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Adipose tissue-derived versus bone marrow-derived cell concentrates for the injective treatment of knee osteoarthritis. Protocol of a prospective randomised controlled trial.

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ABSTRACT

Introduction

Intra-articular injections of mesenchymal stromal cells (MSCs) concentrates showed promising results in the treatment of knee osteoarthritis (OA). Among these, bone marrow aspirate concentrate (BMAC) has been widely adopted in the clinical practice. More recently, micro-fragmented adipose tissue (MFAT) has been proposed as a more suitable solution. However, there is still no high-level evidence demonstrating the superiority of MFAT to BMAC. The aim of this randomised controlled trial is to compare the safety and clinical outcomes of a single intra-articular injection of BMAC versus a single intra-articular injection of MFAT in patients with knee OA.

Methods and analysis

Two hundred four patients aged 20 to 75 years and affected by knee OA are randomised to receive a single injection of BMAC or MFAT in a 1:1 ratio. The primary outcome of the study is the Western Ontario and McMaster University Osteoarthritis index (WOMAC) pain score at 6 months. The secondary outcomes of the study are the WOMAC pain score at 2 and 12 months and the WOMAC subscales, the total WOMAC score, the International Knee Documentation Committee (IKDC) subjective and objective scores, the Knee Injury and Osteoarthritis Outcome score (KOOS), the visual analogue scale (VAS) for pain evaluation, the EuroQol visual analogue scale (EQ-VAS), and the Tegner score at 2, 6, and 12 months. Moreover, the study aims at demonstrating whether these products have disease-modifying effects: radiographs and magnetic resonance (MR) evaluations are performed at baseline and at 12 months of follow-up, while systemic OA biomarkers are evaluated at baseline and after 2, 6, and 12 months. As tertiary outcome, this study aims at identifying the factors

that influence the clinical response, including baseline patient clinical characteristics, biological features of the OA joint, as well as anabolic and anti-inflammatory properties of the injected products.

Ethics and dissemination

The study protocol has been approved by *blinded for review*'s Ethics Committee *blinded for review*. Written informed consent is obtained from all participants. The findings of this study will be disseminated through peer-reviewed publications and conference presentations.

Protocol version

March 2023.

Trial registration number

blinded for review

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the first prospective, randomised controlled trial evaluating the results of intra-articular adipose-tissue derived products injections compared with intra-articular bone marrow concentrate injections in patients with knee osteoarthritis.

⇒ Patients are analysed using Patient Reported Outcome Measures (PROMs), objective measures, x-rays, MRI, and biomarkers evaluation.

⇒ The analysis of patient baseline characteristics and disease-related factors can help better define the aspects that make different individuals more or less responsive to these treatments.

⇒ The main limitation of this RCT is the inability to maintain patients blind to the treatment allocation due to the different incisions performed for each treatment group.

⇒The sample size was calculated on the power analysis based on the primary outcome (WOMAC pain score at 6 months of follow-up), and thus it may be underpowered to detect differences in secondary outcomes.

INTRODUCTION

Knee osteoarthritis (OA) is an increasingly common condition causing joint pain and functional limitation, often requiring invasive surgical procedures like knee replacement. These have long rehabilitation and potential severe morbidity, they often need re-operation, and overall present extensive socio-economic impact [1, 2]. The only partially satisfactory results of conventional treatments led to the development of less invasive procedures such as intra-articular injections of orthobiologics. Among the available options, mesenchymal stromal cells (MSCs) are emerging as a promising solution given their anti-inflammatory and anabolic potential, reported both for cultured cells and minimally manipulated products [3, 4]. Aiming at reducing costs and avoiding regulatory restrictions associated with cell culture approaches, minimally manipulated products have become a popular strategy to exploit the potential of MSCs concentrates directly on-site in a one-step treatment [5, 6]. Among the main treatment modalities emerged in the past few years, bone marrow aspirate concentrate (BMAC) has been widely adopted in the clinical practice as injective approach for degenerative orthopaedic conditions like knee OA [7]. However, despite some clinical benefits, overall results are still suboptimal. More recently, minimally manipulated adipose tissue (MM-AT) derived products, and in particular micro-fragmented adipose tissue (MFAT), have been proposed as a more promising alternative for the treatment of OA, given the advantages of adipose tissue over other MSCs sources [8-12]. MM-AT derived products represent a valid source of MSCs given the tissue abundance, ease of harvesting with little patient discomfort, and high concentration in MSCs [13]. However, as of today, no RCT directly compared the clinical benefit of BMAC and MFAT orthobiologic approaches.

Objectives and trial design

A randomised controlled trial (RCT) was designed to compare the safety and clinical outcomes of a single intra-articular injection of BMAC versus a single intra-articular injection of MFAT to address patients with knee OA, with a 1:1 allocation ratio. As secondary goals, this study aims at demonstrating whether BMAC or MFAT injections can have disease-modifying effects by investigating tissue modifications through combined imaging and biological evaluations. As tertiary goal, this study aims at identifying the factors that influence the clinical response, including baseline patient clinical characteristics, biological features of the OA joint, as well as anabolic and anti-inflammatory properties of the injected products.

METHODS AND ANALYSIS

Study setting

The study is a single-centre RCT, with all activities related to the study performed at a single site (*blinded for review*). This trial protocol is produced according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) reporting guidelines [14].

Patient and public involvement

Patients were not involved in planning research questions, outcome measures or design of the study.

Eligibility criteria

Patients are recruited according to the following criteria.

Inclusion criteria:

- Men or women aged between 40 and 75 years;
- Symptomatic tibio-femoral OA with history of knee pain and/or swelling for at least 6 months;
- Radiographic signs of OA (grade 1 to 4 according to the Kellgren-Lawrence classification);
- Ability and consent to participate to clinical and imaging follow-ups;

- Informed consent signed.

Exclusion criteria:

- Patients mentally incapacitated;
- Patients with active malignancy;
- Patients suffering from rheumatic diseases or arthritis secondary to other inflammatory diseases;
- Human immunodeficiency virus (HIV) infection, viral hepatitis;
- Patients suffering from uncontrolled diabetes or thyroid disorders;
- Patients with a history of alcohol or drug abuse;
- Pregnancy, breast-feeding, or intention to start pregnancy during the study;
- Patients who underwent knee surgery in the previous 12 months;
- Lower limb axial deviation > 5°;
- History of major knee trauma within 6 months prior to the treatment;
- History of intra-articular injections within 6 months prior to the treatment;
- Patients with history of allergic reactions to local anaesthetics used in the procedures;
- Presence of joint infection or other knee lesions causing pain other than OA (e.g. osteochondral lesions, meniscal tears);
- Body mass index >35 kg/m^2.

Intervention

Patients are treated by orthopaedic surgeons with experience in orthobiologics. The procedure is performed in a single step in the operating room with patients in supine position, with a sedative and analgesic oral premedication of tramadol 100mg/ml and bromazepam 2.5mg/ml adjusted for weight (number of oral drops is equal to weight in kg divided by 3) and using local anaesthesia.

In the BMAC group bone marrow is harvested from the anterior iliac crest: a single incision is made at the level of the anterior iliac crest using a dedicated EC approved kit (Isto Biologics Magellan®) and collected using two 30 mL syringes coated with heparin for a total of 60 mL. This anatomical site has been proven to be one of the most appropriate in terms of biological potential [15]. The harvested bone marrow is filtered with a heparin washed filter and then centrifuged through the Magellan® centrifuge (Isto Biologics, Hopkinton, MA, USA, previously Arteriocyte Medical Systems, Hopkinton, MA, USA) at a rate of 3600 RPM for 15 min, thus obtaining 8 mL of BMAC (Fig. 1). The incision is then sutured using a single stitch, that has to be removed 2 weeks after the surgery.

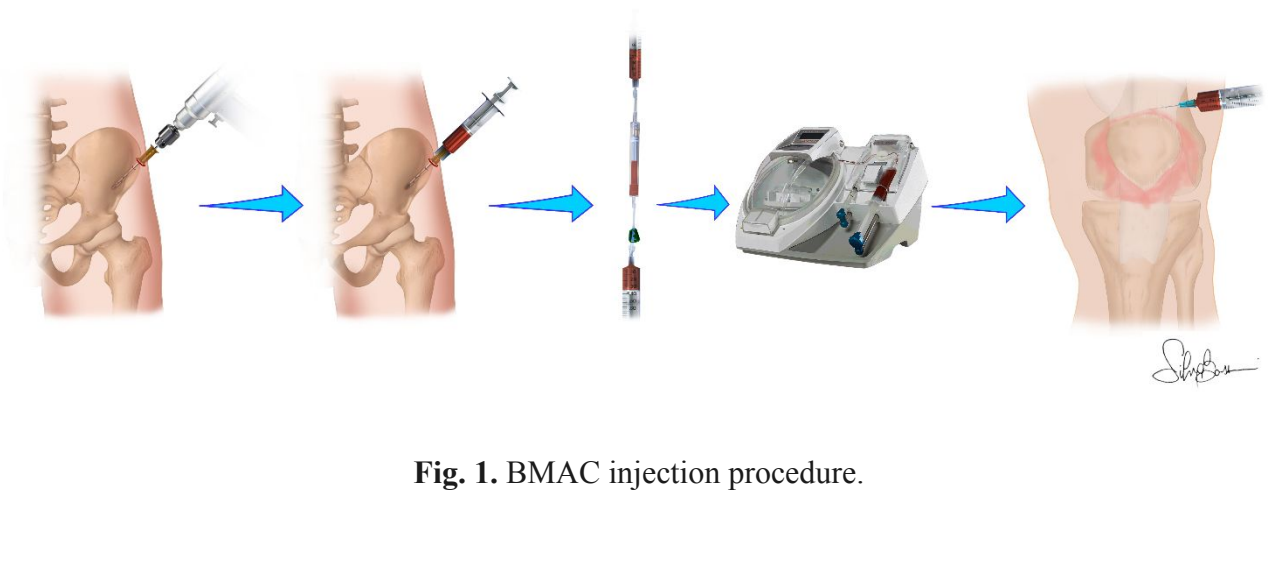

Fig. 1. BMAC injection procedure.

In the MFAT group adipose tissue is harvested from the subcutaneous abdominal fat, as this site proved to be the most appropriate in terms of biological potential and ease of harvesting [8]. After local anaesthesia, a sub-centrimetric incision is performed on both sides of the lower or lateral abdomen. Before harvesting the fat, each side is injected with 180 ml of Klein solution (1 ml 2µg/ml adrenaline and 40 ml 0.02% lidocaine in 500 mL of saline solution) by using a disposable 17-gauge blunt cannula connected to a 60-mL Luerlock syringe. Adipose tissue is then collected using a 13-gauge blunt cannula, for fast and atraumatic suction, connected to a 20-mL Vaclock syringe. The harvested fat is immediately processed using the Lipogems system (Lipogems International Spa, Milan, Italy) as previously described [16]. The entire process is performed in complete immersion in

isotonic solution, thus minimizing cell trauma. The size of adipose tissue clusters is progressively reduced with a mild mechanical action to microspheres, in accordance with the manufacturer's instructions. This process allows for elimination of oily substances, cell debris, and blood residues. Finally, the resulting micro-fragmented tissue (8 mL) is collected in a 10-mL syringe [17]. The process in shown in Fig. 2. The two incisions are then sutured using a single stitch for each side, which has to be removed 2 weeks after the surgery. After the surgery, a girdle must be worn for 10 days.

