	A total of 49 subjects with GTPS will be enrolled.
SELECTION CRITERIA	Inclusion criteria • male and female subjects aged between 18 and 70 years; • subjects with lateral palpatory pain that has appeared for at least 1 month; • subjects with pain symptoms of the hip assessed according to the Numerical rating scale (NRS) ≥ a 5; • subjects who are able to cooperate with the assessments in the investigation plan; • subjects able to understand and sign the informed consent. Exclusion criteria • subjects suffering from true coxalgia (with positive FADDIR) • subjects suffering from ESHS (External Snap Hip Syndrome); • subjects who have already undergone candidate hip replacement surgery; • subjects with radiological and clinical evidence of detachment of the tendons of the lesser and/or middle gluteus with an

	indication for surgical repair; • subjects with evidence of radiographically documented tendon calcifications; • subjects with a degree of coxarthrosis of the hip that is a candidate for treatment according to the Tonnis>1 classification • subjects who have taken fluoroquinolones within 30 days prior to enrolment • subjects who have undergone treatment with hyaluronic acid or corticosteroids in the hip candidate for infiltrative treatment within 4 weeks prior to enrolment • subjects with local or systemic infections of the candidate hip, osteomyelitis or sepsis; • subjects undergoing chronic treatment with corticosteroids or immunosuppressants. • subjects who are drug addicts, alcoholics, suffer from psychiatric disorders or have clinical conditions that may compromise the correct interpretation of PROMs or follow-up; • subjects with coagulopathies, platelet aggregation disorders or those on oral anticoagulants or antiplatelet agents that cannot be discontinued during the study period • pregnant and lactating subjects (female subjects of childbearing age must be tested for pregnancy prior to enrolment) • subjects with an allergy to porcine collagen.
PHASE OF THE STUDY	Fase IV post market
NUMBER OF CENTRES	1 Experimental Centre.
DESCRIPTION OF THE MEDICAL DEVICE UNDER STUDY	MD-Tissue *Collagen Medical Device* is an injectable medical device based on porcine collagen type I; the collagen content is 100µg/2mL.
STUDY DURATION	The total duration of the study will be 10 months. There will be a 4-month subject selection and recruitment period and a 24-week treatment and observation period.
STUDY DURATION PER SUBJECT	The duration of the study for each subject will be in weeks 24.

2. ABBREVIATIONS LIST

AE	Adverse Event
COL-1	Collagen Type I
E-CRF	Electronic Data Collection Form
ECM	Extra Cellular Matrix
ESHS	External Snap Hip Syndrome
ESWT	Extracoroporeal Shock Wave Therapy
FADDIR	Flexion adduction internal rotation
NSAID	Non-steroidal anti-inflammatory drugs
GCP	GCP Good Clinical Practice
GTPS	Painful syndrome of the greater trochanter
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
mRNA	Messenger RNA
MD	Medical Device
mHHS	Modified Harris Hip Score
MMP-1	Matrix Metalloproteinase Type 1
NRS	Numerical rating scale
PRP	Platelet-rich plasma
MRI	Nuclear Magnetic Resonance
RT-PCR	Reverse transciptase-polymerase chain reaction
SAE	Serious Adverse Event
SUSAR	Suspected Serious Adverse Reaction
TIMP-1	Tissue inhibitor metalloproteinase type 1

3. INTRODUCTION

3.1 Tendinous tissue

Tendons are specialised organs that interpose themselves between muscle and bone, mediating the transmission of forces generated by muscles, thus enabling movement. They are mainly composed of specialised fibroblasts, tenocytes and extracellular matrix (ECM).

The complex microenvironment of the ECM, which surrounds and supports the cellular component of the tissue, specifically consists of three main classes of macromolecules: structural proteins (collagen and elastin), specialised proteins (fibrin and fibronectin) and proteoglycans (1).

The correct homeostasis and quantitative relationship between the cellular components of the ECM, as well as the reciprocal interaction between tenocytes and the ECM, are essential for maintaining the correct biomechanical properties of tendons (2). In fact, an alteration of the ECM may be consequent to changes in tenocyte activity, and, reciprocally, changes in the ECM may induce alterations in the tenocyte cell phenotype such as proliferation, migration and apoptosis (3)

Collagen is the main component of the tendon ECM. Although collagen type I (COL-1) is the major determinant of the tendon's biomechanical properties, comprising approximately 65-80% of its dry mass and nearly 95% of the total collagen in normal tendon (4), other types of collagen have been shown to occur in small amounts such as collagen types II, III, IV, V, VI, IX, X, XII, and XIV (Evans et al., 1998; Waggett et al., 1998, Riley, 2005).

Collagen fibres organise into bundles that form the main structural units, aligned along the length of the tendon, giving the tendon the property of resisting tensile forces. The expression of COL-I changes in relation to the tensile load applied to the tendon. The ability of collagen molecules to assemble into fibres by forming cross-links is an important requirement for the development of strength in this tissue, for stabilising the fibres themselves, and for increasing tensile strength.

The maintenance and turnover of the ECM of the tendon are therefore fundamental to the ability of this organ to resist mechanical forces and to respond to damage caused by pathological conditions (5); some authors suggest an alteration in the balance between ECM synthesis and degradation as a determinant of tendon deterioration and degeneration (3).

