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A prospective double-blind randomized controlled trial protocol comparing bone marrow aspirate concentrate intra-articular injection combined with subchondral injection versus intra-articular injection alone for the treatment of symptomatic knee osteoarthritis.

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Keywords: Osteoarthritis; Mesenchymal Stromal Cells; Subchondral injections; Bone marrow aspirate concentrate; BMAC

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ABSTRACT

Introduction: Subchondral and intra-articular injections of bone marrow aspirate concentrate (BMAC) showed promising results for knee OA patients. To date, there is no evidence to demonstrate whether the combination of these treatments provides higher benefits than the intra-articular injection alone.

Methods and analysis: Eighty-six patients with symptomatic knee OA (aged between 40 and 70) are randomized to BMAC intra-articular injection combined with subchondral BMAC injection or BMAC intra-articular injection alone in a ratio of 1:1. The primary outcome is the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), the secondary outcomes the International Knee Documentation Committee (IKDC) Subjective and Objective Knee Evaluation Form, the Tegner activity scale, the EuroQol-visual analogue scale (EQ-VAS), and the health questionnaire EQ-5D score. Additional CT and MRI evaluations will be performed at the baseline assessment and at the final 12-month follow-up. The hypothesis is that the combined injection provides higher knee pain and function improvement compared to BMAC intra-articular injection alone. The primary analysis follows an intention-to-treat principle.

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

Strengths and limitations of this study:

- The study design is a prospective, randomized, double-blinded and controlled trial
- This is the first RCT evaluating results of BMAC intra-articular injection combined with subchondral injection compared to BMAC intra-articular injection alone in knee OA.
- Patients are analyzed using PROMs, objective measures, MRI and CT examination, and biomarker evaluation.
- Patient base-line characteristics and disease-related factors can help to better define the aspects that make different individuals more or less responsive to this type of treatments.
- This study can clarify the benefits, and limitations, of the newly proposed combination of intra-articular and subchondral BMAC injections, providing clear indications for the clinical practice.

Trial registration: Clinical Trials.gov Identifier number NCT03876795

Protocol version: Version 1 (14 May 2018)

Ethical approval: (Prot. n. 0003132) for study protocol Interface (identifier: 207/2018/Sper/IOR) was obtained on 5 May 2018 from the Ethical Committee Area Vasta Emilia Centro (CE-AVEC) of the Emilia-Romagna Region settled at the University General Hospital Sant'Orsola-Malpighi of Bologna.

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INTRODUCTION

Knee osteoarthritis (OA) is a chronic, degenerative disease leading to irreversible structural and functional changes in the entire joint, including subchondral bone sclerosis and cartilage loss, and progressively determines debilitating pain and loss of function.[1-2] It affects a large part of the aging population with a high impact on patients and healthcare costs.[3] Total knee arthroplasty represents a definitive solution to address knee OA, but it is also encumbered by several complications.[4] Conservative approaches, such as physical therapy and anti-inflammatory drugs, should be pursued, but their benefits are generally temporary with short-term relief, and they are not able to affect the natural course of the disease progression.[5] Thus, to delay or avoid the need for arthroplasty, research efforts have been made to find new minimally invasive and more effective procedures to address knee OA.

In this light, the use of orthobiologics is gaining increasing interest due to the availability of several promising products, ranging from blood-derivatives (platelet-rich plasma - PRP) to minimally manipulated mesenchymal stromal cells (MSCs) harvested from bone marrow or adipose tissue. Although the intra-articular use of these products for the treatment of knee OA provided overall positive results, the improvement in terms of pain relief and function remains partial and not always satisfactory.[6] Thus, a new technique has been recently proposed to further exploit the potential of biologic products by targeting the subchondral bone.[7] This strategy is supported by the substantial evidence revealing that subchondral bone alterations may play a critical role in both the pathophysiology and progression of knee OA.[8] However, beside promising early findings and the increasing use of this approach in the clinical practice, there is only limited and low-level evidence, and it would be clinically relevant to evaluate with a high-level study design the real benefit provided by the addition of subchondral injections to improve the results of intra-articular injections for knee OA.

Objectives and trial design

A double-blinded randomized controlled trial (RCT) was designed to compare the efficacy of a combination of intra-articular and subchondral injections of bone marrow aspirate concentrate (BMAC) (treatment group) versus BMAC intra-articular injection alone (control group) to treat knee OA, with a 1:1 allocation ratio. The aim of this superiority trial is to evaluate the safety and the clinical potential of this new treatment approach up to one year of follow-up, and to verify the hypothesis that the combination of subchondral and intra-articular injections provides higher knee pain and function improvement compared to BMAC intra-articular injection alone in knee OA.

METHODS AND ANALYSIS

Study setting

The study is a single center double-blind RCT, with all activities related to the study performed in a single site, the IRCCS Rizzoli Orthopaedic Institute, Bologna, Italy.

This trial protocol is produced according to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) reporting guidelines. [9]

Patient and public involvement

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

Eligibility criteria

Patients are recruited according to the following criteria.

Inclusion criteria:

- Male or female patients, aged between 40 and 70;
- OA of the medial compartment of the knee (grade II or III according to the Kellgren-Lawrence classification);

- Failure after at least 6 months of conservative treatment (drug therapy with NSAIDs and painkillers, hyaluronic acid infiltration, corticosteroid infiltration, PRP);
- Patients' ability and consent to participate in clinical and radiological follow-up;
- Signature of informed consent.

Exclusion criteria:

- Patients with trauma in the 6 months prior to surgery;
- Patients with malignancy;
- Patients suffering from rheumatic diseases;
- Patients suffering from uncompensated diabetes;
- Patients suffering from uncompensated thyroid metabolic disorders;
- Patients abusing alcoholic beverages or drugs;
- Patients with axial deviations > 5°;
- Body Mass Index > 35;
- Patients treated with joint injections in the previous 6 months;
- Patients treated with surgery at the same knee in the previous 12 months.

Intervention

All patients are treated by orthopedic surgeons with established experience in cartilage and osteoarthritis orthobiologic procedures. The procedure is performed in a single step in the operating room with patients in supine position under spinal loco-regional anaesthesia. The ipsilateral hip is sterilely prepared and draped for anterior iliac crest bone marrow aspiration. The anterior superior iliac spine is the anatomical landmark for a small surgical incision. A diamond tip trocar is inserted in this point and then advanced into the bone marrow using a drill. Bone marrow is collected using two 30 ml syringes coated with heparin. The harvested bone marrow is filtered with a heparin washed filter and then centrifuged through the Magellan® centrifuge (Arteriocyte Medical Systems, MA, USA) at a rate of 3600 RPM for approximately 15 minutes, thus obtaining 10 ml of BMAC.

For each patient, BMAC samples that are not used for surgical treatment are sent to the laboratory for the count of mononuclear cells, cell clonogenic ability by colony forming unit-fibroblast test and phenotypical characterization by flow-cytometry evaluation.

Concomitantly with the bone marrow concentration process, all patients undergo an arthroscopic evaluation to confirm lesions grade and site of both medial femoral condyle and medial tibial plateau. Arthroscopy is done using the standard antero-lateral, antero-medial, and supero-medial portals. If the arthroscopic examination reveals intra-articular problems (excluding minor arthroscopic shaving) requiring surgical intervention which may affect the results of the procedure, the patient is excluded from the study.

Once the arthroscopy and the BMAC procedure are completed, the injections are performed. The treatment group receives two 2.5 ml subchondral BMAC injections, that are performed inserting two 8-Gauge trocars through the supero-medial and antero-medial arthroscopic portals and are manually introduced with clockwise and anticlockwise movements, under fluoroscopic control, into the bone of both medial femoral condyle and tibial plateau. Following arthroscopic portals suture, both groups of treatment receive a 3ml intra-articular injection of BMAC using a lateral suprapatellar approach. An elastic bandage is made after wounds medication. The whole procedure is presented in Figure 1.

Postoperatively, patients are discharged on the same day of the procedure or the day after, based on patient condition. Pain control is prescribed as needed with analgesics only in the immediate period after treatment, and thromboembolic prophylaxis is prescribed for two weeks. During the same time, patients are taught to walk with the support of two crutches to allow a partial weight-bearing on the operated limb. Cryotherapy is started within the first 24 hours. Passive mobilisation and quadriceps isometric exercises are started at the second post-operative day. Patients are permitted to return to most of their daily activities as tolerated once they reach full weight-bearing. No other conservative treatments are prescribed during the study period. Joint impacting sport activities are discouraged within the first month after treatment.

Outcomes

The primary outcome is the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), a 24 items self-administered questionnaire taking into account articular pain and stiffness and physical function limitations due to knee OA. It ranges from 0 to 96 points and higher WOMAC scores indicate worse pain, stiffness, and functional limitations.