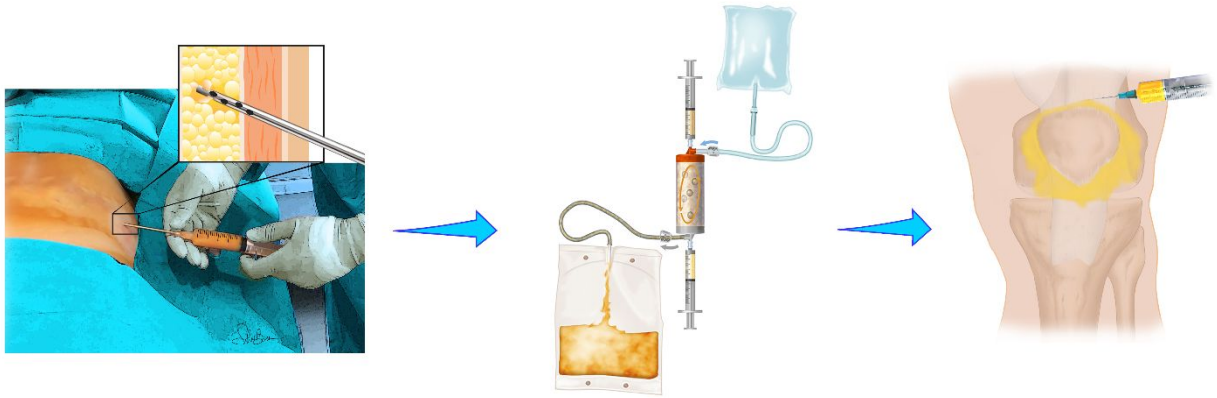

Fig. 2. MFAT injection procedure.

An arthrocentesis is then performed in the affected knee to collect synovial fluid. This will not be possible in all patients as synovial fluid may be absent or insufficient. In addition, if synovitis is present, a synovial membrane biopsy is collected with ultrasound guidance with a dedicated biopsy needle. In both groups, the intra-articular injection of 6 ml of orthobiologic products is then performed with ultrasound guidance in a lateral suprapatellar approach using an 18-gauge needle, with the patient in supine position and the knee fully extended. The remaining 2-ml sample is sent to the laboratory for the in vitro analyses together with the synovial fluid and synovial tissue samples.

At the end of the injection, the patient is encouraged to bend and extend the knee a few times to allow the product to spread throughout the joint. The postoperative protocol includes rest and avoiding high-impact sports activities and strenuous work for 2 weeks, without restrictions in weightbearing

following the procedure. After stitches removal, gradual return to sport is allowed as tolerated, with exercise bike and aquatic therapy being recommended activities.

Outcomes

Patients are evaluated at baseline and at 2, 6, and 12 months with validated questionnaires. The primary outcome of the study is the Western Ontario and McMaster University Osteoarthritis index (WOMAC) pain score at 6 months. The secondary outcomes of the study are the WOMAC pain score at 2 and 12 months and the WOMAC subscales (function, stiffness), the total WOMAC score, the International Knee Documentation Committee (IKDC) subjective and objective scores, the Knee Injury and Osteoarthritis Outcome score (KOOS), the visual analogue scale (VAS) for pain evaluation, the EuroQol visual analogue scale (EQ-VAS) for the overall quality of life evaluation, and the Tegner score to document the activity level of the treated patients at 2, 6, and 12 months.

Imaging evaluation is performed analysing knees with radiographs (antero-posterior and lateral views) at baseline and at 12 months of follow-up to assess the OA grade according to the Kellgren-Lawrence classification. An approved artificial intelligence (AI) imaging analysis system is also used. High-resolution 3.0 Tesla magnetic resonance (MR) imaging is performed at baseline and at 12 months of follow-up, and the Whole-Organ Magnetic Resonance Imaging Score (WORMS) is used to assess articular cartilage morphology, bone marrow oedema, subchondral cysts, articular profile, marginal osteophytes, meniscal integrity, and synovitis.

Systemic OA biomarkers are evaluated at baseline and after 2, 6, and 12 months of follow-up, through circulating miRNAs expression analysis and spontaneous osteoclastogenesis evaluation. Circulating miRNAs from peripheral blood samples are analysed as reported below. An aliquot of peripheral blood sample (approximately 5 ml) is stored at 4°C overnight, then centrifuged, and plasma is then stored at -80 °C. Circulating miRNAs are isolated following the guidelines of commercial kit of extraction and isolation (mirVana™ miRNA Isolation Kit). MiRNAs characterization is performed after retrotranscription of miRNAs isolated using TaqMan™ Advanced miRNA cDNA Synthesis Kit.

MiRNAs involved in OA progression or inflammation are identified and quantified using precast microRNA seq panel analysis (TaqMan Advanced miRNA Human A and B 96-well Plates, fast) [18-22]. Data obtained are analysed with bioinformatic and multivariate analyses. Subsequently, a list of putative biomarkers is identified by means of bioinformatic investigations.

Spontaneous osteoclastogenesis is evaluated. Monocytes from patients with OA display enhanced capacity to generate osteoclasts (OCs) compared to cells from healthy controls [23]. OCs are obtained from approximately 2 ml of peripheral blood from each patient. More precisely, peripheral blood mononuclear cells (PBMCs) are isolated with Ficoll density-gradient centrifugation, and then cultured in alpha-minimum essential medium. After monitoring viability, once a week for 3 weeks through Alamar blue dye test, a differentiation assay is performed after 21 days of culture through tartrate-resistant acid phosphatase (TRAP) staining: the large, multinucleated cells (>3 nuclei), which developed a brown colour, are scored as positive cells and the ratio between the brown-coloured region and total image area is measured using an image analysis system of inverted microscope. In addition, the supernatants of cells, after 21 days of culture, are stored at -80°C and evaluated for Cathepsin K (CTSK), metalloproteinase-7 (MMP-7), and MMP-9 production, with immunoenzymatic ELISA tests, for OC activity assessment. Finally, the resorption assay is carried out by culturing PBMCs on bone slices then stained with toluidine blue staining to reveal pits: an image analysis program evaluates the resorption area.

At baseline, autologous BMAC and MFAT remaining from patient treatment (approximately 2 ml), are collected and transferred aseptically to the laboratory for in vitro analysis. An aliquot is immediately cultured for the evaluation of cell viability and protein production. The Alamar blue dye test is used for cell viability assessment and the production of the most important factors involved in trophic and anti-inflammatory processes and interleukins, such as platelet derived growth factor (PDGF), transforming growth factor β (TGFß), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin like growth factor-I (IGF-I), granulocyte-macrophage colony-

stimulating factor (GMCSF), bone morphogenetic protein 2 (BMP2), BMP7, Interleukin 1β (IL1ß), IL6, IL8, and IL-1ra, evaluated from the supernatant using Bio-Plex ProTM panels.

The remaining aliquot of BMAC and MFAT is employed for MSCs characterisation. After 1 day of culture, the adherent cells are evaluated for: i) surface antigen expression through FACS analysis with fluorescein isothiocyanate (FITC)-conjugated antibodies against CD31,34,44,45,73,90 and 105; ii) colony forming units-fibroblast (CFU-f) capacity through Toluidine blue staining after 10 days of culture: The aggregates with >20 cells are visually scored as colonies and counted; iii) three lineages differentiation after 21 days of culture in culture media. Calcium deposits (osteogenic differentiation), lipid accumulation (adipogenic differentiation), and glycosaminoglycans production (chondrogenic differentiation) are evaluated using Alizarin Red S, Oil Red O, and Alcian blue staining, respectively, and an image analysis program. Then, gene expression of SOX-9, ACAN, COMP, ALPL, BGLAP, COL1A1, OPG, RUNX2, ADIPOQ, and PPARG genes is evaluated through RT-PCR.

Synovial fluid (SF) and the synovial membrane are collected at baseline, when the minimal invasiveness of the biopsy is justified by the concurrent procedure performed to treat the patients, for local biomarkers evaluation. More precisely, approximately 1-2 ml of SF are collected from patients with a 18G syringe and sent to the laboratory for the evaluation of the inflammatory grade of the joint and local biomarkers analysis. In detail, a SF aliquot is immediately used by performing the mucin clot test and the compactness of the clot is evaluated. For biomarkers analysis, the most important "Burden of disease biomarkers" [24], such as IL6, IL8, MMP-1, MMP-13, cartilage oligomeric matrix protein (COMP), VEGF, C-teloprotein of type I collagen (CTX-1), Leptin, and tissue inhibitor of metalloproteinase 1 (TIMP1) are evaluated through Bio-Plex ProTM panels.

Synovial membrane biopsies are collected with ultrasound guidance from patient affected by hypertrophic membrane related to synovitis with a dedicated biopsy needle and sent to the laboratory for miRNA evaluation. After lysis and homogenisation of the biopsies in NucleoZOL reagent, contaminating molecules are precipitated by the addition of water and are removed by centrifugation. RNA is reconstituted by RNase-free water and is stored at -80°C overnight. MiRNAs are

characterised after retrotranscription and their investigation is performed by microRNA seq using a commercial and precast miRNAs panel analysis that permits to identify the expression of 376 miRNAs (TaqMan Advanced miRNA Human A and B 96-well).

Factors that can influence the clinical response to the injections of BMAC or MFAT are investigated to identify aspects predictive of a better outcome.

1) Baseline demographic characteristics are analysed, including sex, age, body mass index (BMI), OA severity, symptom duration, knee alignment, previous knee injective treatment, and previous knee surgery. All these factors are correlated to the different scores used to quantify the different subjective, objective, symptomatic, and functional aspects related to the patient experience in response to the applied treatment. These aspects are investigated both in terms of improvement and benefit duration, as well as adverse events and failures.

Participant timeline

The study has a total duration of 36 months. Patient screening, enrolment, and treatment will last 22 months and started in January 2024. The first patient was treated in January 2024. The follow-up evaluations last 12 months. Clinical evaluation is performed at baseline, 2, 6, and 12 months. Imaging evaluation (MR, radiographs) is performed at baseline and 12 months. The biological analysis on blood samples is performed at baseline, 2, 6, and 12 months. Detailed participant timeline is outlined in Table 1.

Table 1. Study schedule.

	Pre-operatory medical examination (baseline)	Surgical procedure	2-month follow-up	6-month follow-up	12-month follow-up
Inclusion/exclusion criteria evaluation	X				
Informed consent signing	X				
Demographic data	X				
Medical history	X				
Randomisation	X				
WOMAC	X		X		X
IKDC Subjective	X		X		X
IKDC Objective	X		X		X
KOOS	X		X		X
PASS	X		X		X
EQ-VAS	X		X		X
Tegner	X		X		X
MCID			X		X
Patient expectation on the treatment	X				
Patient judgement on the treatment					X
Adverse events reporting		X	X		X
Medication tracking sheet			X		X
Radiographs and KL evaluation	X				X
3T MR and WORMS	X				X
Blood sample collection		X	X		X
SF collection and synovial membrane biopsy		X			

EQ-VAS, EuroQol-Visual Analogue Scale; IKDC, International Knee Documentation Committee; KL, Kellgren-Lawrence; KOOS, Knee Injury and Osteoarthritis Outcome score; MR, Magnetic Resonance; T, Tesla; SF, synovial fluid; WOMAC, Western Ontario and McMaster Universities Arthritis Index; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

Recruitment

Patients undergo an outpatient visit conducted by trained medical staff of the *blinded for review*, which assesses patients' eligibility and informs patients of design and content of the study.

Blinding

This is a RCT with radiologists, biologists, and physicians assessing outcomes being blinded to the treatment allocation. Considering the different nature and harvest source of the injected products it is not possible to blind patients and surgeons performing the procedure. Nevertheless, this should not affect patient expectations and thus study results, being both promising cell-based orthobiologic procedures. Moreover, the statistician involved in the study will be blinded to the treatment groups for data analysis.