3.2 Greater Trochanteric Pain Syndrome

Greater Trochanteric Pain Syndrome, also known as GTPS (Greater Trochanteric Pain Syndrome) is a complex clinical condition characterised by chronic and recurrent pain in the lateral region of the hip, near the greater trochanter of the femur.

In the past, GTPS was exclusively attributed to a state of inflammation of the bursae of the peri-trochanteric space, so much so that the term 'trochanteric bursitis' was confused and identified with it.

More recently, efforts to further study the aetiopathogenesis of lateral hip pain have led to the delineation and more accurate definition of GTPS.

This syndromic condition manifests itself as the result of biomechanical and anatomo-histological alterations of the structures of the peritrochanteric space, in which, given the close anatomo-functional relationships, the origin can be traced back to three different pathological entities that can influence each other and feed the progressive exacerbation of the symptomatology. These are: external trigger hip, trochanteric bursitis and tendinopathies of the tendons of the gluteus medius and gluteus minimus muscles.

Recent studies concerning GTPS (6)(7) have shown that in most cases, this condition is due to a degenerative tendinopathy of the tendons of the gluteus minimus and gluteus medius muscles. Tendinopathy is defined as a pathological condition associated with histological changes that may result in a change in the organisation of collagen fibrils, a relative increase in the percentage of proteoglycans, glycosaminoglycans and non-collagenous components of the ECM accompanied by neo-vascularisation and inflammatory status (8). Tendinopathies thus lead to painful symptoms that very often also result in biomechanical functional deficits.

Clinically, GTPS presents as a pain that is often debilitating and exacerbated by activities such as walking, climbing stairs and lying on the affected side at night, associated with a progressive loss of stenia in hip abduction movements. On objective examination, there is a point of tenderness (trigger point) at the level of the greater trochanter region, which may radiate to the lumbar region and along the lateral aspect of the thigh, up to the ipsilateral knee, and a difficulty on strength versus resistance tests in hip abduction movements.

The diagnosis is predominantly clinical; a highly predictive sign is pain on trochanteric palpation ("Jump Sign"), often associated with the positive Single Leg Stance Test and the positive Ober's Test, which are secondary clinical tests aimed at identifying the aetiopathogenesis. Imaging may be used in the second instance to rule out other pathologies and confirm the diagnosis. Nuclear Magnetic Resonance Imaging (MRI) in particular can highlight the inflammatory state of peritrochanteric soft tissue. (9)

Although it is a very common syndrome(7), the treatment of painful gran trochanter syndrome, as well as that of tendinopathies in general, still represents a major obstacle because the specific cellular pathogenetic and biomechanical etiopathogenetic mechanisms are still partly unknown and many treatments are empirical (10). Traditionally, the treatment of GTPS is initially conservative and includes rest, ice, NSAIDs and physiotherapy with stretching exercises of the fascia lata (10)(11). The use of corticosteroids, with systemic or local infiltration, for the treatment of tendinopathies is highly controversial and in any case does not appear to be effective in the long term (10). Some authors have reported good results in the use of Extracoroporeal Shock Wave Therapy (ESWT) but no Level I study has been published in the literature (11). As far as biological treatments are concerned, a number of studies have been conducted in recent years with particular interest in the use of PRP (Platelet Rich

Plasma) infiltration; however, currently only one study has reported good results at 12 weeks (in terms of pain improvement and according to the mHHS, in comparison with a single corticosteroid injection) (12)(13) (16) (17) (18). In another study, promising results were reported to support the use of injected autologous tenocytes (14). Among the more invasive treatments, surgical release of the ITB and bursectomy of the trochanteric bursa appear to be indicated in particular cases. (14)

3.3 Preclinical studies

In an ex vivo study (15), the effects of MD-Tissue *Collagen Medical Device* (GUNA S.p.a. Milan, Italy) on a culture of human gluteal tenocytes were described, focusing in particular on the collagen turnover pathways, in order to understand how this medical device can influence tendon biology.

MD-Tissue *Collagen Medical Device* is an injectable medical device based on porcine collagen type I; the collagen content is 100µg/2mL. Porcine collagen is similar to human collagen and highly compatible; it has a very low risk of inducing adverse effects and is therefore used in several clinical settings. In the ex vivo study, MD-Tissue *Collagen Medical Device* was used as a substrate for cell cultures of human gluteal tenocytes on culture plates. The results suggest that MD-Tissue is able to induce an anabolic phenotype in the tenocytes by stimulating their proliferation and migration; it would also be able to promote the synthesis, maturation and secretion of COL-I, thus promoting tendon homeostasis and repair. In particular, the modification of gene expression and proteins involved in collagen turnover pathways were analysed by real-time PCR, Slot blot and SDS-zymography. The data of the study showed that tenocytes cultured with MD-Tissue, compared to controls, showed increased secretion and migration of COL-1 and increased mRNA levels of the matrix metallopreotease inhibitor proteins MMP-1 and TIMP-1.

