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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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Manuscripts

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3 1 **A prospective double-blind randomized controlled trial protocol comparing**
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5 2 **bone marrow aspirate concentrate intra-articular injection combined with**
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7 3 **subchondral injection versus intra-articular injection alone**
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10 4 **for the treatment of symptomatic knee osteoarthritis.**
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50
51 22 *Keywords:* Osteoarthritis; Mesenchymal Stromal Cells; Subchondral injections; Bone marrow
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53 23 aspirate concentrate; BMAC
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57
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1
2
3 **27 ABSTRACT**
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6 **28** *Introduction:* Subchondral and intra-articular injections of bone marrow aspirate concentrate
7
8 **29** (BMAC) showed promising results for knee OA patients. To date, there is no evidence to
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10 **30** demonstrate whether the combination of these treatments provides higher benefits than the intra-
11
12 **31** articular injection alone.
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16 **32** *Methods and analysis:* Eighty-six patients with symptomatic knee OA (aged between 40 and 70) are
17
18 **33** randomized to BMAC intra-articular injection combined with subchondral BMAC injection or
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20 **34** BMAC intra-articular injection alone in a ratio of 1:1. The primary outcome is the Western Ontario
21
22 **35** and McMaster Universities Osteoarthritis Index (WOMAC), the secondary outcomes the
23
24 **36** International Knee Documentation Committee (IKDC) Subjective and Objective Knee Evaluation
25
26 **37** Form, the Tegner activity scale, the EuroQol-visual analogue scale (EQ-VAS), and the health
27
28 **38** questionnaire EQ-5D score. Additional CT and MRI evaluations will be performed at the baseline
29
30 **39** assessment and at the final 12-month follow-up. The hypothesis is that the combined injection
31
32 **40** provides higher knee pain and function improvement compared to BMAC intra-articular injection
33
34 **41** alone. The primary analysis follows an intention-to-treat principle.
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40 **42** *Ethics and dissemination:* The study protocol has been approved by the Emilia Wide Area Ethical
41
42 **43** Committee of the Emilia-Romagna Region (CE-AVEC), Bologna, Italy. Written informed consent
43
44 **44** is obtained from all the participants. findings of this study will be disseminated the through peer-
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46 **45** reviewed publications and conference presentations.
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3 47 *Strengths and limitations of this study:*
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- 8 49 • The study design is a prospective, randomized, double-blinded and controlled trial
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10 50 • This is the first RCT evaluating results of BMAC intra-articular injection combined
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12 with subchondral injection compared to BMAC intra-articular injection alone in knee OA.
13 51
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15 52 • Patients are analyzed using PROMs, objective measures, MRI and CT examination,
16
17 and biomarker evaluation.
18 53
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20 54 • Patient base-line characteristics and disease-related factors can help to better define
21
22 the aspects that make different individuals more or less responsive to this type of treatments.
23 55
24
25 56 • This study can clarify the benefits, and limitations, of the newly proposed
26
27 combination of intra-articular and subchondral BMAC injections, providing clear
28
29 indications for the clinical practice.
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34 60 *Trial registration:* Clinical Trials.gov Identifier number NCT03876795
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36 61

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38 62 *Protocol version:* Version 1 (14 May 2018)
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43 64 *Ethical approval:* (Prot. n. 0003132) for study protocol Interface (identifier: 207/2018/Sper/IOR)
44
45 65 was obtained on 5 May 2018 from the Ethical Committee Area Vasta Emilia Centro (CE-AVEC) of
46
47 the Emilia-Romagna Region settled at the University General Hospital Sant'Orsola-Malpighi of
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50 67 Bologna.
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97 INTRODUCTION

98

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

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

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3 123 *Objectives and trial design*
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5 124 A double-blinded randomized controlled trial (RCT) was designed to compare the efficacy of a
6
7
8 125 combination of intra-articular and subchondral injections of bone marrow aspirate concentrate
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10 126 (BMAC) (treatment group) versus BMAC intra-articular injection alone (control group) to treat
11
12 127 knee OA, with a 1:1 allocation ratio. The aim of this superiority trial is to evaluate the safety and
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14
15 128 the clinical potential of this new treatment approach up to one year of follow-up, and to verify the
16
17 129 hypothesis that the combination of subchondral and intra-articular injections provides higher knee
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19 130 pain and function improvement compared to BMAC intra-articular injection alone in knee OA.
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23
24 132 **METHODS AND ANALYSIS**
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26 133 *Study setting*
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28 134 The study is a single center double-blind RCT, with all activities related to the study performed in a
29
30
31 135 single site, the IRCCS Rizzoli Orthopaedic Institute, Bologna, Italy.
32

33 136 This trial protocol is produced according to the SPIRIT (Standard Protocol Items:
34
35 137 Recommendations for Interventional Trials) reporting guidelines. [9]
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40 139 *Patient and public involvement*
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42 140 Patients are not involved in planning of research questions, outcome measures or design of the
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44
45 141 study.
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49 143 *Eligibility criteria*
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51 144 Patients are recruited according to the following criteria.
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53

54 145 Inclusion criteria:

- 55
56 146 - Male or female patients, aged between 40 and 70;
57
58 147 - OA of the medial compartment of the knee (grade II or III according to the Kellgren-
59
60 148 Lawrence classification);

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3 149 - Failure after at least 6 months of conservative treatment (drug therapy with NSAIDs and
4
5 150 painkillers, hyaluronic acid infiltration, corticosteroid infiltration, PRP);
6
7
8 151 - Patients' ability and consent to participate in clinical and radiological follow-up;
9
10 152 - Signature of informed consent.

11
12 153 Exclusion criteria:

- 13
14 154 - Patients with trauma in the 6 months prior to surgery;
15
16
17 155 - Patients with malignancy;
18
19 156 - Patients suffering from rheumatic diseases;
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22 157 - Patients suffering from uncompensated diabetes;
23
24 158 - Patients suffering from uncompensated thyroid metabolic disorders;
25
26 159 - Patients abusing alcoholic beverages or drugs;
27
28
29 160 - Patients with axial deviations $> 5^\circ$;
30
31 161 - Body Mass Index > 35 ;
32
33 162 - Patients treated with joint injections in the previous 6 months;
34
35 163 - Patients treated with surgery at the same knee in the previous 12 months.

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40 165 *Intervention*

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42 166 All patients are treated by orthopedic surgeons with established experience in cartilage and
43
44 167 osteoarthritis orthobiologic procedures. The procedure is performed in a single step in the operating
45
46
47 168 room with patients in supine position under spinal loco-regional anaesthesia. The ipsilateral hip is
48
49 169 sterilely prepared and draped for anterior iliac crest bone marrow aspiration. The anterior superior
50
51 170 iliac spine is the anatomical landmark for a small surgical incision. A diamond tip trocar is inserted
52
53
54 171 in this point and then advanced into the bone marrow using a drill. Bone marrow is collected using
55
56 172 two 30 ml syringes coated with heparin. The harvested bone marrow is filtered with a heparin
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58 173 washed filter and then centrifuged through the Magellan[®] centrifuge (Arteriocyte Medical Systems,
59
60 174 MA, USA) at a rate of 3600 RPM for approximately 15 minutes, thus obtaining 10 ml of BMAC.