The secondary outcomes include the International Knee Documentation Committee (IKDC) Subjective and Objective Knee Evaluation Form (a patient-completed tool taking into account knee symptoms, knee function, and sport activity), the Tegner activity scale (a one-item score based on work and sports activities), the EuroQol-visual analogue scale (EQ-VAS) that provides an assessment of patients global health, the health questionnaire EQ-5D score (a 5 level self-assessed, health related, quality of life questionnaire).

Patients will also undergo MRI and CT assessments. MRI scans are obtained with a high-resolution 3 Tesla MRI scanner with PD-weighted Turbo Spin Echo 3D sequences with and without fat saturation (FS), 3D T2* Gradient Echo (MERGE) with FS, axial PD-weighted Fast Spin Echo sequences with FS, and Multi-Echo T2 Mapping on the sagittal plane with 8 different Echo Times.

The Whole-Organ Magnetic Resonance Imaging Score (WORMS) will be used to assess seven features of the treated knees: articular cartilage morphology, bone marrow oedema, subchondral cysts, articular profile, marginal osteophytes, meniscal integrity, and synovitis.

Articular cartilage morphology will be examined with the 3D MERGE and the T2 Mapping; bone marrow oedema and synovitis with the PD fat sat sequences, the articular profiles with the PD and MERGE sequences, and the meniscal integrity with the DP sequences.

CT knee scans are obtained with a 64-channels CT scanner to better assess the structural resolution of bone trabeculae as well as to assess the presence of osteophytes, calcifications, and cancellous bone microcysts. The images were acquired using a slice thickness of 1.25 mm and an interval of 0.625 mm at 120kV with 250 mA, post-processed with the “Bone” filter, and reformatted in the coronal and sagittal plane.

Blood samples are obtained from participants before treatment and at 2, 6, and 12 months of follow-up. Samples are analysed for inflammatory (IL-1β, TNFα) and OA progression markers (Cleavage of Type II Collagen, Serum C-telopeptide fragments of type II collagen).

Participant timeline

Research assistants first conduct a screening of potential candidates over the telephone. If early checks of study eligibility are favourable, participants are booked in for a face-to-face screening visit with an orthopaedic specialist to confirm eligibility and explain the study protocol. After the screening visit, patients complete the questionnaires, undergo a knee MRI and CT, and sign the informed written consent. Patient enrolment started on November 2019. The first patient was treated in December 2019. Follow-up assessments is performed at 2, 6, and 12 months postoperatively with patient questionnaires and blood samples. At the final 12-month follow-up patients undergo knee MRI and CT scans. Due to operational delays caused by the COVID-19 pandemic, patient treatment is still ongoing; the study conclusion is foreseen before the end of 2023. Participant timeline is outlined in Table 1.

Table 1. The study procedures schedule.

	Before treatment	Treatment	2-month follow-up	6-month follow-up	12-month follow-up
Patient eligibility	X				
Informed consent	X				
WOMAC	X		X	X	X
IKDC score	X		X	X	X
Tegner activity score	X		X	X	X
EQ-5D and EQ-VAS	X		X	X	X
Blood sample		X	X	X	X
BMAC sample		X			
MRI	X				X
CT	X				X
AE reporting		X	X	X	X

AE: Adverse Event; BMAC: Bone Marrow Aspirate Concentrate; CT: Computed tomography; EQ-5D: European Quality of Life Five Dimension; EQ-VAS: European Quality - Visual Analog Scale; IKDC: International Knee Documentation Committee; MRI: magnetic resonance imaging; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index

Recruitment

Patients undergo an outpatient visit conducted by properly trained medical staff belonging to the team of orthopaedic surgeons of the Rizzoli Orthopaedic Institute, which assess patients' eligibility and take care of patient education.

Blinding

This is a double-blind RCT with both participants and physicians assessing outcomes being blinded to treatment allocation. Only after the evaluation at the 12-month follow-up the blinding is opened and it is revealed to the patient which one of the two treatments was administered.

The blindness of treated patients is further guaranteed by the same number of surgical access and by the same length of the surgical incision for both treatments. Early unblinding occurs in case of premature patients drop-out.

Allocation

A total of 86 eligible patients are allocated to receive either a combination of intra articular and subchondral BMAC injection or BMAC intra articular injection alone, in a 1:1 ratio (43 patients for each group of treatment) based on a computer-generated random numbers randomisation. This is conducted by research staff members dedicated to study organization and monitoring with no direct involvement in the study procedures. The randomization list is covered by password and accessible only by staff members with no direct involvement in the study.

Adverse events and assessment process

Adverse events are monitored throughout the study, intraoperatively and at clinical follow-up evaluations. Standard safety and efficacy monitoring is performed through regular face-to-face visits and phone calls between visits. The patients are also requested to report any adverse events to the research staff spontaneously. Every adverse event is recorded in the patient Case Report Form

(CRF). Severe adverse events are considered those resulting in death or being life-threatening, requiring hospitalization or intervention to prevent permanent impairment or damage; they are reported in accordance with the requirements of the Ethical Committee. Use of pain medicines is recorded at all visits.

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 standard protocol book which contains details of standard operating procedures, trial contacts, visits, measurements, monitoring, and case report forms.

Data collection methods

Data are firstly collected on paper-based case report forms, with the help of research trained orthopaedic residents blinded to treatment allocation, and subsequently trained data analysts process data into electronic form for statistical analysis. Baseline and final MRI and CT knee scans are coded and stored at the Rizzoli Orthopaedic Institute to ensure data quality control. Operative data are collected electronically by the respective surgeons shortly after surgery.

Data management

Study data are stored in a password-protected spreadsheet on a server that is hosted at the Rizzoli Orthopaedical Institute. Data transfer is encrypted with all data de-identified. Only trained research personnel specifically dedicated to the data handling can access the database and ensures the correspondence of the electronic data with the original paper-based questionnaires and medical charts.

Statistical methods

We conducted a power analysis (G*Power 3.1.9.2) and using assumptions of 80% of power and 5% of probability of type 1 error (alpha = 0.05), we will need 76 participants. Adjusting for a 10% loss

to follow-up, we will need 86 participants (43 in each arm). With 76 subjects we will have a 80% power to detect a difference between the two groups in terms of WOMAC score at one year follow-up with a moderate size-effect (0.55) determined with the Cohen convention (effects: small ≥ .20, medium ≥ .50, large ≥ .80). The primary analyses will be intention-to-treat analyses of primary and secondary outcomes. Per protocol analyses will be performed as the secondary analyses. All those who have started the treatment are considered part of the research, regardless of whether they will complete it.

Continuous variables will be expressed as means and standard deviations if normally distributed, as medians and range if not. Categorical variables will be expressed as frequencies and percentage. Normality of the distribution will be assessed using the Shapiro Wilks test. The Levene test will be used to assess the homoscedasticity of the data. The Repeated Measures ANOVA, followed by the post hoc Sidak pairwise test will be performed to compare the scores at different follow-up times. The OneWay ANOVA test will be performed to assess the between group differences of continuous and normally distributed and homoscedastic data; the Mann Whitney test will be used otherwise. The ANOVA test followed by the Scheffè post hoc pairwise comparison will be used also to assess the among groups differences of continuous, normally distributed and homoscedastic data, the Kruskal Wallis test followed by the Mann Whitney test with the Bonferroni correction for multiple comparison will be used otherwise. The Monte Carlo method will be used to evaluate the non-parametric tests in case of small size of the sub-groups. Pearson chi square exact test will be performed to investigate relationships between grouping variables. The Spearman rank Correlation will be used to assess correlations between the numerical scores and continuous data. The General linear model or the Generalized linear model in case of not normal distribution, will be used as multivariate analysis to compare the group's outcomes corrected by the influencing factors. The Kaplan Meyer analysis will be performed to assess survival to major adverse events. For all tests $p<0.05$ will be considered significant. SPSS version 19.0 (IBM Corp., Armonk, NY, USA) will be applied for the analyses.

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 Ministry of Health who funded the project. The monitoring personnel belongs to a research structure of the Scientific Direction of the Institution, the Applied and Translational Research Center, and it is independent from the Clinic and the medical personnel performing the study procedures. A further project auditing is performed by another independent entity of the Institution, the Clinical Trial Center. The final study report is also sent to the Ethic Committee.

ETHICS AND DISSEMINATION

Research ethics approval

Ethical approval was obtained on 5 May 2018 from the central Emilia Wide Area Ethical Committee of the Emilia-Romagna Region (CE-AVEC) settled at the University General Hospital Sant'Orsola-Malpighi of Bologna.

Protocol amendments

Minor protocol amendments, for example, database production changes to facilitate monitoring processes or improve outcome assessment by questionnaire, are fully documented. In case of major amendments, for example, changes to the patient information sheet and consent form, change of a local project leader or the inclusion of a new project site, they will be submitted for approval by the lead Ethics Committee as required.