The tenocytes used for the cell cultures were gluteal tenocytes, derived from human gluteal tendon fragments (obtained from subjects without any tendon pathology, who had undergone total hip replacement surgery), which is why it is reasonable to think that the porcine collagen type I compound could be a valid treatment in GTPS. (15)

4. SCIENTIFIC REQUIREMENT

4.1 Title of the Clinical Investigation

Evaluation of the efficacy of the use of a Type I Collagen medical device (MD-Tissue *Collagen Medical Device*) in the infiltrative treatment of Great Trochanter Pain Syndrome. "MEDANTRO PILOT STUDY''.

4.2 Purpose of the research

The results of the preclinical study suggest that MD-Tissue *Collagen Medical Device* can induce an anabolic phenotype in tenocytes by stimulating tenocyte proliferation and the synthesis, maturation and secretion of COL-I, thus promoting tendon repair. As these effects have been evaluated ex vivo on

tenocytes of gluteal muscle tendons, the aim of this study is to evaluate its efficacy in the local infiltrative treatment of GTPS in the peritrochanteric region, in terms of resolution of pain symptoms and recovery of abduction fatigue.

4.3 Disign clinical investigation

This pilot single-centre clinical investigation will be based on a one-sample design. Variables will be assessed at 6 different times: at baseline (T0 time), after week 1, weeks 2, weeks 6, weeks10 and after 24 weeks the start of the Clinical Investigation.

4.4 Duration of the clinical investigation

The total duration of the study will be 10 months. There will be a 4-month subject selection and recruitment period and a 24-week treatment and observation period. (see figure 1).

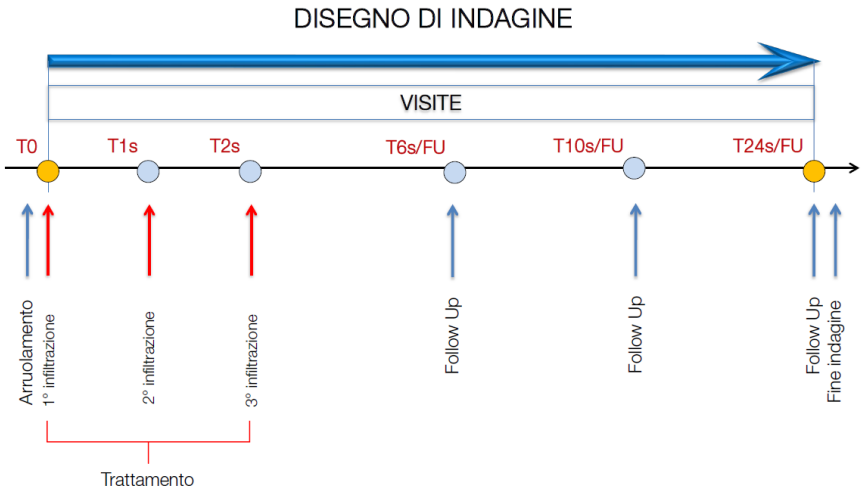

Fig.1 Flow chart of the clinical Investigation

4.5 Participant population

A total of 49 GTPS subjects will be enrolled. The recruitment phase will be closed no earlier than the number of subjects envisaged in the Clinical Investigation Plan has been reached.

Only subjects will be included in the two experimental groups:

- belonging to the U.O.C. 1st Orthopaedic Clinic (Gaetano Pini Orthopaedic Institute, Milan);
- who meet the inclusion criteria and have no exclusion criteria.

4.6 Recruitment

Subjects will be enrolled after approval of the Clinical Investigation Plan by the relevant Ethics Committee of the ASST Istituto Ortopedico Gaetano Pini and notification of the study at the Ministry of Health.

Enrolment will involve subjects affected by GTPS, eligible according to the selection criteria. The diagnosis of GTPS will be made by the Principal Investigator through clinical examination and instrumental investigation of pelvis MRI for hips. The MRI examination must be recent and of good

quality; if the subject does not have one, an MRI examination will be performed prior to diagnosis and enrolment. Eligible subjects will be explained by a Co-Investigator the rationale of the Investigation Plan and the procedures involved. Consent to participate in the study will then be sought.

4.7 Inclusion Criteria

- male and female subjects aged between 18 and 70 years;
- subjects with lateral palpatory pain that has appeared for at least 1 month;
- - subjects with pain symptoms of the hip assessed according to the Numerical rating scale (NRS) ≥ a 5;
- subjects who are able to cooperate with the assessments in the investigation plan;
- Subjects able to understand and sign informed consent.
- (NB. Subjects with bilateral hip pain symptoms will be included for the more symptomatic side and treated monolaterally).