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3 175 For each patient, BMAC samples that are not used for surgical treatment are sent to the laboratory
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5 176 for the count of mononuclear cells, cell clonogenic ability by colony forming unit-fibroblast test and
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8 177 phenotypical characterization by flow-cytometry evaluation.
9

10 178 Concomitantly with the bone marrow concentration process, all patients undergo an arthroscopic
11
12 179 evaluation to confirm lesions grade and site of both medial femoral condyle and medial tibial
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15 180 plateau. Arthroscopy is done using the standard antero-lateral, antero-medial, and supero-medial
16
17 181 portals. If the arthroscopic examination reveals intra-articular problems (excluding minor
18
19 182 arthroscopic shaving) requiring surgical intervention which may affect the results of the procedure,
20
21
22 183 the patient is excluded from the study.
23

24 184 Once the arthroscopy and the BMAC procedure are completed, the injections are performed. The
25
26 185 treatment group receives two 2.5 ml subchondral BMAC injections, that are performed inserting
27
28 186 two 8-Gauge trocars through the supero-medial and antero-medial arthroscopic portals and are
29
30
31 187 manually introduced with clockwise and anticlockwise movements, under fluoroscopic control, into
32
33 188 the bone of both medial femoral condyle and tibial plateau. Following arthroscopic portals suture,
34
35 189 both groups of treatment receive a 3ml intra-articular injection of BMAC using a lateral
36
37
38 190 suprapatellar approach. An elastic bandage is made after wounds medication. The whole procedure
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40 191 is presented in Figure 1.
41

42 192 Postoperatively, patients are discharged on the same day of the procedure or the day after, based on
43
44 193 patient condition. Pain control is prescribed as needed with analgesics only in the immediate period
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46
47 194 after treatment, and thromboembolic prophylaxis is prescribed for two weeks. During the same
48
49 195 time, patients are taught to walk with the support of two crutches to allow a partial weight-bearing
50
51 196 on the operated limb. Cryotherapy is started within the first 24 hours. Passive mobilisation and
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53
54 197 quadriceps isometric exercises are started at the second post-operative day. Patients are permitted to
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56 198 return to most of their daily activities as tolerated once they reach full weight-bearing. No other
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58 199 conservative treatments are prescribed during the study period. Joint impacting sport activities are
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60 200 discouraged within the first month after treatment.

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201 *Outcomes*

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

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

212 Patients will also undergo MRI and CT assessments. MRI scans are obtained with a high-resolution
213 3 Tesla MRI scanner with PD-weighted Turbo Spin Echo 3D sequences with and without fat
214 saturation (FS), 3D T2* Gradient Echo (MERGE) with FS, axial PD-weighted Fast Spin Echo
215 sequences with FS, and Multi-Echo T2 Mapping on the sagittal plane with 8 different Echo Times.

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

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

222 CT knee scans are obtained with a 64-channels CT scanner to better assess the structural resolution
223 of bone trabeculae as well as to assess the presence of osteophytes, calcifications, and cancellous
224 bone microcysts. The images were acquired using a slice thickness of 1.25 mm and an interval of
225 0.625 mm at 120kV with 250 mA, post-processed with the “Bone” filter, and reformatted in the
226 coronal and sagittal plane.

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3 227 Blood samples are obtained from participants before treatment and at 2, 6, and 12 months of follow-
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5 228 up. Samples are analysed for inflammatory (IL-1 β , TNF α) and OA progression markers (Cleavage
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7 229 of Type II Collagen, Serum C-telopeptide fragments of type II collagen).
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11 12 231 *Participant timeline*

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14 232 Research assistants first conduct a screening of potential candidates over the telephone. If early
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16 233 checks of study eligibility are favourable, participants are booked in for a face-to-face screening
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18 234 visit with an orthopaedic specialist to confirm eligibility and explain the study protocol. After the
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20 235 screening visit, patients complete the questionnaires, undergo a knee MRI and CT, and sign the
21
22 236 informed written consent. Patient enrolment started on November 2019. The first patient was
23
24 237 treated in December 2019. Follow-up assessments is performed at 2, 6, and 12 months
25
26 238 postoperatively with patient questionnaires and blood samples. At the final 12-month follow-up
27
28 239 patients undergo knee MRI and CT scans. Due to operational delays caused by the COVID-19
29
30 240 pandemic, patient treatment is still ongoing; the study conclusion is foreseen before the end of
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32 241 2023. Participant timeline is outlined in Table 1.
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39 242 **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

58 243 AE: Adverse Event; BMAC: Bone Marrow Aspirate Concentrate; CT: Computed tomography; EQ-5D: European
59 244 Quality of Life Five Dimension; EQ-VAS: European Quality - Visual Analog Scale; IKDC: International Knee
60 245 Documentation Committee; MRI: magnetic resonance imaging; WOMAC: Western Ontario and McMaster Universities
246 Osteoarthritis Index

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247 *Recruitment*

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

251

252 *Blinding*

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

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

259

260 *Allocation*

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

267

268 *Adverse events and assessment process*

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

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3 273 (CRF). Severe adverse events are considered those resulting in death or being life-threatening,
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5 274 requiring hospitalization or intervention to prevent permanent impairment or damage; they are
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7
8 275 reported in accordance with the requirements of the Ethical Committee. Use of pain medicines is
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10 276 recorded at all visits.

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12 277 To ensure high-quality execution of the trial in accordance with the protocol, all trial staff is trained
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14 278 by the chief investigators and provided with a standard protocol book which contains details of
15
16
17 279 standard operating procedures, trial contacts, visits, measurements, monitoring, and case report
18
19 280 forms.

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23 24 282 *Data collection methods*

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26 283 Data are firstly collected on paper-based case report forms, with the help of research trained
27
28 284 orthopaedic residents blinded to treatment allocation, and subsequently trained data analysts process
29
30
31 285 data into electronic form for statistical analysis. Baseline and final MRI and CT knee scans are
32
33 286 coded and stored at the Rizzoli Orthopaedic Institute to ensure data quality control. Operative data
34
35 287 are collected electronically by the respective surgeons shortly after surgery.

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39 40 289 *Data management*

41
42 290 Study data are stored in a password-protected spreadsheet on a server that is hosted at the Rizzoli
43
44 291 Orthopaedical Institute. Data transfer is encrypted with all data de-identified. Only trained research
45
46
47 292 personnel specifically dedicated to the data handling can access the database and ensures the
48
49 293 correspondence of the electronic data with the original paper-based questionnaires and medical
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51 294 charts.