Consent or assent

All participants will provide informed written consent in Italian and they may dropout the trial at any time during the study course.

Confidentiality

Data are recorded using case report forms and processed centrally at the Rizzoli Orthopedics Institute, Bologna, Italy. The hard copies of case report forms are stored in a locked area with secured and restricted access. The electronic data are stored on password protected servers with restricted access. All data collected are kept strictly confidential. Daily backups of all electronic data occur to minimize any risk of lost data. After study completion, paper copies of data are archived in secure storage. Identifiers are be removed in case follow-up of study patients is necessary; however, electronic data continue to be kept in a secure electronic database. This remains password protected and with access given only to the study investigators unless otherwise authorized by the study team.

Access to data

Only members of the research team who need to contact study patients, enter data, or perform data quality control have access to patient information.

Dissemination policy

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

Scientific relevance and broader impact

This study provides a detailed method of treatment for knee OA and can offer clear indications on the potential and limitations of the combined use of intra-articular and subchondral bone injections of BMAC. The BMAC analysis provides characterisation of this product to shed greater light on the

properties ensuring its effectiveness. Baseline patient-related and disease-related factors analysis can allow to better define those characteristics that make different subjects more or less responsive to this type of treatment.

Contributorship statement

ADM is the principal investigator of this study. SS, LA, AB, DR wrote the manuscript and will conduct the trial. GV and MM are responsible of imaging evaluation. CC and BG are involved in products and patients' characterization. ADM, SZ and GF applied for funding and supervise the trial. All authors read and approved the final protocol.

Competing interest

SZ reports non-financial support from personal fees from I+SRL, grants from FidiaFarmaceutici S.p.A., Cartiheal Ltd, IGEA clinical biophysics, BIOMET, and Kensey Nash, outside the submitted work. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results. The principal investigator and other authors declare no financial and other competing interests.

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Data sharing statement

No data are available

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FIGURE LEGEND

Figure 1 - Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and subchondral Bone Marrow Aspirate Concentrate injections (F).

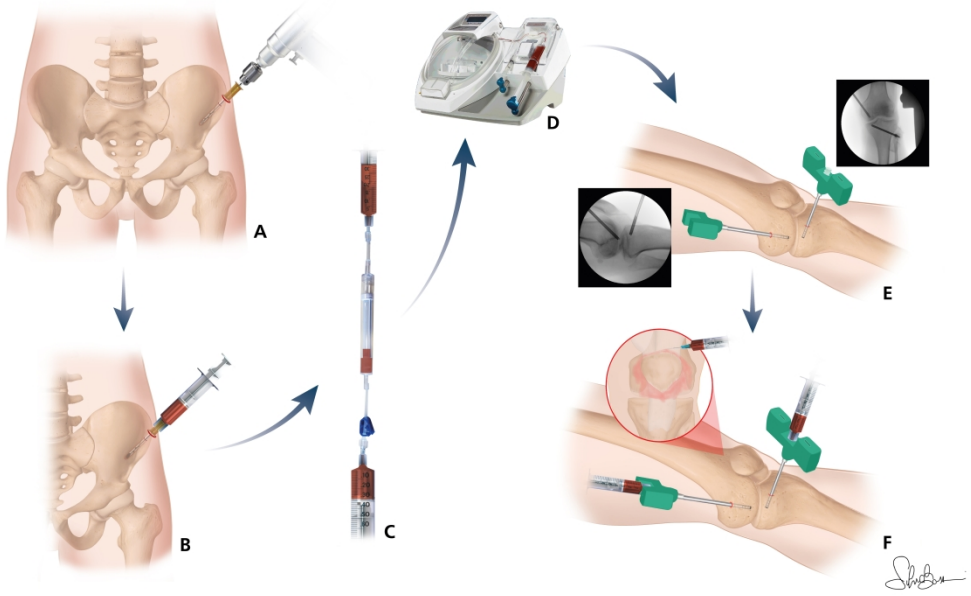

Figure 1: Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and subchondral Bone Marrow Aspirate Concentrate injections (F).

422x279mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered,	1

		name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	1
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	1
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	2
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	1

Introduction

Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg,	4

		surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7

Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9

Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values).	10

		Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial	10

		conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	12
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13
Declaration of	#28	Financial and other competing interests for principal	13

interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	14
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

BMJ Open

A prospective double-blind randomized controlled trial protocol comparing bone marrow aspirate concentrate intra-articular injection combined with subchondral injection versus intra-articular injection alone for the treatment of symptomatic knee osteoarthritis.

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A prospective double-blind randomized controlled trial protocol comparing bone marrow aspirate concentrate intra-articular injection combined with subchondral injection versus intra-articular injection alone for the treatment of symptomatic knee osteoarthritis.

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Keywords: Osteoarthritis; Mesenchymal Stromal Cells; Subchondral injections; Bone marrow aspirate concentrate; BMAC

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ABSTRACT

Introduction: Subchondral and intra-articular injections of bone marrow aspirate concentrate (BMAC) showed promising results for knee OA patients. To date, there is no evidence to demonstrate whether the combination of these treatments provides higher benefits than the intra-articular injection alone.

Methods and analysis: Eighty-six patients with symptomatic knee OA (aged between 40 and 70) are randomized to BMAC intra-articular injection combined with subchondral BMAC injection or BMAC intra-articular injection alone in a ratio of 1:1. The primary outcome is the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), the secondary outcomes the International Knee Documentation Committee (IKDC) Subjective and Objective Knee Evaluation Form, the Tegner activity scale, the EuroQol-visual analogue scale (EQ-VAS), and the health questionnaire EQ-5D score. Additional CT and MRI evaluations are performed at the baseline assessment and at the final 12-month follow-up. The hypothesis is that the combined injection provides higher knee pain and function improvement compared to BMAC intra-articular injection alone. The primary analysis follows an intention-to-treat principle.

Ethics and dissemination: The study protocol has been approved by the Emilia Wide Area Ethical Committee of the Emilia-Romagna Region (CE-AVEC), Bologna, Italy. Written informed consent is obtained from all the participants. Findings of this study will be disseminated the through peer-reviewed publications and conference presentations.

Strengths and limitations of this study:

- This is the first prospective, randomized, double-blind and controlled trial evaluating results of BMAC intra-articular injection combined with subchondral injection compared to BMAC intra-articular injection alone in knee OA.
- Patients are analyzed using PROMs, objective measures, MRI and CT examination, and biomarker evaluation.
- Patient base-line characteristics and disease-related factors can help to better define the aspects that make different individuals more or less responsive to this type of treatments.
- The uncontrolled pain medication use by patients (although being discouraged) could influence the primary outcome and this is a relevant limitation of the study.
- This study can clarify the benefits, and limitations, of the newly proposed combination of intra-articular and subchondral BMAC injections, providing clear indications for the clinical practice.

Trial registration: Clinical Trials.gov Identifier number NCT03876795

Protocol version: Version 1 (14 May 2018)

Ethical approval: (Prot. n. 0003132) for study protocol Interface (identifier: 207/2018/Sper/IOR) was obtained on 5 May 2018 from the Ethical Committee Area Vasta Emilia Centro (CE-AVEC) of the Emilia-Romagna Region settled at the University General Hospital Sant'Orsola-Malpighi of Bologna.

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INTRODUCTION

Knee osteoarthritis (OA) is a chronic, degenerative disease leading to irreversible structural and functional changes in the entire joint, including subchondral bone sclerosis and cartilage loss, and progressively determines debilitating pain and loss of function.[1-2] It affects a large part of the aging population with a high impact on patients and healthcare costs.[3] Total knee arthroplasty represents a definitive solution to address knee OA, but it is also encumbered by several complications.[4] Conservative approaches, such as physical therapy and anti-inflammatory drugs, should be pursued, but their benefits are generally temporary with short-term relief, and they are not able to affect the natural course of the disease progression.[5] Thus, to delay or avoid the need for arthroplasty, research efforts have been made to find new minimally invasive and more effective procedures to address knee OA.

In this light, the use of orthobiologics is gaining increasing interest due to the availability of several promising products, ranging from blood-derivatives (platelet-rich plasma - PRP) to minimally manipulated mesenchymal stromal cells (MSCs) harvested from bone marrow or adipose tissue. Although the intra-articular use of these products for the treatment of knee OA provided overall positive results, the improvement in terms of pain relief and function remains partial and not always satisfactory.[6] Thus, a new approach has been recently proposed to further exploit the potential of biologic products by targeting the subchondral bone.[7] This strategy is supported by the evidence revealing that subchondral bone alterations may play a critical role in both the pathophysiology and progression of knee OA.[8][9] It has been suggested that with age and knee OA the number and functionality of MSCs present in the subchondral bone of the knee may decrease. Therefore, MSCs subchondral injections could address this deficiency underlying the pathophysiology by providing many bioactive mediators which have been shown to exert positive effects on joint tissues.[10] MSCs subchondral bone injections showed to be safe and may provide even better results than MSC intra-articular injections addressing knee OA in terms of survival to knee arthroplasty.[11]

Moreover, the combination of subchondral and intra-articular injections of bone marrow aspirate concentrate (BMAC) already showed promising results in terms of safety and clinical outcomes.[12] However, beside promising early findings and the increasing use of this approach in the clinical practice, there is only limited and low-level evidence, and it would be clinically relevant to evaluate with a high-level study design the real benefit provided by the addition of these subchondral injections to improve the results of BMAC intra-articular injections for knee OA.