4.8 Exclusion Critera

- subjects suffering from true coxalgia (with positive FADDIR)
- subjects suffering from ESHS (external snapping hip syndrome);
- subjects who have already undergone hip replacement surgery as a candidate for treatment;
- subjects with radiological and clinical evidence of detachment of the tendons of the lesser and/or middle gluteus with an indication for surgical repair;
- subjects with evidence of radiographically documented tendon calcifications;
- subjects with a degree of coxarthrosis of the hip that is a candidate for treatment according to the Tonnis>1 classification
- subjects who have taken fluoroquinolones within 30 days prior to enrolment
- subjects who have undergone treatment with hyaluronic acid or corticosteroids in the hip candidate for infiltrative treatment within 4 weeks prior to enrolment
- subjects with local or systemic infections of the candidate hip, osteomyelitis or sepsis;
- subjects undergoing chronic treatment with corticosteroids or immunosuppressants;
- subjects who are drug addicts, alcoholics, suffer from psychiatric disorders or have clinical conditions that may compromise the correct interpretation of PROMs or follow-up;
- subjects with coagulopathies, platelet aggregation disorders or those on oral anticoagulants or antiplatelet agents that cannot be discontinued during the study period
- pregnant and lactating subjects (female subjects of childbearing age must be tested for pregnancy prior to enrolment)
- subjects with an allergy to porcine collagen.

4.9 Coding of the subjects

At the time of enrolment, each subject shall be assigned a complex alphanumeric identification code consisting of the progressive enrolment number followed by an alphanumeric code generated by GUNA S.p.a. through *Random Sequence Generator* (www.random.org).

The complex code complies with the current Privacy Law on the coding of subjects participating in clinical trials.

4.10 Procedures

4.10.1 Evaluation Time

T0 baseline: Enrolment and start of infiltrative treatment

Week T1: Second infiltrative treatment

T2 weeks: Third infiltrative treatment

T6 weeks/FU Follow-up visit

T10 weeks/FU Control visit

T24 weeks/FU Follow-up visit

4.10.2 Visit Paln

T0 BASAL: Enrolment

In the course of daily clinical activity, investigators participating in the Clinical Ivestigation Plan will select subjects with GTPS. The Principal Investigator will be responsible for the correct clinical diagnosis, also making use of the instrumental examinations provided for in the Investigation Plan. Subjects meeting the inclusion and exclusion criteria will thus be identified. After having thoroughly explained the aims, purposes and procedures foreseen by the Investigation Plan, the subject will be proposed to participate in the Investigation itself. Finally, the selected subject will be asked to read the information form and to date and sign the Informed Consent and Consent to the Processing of Personal Data form.

Female subjects will have to provide evidence of a negative pregnancy test in order to be enrolled.

We will then proceed with the objective examination and the collection of the information required by the electronic Data Collection Form appropriately prepared.

This will be followed by:

- pain assessment using the NRS scale;
- assessment of pain using the Modified Harris Hip Score (mHHS) scale;
- clinical assessment of pain on palpation of the trochanter using the NRS scale;
- clinical assessment of stenia in abduction of the lower limb with the dynamometer;
- clinical assessment of phlogistic-degenerative signs by MRI of the peritrochanteric region;

The first infiltration will then be performed by means of ultrasound.

Previous or ongoing pharmacological treatments of any kind will be documented.

In order to monitor the consumption of analgesic (celecoxib 200mg / paracetamol 1000 mg) used during the investigation in the event of the onset of pain, a clinical diary will be given to the subject in which to indicate the day and the dose of drug used.

The enrolled subject will be assigned an identification code of a complex type formed by the progressive enrolment number followed by an alphanumeric code generated by GUNA S.p.a. through *Random Sequence Generator* (www.random.org.)

T0 BASAL: Enrolment

In the course of daily clinical activity, investigators participating in the Survey will select subjects with GTPS. The Principal Investigator will be responsible for the correct clinical diagnosis, also making use of the instrumental examinations provided for in the Investigation Plan. Subjects meeting the inclusion and exclusion criteria will thus be identified. After having fully explained the aims, purposes and procedures foreseen by the Investigation Plan, the subject will be proposed to participate in the Investigation itself. Finally, the selected subject will be asked to read the information form and to date and sign the Informed Consent and Consent to the Processing of Personal Data form.
Female subjects will have to provide evidence of a negative pregnancy test in order to be enrolled.
We will then proceed with the objective examination and the collection of the information required by the electronic Data Collection Form appropriately prepared.
This will be followed by:
- pain assessment using the NRS scale;
- assessment of the Modified Harris Hip Score (mHHS) scale;
- clinical assessment of pain on palpation of the trochanter according to the NRS scale;
- clinical assessment of stenia in abduction of the lower limb with the dynamometer;
- clinical assessment of phlogistic-degenerative signs by MRI of the peritrochanteric region;
The first infiltration will then be performed by means of ultrasound.
Previous or ongoing pharmacological treatments of any kind will be documented.
In order to monitor the consumption of analgesic (celecoxib 200mg / paracetamol 1000 mg) used during the investigation in the event of the onset of pain, a clinical diary will be given to the subject in which to indicate the day and the dose of drug used.
The enrolled subject will be assigned an identification code of a complex type formed by the progressive enrolment number followed by an alphanumeric code generated by GUNA S.p.a. through Random Sequence Generator (www.random.org.)

T1 week:

At 7 days from day 0, the second infiltration will be performed, the use of celecoxib or paracetamol will be assessed through clinical diary analysis and any Adverse Events occurring after the first administration will be evaluated.

T2 weeks:

At 14 days from day 0, the third infiltration will be performed, celecoxib or paracetamol utilisation will be assessed through clinical diary analysis and any Adverse Events occurring since the last administration will be evaluated.