Allocation

A total of 204 eligible patients is allocated to receive either a single BMAC injection or a single MFAT injection, in a 1:1 ratio (102 patients for each group of treatment). The list for treatment allocation is provided by an independent professional statistician (blinded to the treatments) as generated using a random number generator and then kept in a dedicated data manager office. The allocation is managed by research staff members dedicated to study organisation and monitoring with no direct involvement in the clinical procedures. The randomisation list is password-protected and accessible only by staff members with no direct involvement in the treatment and evaluation.

Stratified permuted block randomisation is used to avoid gender imbalances. Therefore, study participants are divided according to the gender, so block randomisation will be used for each gender. This form of randomisation is recommended for such clinical trials, where known factors (such as sex) are believed to influence treatment outcomes. The permuted block randomisation technique randomises patients between groups within a set of study participants, called a block. Treatment allocations within blocks are determined so that they are in random order but that the desired

allocation proportions are achieved exactly within each block. The eligible subjects are randomised in a 1:1 ratio into one of the two treatment groups:

- Group 1: single intra-articular BMAC injection.

- Group 2: single intra-articular MFAT injection.

The randomisation procedure is managed using the website: www.sealedenvelope.com/simple-randomiser; a randomisation list of 6 will be generated.

Adverse events assessment

All adverse events are assessed and recorded in the patient case report form (CRF). Patients are requested to report any adverse event to the research staff and can inform physicians of potential adverse events at all follow-ups (medical examinations) or through patient-physician communication between follow-ups via phone calls or emails. Adverse events are monitored throughout the study, intra-operatively and at all clinical follow-up evaluations. The CRF also includes the use of pain medications (brand drug name or generic substitute, frequency and duration) and is recorded at all medical examinations. Serious adverse events are considered those resulting in death or being life-threatening, requiring hospitalisation or intervention to prevent permanent impairment or damage. Serious adverse events will be communicated to the Ethics Committee. The expected risk/benefit ratio for these procedures is positive, as the most frequently foreseeable adverse events are mild and resolvable adverse events.

To ensure high-quality execution of the trial in accordance with the protocol, all trial staff is trained by the chief investigators and provided with a document outlining the details of the standard operating procedures, trial contacts, and guidelines.

Data collection and management

Data are collected on a paper-based CRF, with the help of research trained orthopaedic surgery residents blinded to treatment allocation. Subsequently, trained data analysts process all data for

statistical analysis. Radiographs and MR scans are stored on *blinded for review*'s Picture Archiving and Communication System (PACS). Surgical data are collected electronically by orthopaedic surgeons shortly after surgery.

Biological data are collected and stored in a password-protected spreadsheet on a server hosted at *blinded for review*. Data transfer is encrypted with all data de-identified. Only trained research personnel specifically dedicated to the data handling can access the database.

Statistical methods

The sample size calculation was performed by an independent statistician and is based on the power analysis of the primary end point (change in the WOMAC-pain subscale at 6-month follow-up compared to baseline). From previous studies, the WOMAC-pain subscale standard deviation at 6 months of follow-up is 4.0 [25] with an MCID of 1.7 [26]. The resulting effect size is therefore 0.425. Assuming to perform an unpaired t test (comparing the improvement of treatment group 1 versus the improvement of treatment group 2) with an alpha error of 0.05 and a power of 0.8, the minimum sample of patients is 88 for each of the two treatment groups, with a total of 176 patients. Considering a 15% possible drop-out, 102 patients per group are needed, with a total of 204 patients. The power analysis was performed using G*Power 3.1.9.2. With the Unpaired t test the improvement of treatment group 1 is compared with the improvement of treatment group 2.

Continuous variables are expressed as means and standard deviations if normally distributed, as medians and ranges otherwise. Categorical variables are expressed as frequencies and percentages. The normality of the distribution is assessed using the Shapiro Wilks test. The Levene's test is used to evaluate the homoscedasticity of the data. Repeated measures ANOVA, followed by post hoc Sidak pairwise test, are performed to compare scores at different follow-ups. The OneWay ANOVA test is performed to evaluate the difference between groups of continuous and normally distributed and homoscedastic data; otherwise the Mann Whitney test is used. The general linear repeated model (for no missing follow-ups and normally distributed and homoscedastic data) or the generalised linear

mixed model (for all the other cases) are used to assess the influences of the groups on the repeated measures of the outcomes. The group is the fixed effect and any correction for confounding factors is considered as a random effect. Pearson's exact chi-square test is performed to study the relationships between the grouping variables. Spearman rank correlation is used to evaluate correlations between numerical scores and continuous data. Kaplan Meier analysis, followed by Log rank test, is performed to evaluate the difference between groups in failure rate. For all tests $p<0.05$ is considered significant.

Data monitoring

A central project data manager is tasked to perform data quality control on all collected data. An interim report and a final report are foreseen, to be submitted to the *blinded for review* which funded the project (*blinded for review*). The monitoring personnel belongs to the *blinded for review*, which is a research division of the *blinded for review* Scientific Direction and it is independent from the medical personnel performing the study procedures. A further project auditing is performed by the Clinical Trial Center, which is another independent entity of the Institute. The final study report is also sent to the Ethics Committee.

ETHICS AND DISSEMINATION

Research ethics approval

Ethical approval was obtained on March 23rd, 2023, from *blinded for review* (protocol number: *blinded for review*).

Consent or assent

All participants give informed written consent prior to enrolment during the baseline outpatient medical examination with the trained medical staff and according to the study protocol and may withdraw from the trial at any time.

Protocol amendments

Minor protocol amendments, for example, database changes to facilitate monitoring processes or to improve outcome assessment by questionnaire, are fully documented. Major amendments (e.g. changes to the patient information sheet and consent form, change of a local project leader or the inclusion of a new project site) are submitted to the Ethics Committee for approval.

Confidentiality and access to data

Data are recorded using CRFs and processed centrally at the *blinded for review*. CRFs hard copies are stored in a locked area with restricted and secured access. Electronic data are stored on password protected servers with restricted access. The collected data are kept confidential. Backups of all electronic data occur daily to minimise risks of data loss. After study completion, data paper-based copies are archived in a secure storage. Identifiers are kept separately and are accessible only to restricted study personnel in case follow-up of study patients is necessary. Only members of the research team who need to contact study patients, enter data, or perform data quality controls have access to the study patient information.

Dissemination policy

This trial is produced according to the SPIRIT international standards. Results will be disseminated through peer-reviewed publications and submitted for presentation at national and international conferences. The authorship is based on International Committee of Medical Journal Editors 2018 Recommendations.

Scientific relevance and broader impact

This RCT will provide definitive evidence on the clinical efficacy and disease-modifying effects of BMAC and MFAT. Current orthobiologic treatments for knee OA offer a partial symptom relief and often require further invasive procedures. BMAC and MFAT contain MSCs, GFs, and cytokines with

anti-inflammatory, regenerative, and immunomodulatory properties that could help delay or possibly avoid the need for joint replacement. However, while these products are extensively used in the clinical practice, there are no high-level trials to guide the treatment choice. This study will provide an innovation in the field by demonstrating if the more recently developed and promising MFAT is able to outperform the more traditional BMAC. In addition, the study will evaluate patient-based determinants for the efficacy of each product. This will be of significant clinical relevance, offering clear and more stratified indications on the most effective solution to treat the challenging patients affected by OA, often doomed to invasive procedures. This project also aims to shed some light about the evolution of knee OA, both from imaging and biomolecular points of view, to better understand the pathogenesis of this debilitating disease and its response to treatment.

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Contributors

Blinded for review is the principal investigator of this study. *Blinded for review* and *blinded for review* wrote the manuscript and will conduct the trial. *Blinded for review* and *blinded for review* are responsible of imaging evaluation. *Blinded for review* and *blinded for review* are involved in products and patients' characterisation. *Blinded for review* and *blinded for review* applied for funding and supervise the trial. All authors read and approved the final protocol.

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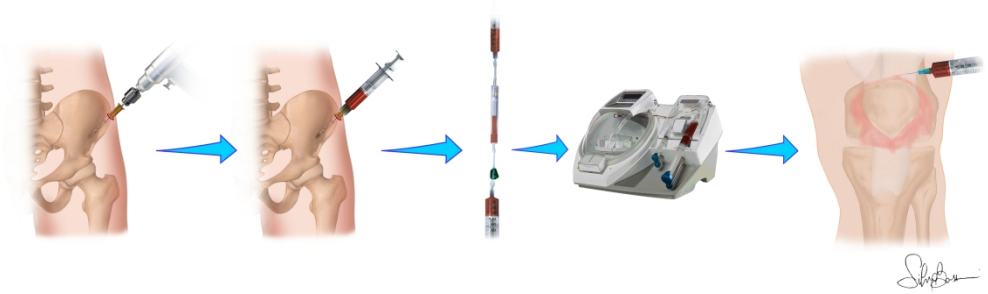

Fig. 1. BMAC injection procedure.

328x122mm (300 x 300 DPI)

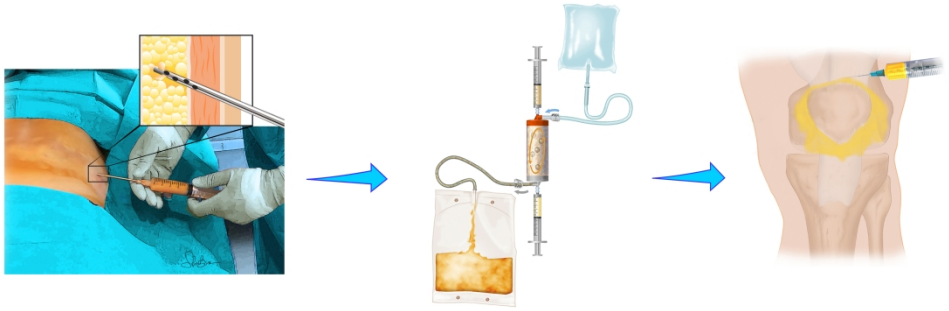

Fig. 2. MFAT injection procedure.

328x122mm (300 x 300 DPI)

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Adipose tissue-derived versus bone marrow-derived cell concentrates for the injective treatment of knee osteoarthritis. Protocol of a prospective randomised controlled trial.

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Adipose tissue-derived versus bone marrow-derived cell concentrates for the injective treatment of knee osteoarthritis. Protocol of a prospective randomised controlled trial.

ABSTRACT

Introduction

Intra-articular injections of mesenchymal stromal cells (MSCs) concentrates showed promising results in the treatment of knee osteoarthritis (OA). Among these, bone marrow aspirate concentrate (BMAC) has been widely adopted in the clinical practice. More recently, micro-fragmented adipose tissue (MFAT) has been proposed as a more suitable solution. However, there is still no high-level evidence demonstrating the superiority of MFAT to BMAC. The aim of this randomised controlled trial is to compare the safety and clinical outcomes of a single intra-articular injection of BMAC versus a single intra-articular injection of MFAT in patients with knee OA.

Methods and analysis

Two hundred four patients aged 40 to 75 years and affected by knee OA are randomised to receive a single injection of BMAC or MFAT in a 1:1 ratio. The primary outcome of the study is the Western Ontario and McMaster University Osteoarthritis index (WOMAC) pain score at 6 months. The secondary outcomes of the study are the WOMAC pain score at 2 and 12 months and the WOMAC subscales, the total WOMAC score, the International Knee Documentation Committee (IKDC) subjective and objective scores, the Knee Injury and Osteoarthritis Outcome score (KOOS), the visual analogue scale (VAS) for pain evaluation, the EuroQol visual analogue scale (EQ-VAS), and the Tegner score at 2, 6, and 12 months. Moreover, the study aims at demonstrating whether these products have disease-modifying effects: radiographs and magnetic resonance (MR) evaluations are performed at baseline and at 12 months of follow-up, while systemic OA biomarkers are evaluated at baseline and after 2, 6, and 12 months. As tertiary outcome, this study aims at identifying the factors

that influence the clinical response, including baseline patient clinical characteristics, biological features of the OA joint, as well as anabolic and anti-inflammatory properties of the injected products.