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55 56 296 *Statistical methods*

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58 297 We conducted a power analysis (G*Power 3.1.9.2) and using assumptions of 80% of power and 5%
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60 298 of probability of type 1 error ($\alpha = 0.05$), we will need 76 participants. Adjusting for a 10% loss

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3 299 to follow-up, we will need 86 participants (43 in each arm). With 76 subjects we will have a 80%
4
5 300 power to detect a difference between the two groups in terms of WOMAC score at one year follow-
6
7
8 301 up with a moderate size-effect (0.55) determined with the Cohen convention (effects: small $\geq .20$,
9
10 302 medium $\geq .50$, large $\geq .80$). The primary analyses will be intention-to-treat analyses of primary and
11
12 303 secondary outcomes. Per protocol analyses will be performed as the secondary analyses. All those
13
14 304 who have started the treatment are considered part of the research, regardless of whether they will
15
16
17 305 complete it.

18
19 306 Continuous variables will be expressed as means and standard deviations if normally distributed,
20
21 307 as medians and range if not. Categorical variables will be expressed as frequencies and
22
23
24 308 percentage. Normality of the distribution will be assessed using the Shapiro Wilks test. The Levene
25
26 309 test will be used to assess the homoscedasticity of the data. The Repeated Measures ANOVA,
27
28 310 followed by the post hoc Sidak pairwise test will be performed to compare the scores at different
29
30
31 311 follow-up times. The OneWay ANOVA test will be performed to assess the between group
32
33 312 differences of continuous and normally distributed and homoscedastic data; the Mann Whitney test
34
35 313 will be used otherwise. The ANOVA test followed by the Scheffè post hoc pairwise comparison
36
37
38 314 will be used also to assess the among groups differences of continuous, normally distributed and
39
40 315 homoscedastic data, the Kruskal Wallis test followed by the Mann Whitney test with the Bonferroni
41
42 316 correction for multiple comparison will be used otherwise. The Monte Carlo method will be used to
43
44
45 317 evaluate the non-parametric tests in case of small size of the sub-groups. Pearson chi square exact
46
47 318 test will be performed to investigate relationships between grouping variables. The Spearman rank
48
49 319 Correlation will be used to assess correlations between the numerical scores and continuous data.
50
51 320 The General linear model or the Generalized linear model in case of not normal distribution, will be
52
53
54 321 used as multivariate analysis to compare the group's outcomes corrected by the influencing factors.
55
56 322 The Kaplan Meyer analysis will be performed to assess survival to major adverse events. For all
57
58 323 tests $p < 0.05$ will be considered significant. SPSS version 19.0 (IBM Corp., Armonk, NY, USA)
59
60 324 will be applied for the analyses.

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2
3 325 *Data monitoring*
4

5 326 A central project data manager is tasked to perform data quality control on all collected data. An
6
7
8 327 interim report and a final report are foreseen, to be submitted to the Ministry of Health who funded
9
10 328 the project. The monitoring personnel belongs to a research structure of the Scientific Direction of
11
12 329 the Institution, the Applied and Translational Research Center, and it is independent from the Clinic
13
14
15 330 and the medical personnel performing the study procedures. A further project auditing is performed
16
17 331 by another independent entity of the Institution, the Clinical Trial Center. The final study report is
18
19 332 also sent to the Ethic Committee.
20

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22 333

23
24 334 **ETHICS AND DISSEMINATION**
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26 335
27
28 336 *Research ethics approval*
29

30 337 Ethical approval was obtained on 5 May 2018 from the central Emilia Wide Area Ethical
31
32
33 338 Committee of the Emilia-Romagna Region (CE-AVEC) settled at the University General Hospital
34
35 339 Sant'Orsola-Malpighi of Bologna.
36

37 340

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40 341 *Protocol amendments*
41

42 342 Minor protocol amendments, for example, database production changes to facilitate monitoring
43
44 343 processes or improve outcome assessment by questionnaire, are fully documented. In case of major
45
46
47 344 amendments, for example, changes to the patient information sheet and consent form, change of a
48
49 345 local project leader or the inclusion of a new project site, they will be submitted for approval by the
50
51 346 lead Ethics Committee as required.
52

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56 348 *Consent or assent*
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58 349 All participants will provide informed written consent in Italian and they may dropout the trial at
59
60 350 any time during the study course.

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3 351 *Confidentiality*

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5 352 Data are recorded using case report forms and processed centrally at the Rizzoli Orthopedics
6
7
8 353 Institute, Bologna, Italy. The hard copies of case report forms are stored in a locked area with
9
10 354 secured and restricted access. The electronic data are stored on password protected servers with
11
12 355 restricted access. All data collected are kept strictly confidential. Daily backups of all electronic
13
14 356 data occur to minimize any risk of lost data. After study completion, paper copies of data are
15
16
17 357 archived in secure storage. Identifiers are be removed in case follow-up of study patients is
18
19 358 necessary; however, electronic data continue to be kept in a secure electronic database. This
20
21
22 359 remains password protected and with access given only to the study investigators unless otherwise
23
24 360 authorized by the study team.

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26 361
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28 362 *Access to data*

29
30 363 Only members of the research team who need to contact study patients, enter data, or perform data
31
32
33 364 quality control have access to patient information.

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35 365
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37 366 *Dissemination policy*

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39
40 367 This trial is produced according to the SPIRIT (Standard Protocol Items: Recommendations for
41
42 368 Interventional Trials) international standards. Results will be disseminated through peer-reviewed
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44
45 369 publications and will be submitted for presentation at national and international conferences. The
46
47 370 authorship will be based on International Committee of Medical Journal Editors 2018
48
49 371 Recommendations.

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51 372
52
53 373 *Scientific relevance and broader impact*

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55
56 374 This study provides a detailed method of treatment for knee OA and can offer clear indications on
57
58 375 the potential and limitations of the combined use of intra-articular and subchondral bone injections
59
60 376 of BMAC. The BMAC analysis provides characterisation of this product to shed greater light on the

1
2
3 377 properties ensuring its effectiveness. Baseline patient-related and disease-related factors analysis
4
5 378 can allow to better define those characteristics that make different subjects more or less responsive
6
7
8 379 to this type of treatment.
9

10 380
11
12 381 *Contributorship statement*

13
14 382 ADM is the principal investigator of this study. SS, LA, AB, DR wrote the manuscript and will
15
16
17 383 conduct the trial. GV and MM are responsible of imaging evaluation. CC and BG are involved in
18
19 384 products and patients' characterization. ADM, SZ and GF applied for funding and supervise the
20
21 385 trial. All authors read and approved the final protocol.
22
23