T6 weeks/FU:

At 6 weeks from day 0, the endpoints in the Investigation Plan will be assessed.

Including:

- pain assessment using the NRS scale;
- assessment of the mHHS scale;
- clinical assessment of pain on palpation of the trochanter using the NRS scale;
- clinical assessment of stenia in abduction of the lower limb with the dynamometer;
- assessment of analgesic drug consumption when pain occurs

through clinical diary;

- evaluation of any Adverse Events.

T10 weeks/FU:

At weeks 10 from day i.e. weeks 8 after the end of the treatment, the assessment of the end points in the Clinical Investigation Plan will be carried out again.

Including:

- pain assessment using the NRS scale;
- assessment of the mHHS scale;
- clinical assessment of pain on palpation of the trochanter using the NRS scale;
- clinical assessment of stenia in abduction of the lower limb with the dynamometer;
- assessment of analgesic drug consumption when pain occurs
- through clinical diary;
- evaluation of any Adverse Events.

T24 weeks/FU:

At 24 weeks from day 0 at the end of the Clinical Investigation, all the endpoints of the Investigation Plan will be assessed.

Including:

- pain assessment using the NRS scale
- assessment of the mHHS scale;
- clinical assessment of pain on palpation of the trochanter using the NRS scale;
- clinical assessment of stenia in abduction of the lower limb with the dynamometer;

- clinical evaluation and recording by MRI of signs of the inflammatory and degenerative state of the peritrochanteric region of the hip affected by GTPS.
- evaluation of analgesic drug consumption when pain occurs
- through clinical diary;
- evaluation of any Adverse Events.

Diagnosis, evaluation of inflammatory and degenerative signs on MRI examination of the hip affected by GTPS and evaluation of pain on palpation of the trochanter will be carried out by Prof. Randelli Filippo. The NRS and mHHS rating scales will be administered to the subject by a Co-Investigator. The evaluation of the lower limb abduction stenia will be carried out by a Co-Investigator, by means of a dynamometer, with which three measurements will be taken (with a recovery of 1 minute between each measurement) from which the average will be derived for a more accurate evaluation. The recording of the data in the clinical diary concerning the intake of celecoxib or paracetamol will be performed by the Co-Investigator.

The occurrence of Adverse Events will be assessed at each visit. All Adverse Events (AEs), Serious Adverse Events (SAEs) and Suspected Serious and Unexpected Adverse Reactions (SUSARs) occurring between the date of signing the Informed Consent and the end of the Clinical Investigation will be recorded and reported.

All data collected will have to be entered by the Principal Investigator or a Co-Investigator identified by the Principal Investigator into the Electronic Data Collection Sheet provided by Guna S.p.a.

In particular, it is planned to obtain a data collection that is schematically described in the following table (Tab. 1), in which the entries to be made at each visit are shown:

PROCEDURE SPERIMENTALI	VISIT	VISIT	VISIT	VISIT	VISIT	VISIT
	T 0	**T 1 WEEKS**	**T 2 WEEKS**	**T 6/FU WEEKS**	**T10/FU WEEKS**	**T24/FU WEEKS**
	enrolment 1° infiltration	Visit 2° infiltration	Visit 3° infiltation	Control visit	Control visit	End of Clinical Investigation
Clinical diagnosis of GTPS	**X**					
Evaluation of Selection Criteria	**X**					
Signature of informed consent	**X**					
Signature of consent to personal data	**X**					

processing						
Collection of socio-demographic data	**X**					
Medical history collection	**X**					
NRS Evaluation	**X**			**X**	**X**	**X**
Assessment of pain on palpation according to NRS	**X**			**X**	**X**	**X**
mHHS assessment	**X**			**X**	**X**	**X**
Assessment of stenia in abduction	**X**			**X**	**X**	**X**
Evaluation of phlogistic and degenerative signs on MRI	**X**					**X**
Infiltrative Treatment	**X**	**X**	**X**			
Evaluation of clinical diary	**X**	**X**	**X**	**X**	**X**	**X**
Adverse Event Evaluation (AE/SAE)	**X**	**X**	**X**	**X**	**X**	**X**

Tab.1 Data collection during the Clinical Investigation Plan

4.11 Materials needed to conduct the Clinical Ivestigation Plan

- *Collagen Medical Device* (MD-Tissue) 2 ml vials.

Such material will be sent by Guna S.p.a. to the pharmacy of the Investigational Site in a quantity suitable for the conduct of the Study."

4.12 Study group

- ***Group (Collagen Medical Device):***

Experimental group will be treated with 2 ml volume ultrasound-guided infiltrations of:

- MD-Tissue (GUNA, Milan-Italy)

Composition per 2 ml: collagen 100 micrograms

Excipients: Ascorbic acid, Magnesium gluconate, Pyridoxine hydrochloride, Riboflavin, Thiamine hydrochloride, NaCl, Water for injection.

Subjects will be treated with 1 ultrasound-guided infiltration per week for 3 consecutive weeks.

4.13 Infiltrative Technique

The infiltrations will be performed in ultrasound mode by a radiologist experienced in musculoskeletal ultrasound techniques. The infiltration technique will be performed as follows:

The subject will be positioned in lateral decubitus with the GTPS-affected hip upwards.