Ethics and dissemination

The study protocol has been approved by Emilia Romagna's Ethics Committee (CE-AVEC), Bologna, Italy. Written informed consent is obtained from all participants. The findings of this study will be disseminated through peer-reviewed publications and conference presentations.

Protocol version

March 2023.

Trial registration number

NCT06040957.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a prospective, randomised controlled trial performed in a highly specialised orthopaedic centre for cartilage preservation procedures and knee osteoarthritis treatment.

⇒ Patients are analysed using Patient Reported Outcome Measures (PROMs), objective measures, x-rays, MRI, and biomarkers evaluation.

⇒ The analysis of patient baseline characteristics and disease-related factors can help better define the aspects that make different individuals more or less responsive to these treatments.

⇒ The main limitation of this RCT is the inability to maintain patients blinded to the treatment allocation due to the different incisions performed for each treatment group.

⇒ The 12 months of follow-up timeframe may not be sufficient to analyse the clinical and biological response to the two treatments and the progression of knee OA over time.

INTRODUCTION

Knee osteoarthritis (OA) is an increasingly common condition causing joint pain and functional limitation, often requiring invasive surgical procedures like knee replacement. These have long rehabilitation and potential severe morbidity, they often need re-operation, and overall present extensive socio-economic impact [1, 2]. The only partially satisfactory results of these treatments led to the development of less invasive procedures such as intra-articular injections of orthobiologics. Among these, mesenchymal stromal cells (MSCs) are emerging as a promising solution given their biologic potential, reported both for cultured cells and minimally manipulated products, with preclinical studies documenting both anti-inflammatory and anabolic properties of MSCs [3, 4, 5]. Aiming at reducing costs and avoiding regulatory restrictions associated with cell culture approaches, minimally manipulated products have become a popular strategy to exploit the potential of MSCs concentrates directly on-site in a one-step treatment, while avoiding isolation and cell-culture before the injection [6, 7]. Among the main treatment modalities emerged in the past few years, bone marrow aspirate concentrate (BMAC) has been proposed as injective approach for degenerative orthopaedic conditions like knee OA [8]. However, despite improvements in clinical and functional scores, overall results are still suboptimal and preclinical studies supporting the rationale of BMAC injections are scarce, with an overall low quality of evidence of the clinical studies [8]. More recently, minimally manipulated adipose tissue (MM-AT) derived products, and in particular micro-fragmented adipose tissue (MFAT), have been proposed as a more promising alternative for the treatment of OA, given the possible advantages of adipose tissue over other sources of MSCs isolated from different tissues such as bone marrow and synovial tissue [9-13]. In fact, MM-AT derived products represent a valid source of MSCs given the tissue abundance, ease of harvesting with little patient discomfort, high concentration in MSCs, but also their ability to respond to an inflammatory environment better than BMAC in preclinical studies [14]. However, as of today, no RCT directly compared the clinical benefit of BMAC and MFAT orthobiologic approaches, and current literature provides poor guidance

being limited by low level studies, heterogeneity in treatment protocols, and overall poor reporting of treatment characteristics and results in terms of both subjective and objective outcomes.

The hypothesis is that MFAT injections can provide better clinical outcomes compared to BMAC injections for the treatment of patients with symptomatic knee OA. This project will provide for the first time high level evidence to confirm this hypothesis in the clinical setting.

Objectives and trial design

A randomised controlled trial (RCT) was designed to compare efficacy and safety of a single intra-articular injection of BMAC versus a single intra-articular injection of MFAT to address patients with knee OA, with a 1:1 allocation ratio. As secondary goals, this study aims at demonstrating whether BMAC or MFAT injections can have disease-modifying effects by investigating tissue modifications through combined imaging and biological evaluations. As tertiary goal, this study aims at identifying the factors that influence the clinical response, including baseline patient clinical characteristics, biological features of the OA joint, as well as anabolic and anti-inflammatory properties of the injected products.

METHODS AND ANALYSIS

Study setting

The study is a single-centre RCT, with all activities related to the study performed at a single site (IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy). This trial protocol is produced according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) reporting guidelines [15].

Patient and public involvement

Patients were not involved in planning research questions, outcome measures or design of the study.

Eligibility criteria

Patients are recruited according to the following criteria.

Inclusion criteria:

- Men or women aged between 40 and 75 years, to reduce heterogeneity and in light of the possible lower potential of cells from older patients [16];
- Symptomatic tibio-femoral OA with history of knee pain and/or swelling for at least 6 months;
- Radiographic signs of OA (grade 1 to 4 according to the Kellgren-Lawrence classification);
- Ability and consent to participate to clinical and imaging follow-ups;
- Informed consent signed.

Exclusion criteria:

- Patients with cognitive impairment or unable to provide informed consent;
- Patients with active malignancy;
- Patients suffering from rheumatic diseases or arthritis secondary to other inflammatory diseases;
- Human immunodeficiency virus (HIV) infection, viral hepatitis;
- Patients suffering from uncontrolled diabetes or thyroid disorders;
- Patients with a history of alcohol or drug abuse;
- Pregnancy, breast-feeding, or intention to start pregnancy during the study;
- Patients who underwent knee surgery in the previous 12 months;
- Lower limb axial deviation > 5° on the frontal plane, evaluated on a full-length standing radiograph;
- History of major knee trauma within 6 months prior to the treatment;
- History of intra-articular injections within 6 months prior to the treatment;
- Patients with history of allergic reactions to local anaesthetics used in the procedures;

- Presence of joint infection or other knee lesions causing pain other than OA (e.g. osteochondral lesions, impairing meniscal tears causing mechanical symptoms);
- Body mass index >35 kg/m^2, to avoid the possible proinflammatory state related to the adipose tissue from more obese patients [17].

Intervention

Patients are treated by orthopaedic surgeons with experience in orthobiologics. The procedure is performed in a single step in the operating room with patients in supine position, with a sedative and analgesic oral premedication of tramadol 100mg/ml and bromazepam 2.5mg/ml adjusted for weight (number of oral drops is equal to weight in kg divided by 3) and using local anaesthesia.

In the BMAC group bone marrow is harvested from the anterior iliac crest: a single incision is made at the level of the anterior iliac crest using a dedicated EC approved kit (Isto Biologics Magellan®) and collected using two 30 mL syringes coated with heparin for a total of 60 mL. This anatomical site has been proven to be one of the most appropriate in terms of biological potential [16]. The harvested bone marrow is filtered with a heparin washed filter and then centrifuged through the Magellan® centrifuge (Isto Biologics, Hopkinton, MA, USA, previously Arteriocyte Medical Systems, Hopkinton, MA, USA) at a rate of 3600 RPM for 15 min, thus obtaining 8 mL of BMAC (Fig. 1). The incision is then sutured using a single stitch, that has to be removed 2 weeks after the surgery.

Fig. 1. BMAC injection procedure.

In the MFAT group adipose tissue is harvested from the subcutaneous abdominal fat, as this site proved to be the most appropriate in terms of biological potential and ease of harvesting [9]. After local anaesthesia, a sub-centrimetric incision is performed on both sides of the lower or lateral abdomen. Before harvesting the fat, each side is injected with 180 ml of Klein solution (1 ml 2µg/ml adrenaline and 40 ml 0.02% lidocaine in 500 mL of saline solution) by using a disposable 17-gauge

blunt cannula connected to a 60-mL Luerlock syringe. Adipose tissue is then collected using a 13-gauge blunt cannula, for fast and atraumatic suction, connected to a 20-mL Vaclock syringe. The harvested fat is immediately processed using the Lipogems system (Lipogems International Spa, Milan, Italy) as previously described [18]. The entire process is performed in complete immersion in isotonic solution, thus minimizing cell trauma. The size of adipose tissue clusters is progressively reduced with a mild mechanical action to microspheres, in accordance with the manufacturer's instructions. This process allows for elimination of oily substances, cell debris, and blood residues. Finally, the resulting micro-fragmented tissue (8 mL) is collected in a 10-mL syringe [19]. The process in shown in Fig. 2. The two incisions are then sutured using a single stitch for each side, which has to be removed 2 weeks after the surgery. After the surgery, a girdle must be worn for 10 days.

Fig. 2. MFAT injection procedure.

An arthrocentesis is then performed in the affected knee to collect synovial fluid. This will not be possible in all patients as synovial fluid may be absent or insufficient. In addition, if synovitis is present, a synovial membrane biopsy is collected with ultrasound guidance with a dedicated biopsy needle. In both groups, the intra-articular injection of 6 ml of orthobiologic products is then performed with ultrasound guidance in a lateral suprapatellar approach using an 18-gauge needle, with the patient in supine position and the knee fully extended. The remaining 2-ml sample is sent to the laboratory for the in vitro analyses together with the synovial fluid and synovial tissue samples.

At the end of the injection, the patient is encouraged to bend and extend the knee a few times to allow the product to spread throughout the joint. The postoperative protocol includes rest and avoiding high-impact sports activities and strenuous work for 2 weeks, without restrictions in weightbearing following the procedure. After stitches removal, gradual return to sport is allowed as tolerated, with exercise bike and aquatic therapy being recommended activities.

Outcomes

Patients are evaluated at baseline and at 2, 6, and 12 months with validated questionnaires. The primary outcome of the study is the Western Ontario and McMaster University Osteoarthritis index (WOMAC) pain score at 6 months. The secondary outcomes of the study are the WOMAC pain score at 2 and 12 months and the WOMAC subscales (function, stiffness), the total WOMAC score, the International Knee Documentation Committee (IKDC) subjective and objective scores, the Knee Injury and Osteoarthritis Outcome score (KOOS), the visual analogue scale (VAS) for pain evaluation, the EuroQol visual analogue scale (EQ-VAS) for the overall quality of life evaluation, and the Tegner score to document the activity level of the treated patients at 2, 6, and 12 months.

Imaging evaluation is performed analysing knees with radiographs (antero-posterior and lateral views) at baseline and at 12 months of follow-up to assess the OA grade according to the Kellgren-Lawrence classification. An approved artificial intelligence (AI) imaging analysis system is also used to determine the Kellgren-Lawrence grade. High-resolution 3.0 Tesla magnetic resonance (MR) imaging is performed at baseline and at 12 months of follow-up, and the Whole-Organ Magnetic Resonance Imaging Score (WORMS) is used to assess articular cartilage morphology, bone marrow oedema, subchondral cysts, articular profile, marginal osteophytes, meniscal integrity, and synovitis. Systemic OA biomarkers are evaluated at baseline and after 2, 6, and 12 months of follow-up, through circulating miRNAs expression analysis and spontaneous osteoclastogenesis evaluation. Circulating miRNAs from peripheral blood samples are analysed as reported below. An aliquot of peripheral blood sample (approximately 5 ml) is stored at 4°C overnight, then centrifuged, and plasma is then stored at -80 °C. Circulating miRNAs are isolated following the guidelines of commercial kit of extraction and isolation (mirVana™ miRNA Isolation Kit), which can provide highly sensitive results by enabling the miRNA detection from just 1 pg starting material. MiRNAs characterization is performed after retrotranscription of miRNAs isolated using TaqMan™ Advanced miRNA cDNA Synthesis Kit. MiRNAs involved in OA progression or inflammation are identified and quantified

using precast microRNA seq panel analysis (TaqMan Advanced miRNA Human A and B 96-well Plates, fast) [20-24]. Data obtained are analysed with bioinformatic and multivariate analyses. Subsequently, a list of putative biomarkers is identified by means of bioinformatic investigations.