Objectives and trial design

A double-blinded randomized controlled trial (RCT) was designed to compare the efficacy of a combination of intra-articular and subchondral injections of BMAC (treatment group) versus BMAC intra-articular injection alone (control group) to treat knee OA, with a 1:1 allocation ratio. The aim of this superiority trial is to evaluate the safety and the clinical potential of this new treatment approach up to one year of follow-up, and to verify the hypothesis that the combination of subchondral and intra-articular injections provides higher knee pain and function improvement compared to BMAC intra-articular injection alone in knee OA.

METHODS AND ANALYSIS

Study setting

The study is a single center double-blind RCT, with all activities related to the study performed in a single site, the IRCCS Rizzoli Orthopaedic Institute, Bologna, Italy.

This trial protocol is produced according to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) reporting guidelines. [13]

Patient and public involvement

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

Eligibility criteria

Patients are recruited according to the following criteria.

Inclusion criteria:

- Male or female patients, aged between 40 and 70 years old;
- OA of the medial compartment of the knee (grade II or III according to the Kellgren-Lawrence classification);
- Failure after at least 6 months of conservative treatment (drug therapy with NSAIDs and painkillers, hyaluronic acid infiltration, corticosteroid infiltration, PRP);
- Patients' ability and consent to participate in clinical and radiological follow-up;
- Signature of informed consent.

Exclusion criteria:

- Patients with trauma in the 6 months prior to surgery;
- Patients with malignancy;
- Patients suffering from rheumatic diseases;
- Patients suffering from uncompensated diabetes;
- Patients suffering from uncompensated thyroid metabolic disorders;
- Patients abusing alcoholic beverages or drugs;
- Patients with axial deviations > 5°;
- Body Mass Index > 35;
- Patients treated with joint injections in the previous 6 months;
- Patients treated with surgery at the same knee in the previous 12 months.

Intervention

All patients are treated by orthopedic surgeons with established experience in cartilage and osteoarthritis orthobiologic procedures. The procedure is performed in a single step in the operating

room with patients in supine position under spinal loco-regional anaesthesia. The ipsilateral hip is sterilely prepared and draped for anterior iliac crest bone marrow aspiration. The anterior superior iliac spine is the anatomical landmark for a small surgical incision. A diamond tip trocar is inserted in this point and then advanced into the bone marrow using a drill. Bone marrow is collected using two 30 ml syringes coated with heparin for a total of 60 ml. The harvested bone marrow is filtered with a heparin washed filter and then centrifuged through the Magellan® centrifuge (Arteriocyte Medical Systems, MA, USA) at a rate of 3600 RPM for approximately 15 minutes, thus obtaining 10 ml of BMAC. The BMAC procedure involved a kit available in the clinical practice. In fact, the purpose of the study was not to evaluate a new product, but rather to explore the potential of applying BMAC also at the subchondral bone level, to give indications on the potential of this approach for physicians considering this technique for their clinical practice.

For each patient, BMAC samples that are not used for surgical treatment are sent to the laboratory for the count of mononuclear cells, cell clonogenic ability by colony forming unit-fibroblast test and phenotypical characterization by flow-cytometry evaluation.

Concomitantly with the bone marrow concentration process, all patients undergo an arthroscopic evaluation to confirm the location on both medial femoral condyle and medial tibial plateau involved by osteoarthritic lesions. Arthroscopy is done using the standard antero-lateral, antero-medial, and supero-medial portals. If the arthroscopic examination reveals intra-articular problems (excluding minor arthroscopic shaving) requiring surgical intervention which may affect the results of the procedure, the patient is excluded from the study.

Once the arthroscopy and the BMAC procedure are completed, the injections are performed. The treatment group receives two 2.5 ml subchondral BMAC injections, that are performed inserting two 8-Gauge trocars through the supero-medial and antero-medial arthroscopic portals and are manually introduced with clockwise and anticlockwise movements, under fluoroscopic control, into the bone of both medial femoral condyle and tibial plateau. Following arthroscopic portals suture, both groups of treatment receive a 3ml intra-articular injection of BMAC using a lateral

suprapatellar approach. An elastic bandage is made after wounds medication. The whole procedure is presented in Figure 1.

Postoperatively, patients are discharged on the same day of the procedure or the day after, based on patient condition. Pain control is prescribed as needed with analgesics only in the immediate period after treatment, and thromboembolic prophylaxis is prescribed for two weeks. During the same time, patients are taught to walk with the support of two crutches to allow a partial weight-bearing on the operated limb. Cryotherapy is started within the first 24 hours. Passive mobilisation and quadriceps isometric exercises are started at the second post-operative day. Patients are permitted to return to most of their daily activities as tolerated once they reach full weight-bearing. No other conservative treatments are prescribed during the study period. Joint impacting sport activities are discouraged within the first month after treatment.

Outcomes

The primary outcome is the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), a 24 items self-administered questionnaire taking into account articular pain and stiffness and physical function limitations due to knee OA. It ranges from 0 to 96 points and higher WOMAC scores indicate worse pain, stiffness, and functional limitations. The total WOMAC score was chosen as primary outcome aiming at capturing a more comprehensive assessment of symptoms and function benefits offered by the treatments.

The secondary outcomes include the International Knee Documentation Committee (IKDC) Subjective and Objective Knee Evaluation Form (a patient-completed tool taking into account knee symptoms, knee function, and sport activity), the Tegner activity scale (a one-item score based on work and sports activities), the EuroQol-visual analogue scale (EQ-VAS) that provides an assessment of patients global health, the health questionnaire EQ-5D score (a 5 level self-assessed, health related, quality of life questionnaire).

Patients also undergo MRI and CT assessments. MRI scans are obtained with a high-resolution 3 Tesla MRI scanner with PD-weighted Turbo Spin Echo 3D sequences with and without fat saturation (FS), 3D T2* Gradient Echo (MERGE) with FS, axial PD-weighted Fast Spin Echo sequences with FS, and Multi-Echo T2 Mapping on the sagittal plane with 8 different Echo Times.

The Whole-Organ Magnetic Resonance Imaging Score (WORMS) is used to assess seven features of the treated knees: articular cartilage morphology, bone marrow oedema, subchondral cysts, articular profile, marginal osteophytes, meniscal integrity, and synovitis.

Articular cartilage morphology is examined with the 3D MERGE and the T2 Mapping; bone marrow oedema and synovitis with the PD fat sat sequences, the articular profiles with the PD and MERGE sequences, and the meniscal integrity with the DP sequences.

CT knee scans are obtained with a 64-channels CT scanner to better assess the structural resolution of bone trabeculae as well as to assess the presence of osteophytes, calcifications, and cancellous bone microcysts. The images are acquired using a slice thickness of 1.25 mm and an interval of 0.625 mm at 120kV with 250 mA, post-processed with the "Bone" filter, and reformatted in the coronal and sagittal plane.

Blood samples are obtained from participants before treatment and at 2, 6, and 12 months of follow-up. Samples are analysed for inflammatory (IL-1β, TNFα) and OA progression markers (Cleavage of Type II Collagen, Serum C-telopeptide fragments of type II collagen).

Participant timeline

Research assistants first conduct a screening of potential candidates over the telephone. If early checks of study eligibility are favourable, participants are booked in for a face-to-face screening visit with an orthopaedic specialist to confirm eligibility and explain the study protocol. After the screening visit, patients complete the questionnaires, undergo a knee MRI and CT, and sign the informed written consent. Patient enrolment started on November 2019. The first patient was treated in December 2019. Follow-up assessments is performed at 2, 6, and 12 months

postoperatively with patient questionnaires and blood samples. At the final 12-month follow-up patients undergo knee MRI and CT scans. Due to operational delays caused by the COVID-19 pandemic, patient treatment is still ongoing; the study conclusion is foreseen before the end of 2023. Participant timeline is outlined in Table 1.

Table 1. The study procedures schedule.