A sterile field will be set up with disposable drapes and careful skin disinfection with 2% chlorhexidine and betadine.

Using a Convex probe, fitted with a sterile sheath, the greater trochanter, the bursa trochanteris and the tendons of the small and middle gluteal muscles will first be located, identifying the areas most affected by degenerative processes.

The infiltration will then be performed in complete asepsis, using a 20G disposable spinal needle.

MD-Tissue *Collagen Medical Device* will be infiltrated into the trochanteric bursa and at the level of the tendons of the gluteus minimus and gluteus medius, particularly at the level of the most degenerated insertional areas.

4.14 Criteria for effectiveness

4.14.1 End Point Primario

The objective of this study is to evaluate the efficacy of the experimental treatment in terms of reduction of referred pain in the peritrochanteric area, of the hip affected by GTPS.

The primary endpoint will be assessed at 10 weeks and will include:

- Evaluation of the NRS score at time 10 weeks compared to time T0. It can be considered clinically significant if at least 50% of the treated subjects show a reduction of at least 3 points on the NRS scale.

4.14.2 Secondary End Points

- Evaluation of the NRS score at time weeks 6 and weeks 24 compared to time day 0.
- Evaluation of mHHS score at time weeks 6, weeks 10, and weeks 24 compared to time day 0.
-
- Clinical evaluation of abduction stenia at time weeks 6, weeks 10, and weeks 24 compared to time day 0.

  [the stenia will be assessed, with the subject standing on the healthy limb, through measurements of the abduction force of the GTPS-affected hip, using a dynamometer]
- Evidence of resolution or decrease in inflammatory and degenerative signs of the peritrochanteric region of the GTPS-affected hip on MRI investigation at weeks 24 compared to day 0.
- Assessment of analgesic drug unit consumption based on clinical diary at day 0, week 1, week 2, weeks 6, weeks 10 and weeks 24.

  [Celecoxib 200mg cpr 1 tablet/day will be used as a pain killer, in case of onset/recurrence of pain; if the subject is hypertensive or intolerant Paracetamol 1000mg cpr 2 tablets/day].
- Evaluation of the fraction of subjects who drop out of the Clinical Investigation Plan early in relation to Adverse Events (AE/SAE/SUSAR).

4.15 Data collection

All data resulting from the Clinical Investigation will be entered, within seven days of their collection, into the electronic Data Collection Form (e-CRF) "Pheedit Dedagroup" provided by the

Sponsor. Radiological data will be collected and stored digitally on the PACS system. Data entry procedures will be performed by the Data Manager.

4.16 Early abandonment of the Clinical Study

I Subjects may leave the Clinical study for the following reasons:

a. at their own request (even without justification);

b. at the investigator's discretion;

c. if they experience an Adverse Event.

The reason for abandonment will be stated in the e-CRF.

5. STATISTICS

5.1 Dimensione del campione

This is a one-sample study. It is assumed that the proportion of successes (patients who have a reduction of at least 3 points on the NRS scale) in the absence of treatment cannot exceed 25%, (H0) while, in section 4.14.1, a success rate of at least 50% (HA) in treated subjects was assumed to be clinically significant.

Under these assumptions, an exact one-tailed binomial test applied to a sample of 49 subjects achieves a power of 95.7% in discriminating the 25% difference between the proportion predicted by HA and that predicted by H0, at a significance level of 0.025 (the significance level calculated by simulation is instead 0.023, thus below the target for the one-tailed test).

There is no inflation of the sample size determined by possible dropouts, as every such subject will automatically be considered a failure.

Incidentally, it is also pointed out that by the time the 19th success is reached, the null hypothesis can already be rejected, as 19 successes out of 49 would represent a proportion of 38.776%, which has an exact 95% binomial confidence interval ranging from 25.197% to 53.761%, so that the maximum proportion of 25% predicted by H0 would be below the lower margin of the confidence interval.

5.2 Statistical Analysis Plan

5.2.1 Descriptive statistics

All variables will be subjected to the appropriate descriptive analyses after validation of the input data. Continuous variables: mean ± standard deviation or median and range, depending on the distribution; categorical variables: absolute and relative frequency tables.

5.2.2 Primary Endpoint

The primary endpoint will be assessed with one-tailed exact binomial tests, as described in section.

5.2.3 Secondary Endpoints

Foreword: for all continuous variables, an attempt will always be made to use the parametric test; in the case of non-Gaussian distribution (possibly assessed after Shapiro-Wilk tests) or non-

homogeneous variance, an attempt will however be made to modify the variable with the use of a nonlinear transform (logarithm, ladder, etc.) before using the non-parametric equivalents.