Spontaneous osteoclastogenesis is evaluated. Monocytes from patients with OA display enhanced capacity to generate osteoclasts (OCs) compared to cells from healthy controls [25]. OCs are obtained from approximately 2 ml of peripheral blood from each patient. More precisely, peripheral blood mononuclear cells (PBMCs) are isolated with Ficoll density-gradient centrifugation, and then cultured in alpha-minimum essential medium. After monitoring viability, once a week for 3 weeks through Alamar blue dye test, a differentiation assay is performed after 21 days of culture through tartrate-resistant acid phosphatase (TRAP) staining: the large, multinucleated cells (>3 nuclei), which developed a brown colour, are scored as positive cells and the ratio between the brown-coloured region and total image area is measured using an image analysis system of inverted microscope. In addition, the supernatants of cells, after 21 days of culture, are stored at -80°C and evaluated for Cathepsin K (CTSK), metalloproteinase-7 (MMP-7), and MMP-9 production, with immunoenzymatic ELISA tests (minimum detectable dose less than 0.057 ng/ml), for OC activity assessment. Finally, the resorption assay is carried out by culturing PBMCs on bone slices then stained with toluidine blue staining to reveal pits: an image analysis program evaluates the resorption area.

At baseline, autologous BMAC and MFAT remaining from patient treatment (approximately 2 ml), are collected and transferred aseptically to the laboratory for in vitro analysis. An aliquot is immediately cultured for the evaluation of cell viability and protein production. The Alamar blue dye test is used for cell viability assessment and the production of the most important factors involved in trophic and anti-inflammatory processes and interleukins, such as platelet derived growth factor (PDGF), transforming growth factor β (TGFß), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin like growth factor-I (IGF-I), granulocyte-macrophage colony-

stimulating factor (GMCSF), bone morphogenetic protein 2 (BMP2), BMP7, Interleukin 1β (IL1ß), IL6, IL8, and IL-1ra, evaluated from the supernatant using Bio-Plex ProTM panels.

The remaining aliquot of BMAC and MFAT is employed for MSCs characterisation. After 1 day of culture, the adherent cells are evaluated for: i) surface antigen expression through FACS analysis with fluorescein isothiocyanate (FITC)-conjugated antibodies against CD31,34,44,45,73,90 and 105; ii) colony forming units-fibroblast (CFU-f) capacity through Toluidine blue staining after 10 days of culture: The aggregates with >20 cells are visually scored as colonies and counted; iii) three lineages differentiation after 21 days of culture in culture media. Calcium deposits (osteogenic differentiation), lipid accumulation (adipogenic differentiation), and glycosaminoglycans production (chondrogenic differentiation) are evaluated using Alizarin Red S, Oil Red O, and Alcian blue staining, respectively, and an image analysis program. Then, gene expression of SOX-9, ACAN, COMP, ALPL, BGLAP, COL1A1, OPG, RUNX2, ADIPOQ, and PPARG genes is evaluated through RT-PCR.

Synovial fluid (SF) and the synovial membrane are collected at baseline, when the minimal invasiveness of the biopsy is justified by the concurrent procedure performed to treat the patients, for local biomarkers evaluation. More precisely, approximately 1-2 ml of SF are collected from patients with a 18G syringe and sent to the laboratory for the evaluation of the inflammatory grade of the joint and local biomarkers analysis. In detail, a SF aliquot is immediately used by performing the mucin clot test and the compactness of the clot is evaluated. For biomarkers analysis, the most important "Burden of disease biomarkers" [26], such as IL6, IL8, MMP-1, MMP-13, cartilage oligomeric matrix protein (COMP), VEGF, C-teloprotein of type I collagen (CTX-1), Leptin, and tissue inhibitor of metalloproteinase 1 (TIMP1) are evaluated through Bio-Plex ProTM panels (minimum detectable dose less than 2.9 pg/ml).

Synovial membrane biopsies are collected with ultrasound guidance from patient affected by hypertrophic membrane related to synovitis with a dedicated biopsy needle and sent to the laboratory for miRNA evaluation. After lysis and homogenisation of the biopsies in NucleoZOL reagent, contaminating molecules are precipitated by the addition of water and are removed by centrifugation.

RNA is reconstituted by RNase-free water and is stored at -80°C overnight. MiRNAs are characterised after retrotranscription and their investigation is performed by microRNA seq using a commercial and precast miRNAs panel analysis that permits to identify the expression of 376 miRNAs (TaqMan Advanced miRNA Human A and B 96-well) providing highly sensitive results by enabling the miRNA detection from just 1 pg starting material.

Factors that can influence the clinical response to the injections of BMAC or MFAT are investigated to identify aspects predictive of a better outcome.

1) Baseline demographic characteristics are analysed, including sex, age, body mass index (BMI), OA severity, symptom duration, knee alignment, previous knee injective treatment, and previous knee surgery. All these factors are correlated to the different scores used to quantify the different subjective, objective, symptomatic, and functional aspects related to the patient experience in response to the applied treatment. These aspects are investigated both in terms of improvement and benefit duration, as well as adverse events and failures.

Participant timeline

The study has a total duration of 36 months. Patient screening, enrolment, and treatment will last 22 months and started in January 2024. The first patient was treated in January 2024. The follow-up evaluations last 12 months. Clinical evaluation is performed at baseline, 2, 6, and 12 months. Imaging evaluation (MR, radiographs) is performed at baseline and 12 months. The biological analysis on blood samples is performed at baseline, 2, 6, and 12 months. Detailed participant timeline is outlined in Table 1.

Table 1. Study schedule.

	Pre-operatory medical examination (baseline)	Surgical procedure	2-month follow-up	6-month follow-up	12-month follow-up
Inclusion/exclusion criteria evaluation	X				
Informed consent signing	X				
Demographic data	X				
Medical history	X				
Randomisation	X				
WOMAC	X		X		X
IKDC Subjective	X		X		X
IKDC Objective	X		X		X
KOOS	X		X		X
PASS	X		X		X
EQ-VAS	X		X		X
Tegner	X		X		X
MCID			X		X
Patient expectation on the treatment	X				
Patient judgement on the treatment					X
Adverse events reporting		X	X		X
Medication tracking sheet			X		X
Radiographs and KL evaluation	X				X
3T MR and WORMS	X				X
Blood sample collection		X	X		X
SF collection and synovial membrane biopsy		X			

EQ-VAS, EuroQol-Visual Analogue Scale; IKDC, International Knee Documentation Committee; KL, Kellgren-Lawrence; KOOS, Knee Injury and Osteoarthritis Outcome score; MR, Magnetic Resonance; T, Tesla; SF, synovial fluid; WOMAC, Western Ontario and McMaster Universities Arthritis Index; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

Recruitment

Patients undergo an outpatient visit conducted by trained medical staff of the IRCCS Istituto Ortopedico Rizzoli, which assesses patients' eligibility and informs patients of design and content of the study.

Blinding

This is a RCT with radiologists, biologists, and physicians assessing outcomes being blinded to the treatment allocation. Considering the different nature and harvest source of the injected products it is not possible to blind patients and surgeons performing the procedure. Nevertheless, this should not affect patient expectations and thus study results, being both promising cell-based orthobiologic procedures. Moreover, the statistician involved in the study will be blinded to the treatment groups for data analysis.

Allocation

A total of 204 eligible patients is allocated to receive either a single BMAC injection or a single MFAT injection, in a 1:1 ratio (102 patients for each group of treatment). The list for treatment allocation is provided by an independent professional statistician (blinded to the treatments) as generated using a random number generator and then kept in a dedicated data manager office. The allocation is managed by research staff members dedicated to study organisation and monitoring with no direct involvement in the clinical procedures. The randomisation list is password-protected and accessible only by staff members with no direct involvement in the treatment and evaluation.

Stratified permuted block randomisation is used to avoid gender imbalances. Therefore, study participants are divided according to the gender, so block randomisation will be used for each gender. This form of randomisation is recommended for such clinical trials, where known factors (such as sex) are believed to influence treatment outcomes. The permuted block randomisation technique randomises patients between groups within a set of study participants, called a block. Treatment

allocations within blocks are determined so that they are in random order but that the desired allocation proportions are achieved exactly within each block. The randomisation procedure is managed using the website: www.sealedenvelope.com/simple-randomiser; a randomisation list with a block size of 6 will be generated. The eligible subjects are randomised in a 1:1 ratio into one of the two treatment groups:

- Group 1: single intra-articular BMAC injection.

- Group 2: single intra-articular MFAT injection.

Adverse events assessment

All adverse events are assessed and recorded in the patient case report form (CRF) and will be evaluated to define whether they may be related to the study intervention or not. Patients are requested to report any adverse event to the research staff and can inform physicians of potential adverse events at all follow-ups (medical examinations) or through patient-physician communication between follow-ups via phone calls or emails. Adverse events are monitored throughout the study, intra-operatively and at all clinical follow-up evaluations. The CRF also includes the use of pain medications (brand drug name or generic substitute, frequency and duration) and is recorded at all medical examinations. Serious adverse events are considered those resulting in death or being life-threatening, requiring hospitalisation or intervention to prevent permanent impairment or damage. Serious adverse events will be communicated to the Ethics Committee. The expected risk/benefit ratio for these procedures is positive, as the most frequently foreseeable adverse events are mild and resolvable adverse events. In case patients will undergo procedures such as intraarticular injections, radiofrequency of the genicular nerves or even surgery during the follow-up period, they will be considered failures.

To ensure high-quality execution of the trial in accordance with the protocol, all trial staff is trained by the chief investigators and provided with a document outlining the details of the standard operating procedures, trial contacts, and guidelines.

Data collection and management

Data are collected on a paper-based CRF, with the help of research trained orthopaedic surgery residents blinded to treatment allocation. Subsequently, trained data analysts process all data for statistical analysis. Radiographs and MR scans are stored on IRCCS Istituto Ortopedico Rizzoli's Picture Archiving and Communication System (PACS). Surgical data are collected electronically by orthopaedic surgeons shortly after surgery.

Biological data are collected and stored in a password-protected spreadsheet on a server hosted at IRCCS Istituto Ortopedico Rizzoli. Data transfer is encrypted with all data de-identified. Only trained research personnel specifically dedicated to the data handling can access the database.

Statistical methods

The sample size calculation was performed by an independent statistician and is based on the power analysis of the primary end point (change in the WOMAC-pain subscale at 6-month follow-up compared to baseline). From previous studies, the WOMAC-pain subscale standard deviation at 6 months of follow-up is 4.0 [27] with an MCID of 1.7 [28]. The resulting effect size is therefore 0.425. Assuming to perform an unpaired t test (comparing the improvement of treatment group 1 versus the improvement of treatment group 2) with an alpha error of 0.05 and a power of 0.8, the minimum sample of patients is 88 for each of the two treatment groups, with a total of 176 patients. Considering a 15% possible drop-out, 102 patients per group are needed, with a total of 204 patients. The power analysis was performed using G*Power 3.1.9.2. With the Unpaired t test the improvement of treatment group 1 is compared with the improvement of treatment group 2. The primary analysis on the primary outcome is performed with a per-protocol approach as well as with an intention to treat

analysis. The multiple imputation method will be used for missing data. Per-protocol analyses will be performed for the secondary outcomes. Continuous variables are expressed as means and standard deviations if normally distributed, as medians and ranges otherwise. Categorical variables are expressed as frequencies and percentages. The normality of the distribution is assessed using the Shapiro Wilks test. The Levene's test is used to evaluate the homoscedasticity of the data. Repeated measures ANOVA, followed by post hoc Sidak pairwise test, are performed to compare scores at different follow-ups. The OneWay ANOVA test is performed to evaluate the difference between groups of continuous and normally distributed and homoscedastic data; otherwise the Mann Whitney test is used. The general linear repeated model (for no missing follow-ups and normally distributed and homoscedastic data) or the generalised linear mixed model (for all the other cases) are used to assess the influences of the groups on the repeated measures of the outcomes. The group is the fixed effect and any correction for confounding factors is considered as a random effect. Pearson's exact chi-square test is performed to study the relationships between the grouping variables. Spearman rank correlation is used to evaluate correlations between numerical scores and continuous data. Kaplan Meier analysis, followed by Log rank test, is performed to evaluate the difference between groups in failure rate. For all tests $p<0.05$ is considered significant.