	Before treatment	Treatment	2-month follow-up	6-month follow-up	12-month follow-up
Patient eligibility	X				
Informed consent	X				
WOMAC	X		X	X	X
IKDC score	X		X	X	X
Tegner activity score	X		X	X	X
EQ-5D and EQ-VAS	X		X	X	X
Blood sample		X	X	X	X
BMAC sample		X			
MRI	X				X
CT	X				X
AE reporting		X	X	X	X

AE: Adverse Event; BMAC: Bone Marrow Aspirate Concentrate; CT: Computed tomography; EQ-5D: European Quality of Life Five Dimension; EQ-VAS: European Quality - Visual Analog Scale; IKDC: International Knee Documentation Committee; MRI: magnetic resonance imaging; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index

Recruitment

Patients undergo an outpatient visit conducted by properly trained medical staff belonging to the team of orthopaedic surgeons of the Rizzoli Orthopaedic Institute, which assess patients' eligibility and take care of patient education.

Blinding

This is a double-blind RCT with both participants and physicians assessing outcomes being blinded to treatment allocation. Only after the evaluation at the 12-month follow-up the blinding is opened and it is revealed to the patient which one of the two treatments was administered.

The blindness of treated patients is further guaranteed by the same number of surgical access and by the same length of the surgical incision for both treatments. Early unblinding occurs in case of premature patients drop-out. The level of blinding prevents from the enhanced placebo effect that a subchondral injection could add to the placebo effect of the intra-articular injection alone.[14]
Imaging evaluation is provided by experienced radiologists which are blinded as well to the type of treatment that the patients have received.

Allocation

A total of 86 eligible patients are allocated to receive either a combination of intra articular and subchondral BMAC injection or BMAC intra articular injection alone, in a 1:1 ratio (43 patients for each group of treatment) based on a computer-generated random numbers randomisation. This is conducted by research staff members dedicated to study organization and monitoring with no direct involvement in the study procedures. The randomization list is covered by password and accessible only by staff members with no direct involvement in the study.

Adverse events and assessment process

Adverse events are monitored throughout the study, intraoperatively and at clinical follow-up evaluations. Standard safety and efficacy monitoring is performed through regular face-to-face visits and phone calls between visits. The patients are also requested to report any adverse events to the research staff spontaneously. Every adverse event is recorded in the patient Case Report Form (CRF). Serious adverse events are considered those resulting in death or being life-threatening, requiring hospitalization or intervention to prevent permanent impairment or damage; they are reported in accordance with the requirements of the Ethical Committee. Use of rescue pain medication is recorded at all visits without a diary and without homogenizing the type of medication, which is decided by patients autonomously (although discouraged for study purposes).

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 standard protocol book which contains details of standard operating procedures, trial contacts, visits, measurements, monitoring, and case report forms.

Data collection methods

Data are firstly collected on paper-based case report forms, with the help of research trained orthopaedic residents blinded to treatment allocation, and subsequently trained data analysts process data into electronic form for statistical analysis. Baseline and final MRI and CT knee scans are coded and stored at the Rizzoli Orthopaedic Institute to ensure data quality control. Operative data are collected electronically by the respective surgeons shortly after surgery.

Data management

Study data are stored in a password-protected spreadsheet on a server that is hosted at the Rizzoli Orthopaedical Institute. Data transfer is encrypted with all data de-identified. Only trained research personnel specifically dedicated to the data handling can access the database and ensures the correspondence of the electronic data with the original paper-based questionnaires and medical charts.

Statistical methods

A power analysis (G*Power 3.1.9.2) was conducted using assumptions of 90% of power and 5% of probability of type 1 error (alpha = 0.05), with a SD of 18.2 points based on a pilot study and a hypothesized 10-point difference in total WOMAC score between treatments. Accordingly, 76 participants are needed. This leads to a moderate size-effect (0.55) as per the Cohen convention (effects: small ≥ .20, medium ≥ .50, large ≥ .80), and is in line with other effect sizes and SD reported in the literature. We increased the number of participants to a total of 86 patients (43 in

each arm) to account for a possible 10% loss to follow-up. The primary analyses are intention-to-treat analyses of primary and secondary outcomes. Per protocol analyses will be performed as the secondary analyses. All those who have started the treatment are considered part of the research, regardless of whether they will complete it. For the missing data, they will be analyzed using the multiple imputation analysis, performed by filling the missing data with random values from the distribution of the variable.

Continuous variables are be expressed as means and standard deviations if normally distributed, as medians and range if not. Categorical variables are be expressed as frequencies and percentage. Normality of the distribution is be assessed using the Shapiro Wilks test. The Levene test is be used to assess the homoscedasticity of the data. The Repeated Measures ANOVA, followed by the post hoc Sidak pairwise test is performed to compare the scores at different follow-up times. The OneWay ANOVA test is performed to assess the between group differences of continuous and normally distributed and homoscedastic data; the Mann Whitney test is used otherwise. The ANOVA test, followed by the Scheffè post hoc pairwise comparison, is used also to assess the among groups differences of continuous, normally distributed and homoscedastic data; the Kruskal Wallis, test followed by the Mann Whitney test with the Bonferroni correction for multiple comparison, is used otherwise. The Monte Carlo method is used to evaluate the non-parametric tests in case of small size of the sub-groups. Pearson chi square exact test is performed to investigate relationships between grouping variables. The Spearman rank Correlation is used to assess correlations between the numerical scores and continuous data. The General linear model, or the Generalized linear model in case of not normal distribution, is used as multivariate analysis to compare the group's outcomes corrected by the influencing factors. The Kaplan Meyer analysis is performed to assess survival to major adverse events. For all tests $p<0.05$ is considered significant. SPSS version 19.0 (IBM Corp., Armonk, NY, USA) is applied for the analyses.

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 Ministry of Health who funded the project. The monitoring personnel belongs to a research structure of the Scientific Direction of the Institution, the Applied and Translational Research Center, and it is independent from the Clinic and the medical personnel performing the study procedures. A further project auditing is performed by another independent entity of the Institution, the Clinical Trial Center. The final study report is also sent to the Ethic Committee.

ETHICS AND DISSEMINATION

Research ethics approval

Ethical approval was obtained on 5 May 2018 from the central Emilia Wide Area Ethical Committee of the Emilia-Romagna Region (CE-AVEC) settled at the University General Hospital Sant'Orsola-Malpighi of Bologna.

Protocol amendments

Minor protocol amendments, for example, database production changes to facilitate monitoring processes or improve outcome assessment by questionnaire, are fully documented. In case of major amendments, for example, changes to the patient information sheet and consent form, change of a local project leader or the inclusion of a new project site, they are submitted for approval by the lead Ethics Committee as required.

Consent or assent

All participants will provide informed written consent in Italian and they may dropout the trial at any time during the study course.

Confidentiality

Data are recorded using case report forms and processed centrally at the Rizzoli Orthopedics Institute, Bologna, Italy. The hard copies of case report forms are stored in a locked area with secured and restricted access. The electronic data are stored on password protected servers with restricted access. All data collected are kept strictly confidential. Daily backups of all electronic data occur to minimize any risk of lost data. After study completion, paper copies of data are archived in secure storage. Identifiers are be removed in case follow-up of study patients is necessary; however, electronic data continue to be kept in a secure electronic database. This remains password protected and with access given only to the study investigators unless otherwise authorized by the study team.

Access to data

Only members of the research team who need to contact study patients, enter data, or perform data quality control have access to patient information.

Dissemination policy

This trial is produced according to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) international standards. Results are disseminated through peer-reviewed publications and will be 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 study provides a detailed method of treatment for knee OA and can offer clear indications on the potential and limitations of the combined use of intra-articular and subchondral bone injections of BMAC. The BMAC analysis provides characterisation of this product to shed greater light on the properties ensuring its effectiveness. Baseline patient-related and disease-related factors analysis

can allow to better define those characteristics that make different subjects more or less responsive to this type of treatment.

Contributorship statement

ADM is the principal investigator of this study. SS, LA, AB, DR wrote the manuscript and will conduct the trial. GV and MM are responsible of imaging evaluation. CC and BG are involved in products and patients' characterization. ADM, SZ and GF applied for funding and supervise the trial. All authors read and approved the final protocol.

Competing interest

SZ reports non-financial support from personal fees from I+SRL, grants from FidiaFarmaceutici S.p.A., Cartiheal Ltd, IGEA clinical biophysics, BIOMET, and Kensey Nash, outside the submitted work. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results. The principal investigator and other authors declare no financial and other competing interests.

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Data sharing statement

No data are available

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FIGURE LEGEND

Figure 1 - Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and subchondral Bone Marrow Aspirate Concentrate injections (F).