24 386
25
26 387 *Competing interest*

27
28 388 SZ reports non-financial support from personal fees from I+SRL, grants from FidiaFarmaceutici
29
30 389 S.p.A., Cartiheal Ltd, IGEA clinical biophysics, BIOMET, and Kensey Nash, outside the submitted
31
32
33 390 work. The funders had no role in the design of the study, in the collection, analyses, or
34
35 391 interpretation of data, in the writing of the manuscript, or in the decision to publish the results. The
36
37 392 principal investigator and other authors declare no financial and other competing interests.
38
39

40 393
41
42 394 *Funding:* The study is funded by Italian Health Ministry in the Project "Giovane Ricercatore" (GR-
43
44 395 2016-02361990).
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49 397 *Data sharing statement*

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51 398 No data are available
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2
3 429 **FIGURE LEGEND**
4

5 430 Figure 1 - Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration
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8 431 (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and
9
10 432 subchondral Bone Marrow Aspirate Concentrate injections (F).
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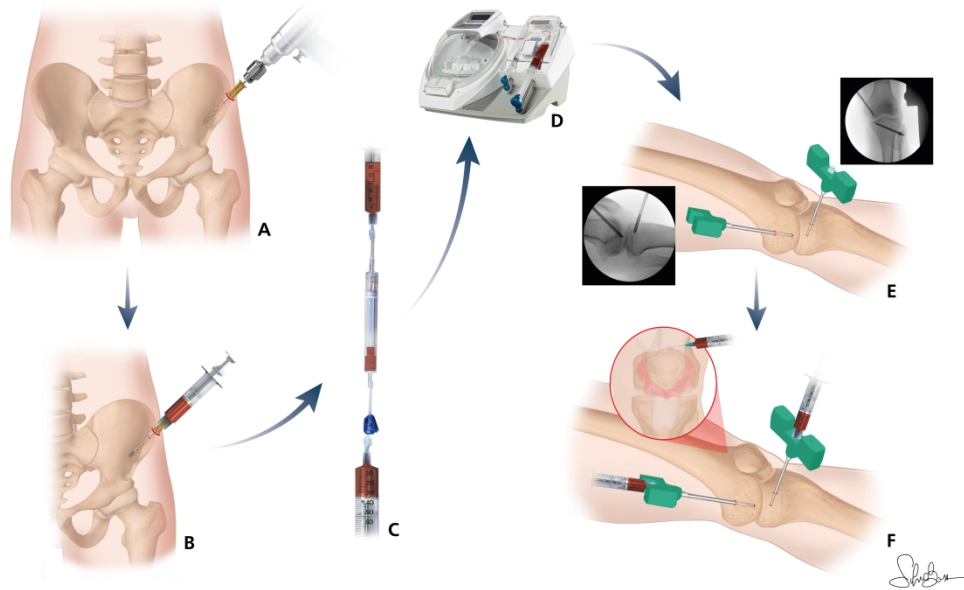


Figure 1: Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and subchondral Bone Marrow Aspirate Concentrate injections (F).

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.

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

In your methods section, say that you used the SPIRIT reporting 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

			Page
		Reporting Item	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

1		name of intended registry	
2			
3			
4	Trial registration: data	#2b All items from the World Health Organization Trial	1
5			
6	set	Registration Data Set	
7			
8			
9	Protocol version	#3 Date and version identifier	1
10			
11			
12	Funding	#4 Sources and types of financial, material, and other support	1
13			
14			
15	Roles and	#5a Names, affiliations, and roles of protocol contributors	2
16			
17	responsibilities:		
18			
19	contributorship		
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23	Roles and	#5b Name and contact information for the trial sponsor	1
24			
25	responsibilities:		
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27	sponsor contact		
28			
29	information		
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33	Roles and	#5c Role of study sponsor and funders, if any, in study design;	1
34			
35	responsibilities:	collection, management, analysis, and interpretation of	
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37	sponsor and funder	data; writing of the report; and the decision to submit the	
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39		report for publication, including whether they will have	
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41		ultimate authority over any of these activities	
42			
43			
44			
45	Roles and	#5d Composition, roles, and responsibilities of the coordinating	1
46			
47	responsibilities:	centre, steering committee, endpoint adjudication	
48			
49	committees	committee, data management team, and other individuals	
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51		or groups overseeing the trial, if applicable (see Item 21a	
52			
53		for data monitoring committee)	
54			
55			
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57	Introduction		
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60			

1	Background and	#6a	Description of research question and justification for	3
2				
3	rationale		undertaking the trial, including summary of relevant studies	
4			(published and unpublished) examining benefits and harms	
5			for each intervention	
6				
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11	Background and	#6b	Explanation for choice of comparators	3
12				
13	rationale: choice of			
14				
15	comparators			
16				
17				
18	Objectives	#7	Specific objectives or hypotheses	4
19				
20				
21				
22	Trial design	#8	Description of trial design including type of trial (eg, parallel	4
23			group, crossover, factorial, single group), allocation ratio,	
24			and framework (eg, superiority, equivalence, non-inferiority,	
25			exploratory)	
26				
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31	Methods:			
32				
33	Participants,			
34				
35	interventions, and			
36				
37	outcomes			
38				
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40				
41	Study setting	#9	Description of study settings (eg, community clinic,	4
42			academic hospital) and list of countries where data will be	
43			collected. Reference to where list of study sites can be	
44			obtained	
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51	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	4
52			applicable, eligibility criteria for study centres and	
53			individuals who will perform the interventions (eg,	
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1		surgeons, psychotherapists)	
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3			
4	Interventions:	#11a Interventions for each group with sufficient detail to allow	5
5			
6	description	replication, including how and when they will be	
7			
8		administered	
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11	Interventions:	#11b Criteria for discontinuing or modifying allocated	6
12			
13	modifications	interventions for a given trial participant (eg, drug dose	
14			
15		change in response to harms, participant request, or	
16			
17		improving / worsening disease)	
18			
19			
20			
21	Interventions:	#11c Strategies to improve adherence to intervention protocols,	9
22			
23	adherence	and any procedures for monitoring adherence (eg, drug	
24			
25		tablet return; laboratory tests)	
26			
27			
28			
29	Interventions:	#11d Relevant concomitant care and interventions that are	6
30			
31	concomitant care	permitted or prohibited during the trial	
32			
33			
34	Outcomes	#12 Primary, secondary, and other outcomes, including the	7
35			
36		specific measurement variable (eg, systolic blood	
37			
38		pressure), analysis metric (eg, change from baseline, final	
39			
40		value, time to event), method of aggregation (eg, median,	
41			
42		proportion), and time point for each outcome. Explanation	
43			
44		of the clinical relevance of chosen efficacy and harm	
45			
46		outcomes is strongly recommended	
47			
48			
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51	Participant timeline	#13 Time schedule of enrolment, interventions (including any	7
52			
53		run-ins and washouts), assessments, and visits for	
54			
55		participants. A schematic diagram is highly recommended	
56			
57		(see Figure)	
58			
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60			