Data monitoring

A central project data manager is tasked to perform data quality control on all collected data. An interim report and a final report are foreseen, to be submitted to the Italian Ministry of Health which funded the project (GR-2021-12374140). The monitoring personnel belongs to the Applied and Translational Research center, which is a research division of IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy Scientific Direction and it is independent from the medical personnel performing the study procedures. A further project auditing is performed by the Clinical Trial Center, which is another independent entity of the Institute. The final study report is also sent to the Ethics Committee.

ETHICS AND DISSEMINATION

Research ethics approval

Ethical approval was obtained on March 23rd, 2023, from Emilia Romagna's Ethics Committee (CE-AVEC), Bologna, Italy (protocol number: 150/2023/Sper/IOR).

Consent or assent

All participants give informed written consent prior to enrolment during the baseline outpatient medical examination with the trained medical staff and according to the study protocol and may withdraw from the trial at any time. A translated copy of the official patient consent form originally written in Italian is attached as supplementary file.

Protocol amendments

Minor protocol amendments, for example, database changes to facilitate monitoring processes or to improve outcome assessment by questionnaire, are fully documented. Major amendments (e.g. changes to the patient information sheet and consent form, change of a local project leader or the inclusion of a new project site) are submitted to the Ethics Committee for approval.

Confidentiality and access to data

Data are recorded using CRFs and processed centrally at the IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy . CRFs hard copies are stored in a locked area with restricted and secured access. Electronic data are stored on password protected servers with restricted access. The collected data are kept confidential. Backups of all electronic data occur daily to minimise risks of data loss. After study completion, data paper-based copies are archived in a secure storage. Identifiers are kept separately and are accessible only to restricted study personnel in case follow-up of study patients is necessary. To protect patient privacy, only members of the research team who need to contact study patients,

enter data, or perform data quality controls have access to the study patient information, besides the auditing personnel from the Institution and Ethics Committee.

Dissemination policy

This trial is produced according to the SPIRIT international standards. Results will be disseminated through peer-reviewed publications and submitted for presentation at national and international conferences. The authorship is based on International Committee of Medical Journal Editors 2018 Recommendations.

Scientific relevance and broader impact

This RCT will provide definitive evidence on the clinical efficacy and disease-modifying effects of BMAC and MFAT. Current orthobiologic treatments for knee OA offer a partial symptom relief and often require further invasive procedures. BMAC and MFAT contain MSCs, GFs, and cytokines with anti-inflammatory, regenerative, and immunomodulatory properties that could help delay or possibly avoid the need for joint replacement. However, while these products are extensively used in the clinical practice, there are no high-level trials to guide the treatment choice. This study will provide an innovation in the field by demonstrating if the more recently developed and promising MFAT is able to outperform the more traditional BMAC. In addition, the study will evaluate patient-based determinants for the efficacy of each product. This will be of significant clinical relevance, offering clear and more stratified indications on the most effective solution to treat the challenging patients affected by OA, often doomed to invasive procedures. This project also aims to shed some light about the evolution of knee OA, both from imaging and biomolecular points of view, to better understand the pathogenesis of this debilitating disease and its response to treatment.

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Contributors

LA is the principal investigator of this study. LZ and MF wrote the manuscript and will conduct the trial. MM and PS are responsible of imaging evaluation. FV, VC and GG are involved in products and patients' characterisation. SZ and GF applied for funding and supervise the trial. All authors read and approved the final protocol.

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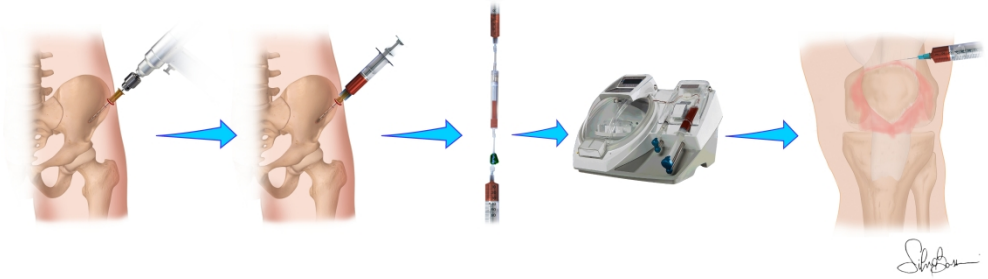

Fig. 1. BMAC injection procedure.

328x122mm (400 x 400 DPI)

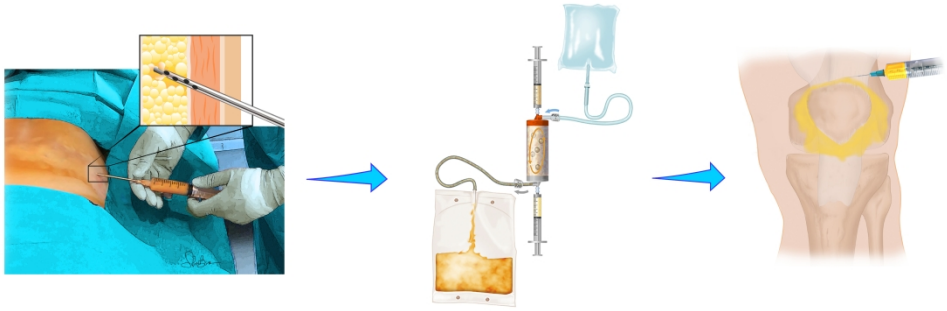

Fig. 2. MFAT injection procedure.

328x122mm (400 x 400 DPI)

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Istituto di Ricovero e Cura a Carattere Scientifico

INFORMATION SHEET AND INFORMED CONSENT FORM FOR PATIENT PARTICIPATION IN A CLINICAL TRIAL

Official title of the study: BONE MARROW VERSUS ADIPOSE TISSUE AS A CELL SOURCE FOR INJECTIVE TREATMENT OF KNEE OSTEOARTHRITIS: A RANDOMISED CLINICAL TRIAL
Official title of the trial in more patient-friendly terms: Treatment of knee osteoarthritis with an injection of mesenchymal cells taken from bone marrow or adipose tissue: a randomised study.
Structure-context in which the experimentation will take place IRCCS Rizzoli Orthopaedic Institute, Via Pupilli 1, 40136, Bologna
Registry in which the trial has been or will be registered (if applicable) and any identification code if available Identification code NCT06040957 ClinicalTrial.gov Registry
Principal Investigator Name Dr. Luca Andriolo Affiliation: IRCCS Rizzoli Orthopaedic Institute, Orthopaedic and Traumatology Clinic 2
Promoter: IRCCS Rizzoli Orthopedic Institute
Ethics Committee: CE-AVEC (Area Vasta Emilia Centro)

This document is composed of the following sections:

A. INTRODUCTION
B. INFORMATION SECTION. TRIAL SUMMARY: KEY INFORMATION
C. INFORMATION SECTION. FURTHER INSIGHTS
D. EXPRESSION OF CONSENT SECTION

ATTACHMENTS

ADDITIONAL DOCUMENTS

Dear Madam/Sir., the information contained in the following information sheet is very detailed. We ask you to agree to participate in the trial ONLY after having carefully read this information sheet and having had an EXHAUSTIVE INTERVIEW with a member of the trial group who will dedicate the NECESSARY TIME to fully explain what is being proposed to you.

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A. PREMISE

Dear Madam/Sir,

We propose that you participate in the clinical trial, which we illustrate below.

You have the right to be informed about the purpose and characteristics of the trial so that you can decide freely and consciously whether to participate.

This document aims to inform you about the nature of the trial, its purpose, what the participation will entail for you, including your rights and responsibilities.

We invite you to read carefully the following pages. The researchers involved in this project, listed at the beginning of this document, are available to answer your questions. No question that comes to mind is trivial: do not be afraid to ask it!

In addition to us, you can discuss the proposal contained in this document with your GP, your family members and other people you trust. Take all the time you need to decide. You can take an unsigned copy of this document home to think about it or discuss it with others before making a decision.

Participation in the trial is voluntary. If you decide not to participate in the trial, you will still receive the best care possible for patients with your condition/disease.

A refusal will in no way be interpreted as a lack of trust.

Once you have read this form, received answers to any questions you may have and have decided to take part in the trial, you will be asked to sign a consent form, of which you will receive a paper copy.

The Principal Investigator

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B. INFORMATION SECTION.
OVERVIEW OF THE TRIAL: KEY INFORMATION

This section aims to present in a concise manner the key aspects of the trial we are proposing you to join. The following sections will provide more details in order to give you the possibility to express or not a fully informed consent to your participation in the trial.

- Why am I being asked to participate in this experiment?

We are asking you to participate in a clinical trial at the Rizzoli Orthopaedic Institute because you suffer from knee osteoarthritis and we have the opportunity to offer you an innovative treatment within this clinical trial that aims to compare the safety and efficacy of a single intra-articular injection of cellular material obtained from bone marrow or adipose tissue in order to determine which of the two products is more effective in the treatment of patients with knee OA.

You have been included among those asked to participate in this trial because you present some clinical characteristics that will be better specified in section C.

- What are the objectives of the trial? How many centers and patients will participate?

The trial is conducted to evaluate and compare the clinical results of an injection of autologous marrow concentrate (BMAC) versus an injection of minimally manipulated adipose tissue (MM-AT) for the treatment of patients with knee osteoarthritis . The study also aims to compare the effect of the two different orthobiological products obtained from bone marrow or adipose tissue on the evolution of osteoarthritis, assessed with radiographic and magnetic resonance imaging examinations and with blood tests . Finally, the tertiary objective is to characterise the two cellular products and identify the factors that influence the clinical response to treatment (baseline characteristics of the patients, biological characteristics of the treated knee, characteristics of the cellular products).

This study will be conducted in a single center (Rizzoli Orthopedic Institute in Bologna) and will include 204 patients.

- What is the routine care approach for the treatment of knee osteoarthritis?

Currently, for the treatment of knee osteoarthritis in normal clinical practice, physical therapies such as physiotherapy sessions are used. For subjects with a body mass index above the norm, weight loss is recommended. Alternatively, it may be recommended to take an oral anti-inflammatory drugs or an injection therapy with hyaluronic acid.

- Is it my free choice whether or not to participate?

You can freely choose whether or not to participate in the study. Even after accepting, you can change your mind at any time.

- If I decide not to give my consent to participate in the trial, what choices do I have?

If you decide not to join the trial, you will be followed by the clinical center that is treating you and will be treated using the best approved therapeutic methods (not experimental) for your disease.

Alternatives to the experimental treatment include physiotherapy exercises, local or systemic painkillers/anti-inflammatory drugs, and infiltrative treatments based on hyaluronic acid. If these conservative therapies are ineffective, a partial or total knee replacement will be considered.

Furthermore, you may participate in any other trials that may be underway.

- What happens if I decide to participate in the trial?

The study in which we propose you to participate is characterized by the concept of "randomization": the patients included in the study will be divided into two treatment groups thanks to the use of a particular software that randomly assigns the patient to one of the two different treatments . The patients, therefore, will receive an injection of autologous marrow concentrate (BMAC) or an injection of minimally manipulated adipose tissue (MM-AT).

The treatment administered will be communicated to the patient upon admission to the hospital.