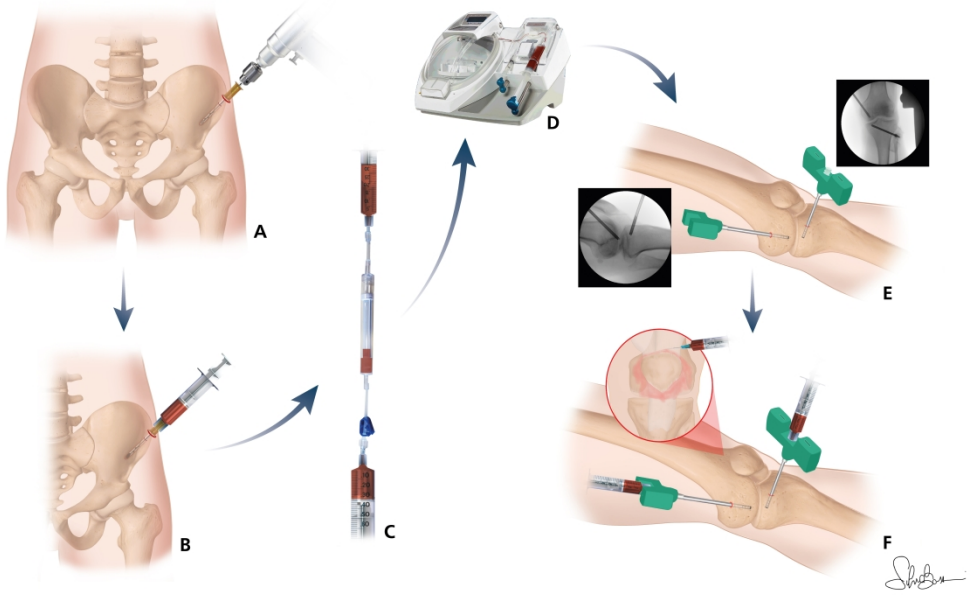

Figure 1: Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and subchondral Bone Marrow Aspirate Concentrate injections (F).

422x279mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

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Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered,	1

		name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	1
Protocol version	#3	Date and version identifier	3
Funding	#4	Sources and types of financial, material, and other support	17
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	4
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	1

Introduction

Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg,	7

		surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10

Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13

Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values).	13

		Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	15
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial	12

		conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	15
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	15
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	15
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16
Declaration of	#28	Financial and other competing interests for principal	17

interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	16
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

BMJ Open

A prospective double-blind randomized controlled trial protocol comparing bone marrow aspirate concentrate intra-articular injection combined with subchondral injection versus intra-articular injection alone for the treatment of symptomatic knee osteoarthritis.

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A prospective double-blind randomized controlled trial protocol comparing bone marrow aspirate concentrate intra-articular injection combined with subchondral injection versus intra-articular injection alone for the treatment of symptomatic knee osteoarthritis.

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ABSTRACT

Introduction: Subchondral and intra-articular injections of bone marrow aspirate concentrate (BMAC) showed promising results for knee OA patients. To date, there is no evidence to demonstrate whether the combination of these treatments provides higher benefits than the intra-articular injection alone.

Methods and analysis: Eighty-six patients with symptomatic knee OA (aged between 40 and 70) are randomized to BMAC intra-articular injection combined with subchondral BMAC injection or BMAC intra-articular injection alone in a ratio of 1:1. The primary outcome is the total Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), the secondary outcomes the International Knee Documentation Committee (IKDC) Subjective and Objective Knee Evaluation Form, the Tegner activity scale, the EuroQol-visual analogue scale (EQ-VAS), and the health questionnaire EQ-5D score. Additional CT and MRI evaluations are performed at the baseline assessment and at the final 12-month follow-up. The hypothesis is that the combined injection provides higher knee pain and function improvement compared to BMAC intra-articular injection alone. The primary analysis follows an intention-to-treat principle.

Ethics and dissemination: The study protocol has been approved by the Emilia Wide Area Ethical Committee of the Emilia-Romagna Region (CE-AVEC), Bologna, Italy. Written informed consent is obtained from all the participants. Findings of this study will be disseminated the through peer-reviewed publications and conference presentations.

Strengths and limitations of this study:

- This is the first prospective, randomized, double-blind and controlled trial evaluating results of BMAC intra-articular injection combined with subchondral injection compared to BMAC intra-articular injection alone in knee OA.
- Patients are analyzed using PROMs, objective measures, MRI and CT examination, and biomarker evaluation.
- Patient base-line characteristics and disease-related factors can help to better define the aspects that make different individuals more or less responsive to this type of treatments.
- The uncontrolled pain medication use by patients (although being discouraged) could influence the primary outcome and this is a relevant limitation of the study.
- This study can clarify the benefits, and limitations, of the newly proposed combination of intra-articular and subchondral BMAC injections, providing clear indications for the clinical practice.

Trial registration: Clinical Trials.gov Identifier number NCT03876795

Protocol version: Version 1 (14 May 2018)

Ethical approval: (Prot. n. 0003132) for study protocol Interface (identifier: 207/2018/Sper/IOR) was obtained on 5 May 2018 from the Ethical Committee Area Vasta Emilia Centro (CE-AVEC) of the Emilia-Romagna Region settled at the University General Hospital Sant'Orsola-Malpighi of Bologna.

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INTRODUCTION

Knee osteoarthritis (OA) is a chronic, degenerative disease leading to irreversible structural and functional changes in the entire joint, including subchondral bone sclerosis and cartilage loss, and progressively determines debilitating pain and loss of function.[1-2] It affects a large part of the aging population with a high impact on patients and healthcare costs.[3] Total knee arthroplasty represents a definitive solution to address knee OA, but it is also encumbered by several complications.[4] Conservative approaches, such as physical therapy and anti-inflammatory drugs, should be pursued, but their benefits are generally temporary with short-term relief, and they are not able to affect the natural course of the disease progression.[5] Thus, to delay or avoid the need for arthroplasty, research efforts have been made to find new minimally invasive and more effective procedures to address knee OA.

In this light, the use of orthobiologics is gaining increasing interest due to the availability of several promising products, ranging from blood-derivatives (platelet-rich plasma - PRP) to minimally manipulated mesenchymal stromal cells (MSCs) harvested from bone marrow or adipose tissue. Although the intra-articular use of these products for the treatment of knee OA provided overall positive results, the improvement in terms of pain relief and function remains partial and not always satisfactory.[6] Thus, a new approach has been recently proposed to further exploit the potential of biologic products by targeting the subchondral bone.[7] This strategy is supported by the evidence revealing that subchondral bone alterations may play a critical role in both the pathophysiology and progression of knee OA.[8][9] It has been suggested that with age and knee OA the number and functionality of MSCs present in the subchondral bone of the knee may decrease. Therefore, MSCs subchondral injections could address this deficiency underlying the pathophysiology by providing many bioactive mediators which have been shown to exert positive effects on joint tissues.[10] MSCs subchondral bone injections showed to be safe and may provide even better results than MSC intra-articular injections addressing knee OA in terms of survival to knee arthroplasty.[11]

Moreover, the combination of subchondral and intra-articular injections of bone marrow aspirate concentrate (BMAC) already showed promising results in terms of safety and clinical outcomes.[12] However, beside promising early findings and the increasing use of this approach in the clinical practice, there is only limited and low-level evidence, and it would be clinically relevant to evaluate with a high-level study design the real benefit provided by the addition of these subchondral injections to improve the results of BMAC intra-articular injections for knee OA.

Objectives and trial design

A double-blinded randomized controlled trial (RCT) was designed to compare the efficacy of a combination of intra-articular and subchondral injections of BMAC (treatment group) versus BMAC intra-articular injection alone (control group) to treat knee OA, with a 1:1 allocation ratio. The aim of this superiority trial is to evaluate the safety and the clinical potential of this new treatment approach up to one year of follow-up, and to verify the hypothesis that the combination of subchondral and intra-articular injections provides higher knee pain and function improvement compared to BMAC intra-articular injection alone in knee OA.

METHODS AND ANALYSIS

Study setting

The study is a single center double-blind RCT, with all activities related to the study performed in a single site, the IRCCS Rizzoli Orthopaedic Institute, Bologna, Italy.

This trial protocol is produced according to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) reporting guidelines. [13]

Patient and public involvement

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

Eligibility criteria

Patients are recruited according to the following criteria.

Inclusion criteria:

- Male or female patients, aged between 40 and 70 years old;
- OA of the medial compartment of the knee (grade II or III according to the Kellgren-Lawrence classification);
- Failure after at least 6 months of conservative treatment (drug therapy with NSAIDs and painkillers, hyaluronic acid infiltration, corticosteroid infiltration, PRP);
- Patients' ability and consent to participate in clinical and radiological follow-up;
- Signature of informed consent.

Exclusion criteria:

- Patients with trauma in the 6 months prior to surgery;
- Patients with malignancy;
- Patients suffering from rheumatic diseases;
- Patients suffering from uncompensated diabetes;
- Patients suffering from uncompensated thyroid metabolic disorders;
- Patients abusing alcoholic beverages or drugs;
- Patients with axial deviations > 5°;
- Body Mass Index > 35;
- Patients treated with joint injections in the previous 6 months;
- Patients treated with surgery at the same knee in the previous 12 months.