1	Sample size	#14	Estimated number of participants needed to achieve study	10
2			objectives and how it was determined, including clinical and	
3			statistical assumptions supporting any sample size	
4			calculations	
5				
6				
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10				
11	Recruitment	#15	Strategies for achieving adequate participant enrolment to	9
12			reach target sample size	
13				
14				
15				
16	Methods: Assignment			
17	of interventions (for			
18	controlled trials)			
19				
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24	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	9
25	generation		computer-generated random numbers), and list of any	
26			factors for stratification. To reduce predictability of a	
27			random sequence, details of any planned restriction (eg,	
28			blocking) should be provided in a separate document that is	
29			unavailable to those who enrol participants or assign	
30			interventions	
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41	Allocation	#16b	Mechanism of implementing the allocation sequence (eg,	9
42	concealment		central telephone; sequentially numbered, opaque, sealed	
43	mechanism		envelopes), describing any steps to conceal the sequence	
44			until interventions are assigned	
45				
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51	Allocation:	#16c	Who will generate the allocation sequence, who will enrol	9
52	implementation		participants, and who will assign participants to	
53			interventions	
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1	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg,	9
2			trial participants, care providers, outcome assessors, data	
3			analysts), and how	
4				
5				
6				
7				
8	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is	9
9	emergency		permissible, and procedure for revealing a participant's	
10			allocated intervention during the trial	
11	unblinding			
12				
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16	Methods: Data			
17	collection,			
18	management, and			
19	analysis			
20				
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26	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline,	10
27			and other trial data, including any related processes to	
28			promote data quality (eg, duplicate measurements, training	
29			of assessors) and a description of study instruments (eg,	
30			questionnaires, laboratory tests) along with their reliability	
31			and validity, if known. Reference to where data collection	
32			forms can be found, if not in the protocol	
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43	Data collection plan:	#18b	Plans to promote participant retention and complete follow-	9
44	retention		up, including list of any outcome data to be collected for	
45			participants who discontinue or deviate from intervention	
46			protocols	
47				
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53	Data management	#19	Plans for data entry, coding, security, and storage,	10
54			including any related processes to promote data quality	
55			(eg, double data entry; range checks for data values).	
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1		Reference to where details of data management	
2			
3		procedures can be found, if not in the protocol	
4			
5			
6	Statistics: outcomes	#20a Statistical methods for analysing primary and secondary	11
7			
8		outcomes. Reference to where other details of the	
9			
10		statistical analysis plan can be found, if not in the protocol	
11			
12			
13	Statistics: additional	#20b Methods for any additional analyses (eg, subgroup and	11
14			
15	analyses	adjusted analyses)	
16			
17			
18	Statistics: analysis	#20c Definition of analysis population relating to protocol non-	11
19			
20	population and	adherence (eg, as randomised analysis), and any statistical	
21			
22	missing data	methods to handle missing data (eg, multiple imputation)	
23			
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25			
26	Methods: Monitoring		
27			
28			
29	Data monitoring:	#21a Composition of data monitoring committee (DMC);	12
30			
31	formal committee	summary of its role and reporting structure; statement of	
32			
33		whether it is independent from the sponsor and competing	
34			
35		interests; and reference to where further details about its	
36			
37		charter can be found, if not in the protocol. Alternatively, an	
38			
39		explanation of why a DMC is not needed	
40			
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44	Data monitoring:	#21b Description of any interim analyses and stopping	12
45			
46	interim analysis	guidelines, including who will have access to these interim	
47			
48		results and make the final decision to terminate the trial	
49			
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51	Harms	#22 Plans for collecting, assessing, reporting, and managing	10
52			
53		solicited and spontaneously reported adverse events and	
54			
55		other unintended effects of trial interventions or trial	
56			
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1		conduct	
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4	Auditing	#23 Frequency and procedures for auditing trial conduct, if any,	12
5			
6		and whether the process will be independent from	
7			
8		investigators and the sponsor	
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11	Ethics and		
12			
13	dissemination		
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16	Research ethics	#24 Plans for seeking research ethics committee / institutional	12
17			
18	approval	review board (REC / IRB) approval	
19			
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22	Protocol	#25 Plans for communicating important protocol modifications	12
23			
24	amendments	(eg, changes to eligibility criteria, outcomes, analyses) to	
25			
26		relevant parties (eg, investigators, REC / IRBs, trial	
27			
28		participants, trial registries, journals, regulators)	
29			
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31			
32	Consent or assent	#26a Who will obtain informed consent or assent from potential	13
33			
34		trial participants or authorised surrogates, and how (see	
35			
36		Item 32)	
37			
38			
39	Consent or assent:	#26b Additional consent provisions for collection and use of	n/a
40			
41	ancillary studies	participant data and biological specimens in ancillary	
42			
43		studies, if applicable	
44			
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47	Confidentiality	#27 How personal information about potential and enrolled	13
48			
49		participants will be collected, shared, and maintained in	
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51		order to protect confidentiality before, during, and after the	
52			
53		trial	
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57	Declaration of	#28 Financial and other competing interests for principal	13
58			
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1	interests		investigators for the overall trial and each study site	
2				
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4	Data access	#29	Statement of who will have access to the final trial dataset,	13
5			and disclosure of contractual agreements that limit such	
6			access for investigators	
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11	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial care, and for	n/a
12			compensation to those who suffer harm from trial	
13	trial care		participation	
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19	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial	14
20			results to participants, healthcare professionals, the public,	
21	trial results		and other relevant groups (eg, via publication, reporting in	
22			results databases, or other data sharing arrangements),	
23			including any publication restrictions	
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31	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	14
32			professional writers	
33	authorship			
34				
35				
36	Dissemination policy:	#31c	Plans, if any, for granting public access to the full protocol,	n/a
37			participant-level dataset, and statistical code	
38	reproducible research			
39				
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42	Appendices			
43				
44				
45	Informed consent	#32	Model consent form and other related documentation given	n/a
46			to participants and authorised surrogates	
47	materials			
48				
49				
50	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of	n/a
51			biological specimens for genetic or molecular analysis in	
52			the current trial and for future use in ancillary studies, if	
53			applicable	
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2 Commons Attribution License CC-BY-NC. This checklist was completed on 04. March 2022 using
3 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
4 [Penelope.ai](#)
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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.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-062632.R1
Article Type:	Protocol
Date Submitted by the Author:	30-Jul-2022
Complete List of Authors:	Silva, Simone; IRCCS Istituto Ortopedico Rizzoli, II Orthopaedic and Traumatologic Clinic Andriolo, Luca; IRCCS Istituto Ortopedico Rizzoli, II Orthopaedic and Traumatologic Clinic Boffa, Angelo ; IRCCS Istituto Ortopedico Rizzoli, II Orthopaedic and Traumatologic Clinic Di Martino, Alessandro; IRCCS Istituto Ortopedico Rizzoli, II Orthopaedic and Traumatologic Clinic Reale, Davide; Istituto Ortopedico Rizzoli Istituto di Ricovero e Cura a Carattere Scientifico, II Orthopaedic and Traumatologic Clinic Vara, Giulio; IRCCS Istituto Ortopedico Rizzoli, Diagnostic and Interventional Radiology Marco, Miceli; IRCCS Istituto Ortopedico Rizzoli, Diagnostic and Interventional Radiology Cavallo, Carola; IRCCS Istituto Ortopedico Rizzoli, Laboratory RAMSES, Research & Innovation Technology Department Grigolo, Brunella; IRCCS Istituto Ortopedico Rizzoli, Laboratory RAMSES, Research & Innovation Technology Department Zaffagnini, Stefano ; IRCCS Istituto Ortopedico Rizzoli, II Orthopaedic and Traumatologic Clinic Filardo, Giuseppe; IRCCS Istituto Ortopedico Rizzoli, Applied and Translational Research Center
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Evidence based practice
Keywords:	ORTHOPAEDIC & TRAUMA SURGERY, Adult orthopaedics < ORTHOPAEDIC & TRAUMA SURGERY, Orthopaedic & trauma surgery < SURGERY, Knee < ORTHOPAEDIC & TRAUMA SURGERY