The tests provided for the secondary endpoints are:

- NRS score assessment at time weeks 6 and weeks 24 compared to time day0: anova to repeated measures within subject or Friedman test.
- Evaluation of mHHS score time weeks 6, weeks 10, and weeks 24 compared to time day 0. Test Anova to repeated measures within subject or Friedman test.
- Clinical evaluation of stenia in abduction at time weeks 6, weeks 10, and weeks 24 compared to time day 0: Anova at repeated measurements within subject or Friedman's test
- Evidence of resolution or decrease in inflammatory and degenerative signs of the peritrochanteric region of the GTPS-affected hip, on MRI investigation at weeks 24 compared to day 0: Student's t-test for paired data or Wilcoxon test.
- Assessment of analgesic drug unit consumption based on clinical diary at day 0, week 1, weeks 2, weeks weeks 10, and weeks 24 compared to time day 0: repeated measures Anova or Friedman test.
- Assessment of the fraction of subjects leaving the clinical investigational early in relation to Adverse Events (AE/SAE/SUSAR): measurement of absolute and relative frequency and 95% confidence interval.

5.3 Ethical Authorisation

The Clinical Investigation Plan will be submitted to the relevant Ethics Committee for approval.

5.4 Amendments to the Clinical Investigation Plan

Any amendments to this Investigation Plan will be submitted by the Sponsor, in the form of an amendment, to the relevant Ethics Committee for approval.

5.5 Information of the subject

The purposes and modalities of the Investigation will be explained to each subject by means of an information document (Informed Consent), containing what is verbally stated by the clinician. The subject must date and sign the consent document.

A copy of the document will be given to the subject while the original will be kept by the investigator.

5.6 Adverse Events

An Adverse Event is defined as any harmful clinical event that will occur during the course of the clinical investigation in one of the subjects comprising the study population who has undergone one of the treatments in the investigation plan.

Adverse events will be classified into serious (SAE) non-serious (AE) and Suspected Serious and Unexpected Adverse Reactions (SUSAR).

An Adverse Event is defined as serious if (ICH-GCP DM 15/07/1997):

- it causes the death of the subject involved in the investigation
- places the subject's life in danger
- is such as to require hospitalization
- is such as to require prolonged hospitalization
- is such as to cause permanent or temporary disability
- leads to a congenital anomaly or birth defect

In all other cases, the Adverse Event will be non-serious and will be classified as:

- *mild* does not interfere with normal activities of daily living and resolves spontaneously;

- *moderate*, interferes with activities of daily living but resolves spontaneously;

- *severe*, prevents daily life activities and does not resolve spontaneously.

All personnel who come in contact with study subjects must report Adverse Events reported by subjects to the Investigator, who has a duty to collect all possible data about them.

- Adverse events (AEs) will be reported within 48 h by the Principal Investigator to the Clinical Research Unit of Guna S.p.a, through telephone, email, appropriate form and simultaneously through e-CRF.

- Serious Adverse Events (SAEs) and Suspected Serious and Unexpected Adverse Reactions (SUSARs) will have to be reported to the Sponsor immediately (within 24 h maximum) through telephone, email, appropriate form and simultaneously through e-CRF by the Principal Investigator and communicated to the Ethics Committee and the Ministry of Health.

GUNA S.p.a. Clinical Research Units

tel: 02.28018358 / 02.28018359

mail: v.miranda@guna.it

k.laarej@guna.it

clinical@guna.it

GUNA S.p.a. Pharmacovigilance Office

Tel: 02.28018459 / 345.5884590

mail: m.rossi@guna.it

vigilanza@guna.it

The investigator should ensure that procedures are in place to ensure that the event is resolved.

In the case of nonserious Adverse Events (AEs), it is necessary to keep in mind that they can still give rise to serious events, which is why each Adverse Event must be monitored.

Adverse Events that have not yet resolved at the end of the study will be followed up by the Investigator; each subject who has experienced an Adverse Event will be contacted at least once a month after the conclusion of the study for as many months as deemed appropriate.

5.7 Privacy protection

The identities of data subjects will be known only to the Investigators and the Clinical Monitor in charge of monitoring the investigation. Reference will be made to Regulation (EU) 2017/745.

Full respect for the anonymity of the subjects participating in the Clinical study will be ensured. Data collection, data processing, any scientific publications or congress presentations of the results of the Clinical Investigation will be conducted in accordance with the current Privacy Law - Regulation (EU) 2017/745.

5.8 Access to data

In compliance with the regulations in force concerning Clinical Investigations on medical devices, the Institute and the investigators will allow monitoring of the study according to the monitoring plan drawn up by the Sponsor. In addition, they will allow the Competent Authorities direct access to the study documentation being audited.

5.9 Data ownership

All data collected will belong to Guna S.p.a. Milan, Italy, Sponsor of the Clinical study.

Further information on data ownership can be found in the Economic Agreement document.

5.10 Data processing and publication

The results of this study will be summarised and presented in a final report that will aim to draw reliable conclusions regarding the clinical efficacy of the infiltrative treatment of the painful large trochanter syndrome (GTPS) by means of an infiltrative cycle with an injectable MD Tissue *Collagen Medical Device*. The scientific article will then be edited and sent to a peer reviewed journal in the field for publication in order to disseminate the results obtained to the scientific community.

5.11 Financing

This research is financed by GUNA S.p.a.

Please refer to the Economic Agreement for further information.

5.12 Reference Code of Ethics

Reference is made to the Declaration of Helsinki (Fortaleza 64th 2013) and the principles of Good Clinical Practice (GCP) are to be followed (D.leg. 24 June 2003 - n.211 in G.U. n.184 of 09/08/2003).