Intervention

All patients are treated by orthopedic surgeons with established experience in cartilage and osteoarthritis orthobiologic procedures. The procedure is performed in a single step in the operating

room with patients in supine position under spinal loco-regional anaesthesia. The ipsilateral hip is sterilely prepared and draped for anterior iliac crest bone marrow aspiration. The anterior superior iliac spine is the anatomical landmark for a small surgical incision. A diamond tip trocar is inserted in this point and then advanced into the bone marrow using a drill. Bone marrow is collected using two 30 ml syringes coated with heparin for a total of 60 ml. The harvested bone marrow is filtered with a heparin washed filter and then centrifuged through the Magellan® centrifuge (Arteriocyte Medical Systems, MA, USA) at a rate of 3600 RPM for approximately 15 minutes, thus obtaining 10 ml of BMAC. The BMAC procedure involved a kit available in the clinical practice. In fact, the purpose of the study was not to evaluate a new product, but rather to explore the potential of applying BMAC also at the subchondral bone level, to give indications on the potential of this approach for physicians considering this technique for their clinical practice.

For each patient, BMAC samples that are not used for surgical treatment are sent to the laboratory for the count of mononuclear cells, cell clonogenic ability by colony forming unit-fibroblast test and phenotypical characterization by flow-cytometry evaluation.

Concomitantly with the bone marrow concentration process, all patients undergo an arthroscopic evaluation to confirm the location on both medial femoral condyle and medial tibial plateau involved by osteoarthritic lesions. Arthroscopy is done using the standard antero-lateral, antero-medial, and supero-medial portals. The same portals are used to access the subchondral bone in the experimental group in order to maintain blinding. If the arthroscopic examination reveals intra-articular problems (excluding minor arthroscopic shaving) requiring surgical intervention which may affect the results of the procedure, the patient is excluded from the study.

Once the arthroscopy and the BMAC procedure are completed, the injections are performed. The treatment group receives two 2.5 ml subchondral BMAC injections, that are performed inserting two 8-Gauge trocars through the supero-medial and antero-medial arthroscopic portals and are manually introduced with clockwise and anticlockwise movements, under fluoroscopic control, into the bone of both medial femoral condyle and tibial plateau. Following arthroscopic portals suture,

both groups of treatment receive a 3ml intra-articular injection of BMAC using a lateral suprapatellar approach. An elastic bandage is made after wounds medication. The whole procedure is presented in Figure 1.

Postoperatively, patients are discharged on the same day of the procedure or the day after, based on patient condition. Pain control is prescribed as needed with analgesics only in the immediate period after treatment, and thromboembolic prophylaxis is prescribed for two weeks. During the same time, patients are taught to walk with the support of two crutches to allow a partial weight-bearing on the operated limb. Cryotherapy is started within the first 24 hours. Passive mobilisation and quadriceps isometric exercises are started at the second post-operative day. Patients are permitted to return to most of their daily activities as tolerated once they reach full weight-bearing. No other conservative treatments are prescribed during the study period. Joint impacting sport activities are discouraged within the first month after treatment.

Outcomes

The primary outcome is the total Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) at 12 month, a 24 items self-administered questionnaire taking into account articular pain and stiffness and physical function limitations due to knee OA. It ranges from 0 to 96 points and higher WOMAC scores indicate worse pain, stiffness, and functional limitations. The total WOMAC score was chosen as primary outcome aiming at capturing a more comprehensive assessment of symptoms and function benefits offered by the treatments.

The secondary outcomes include the total WOMAC score at other follow-ups, the WOMAC subscales (pain, stiffness, and physical function), as well as the International Knee Documentation Committee (IKDC) Subjective and Objective Knee Evaluation Form (a patient-completed tool taking into account knee symptoms, knee function, and sport activity), the Tegner activity scale (a one-item score based on work and sports activities), the EuroQol-visual analogue scale (EQ-VAS)

that provides an assessment of patients global health, and the health questionnaire EQ-5D score (a 5 level self-assessed, health related, quality of life questionnaire).

Patients also undergo MRI and CT assessments. MRI scans are obtained with a high-resolution 3 Tesla MRI scanner with PD-weighted Turbo Spin Echo 3D sequences with and without fat saturation (FS), 3D T2* Gradient Echo (MERGE) with FS, axial PD-weighted Fast Spin Echo sequences with FS, and Multi-Echo T2 Mapping on the sagittal plane with 8 different Echo Times.

The Whole-Organ Magnetic Resonance Imaging Score (WORMS) is used to assess seven features of the treated knees: articular cartilage morphology, bone marrow oedema, subchondral cysts, articular profile, marginal osteophytes, meniscal integrity, and synovitis.

Articular cartilage morphology is examined with the 3D MERGE and the T2 Mapping; bone marrow oedema and synovitis with the PD fat sat sequences, the articular profiles with the PD and MERGE sequences, and the meniscal integrity with the DP sequences.

CT knee scans are obtained with a 64-channels CT scanner to better assess the structural resolution of bone trabeculae as well as to assess the presence of osteophytes, calcifications, and cancellous bone microcysts. The images are acquired using a slice thickness of 1.25 mm and an interval of 0.625 mm at 120kV with 250 mA, post-processed with the “Bone” filter, and reformatted in the coronal and sagittal plane.

Blood samples are obtained from participants before treatment and at 2, 6, and 12 months of follow-up. Samples are analysed for inflammatory (IL-1β, TNFα) and OA progression markers (Cleavage of Type II Collagen, Serum C-telopeptide fragments of type II collagen).

Participant timeline

Research assistants first conduct a screening of potential candidates over the telephone. If early checks of study eligibility are favourable, participants are booked in for a face-to-face screening visit with an orthopaedic specialist to confirm eligibility and explain the study protocol. After the screening visit, patients complete the questionnaires, undergo a knee MRI and CT, and sign the

informed written consent. Patient enrolment started on November 2019. The first patient was treated in December 2019. Follow-up assessments is performed at 2, 6, and 12 months postoperatively with patient questionnaires and blood samples. At the final 12-month follow-up patients undergo knee MRI and CT scans. Due to operational delays caused by the COVID-19 pandemic, patient treatment is still ongoing; the study conclusion is foreseen before the end of 2023. Participant timeline is outlined in Table 1.

Table 1. The study procedures schedule.

	Before treatment	Treatment	2-month follow-up	6-month follow-up	12-month follow-up
Patient eligibility	X				
Informed consent	X				
WOMAC (total and subscale)	X		X	X	X
IKDC score	X		X	X	X
Tegner activity score	X		X	X	X
EQ-5D and EQ-VAS	X		X	X	X
Blood sample		X	X	X	X
BMAC sample		X			
MRI	X				X
CT	X				X
AE reporting		X	X	X	X

AE: Adverse Event; BMAC: Bone Marrow Aspirate Concentrate; CT: Computed tomography; EQ-5D: European Quality of Life Five Dimension; EQ-VAS: European Quality - Visual Analog Scale; IKDC: International Knee Documentation Committee; MRI: magnetic resonance imaging; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index

Recruitment

Patients undergo an outpatient visit conducted by properly trained medical staff belonging to the team of orthopaedic surgeons of the Rizzoli Orthopaedic Institute, which assess patients' eligibility and take care of patient education.

Blinding

This is a double-blind RCT with both participants and physicians assessing outcomes being blinded to treatment allocation. Only after the evaluation at the 12-month follow-up the blinding is opened and it is revealed to the patient which one of the two treatments was administered.

The blindness of treated patients is further guaranteed by the same number of surgical access and by the same length of the surgical incision for both treatments. For ethical reason, no bone puncturing and injection was performed in the control group. This, however, did not compromise blinding since patients presented the same number and type of surgical incisions. Early unblinding occurs in case of premature patients drop-out. The level of blinding prevents from the enhanced placebo effect that a subchondral injection could add to the placebo effect of the intra-articular injection alone.[14]

Imaging evaluation is provided by experienced radiologists which are blinded as well to the type of treatment that the patients have received and evaluation time.

Allocation

A total of 86 eligible patients are allocated to receive either a combination of intra articular and subchondral BMAC injection or BMAC intra articular injection alone, in a 1:1 ratio (43 patients for each group of treatment) based on a computer-generated random numbers randomisation. This is conducted by research staff members dedicated to study organization and monitoring with no direct involvement in the study procedures. The randomization list is covered by password and accessible only by staff members with no direct involvement in the study.

Adverse events and assessment process

Adverse events are monitored throughout the study, intraoperatively and at clinical follow-up evaluations. Standard safety and efficacy monitoring is performed through regular face-to-face visits and phone calls between visits. The patients are also requested to report any adverse events to the research staff spontaneously. Every adverse event is recorded in the patient Case Report Form

(CRF). Serious adverse events are considered those resulting in death or being life-threatening, requiring hospitalization or intervention to prevent permanent impairment or damage; they are reported in accordance with the requirements of the Ethical Committee. Use of rescue pain medication is recorded at all visits without a diary and without homogenizing the type of medication, which is decided by patients autonomously (although discouraged for study purposes).