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3 1 **A prospective double-blind randomized controlled trial protocol comparing**
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5 2 **bone marrow aspirate concentrate intra-articular injection combined with**
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7 3 **subchondral injection versus intra-articular injection alone**
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9 4 **for the treatment of symptomatic knee osteoarthritis.**
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51 22 aspirate concentrate; BMAC
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2
3 27 **ABSTRACT**
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6 28 *Introduction:* Subchondral and intra-articular injections of bone marrow aspirate concentrate
7
8 29 (BMAC) showed promising results for knee OA patients. To date, there is no evidence to
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10 30 demonstrate whether the combination of these treatments provides higher benefits than the intra-
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12 31 articular injection alone.
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16 32 *Methods and analysis:* Eighty-six patients with symptomatic knee OA (aged between 40 and 70) are
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18 33 randomized to BMAC intra-articular injection combined with subchondral BMAC injection or
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20 34 BMAC intra-articular injection alone in a ratio of 1:1. The primary outcome is the Western Ontario
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22 35 and McMaster Universities Osteoarthritis Index (WOMAC), the secondary outcomes the
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24 36 International Knee Documentation Committee (IKDC) Subjective and Objective Knee Evaluation
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26 37 Form, the Tegner activity scale, the EuroQol-visual analogue scale (EQ-VAS), and the health
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28 38 questionnaire EQ-5D score. Additional CT and MRI evaluations are performed at the baseline
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30 39 assessment and at the final 12-month follow-up. The hypothesis is that the combined injection
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32 40 provides higher knee pain and function improvement compared to BMAC intra-articular injection
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34 41 alone. The primary analysis follows an intention-to-treat principle.
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40 42 *Ethics and dissemination:* The study protocol has been approved by the Emilia Wide Area Ethical
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42 43 Committee of the Emilia-Romagna Region (CE-AVEC), Bologna, Italy. Written informed consent
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44 44 is obtained from all the participants. Findings of this study will be disseminated the through peer-
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46 45 reviewed publications and conference presentations.
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3 47 *Strengths and limitations of this study:*
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- 8 49 • This is the first prospective, randomized, double-blind and controlled trial evaluating
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10 50 results of BMAC intra-articular injection combined with subchondral injection compared to
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12 51 BMAC intra-articular injection alone in knee OA.
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15 52 • Patients are analyzed using PROMs, objective measures, MRI and CT examination,
16
17 53 and biomarker evaluation.
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20 54 • Patient base-line characteristics and disease-related factors can help to better define
21
22 55 the aspects that make different individuals more or less responsive to this type of treatments.
23
24 56 • The uncontrolled pain medication use by patients (although being discouraged) could
25
26 57 influence the primary outcome and this is a relevant limitation of the study.
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29 58 • This study can clarify the benefits, and limitations, of the newly proposed
30
31 59 combination of intra-articular and subchondral BMAC injections, providing clear
32
33 60 indications for the clinical practice.
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38 62 *Trial registration:* Clinical Trials.gov Identifier number NCT03876795
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43 64 *Protocol version:* Version 1 (14 May 2018)
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47 66 *Ethical approval:* (Prot. n. 0003132) for study protocol Interface (identifier: 207/2018/Sper/IOR)
48
49 67 was obtained on 5 May 2018 from the Ethical Committee Area Vasta Emilia Centro (CE-AVEC) of
50
51 68 the Emilia-Romagna Region settled at the University General Hospital Sant'Orsola-Malpighi of
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53 69 Bologna.
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59 71 *Roles and responsibilities:*
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94 INTRODUCTION

95
96 Knee osteoarthritis (OA) is a chronic, degenerative disease leading to irreversible structural and
97 functional changes in the entire joint, including subchondral bone sclerosis and cartilage loss, and
98 progressively determines debilitating pain and loss of function.[1-2] It affects a large part of the
99 aging population with a high impact on patients and healthcare costs.[3] Total knee arthroplasty
100 represents a definitive solution to address knee OA, but it is also encumbered by several
101 complications.[4] Conservative approaches, such as physical therapy and anti-inflammatory drugs,
102 should be pursued, but their benefits are generally temporary with short-term relief, and they are not
103 able to affect the natural course of the disease progression.[5] Thus, to delay or avoid the need for
104 arthroplasty, research efforts have been made to find new minimally invasive and more effective
105 procedures to address knee OA.

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

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3 120 Moreover, the combination of subchondral and intra-articular injections of bone marrow aspirate
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5 121 concentrate (BMAC) already showed promising results in terms of safety and clinical
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8 122 outcomes.[12] However, beside promising early findings and the increasing use of this approach in
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10 123 the clinical practice, there is only limited and low-level evidence, and it would be clinically relevant
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12 124 to evaluate with a high-level study design the real benefit provided by the addition of these
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14
15 125 subchondral injections to improve the results of BMAC intra-articular injections for knee OA.
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17 126 18 19 127 *Objectives and trial design*

20
21 128 A double-blinded randomized controlled trial (RCT) was designed to compare the efficacy of a
22
23
24 129 combination of intra-articular and subchondral injections of BMAC (treatment group) versus
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26 130 BMAC intra-articular injection alone (control group) to treat knee OA, with a 1:1 allocation ratio.
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28
29 131 The aim of this superiority trial is to evaluate the safety and the clinical potential of this new
30
31 132 treatment approach up to one year of follow-up, and to verify the hypothesis that the combination of
32
33 133 subchondral and intra-articular injections provides higher knee pain and function improvement
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35 134 compared to BMAC intra-articular injection alone in knee OA.
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37 135 38 39 136 **METHODS AND ANALYSIS**

40 137 *Study setting*

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44 138 The study is a single center double-blind RCT, with all activities related to the study performed in a
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47 139 single site, the IRCCS Rizzoli Orthopaedic Institute, Bologna, Italy.