Before taking part in the trial, the doctor will ask you to perform some tests and will check whether you have the characteristics required to take part in the study. During the trial, in patients randomized in the BMAC group, the procedure involves a bone marrow sampling that will be performed from the iliac crest, while in patients randomized in the MM-AT group the adipose tissue sampling will be performed from the abdominal subcutaneous fat. Before the injection, a peripheral venous blood sampling will be performed and, where possible, synovial fluid will be collected from the treated knee and a synovial biopsy will be performed. Patients will be clinically evaluated with the administration of questionnaires before the procedure and 2, 6, and 12 months after the treatment by specialised medical personnel.

The entire program of visits and tests planned during the trial is reported in the next section "What tests, examinations and procedures are planned in the trial?"

- What are the risks and benefits if I participate in the trial?

There may be both risks and benefits to participating in this trial. It is important to weigh these carefully before making a decision.

Expected benefits

BENEFITS FOR THE PATIENT WHO WILL PARTICIPATE IN THE TRIAL: *the aim of the therapy consists in the elimination or reduction of pain and inflammatory manifestations, in preventing functional limitation by accelerating the favorable evolution of the process, with the aim of delaying or making surgical treatments unnecessary.*

Potential risks

We want to make sure you understand some of the possible risks right from the start: additional information can be found in the next section "What risks might I face if I take part in this trial?"

The procedure involves either a bone marrow sample that will be taken from the iliac crest or a fat sample that will be taken from the abdominal subcutaneous fat.

We list below some possible risks associated with the treatments:

Intra-articular injection of minimally manipulated adipose tissue/concentrated bone marrow aspirate:

- *Allergic reaction and/or synovitis and pseudoseptic reaction (non-infectious reaction simulating an infectious reaction).*
- *Inflammation of the joint and surrounding tissues with pain, redness and swelling.*
- *Pain, including joint pain and/or reflex sympathetic dystrophy (pain is not caused by abnormalities in the operated area but is apparently due to abnormal nerve firing). This is rare.*
- *Joint stiffening, resulting in limited mobility.*
- *Joint effusion and swelling, due to the presence of fluids.*
- *Formation of cysts in bone or joint tissue, which can limit joint movement and cause pain.*
- *Risk of intra-articular infection following the injection.*

Bone marrow aspiration/liposuction:

- *Pain in the perioperative period, only rarely does the pain persist longer.*
- *Alteration of sensation, mostly transitory, affecting the skin of the sampling area. They can manifest as "paresthesias", with a temporary or permanent alteration of sensation (at the site of the bone marrow aspiration), which can be increased (pain) or decreased (numbness of the area).*
- *Allergic or toxic reaction to drugs used during anesthesia.*
- *Blood loss, which is always present in limited quantities and rarely more significant.*
- *Hematoma: usually small and tends to resolve in about 14 days. In some cases it can persist longer, up to several months.*
- *Dizziness.*
- *Wound complications, including but not limited to infection. Infection is an inflammation caused by bacteria entering the body. It can be superficial (the wound area) or deep (deep tissues, including joints and bones). There is also a risk of septicaemia (generalized infection caused by the presence of an infectious agent in the blood), reopening of the wound, pain, tissue swelling, adhesions (these occur when a bundle of abnormal tissue joins two different anatomical planes, limiting their movement) and other reactions, such as allergy and inflammation.*

- *Deep vein thrombosis*
- *Seroma: an accumulation of serum may occur in areas where fatty tissue has been removed.*
- *Skin Surface Irregularities: the skin may appear uneven or sagging due to uneven fat removal, poor skin elasticity, or unusual healing. Sagging occurs when the skin in the liposuction area does not tighten around the new contours. Patients with cellulite may develop uneven skin due to over- or under-correction of localized fat deposits. These changes may be permanent, even if the amount of fat removed for treatment is relatively small.*
- *Abrasion of surrounding tissue (damage caused by friction during surgery), likely to trigger pain, damage tissue.*
- *Wound complications, including but not limited to infections. Infection is an inflammation caused by bacteria entering the body. It can be superficial (area of the wound) or deep (deep tissues, including joints and bones). There is also the risk of septicemia (generalized infection caused by the presence of an infectious agent in the blood), reopening of the wound, pain, tissue swelling,*

adhesions (occur when a bundle of abnormal tissue joins two different anatomical planes, limiting their movement) and other reactions, such as allergy and inflammation.

- *Deep vein thrombosis (blockage of blood flow in the veins). The risk of this complication is present in any type of surgery and can cause serious problems, including swelling of the limbs. This complication can also require hospitalization and cause other health problems, and even death.*
- *Lack of efficacy of the experimental treatment (even if we believe that the new treatment may act on osteoarthritis better than those already available, we cannot exclude that it may be ineffective in you);*

Radiograph (X-ray):

The x-rays to be acquired during the 0 and 12 month visits will involve exposure to radiation. The risks associated with exposure to ionizing radiation are linked to DNA alterations with possible onset of mutations and neoplasms. Such occurrences are exclusively attributable to massive doses of radiation and prolonged exposure. The examination lasts approximately 5 seconds.

The radiation used during the study could cause harm to your health. However, the risk is very low.

Magnetic resonance imaging (MRI):

Magnetic resonance imaging is a non-invasive medical examination that is performed under the supervision of a radiologist, who also takes care of the subsequent reporting. This examination helps diagnose pathologies that cannot be adequately assessed with other diagnostic imaging techniques, such as x-rays, ultrasounds or computerized axial tomography (better known as CT).

Magnetic resonance imaging is a safe and painless diagnostic procedure. However, you may feel a slight discomfort due to the noise of the scanner, similar to the sound produced when knocking on a door. MRI uses a powerful magnet and radio waves to produce detailed images of organs and anatomical structures without using different types of radiation. A computer converts the signals from the MRI scan into extremely clear cross-sectional images of the part of the body being examined. Each image represents a portion of the scanned body part. Numerous images are created that can clearly show all the characteristics of that specific part of the body. As in all cases, before an MRI, the doctor will check, using a specific questionnaire, that you do not have metal particles of any size inside your body. These particles (if they are of certain types of metal) may not be able to withstand the magnetic field that the resonance machine produces, so you may not be suitable for the examination and therefore not included in the study protocol.

Venous blood sampling:

- *Subcutaneous hematoma (accumulation of blood within the tissue)*
- *Vagal syndrome (feeling of dizziness)*

Synovial biopsy:

- *Vagal syndrome (feeling of dizziness)*
- *Subcutaneous hematoma (accumulation of blood within the tissue)*
- *Hemarthrosis ((a buildup of blood into a joint, rarely a significant amount)*
- *Pain during the procedure*

- Is the consent final? Can I decide to withdraw from the clinical trial (voluntary withdrawal)?

You can decide to withdraw from the trial at any time and for any reason, without having to justify your decision.

If you decide not to participate, let one of the trial doctors know as soon as possible: it is important to stop treatment safely. Your doctor may decide that a final follow-up visit/exam is appropriate.

Your doctor will keep you informed of any changes in the trial that may affect your willingness to participate.

In case of early withdrawal, the data collected up to that point will be used by the researchers.

- Are there reasons why the trial could be interrupted against my will (early termination)?

Yes, the investigating doctor may decide to discontinue his/her participation in the trial if:

- *If your health conditions change and participating in the trial is potentially harmful*
- *If new information becomes available and the trial is no longer in your best interest*
- *You did not follow the agreed rules for participation in the trial*
- *For women: if you happen to become pregnant during the trial*

C. INFORMATION SECTION . FURTHER INSIGHTS

1. What is the purpose of the experiment?

The aim of the study is to evaluate, through a randomized clinical trial, the clinical outcomes of an injection of autologous marrow concentrate (BMAC) compared to an injection of minimally manipulated adipose tissue (MM-AT) for the treatment of patients with knee osteoarthritis. The study also aims to compare the effect of the two different orthobiological products obtained from bone marrow or adipose tissue on the evolution of osteoarthritis, assessed with radiographic and magnetic resonance imaging examinations and with blood tests. Finally, the tertiary objective is to characterise the two cellular products and identify the factors that influence the clinical response to treatment (baseline characteristics of the patients, biological characteristics of the treated knee, characteristics of the cellular products).

2. What are the patient groups being compared? What is the intervention being tested?

Patients with knee osteoarthritis will be divided into two treatment groups using a special software that randomly assigns the patient to one of two different treatments. Patients will then receive either an injection of autologous bone marrow concentrate (BMAC) or an injection of minimally manipulated adipose tissue (MM-AT).

INCLUSION CRITERIA:

PATIENTS WITH SYMPTOMATIC UNILATERAL KNEE OSTEOARTHRITIS WITH:

1. MEN OR WOMEN BETWEEN THE AGES OF 40 AND 75;
2. SIGNS AND SYMPTOMS OF OA WITH A HISTORY OF KNEE PAIN OR SWELLING FOR AT LEAST 6 MONTHS;
3. RADIOGRAPHIC SIGNS OF OA (KELLGREN -LAWRENCE GRADE 1-4);

4. NO BENEFIT AFTER AT LEAST 4 MONTHS OF CONSERVATIVE TREATMENT;

5. ABILITY AND CONSENT OF PATIENTS TO ACTIVELY PARTICIPATE IN CLINICAL AND RADIOLOGICAL FOLLOW-UP (X-RAY AND MRI);

6. SIGNING OF INFORMED CONSENT.

EXCLUSION CRITERIA:

1. PATIENTS UNABLE TO GIVE CONSENT;

2. PATIENTS AGED < 40 AND > 75 YEARS;

3. PATIENTS WITH AXIAL DEVIATIONS > 5°;

4. PATIENTS WITH HISTORY OF KNEE TRAUMA OR WHO HAVE UNDERGONE INTRA-ARTICULAR INJECTION OF ANOTHER SUBSTANCE IN THE PREVIOUS 6 MONTHS;

5. PATIENTS WHO HAVE UNDERGONE KNEE SURGERY IN THE PREVIOUS 12 MONTHS;

6. PATIENTS WITH CONCOMITANT KNEE INJURIES CAUSING JOINT PAIN OR SWELLING (FOCAL FULL-THICKNESS OSTEOCHONDRAL DEFECTS, ACUTE MENISCAL LESIONS).

7. PATIENTS WITH MALIGNANT NEOPLASMS;

8. PATIENTS WITH UNCOMPENSATED SYSTEMIC DISEASES (RHEUMATIC DISEASES, METABOLIC DISORDERS SUCH AS DIABETES AND THYROID DISEASES);

9. PATIENTS SUFFERING FROM INFECTIOUS DISEASES;

10. PATIENTS WITH A HISTORY OF ALCOHOL OR DRUG ABUSE.

11. PREGNANT PATIENTS

12. PATIENTS WITH ALLERGIES TO THE ANESTHETICS USED IN THE PROCEDURE.

Eligible subjects will be randomized 1:1 to one of two treatment arms:

- *Treatment group 1: single intra-articular injection of BMAC.*
- *Treatment group 2: single intra-articular injection of MM-AT*

In case further investigations with that particular product are abandoned for your illness, your doctor will re-evaluate the possible treatment options.

3. What tests, exams and procedures will I undergo if I participate in the trial?

The protocol provides that the surgical procedure will be performed in a single stage in an outpatient surgical setting (about 45 minutes) in accordance with common hospital practices. The patient's stay in hospital will be about 2 hours. The investigator and all the personnel involved will be appropriately trained regarding the surgical and study protocol.

In patients randomized to the BMAC group, bone marrow sampling will be performed under local anesthesia from the anterior iliac crest with a dedicated needle. In patients randomized to the MM-AT

group, adipose tissue sampling will be performed from the abdominal subcutaneous fat, under local anesthesia and using specific cannulas. Depending on the randomization, approximately 60 mL of bone marrow or adipose tissue will be collected, which will then be processed to obtain 8-10 mL of minimally manipulated product (BMAC or MM-AT).