5.13 Subjects insurance

Insurance cover will be provided by GUNA S.p.a. for the entire duration of the clinical investigation project for all subjects enrolled.

6. PREMATURE TERMINATION OF THE CLINICAL INVESTIGATION

The Investigators reserve the right to discontinue the investigation at any time for reasonable medical and/or administrative reasons. Reasons for discontinuation will be documented; the Ethics Committee and the Ministry of Health will be informed of the decision.

7. GLOSSARY

Medical device: Any instrument, apparatus, implant, substance or other product, whether used alone or in combination, including computer software used for proper functioning, and intended by the manufacturer for use in man for the purpose of diagnosis prevention, control, therapy or mitigation of a disease; diagnosis, control, therapy, mitigation or compensation for an injury or handicap of study, replacement or modification of anatomy or a physiological process; of intervention in conception, which product does not exert its principal action, in or on the human body, for which it is intended, by pharmacological or immunological means, nor by metabolic process but whose function may be assisted by such means.

8. ALLEGATI AL PIANO DI INDAGINE

A. Synopsis of the Clinical Investigation
B. Informed Consent (information and consent)
C. Consent to the processing of Personal data (Information and consent)
D. NRS Pain Rating Scale
E. Non-arthritic hip disease rating scale mHHS
F. Clinical Diary

9. BIBLIOGRAFIA

1. Bosman FT, Stamenkovic I. Functional structure and composition of the extracellular matrix. Journal of Pathology. 2003.
2. Silver FH, Freeman JW, Seehra GP. Collagen self-assembly and the development of tendon mechanical properties. J Biomech. 2003;
3. Arnoczky SP, Lavagnino M, Egerbacher M, Caballero O, Gardner K. Matrix metalloproteinase inhibitors prevent a decrease in the mechanical properties of stress-deprived tendons: An in vitro experimental study. Am J Sports Med. 2007;
4. Kannus P. Structure of the tendon connective tissue. Scand J Med Sci Sport. 2000;
5. PDF; KJAER M. Role of Extracellular Matrix in Adaptation of Tendon and Skeletal Muscle to Mechanical Loading. Physiol Rev. 2004;
6. Ejnisman L, Safran MR. Biologics in hip preservation. Ann Jt. 2018;
7. Williams BS, Cohen SP. Greater trochanteric pain syndrome: A review of anatomy, diagnosis and treatment. Anesthesia and Analgesia. 2009.
8. Karpinski MRK, Piggott H. Greater trochanteric pain syndrome. A report of 15 cases. J Bone Jt Surg - Ser B. 1985;
9. Speers CJB, Bhogal GS. Greater trochanteric pain syndrome: A review of diagnosis and management in general practice. Br J Gen Pract. 2017;67(663):479–80.
10. Frizziero A, Vittadini F, Pignataro A, Gasparre G, Biz C, Ruggieri P, et al. Conservative management of tendinopathies around hip. Muscle Ligaments Tendons J. 2019;
11. Grimaldi A, Mellor R, Hodges P, Bennell K, Wajswelner H, Vicenzino B. Gluteal Tendinopathy: A Review of Mechanisms, Assessment and Management. Sports Medicine. 2015.
12. Ali M, Oderuth E, Atchia I, Malviya A. The use of platelet-rich plasma in the treatment of greater trochanteric pain syndrome: a systematic literature review. J Hip Preserv Surg. 2018;
13. Fitzpatrick J, Bulsara MK, O'Donnell J, McCrory PR, Zheng MH. The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendinopathy: A Randomized, Double-Blind Controlled Trial Comparing a Single Platelet-Rich Plasma Injection With a Single Corticosteroid Injection. Am J Sports Med. 2018;
14. Reid D. The management of greater trochanteric pain syndrome: A systematic literature review. J Orthop [Internet]. 2016;13(1):15–28. Available from: http://dx.doi.org/10.1016/j.jor.2015.12.006
15. Randelli F, Menon A, Giai Via A, Mazzoleni M, Sciancalepore F, Brioschi M, et al. Effect of a Collagen-Based Compound on Morpho-Functional Properties of Cultured Human Tenocytes. Cells. 2018;7(12):246.
16. Singh JA, Schleck C, Harmsen S, Lewallen D. Clinically important improvement thresholds for Harris Hip Score and its ability to predict revision risk after primary total hip arthroplasty. BMC

Musculoskelet Disord. 2016;

17. Galea VP, Florissi I, Rojanasopondist P, Connelly JW, Ingelsrud LH, Bragdon C, et al. The Patient Acceptable Symptom State for the Harris Hip Score Following Total Hip Arthroplasty: Validated Thresholds at 3-Month, 1-, 3-, 5-, and 7-Year Follow-Up. J Arthroplasty [Internet]. 2020;35(1):145-152.e2. Available from: https://doi.org/10.1016/j.arth.2019.08.037
18. Dettoni F, Pellegrino P, La Russa MR, Bonasia DE, Blonna D, Bruzzone M, et al. Validation and cross cultural adaptation of the Italian version of the harris hip score. HIP Int. 2015;