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49 140 This trial protocol is produced according to the SPIRIT (Standard Protocol Items:
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51 141 Recommendations for Interventional Trials) reporting guidelines. [13]
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53 142 54 55 143 *Patient and public involvement*

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58 144 Patients are not involved in planning of research questions, outcome measures or design of the
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60 145 study.

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45 147 *Eligibility criteria*6
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8 148 Patients are recruited according to the following criteria.9
10 149 Inclusion criteria:

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- 12 150 - Male or female patients, aged between 40 and 70 years old;
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- 15 151 - OA of the medial compartment of the knee (grade II or III according to the Kellgren-
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- 17 152 Lawrence classification);
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- 19 153 - Failure after at least 6 months of conservative treatment (drug therapy with NSAIDs and
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- 22 154 painkillers, hyaluronic acid infiltration, corticosteroid infiltration, PRP);
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- 24 155 - Patients' ability and consent to participate in clinical and radiological follow-up;
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- 26 156 - Signature of informed consent.

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28 157 Exclusion criteria:

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- 31 158 - Patients with trauma in the 6 months prior to surgery;
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- 33 159 - Patients with malignancy;
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- 35 160 - Patients suffering from rheumatic diseases;
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- 38 161 - Patients suffering from uncompensated diabetes;
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- 40 162 - Patients suffering from uncompensated thyroid metabolic disorders;
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- 42 163 - Patients abusing alcoholic beverages or drugs;
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- 45 164 - Patients with axial deviations
- $> 5^\circ$
- ;
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- 47 165 - Body Mass Index
- > 35
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- 49 166 - Patients treated with joint injections in the previous 6 months;
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- 51 167 - Patients treated with surgery at the same knee in the previous 12 months.

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56 169 *Intervention*57
58 170 All patients are treated by orthopedic surgeons with established experience in cartilage and
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60 171 osteoarthritis orthobiologic procedures. The procedure is performed in a single step in the operating

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3 172 room with patients in supine position under spinal loco-regional anaesthesia. The ipsilateral hip is
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5 173 sterilely prepared and draped for anterior iliac crest bone marrow aspiration. The anterior superior
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8 174 iliac spine is the anatomical landmark for a small surgical incision. A diamond tip trocar is inserted
9
10 175 in this point and then advanced into the bone marrow using a drill. Bone marrow is collected using
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12 176 two 30 ml syringes coated with heparin for a total of 60 ml. The harvested bone marrow is filtered
13
14
15 177 with a heparin washed filter and then centrifuged through the Magellan® centrifuge (Arteriocyte
16
17 178 Medical Systems, MA, USA) at a rate of 3600 RPM for approximately 15 minutes, thus obtaining
18
19 179 10 ml of BMAC. The BMAC procedure involved a kit available in the clinical practice. In fact, the
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21
22 180 purpose of the study was not to evaluate a new product, but rather to explore the potential of
23
24 181 applying BMAC also at the subchondral bone level, to give indications on the potential of this
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26 182 approach for physicians considering this technique for their clinical practice.
27
28 183 For each patient, BMAC samples that are not used for surgical treatment are sent to the laboratory
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31 184 for the count of mononuclear cells, cell clonogenic ability by colony forming unit-fibroblast test and
32
33 185 phenotypical characterization by flow-cytometry evaluation.
34
35 186 Concomitantly with the bone marrow concentration process, all patients undergo an arthroscopic
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38 187 evaluation to confirm the location on both medial femoral condyle and medial tibial plateau
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40 188 involved by osteoarthritic lesions. Arthroscopy is done using the standard antero-lateral, antero-
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42 189 medial, and supero-medial portals. If the arthroscopic examination reveals intra-articular problems
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45 190 (excluding minor arthroscopic shaving) requiring surgical intervention which may affect the results
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47 191 of the procedure, the patient is excluded from the study.
48
49 192 Once the arthroscopy and the BMAC procedure are completed, the injections are performed. The
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51
52 193 treatment group receives two 2.5 ml subchondral BMAC injections, that are performed inserting
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54 194 two 8-Gauge trocars through the supero-medial and antero-medial arthroscopic portals and are
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56 195 manually introduced with clockwise and anticlockwise movements, under fluoroscopic control, into
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58 196 the bone of both medial femoral condyle and tibial plateau. Following arthroscopic portals suture,
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197 both groups of treatment receive a 3ml intra-articular injection of BMAC using a lateral

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3 198 suprapatellar approach. An elastic bandage is made after wounds medication. The whole procedure
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5 199 is presented in Figure 1.

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8 200 Postoperatively, patients are discharged on the same day of the procedure or the day after, based on
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10 201 patient condition. Pain control is prescribed as needed with analgesics only in the immediate period
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12 202 after treatment, and thromboembolic prophylaxis is prescribed for two weeks. During the same
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14 203 time, patients are taught to walk with the support of two crutches to allow a partial weight-bearing
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17 204 on the operated limb. Cryotherapy is started within the first 24 hours. Passive mobilisation and
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19 205 quadriceps isometric exercises are started at the second post-operative day. Patients are permitted to
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21 206 return to most of their daily activities as tolerated once they reach full weight-bearing. No other
22
23
24 207 conservative treatments are prescribed during the study period. Joint impacting sport activities are
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26 208 discouraged within the first month after treatment.

27 28 29 209 30 31 210 *Outcomes*

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33 211 The primary outcome is the Western Ontario and McMaster Universities Osteoarthritis Index
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35 212 (WOMAC), a 24 items self-administered questionnaire taking into account articular pain and
36
37 213 stiffness and physical function limitations due to knee OA. It ranges from 0 to 96 points and higher
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40 214 WOMAC scores indicate worse pain, stiffness, and functional limitations. The total WOMAC score
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42 215 was chosen as primary outcome aiming at capturing a more comprehensive assessment of
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44 216 symptoms and function benefits offered by the treatments.

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47 217 The secondary outcomes include the International Knee Documentation Committee (IKDC)
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49 218 Subjective and Objective Knee Evaluation Form (a patient-completed tool taking into account knee
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51 219 symptoms, knee function, and sport activity), the Tegner activity scale (a one-item score based on
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54 220 work and sports activities), the EuroQol-visual analogue scale (EQ-VAS) that provides an
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56 221 assessment of patients global health, the health questionnaire EQ-5D score (a 5 level self-assessed,
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58 222 health related, quality of life questionnaire).