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 standard protocol book which contains details of standard operating procedures, trial contacts, visits, measurements, monitoring, and case report forms.

Data collection methods

Data are firstly collected on paper-based case report forms, with the help of research trained orthopaedic residents blinded to treatment allocation, and subsequently trained data analysts process data into electronic form for statistical analysis. Baseline and final MRI and CT knee scans are coded and stored at the Rizzoli Orthopaedic Institute to ensure data quality control. Operative data are collected electronically by the respective surgeons shortly after surgery.

Data management

Study data are stored in a password-protected spreadsheet on a server that is hosted at the Rizzoli Orthopaedical Institute. Data transfer is encrypted with all data de-identified. Only trained research personnel specifically dedicated to the data handling can access the database and ensures the correspondence of the electronic data with the original paper-based questionnaires and medical charts.

Statistical methods

A power analysis (G*Power 3.1.9.2) was conducted using assumptions of 90% of power and 5% of probability of type 1 error (alpha = 0.05), with a SD of 18.2 points based on a pilot study and a hypothesized 10-point difference in total WOMAC score at 12 months between treatments. Accordingly, 76 participants are needed. This leads to a moderate size-effect (0.55) as per the Cohen convention (effects: small ≥ .20, medium ≥ .50, large ≥ .80), and is in line with other effect sizes and SD reported in the literature. We increased the number of participants to a total of 86 patients (43 in each arm) to account for a possible 10% loss to follow-up. The primary analyses are intention-to-treat analyses of primary and secondary outcomes. Per protocol analyses will be performed as the secondary analyses. All those who have started the treatment are considered part of the research, regardless of whether they will complete it. For the missing data, they will be analyzed using the multiple imputation analysis, performed by filling the missing data with random values from the distribution of the variable.

Continuous variables are be expressed as means and standard deviations if normally distributed, as medians and range if not. Categorical variables are be expressed as frequencies and percentage. Normality of the distribution is be assessed using the Shapiro Wilks test. The Levene test is be used to assess the homoscedasticity of the data. The Repeated Measures ANOVA, followed by the post hoc Sidak pairwise test is performed to compare the scores at different follow-up times. The OneWay ANOVA test is performed to assess the between group differences of continuous and normally distributed and homoscedastic data; the Mann Whitney test is used otherwise. The ANOVA test, followed by the Scheffè post hoc pairwise comparison, is used also to assess the among groups differences of continuous, normally distributed and homoscedastic data; the Kruskal Wallis, test followed by the Mann Whitney test with the Bonferroni correction for multiple comparison, is used otherwise. The Monte Carlo method is used to evaluate the non-parametric tests in case of small size of the sub-groups. Pearson chi square exact test is performed to investigate relationships between grouping variables. The Spearman rank Correlation is used to assess correlations between the numerical scores and continuous data. The General linear model, or the

Generalized linear model in case of not normal distribution, is used as multivariate analysis to compare the group's outcomes corrected by the influencing factors. The Kaplan Meyer analysis is performed to assess survival to major adverse events. For all tests $p<0.05$ is considered significant. SPSS version 19.0 (IBM Corp., Armonk, NY, USA) is applied for the analyses.

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 Ministry of Health who funded the project. The monitoring personnel belongs to a research structure of the Scientific Direction of the Institution, the Applied and Translational Research Center, and it is independent from the Clinic and the medical personnel performing the study procedures. A further project auditing is performed by another independent entity of the Institution, the Clinical Trial Center. The final study report is also sent to the Ethic Committee.

ETHICS AND DISSEMINATION

Research ethics approval

Ethical approval was obtained on 5 May 2018 from the central Emilia Wide Area Ethical Committee of the Emilia-Romagna Region (CE-AVEC) settled at the University General Hospital Sant'Orsola-Malpighi of Bologna.

Protocol amendments

Minor protocol amendments, for example, database production changes to facilitate monitoring processes or improve outcome assessment by questionnaire, are fully documented. In case of major amendments, for example, changes to the patient information sheet and consent form, change of a local project leader or the inclusion of a new project site, they are submitted for approval by the lead Ethics Committee as required.

Consent or assent

All participants will provide informed written consent in Italian and they may dropout the trial at any time during the study course.

Confidentiality

Data are recorded using case report forms and processed centrally at the Rizzoli Orthopedics Institute, Bologna, Italy. The hard copies of case report forms are stored in a locked area with secured and restricted access. The electronic data are stored on password protected servers with restricted access. All data collected are kept strictly confidential. Daily backups of all electronic data occur to minimize any risk of lost data. After study completion, paper copies of data are archived in secure storage. Identifiers are be kept separately and accessible only to restricted study personnel in case follow-up of study patients is necessary; however, electronic data continue to be kept in a secure electronic database. This remains password protected and with access given only to the study investigators unless otherwise authorized by the study team.

Access to data

Only members of the research team who need to contact study patients, enter data, or perform data quality control have access to patient information.

Dissemination policy

This trial is produced according to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) international standards. Results are disseminated through peer-reviewed publications and will be 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 study provides a detailed method of treatment for knee OA and can offer clear indications on the potential and limitations of the combined use of intra-articular and subchondral bone injections of BMAC. The BMAC analysis provides characterisation of this product to shed greater light on the properties ensuring its effectiveness. Baseline patient-related and disease-related factors analysis can allow to better define those characteristics that make different subjects more or less responsive to this type of treatment.

Contributorship statement

ADM is the principal investigator of this study. SS, LA, AB, DR wrote the manuscript and will conduct the trial. GV and MM are responsible of imaging evaluation. CC and BG are involved in products and patients' characterization. ADM, SZ and GF applied for funding and supervise the trial. All authors read and approved the final protocol.

Competing interest

SZ reports non-financial support from personal fees from I+SRL, grants from FidiaFarmaceutici S.p.A., Cartiheal Ltd, IGEA clinical biophysics, BIOMET, and Kensey Nash, outside the submitted work. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results. The principal investigator and other authors declare no financial and other competing interests.

Funding

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Data sharing statement

Data sharing not applicable because no data are available in this protocol study.

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FIGURE LEGEND

Figure 1 - Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and subchondral Bone Marrow Aspirate Concentrate injections (F).

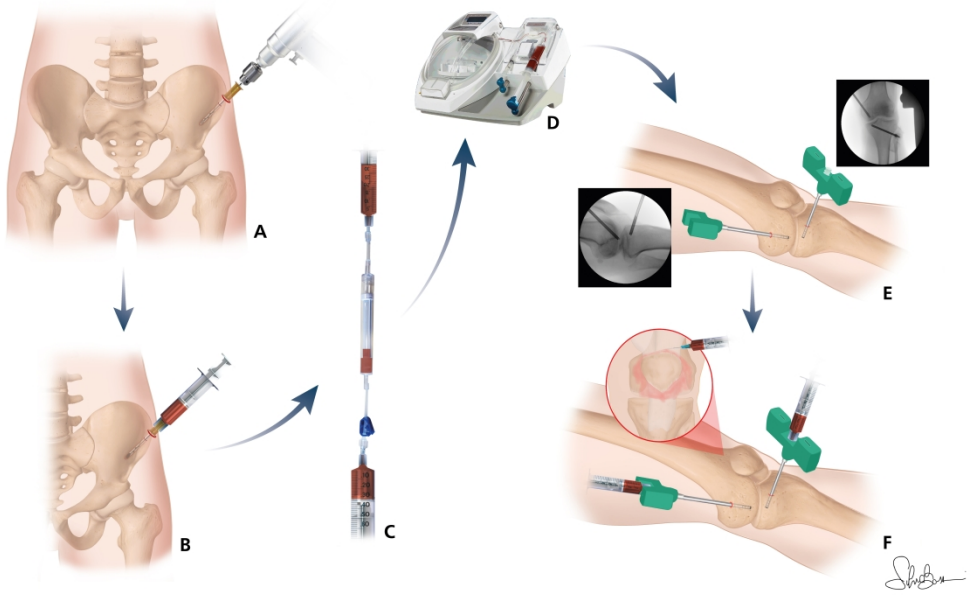

Figure 1: Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and subchondral Bone Marrow Aspirate Concentrate injections (F).

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered,	1

		name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	1
Protocol version	#3	Date and version identifier	3
Funding	#4	Sources and types of financial, material, and other support	17
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	4
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	1

Introduction

Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg,	7

		surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10

Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13

Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values).	13

		Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	15
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial	12

		conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	15
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	15
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	15
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16
Declaration of	#28	Financial and other competing interests for principal	17

interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	16
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a