Both procedures will be performed under local anesthesia in accordance with common hospital practices. After the collection and processing of the bone marrow or adipose tissue, an intra-articular injection of the obtained product (BMAC or MM-AT) will be performed. Before the injection, a peripheral venous blood sample will be taken and, where possible, synovial fluid will be collected from the treated knee and a synovial biopsy will be performed.

Patients will be clinically evaluated with questionnaires before the procedure and 2, 6, and 12 months after the treatment by specialised medical personnel. During the follow-up visits, any adverse events to the treatment will also be evaluated. The duration and extent of joint swelling and pain following the injection will be reported, and any pharmacological therapies administered by the patient will be recorded. A peripheral blood sample will be collected at 0-2-6-12 months of follow-up, and imaging evaluations (X-ray and MRI) will be performed at 0 and 12 months of follow-up.

What risks might I face if I participate in the trial?

There are no additional risks to those already reported in the "Potential Risks" section.

4. Is it useful/necessary to inform the general practitioner/pediatrician of free choice?

Given the study design, if you decide to participate it is important that you inform your GP. To this end, we have prepared a letter that you can give to him, in which the study procedures are explained.

5. What will be my commitment and what are my responsibilities if I decide to participate?

- *Strictly observe the instructions and requests of the healthcare personnel following the trial and ensure attendance at appointments.*
- *Inform the doctor who is following the trial:*

of all the medicines you are taking including non-conventional medicine drugs,
of any side effects that arise during the trial,
of any hospital visits or admissions to facilities other than the experimental center,
of current or previous participation in other clinical trials.

- *(* For women *): Avoid pregnancy or breastfeeding during the trial.*
- *(* For all, where appropriate *): Tell your doctor promptly if you or your partner think you may become pregnant during the trial.*

For a female patient: "We remind you that the treatment provided for in the trial could harm the fetus. It is therefore expected that you will subsequently commit not to become pregnant. If you agree to participate in this trial, you must therefore use a safe method of contraception during the trial period and for 12 months after the injection.

6. Will I incur any costs for participating in the trial? Will I be reimbursed for any expenses? Will I receive compensation?

There are no costs to be borne by you arising from participation in the trial as these are entirely covered by the trial centre.

Furthermore, no financial compensation is foreseen for participation in the experiment.

7. What happens if I suffer harm as a result of participating in the trial?

Participation in a clinical trial may involve inconveniences and risks that cannot be determined a priori. For this reason, the clinical trial provides insurance coverage to protect your participation.

In compliance with the laws in force, insurance is arranged to cover any damages suffered due to participation in the trial, for the entire period of the trial, to cover the civil liability of the experimenter and the promoter. A copy is attached.

It should be noted that, according to the Ministerial Decree of 14 July 2009, the insurance policy does not cover the value exceeding the maximum limit and is only valid for damages for which the request for compensation has been submitted no later than the period provided for in the policy. This limitation does not, however, compromise your right to obtain compensation from the person responsible for any damage (to protect the subject of the trial).

Contact the Principal Investigator Dr. Luca Andriolo in case of any study-related harm.

8. How will my health data, including identifying information, be treated and who will have access to it during the trial?

Your data, in particular personal data and health data and only to the extent that they are essential in relation to the objective of the trial, will be processed in compliance with EU Regulation 2016/679, known as GDPR (General Data Protection Regulation). Regulation) and Legislative Decree 10 August 2018, n. 101. In practical terms, the documents relating to the participant will be kept in a safe place and will not show his name in clear text, known only to researchers, but an identification code.

The data, in pseudo-anonymized form (i.e. associated with a code and not with your name), may be subject to control by regulatory bodies and used for scientific publications (journals, conferences).

Your clinical data collected for the purposes of the trial, as well as the results of the tests performed, will be stored for the time required by law and subsequently destroyed. They will not be destroyed only in the event that a) it is no longer possible to trace them back to your identity, because they are anonymised during the trial itself; b) in the presence of your specific informed consent.

If personal data is transferred to a third country or to an international organisation, all the guarantees provided for by Article 46 of GDPR 679/2016 relating to the transfer will be adopted.

Further information is included in the attached data processing authorization form.

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9. How will my biological samples collected for the purposes of experimentation be treated and who will have access to them?

As with your health data, your biological samples, pseudo-anonymized (a technique that allows you to modify and mask the personal and sensitive data of a natural person, in order to prevent them from being directly and easily attributable to the same person), will also be used for the purposes of the study.

Further information is included in the attached data processing authorization form.

10. How will I have access to the trial results?

Once the trial is concluded and all the resulting data has been collected, they will be analyzed to draw conclusions. The experimenters and the promoter will make them available to the scientific community.

The law provides for the possibility of participants to have access to the results of the trial. Therefore, you may ask the experimenting doctor to communicate to you the general results of the trial.

11. Was the trial approved by the Ethics Committee?

The trial protocol that has been proposed to you has been examined and approved by the Ethics Committee Area Vasta Emilia Centro (CE AVEC). The Ethics Committee has, among other things, verified that the trial complies with the Rules of Good Clinical Practice and the ethical principles expressed in the Declaration of Helsinki and that your safety, rights and well-being have been protected.

12. Who can I contact to obtain more information about the clinical trial I am invited to participate in?

For further information and communications during the study the following staff will be available:

Dr. Luca Andriolo, 051/6366567, luca.andriolo@ior.it

Dr. Alessandro Di Martino, Dr. Angelo Boffa , [redacted]

You can request information on the rights of study participants from the Local Office of the AVEC Ethics Committee, Istituto Ortopedico Rizzoli 40136 Bologna, - E-mail: [redacted]

13. If I join the trial, who can I contact in case of need?

For any doubts and unplanned or unscheduled events during the trial (doubts relating to the ongoing treatment, side effects, decision to abandon the trial, etc.), you can contact:

[redacted]

If you deem it appropriate to report events or facts relating to the trial you have joined to subjects not directly involved in the trial itself, you can refer to the AVEC Ethics Committee Office, Rizzoli Orthopaedic Institute 40136 Bologna, - E-mail: [redacted]

_____ ___/___/_____ _____ _____

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Full name of the doctor who delivered the information

Date Time Signature

Attachments

- Insurance policy
- Form for consent to the processing of personal data

Additional documents:

- Letter for the general practitioner

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EXPRESSION OF CONSENT SECTION

(Note: 1 copy for the participant, 1 copy for the principal investigator)

Title of the trial: BONE MARROW VERSUS ADIPOSE TISSUE AS A CELL SOURCE FOR INJECTIVE TREATMENT OF KNEE OSTEOARTHRITIS: A RANDOMIZED CLINICAL TRIAL

Protocol Code, version and date: MAST-GR, v1.1 of 02/03/2023

Promoter of the experiment/sponsor/funding body: IRCCS Istituto Ortopedico Rizzoli

Principal Investigator (NAME, AFFILIATION, REFERENCES): DR Luca Andriolo, Orthopaedic and Traumatology Clinic 2

I, the undersigned _____

born_____ on ___/___/_____

I DECLARE

☐ that I have received from Doctor _____ comprehensive explanations regarding the request to participate in the research in question, as reported in the information section, which is part of this consent, a copy of which was given to me on _____;

☐ that the nature, purposes, procedures, expected benefits, possible risks and inconveniences and alternative treatment methods compared to the proposed clinical trial have been clearly explained to me and I have understood;

☐ to have had the opportunity to ask any questions to the study investigator and to have received satisfactory answers;

☐ to have had sufficient time to reflect on the information received;

☐ to have had sufficient time to discuss it with third parties;

☐ to have been informed that the trial protocol and all the modules used have received a favorable opinion from the competent Ethics Committee;

☐ to be aware that the research may be interrupted at any time, by decision of the research manager;

☐ that I have been informed that I will be informed of any new data that may compromise the safety of the research and that, for any problems or further questions, I can contact the experimenters;

☐ that for the best protection of my health I am aware of the importance (and my responsibility) of informing the general practitioner of the trial in which I agree to participate. I am aware of the importance of providing all information (drugs, side effects, etc.) concerning me to the experimenter;

☐ that I have been informed that the results of the study will be made known to the scientific community, protecting my identity in accordance with current privacy legislation;

☐ to be aware that any choice expressed in this consent form may be revoked at any time and without any justification;

☐ that you have received a copy of this consent form.

I therefore DECLARE that I

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☐ want to participate in the experiment

☐ want ☐ do NOT want to be informed of all unexpected news relating to my present or future health that may incidentally emerge from the investigations foreseen by the trial, including genetic ones, when this could lead to possible benefits

☐ want ☐ do NOT want to be informed of unexpected news regarding my present or future health only when this may be useful for my health care or to allow me to make informed reproductive choices

☐ want ☐ do NOT want to be contacted again after the end of the trial to provide information on my health status (applies only to contacts not foreseen as follow-up by the study protocol)

☐ accept ☐ do NOT accept to undergo tests to determine the presence of antibodies against HIV, for the purposes of my participation in the study, in compliance with the guarantees that have been explained to me and, in particular, the direct communication of the test result.

If applicable:

☐ accept ☐ DO NOT accept the use of contraceptive drugs

_____ ___/___/___ _____

Full name of the patient Date Signature

Only if the experiment recruits only or also minors, insert a field for the name and signature of both parents

_____ ___/___/___ _____

Full name of parent/legal representative Date Signature

_____ ___/___/___ _____

Full name of parent/legal representative Date Signature

Only in case the experiment recruits only or also incapable patients,

_____ ___/___/___ _____

Full name of the legal representative Date Signature

INDEPENDENT WITNESS

In case the patient/parent/legal representative is unable to read or sign[1]

I, the undersigned: _____

I confirm that I attended the information interview between Doctor _____ and Mr/Mrs. _____ and I confirm that the latter is unable to express his/her consent in writing.

_____ ___/___/___ _____

Full name of impartial witness Date Signature

[1]If the patient or the subject's legally acceptable representative is unable to read, a witness who is independent of the investigator and sponsor must be present during the entire informed consent discussion. The witness must personally sign and date the informed consent statement after the informed consent form and any other written information have been read and explained to the subject or the subject's legally acceptable representative and the subject has given verbal consent to participate in the study.

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STATEMENT OF THE DOCTOR WHO COLLECTED THE CONSENT

(Patient's name, place and date of birth)

Title of the trial: BONE MARROW VERSUS ADIPOSE TISSUE AS A CELL SOURCE FOR INJECTIVE TREATMENT OF KNEE OSTEOARTHRITIS: A RANDOMIZED CLINICAL TRIAL

Protocol Code, version and date: MAST-GR v1.1 of 02/03/2023

Promoter of the experiment/sponsor/funding body: IRCCS Istituto Ortopedico Rizzoli

Principal Investigator (NAME, AFFILIATION, REFERENCES): DR. LUCA ANDRIOLO, ORTHOPEDIC AND TRAUMATOLOGY CLINIC 2

I, the undersigned Prof./Dr. …… …… in my capacity as Experimenter
Surname Name
principal (or delegate of the principal investigator)

I DECLARE

that the Patient has spontaneously consented to his/her participation in the trial

I also declare that:

- ☐ have provided comprehensive explanations regarding the purposes of the trial, the procedures, the possible risks and benefits and its possible alternatives;
- ☐ have verified that the participant has sufficiently understood the information provided to him/her
- ☐ have left the necessary time and the possibility to ask questions about the experiment
- ☐ to have clearly explained the possibility of withdrawing from the experiment at any time or of modifying the choices made
- ☐ not having exercised any coercion or undue influence in seeking this consent
- ☐ have provided information on how the results of the trial will be made known to him/her

_____ _____
Place and date Time

_____ _____
Name Surname (block letters) of the doctor who provided the information and who has collected consent

Signature (and stamp)

This form is an integral part and must be kept together

to the information form for informed consent