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3 223 Patients also undergo MRI and CT assessments. MRI scans are obtained with a high-resolution 3
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5 224 Tesla MRI scanner with PD-weighted Turbo Spin Echo 3D sequences with and without fat
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8 225 saturation (FS), 3D T2* Gradient Echo (MERGE) with FS, axial PD-weighted Fast Spin Echo
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10 226 sequences with FS, and Multi-Echo T2 Mapping on the sagittal plane with 8 different Echo Times.
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12 227 The Whole-Organ Magnetic Resonance Imaging Score (WORMS) is used to assess seven features
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15 228 of the treated knees: articular cartilage morphology, bone marrow oedema, subchondral cysts,
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17 229 articular profile, marginal osteophytes, meniscal integrity, and synovitis.
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19 230 Articular cartilage morphology is examined with the 3D MERGE and the T2 Mapping; bone
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22 231 marrow oedema and synovitis with the PD fat sat sequences, the articular profiles with the PD and
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24 232 MERGE sequences, and the meniscal integrity with the DP sequences.
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26 233 CT knee scans are obtained with a 64-channels CT scanner to better assess the structural resolution
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29 234 of bone trabeculae as well as to assess the presence of osteophytes, calcifications, and cancellous
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31 235 bone microcysts. The images are acquired using a slice thickness of 1.25 mm and an interval of
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33 236 0.625 mm at 120kV with 250 mA, post-processed with the “Bone” filter, and reformatted in the
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35 237 coronal and sagittal plane.
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38 238 Blood samples are obtained from participants before treatment and at 2, 6, and 12 months of follow-
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40 239 up. Samples are analysed for inflammatory (IL-1 β , TNF α) and OA progression markers (Cleavage
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42 240 of Type II Collagen, Serum C-telopeptide fragments of type II collagen).
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47 242 *Participant timeline*
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49 243 Research assistants first conduct a screening of potential candidates over the telephone. If early
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51 244 checks of study eligibility are favourable, participants are booked in for a face-to-face screening
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54 245 visit with an orthopaedic specialist to confirm eligibility and explain the study protocol. After the
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56 246 screening visit, patients complete the questionnaires, undergo a knee MRI and CT, and sign the
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58 247 informed written consent. Patient enrolment started on November 2019. The first patient was
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60 248 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.

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

Allocation

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

Adverse events and assessment process

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

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3 293 To ensure high-quality execution of the trial in accordance with the protocol, all trial staff is trained
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5 294 by the chief investigators and provided with a standard protocol book which contains details of
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8 295 standard operating procedures, trial contacts, visits, measurements, monitoring, and case report
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10 296 forms.

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13 14 15 298 *Data collection methods*

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17 299 Data are firstly collected on paper-based case report forms, with the help of research trained
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19 300 orthopaedic residents blinded to treatment allocation, and subsequently trained data analysts process
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21 301 data into electronic form for statistical analysis. Baseline and final MRI and CT knee scans are
22
23
24 302 coded and stored at the Rizzoli Orthopaedic Institute to ensure data quality control. Operative data
25
26 303 are collected electronically by the respective surgeons shortly after surgery.

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30 31 305 *Data management*

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33 306 Study data are stored in a password-protected spreadsheet on a server that is hosted at the Rizzoli
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35 307 Orthopaedic Institute. Data transfer is encrypted with all data de-identified. Only trained research
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38 308 personnel specifically dedicated to the data handling can access the database and ensures the
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40 309 correspondence of the electronic data with the original paper-based questionnaires and medical
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42 310 charts.

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46 47 312 *Statistical methods*

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49 313 A power analysis (G*Power 3.1.9.2) was conducted using assumptions of 90% of power and 5% of
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51 314 probability of type 1 error ($\alpha = 0.05$), with a SD of 18.2 points based on a pilot study and a
52
53
54 315 hypothesized 10-point difference in total WOMAC score between treatments. Accordingly, 76
55
56 316 participants are needed. This leads to a moderate size-effect (0.55) as per the Cohen convention
57
58 317 (effects: small $\geq .20$, medium $\geq .50$, large $\geq .80$), and is in line with other effect sizes and SD
59
60 318 reported in the literature. We increased the number of participants to a total of 86 patients (43 in

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3 319 each arm) to account for a possible 10% loss to follow-up. The primary analyses are intention-to-
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5 320 treat analyses of primary and secondary outcomes. Per protocol analyses will be performed as the
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8 321 secondary analyses. All those who have started the treatment are considered part of the research,
9
10 322 regardless of whether they will complete it. For the missing data, they will be analyzed using the
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12 323 multiple imputation analysis, performed by filling the missing data with random values from the
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15 324 distribution of the variable.

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17 325 Continuous variables are be expressed as means and standard deviations if normally distributed,
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19 326 as medians and range if not. Categorical variables are be expressed as frequencies and percentage.
20
21
22 327 Normality of the distribution is be assessed using the Shapiro Wilks test. The Levene test is be used
23
24 328 to assess the homoscedasticity of the data. The Repeated Measures ANOVA, followed by the post
25
26 329 hoc Sidak pairwise test is performed to compare the scores at different follow-up times. The
27
28
29 330 OneWay ANOVA test is performed to assess the between group differences of continuous and
30
31 331 normally distributed and homoscedastic data; the Mann Whitney test is used otherwise. The
32
33 332 ANOVA test, followed by the Scheffè post hoc pairwise comparison, is used also to assess the
34
35 333 among groups differences of continuous, normally distributed and homoscedastic data; the Kruskal
36
37
38 334 Wallis, test followed by the Mann Whitney test with the Bonferroni correction for multiple
39
40 335 comparison, is used otherwise. The Monte Carlo method is used to evaluate the non-parametric tests
41
42 336 in case of small size of the sub-groups. Pearson chi square exact test is performed to investigate
43
44
45 337 relationships between grouping variables. The Spearman rank Correlation is used to assess
46
47 338 correlations between the numerical scores and continuous data. The General linear model, or the
48
49 339 Generalized linear model in case of not normal distribution, is used as multivariate analysis to
50
51 340 compare the group's outcomes corrected by the influencing factors. The Kaplan Meyer analysis is
52
53
54 341 performed to assess survival to major adverse events. For all tests $p < 0.05$ is considered significant.
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56 342 SPSS version 19.0 (IBM Corp., Armonk, NY, USA) is applied for the analyses.

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60 344 *Data monitoring*

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3 345 A central project data manager is tasked to perform data quality control on all collected data. An
4
5 346 interim report and a final report are foreseen, to be submitted to the Ministry of Health who funded
6
7
8 347 the project. The monitoring personnel belongs to a research structure of the Scientific Direction of
9
10 348 the Institution, the Applied and Translational Research Center, and it is independent from the Clinic
11
12 349 and the medical personnel performing the study procedures. A further project auditing is performed
13
14
15 350 by another independent entity of the Institution, the Clinical Trial Center. The final study report is
16
17 351 also sent to the Ethic Committee.

21 353 **ETHICS AND DISSEMINATION**

23 354 *Research ethics approval*

25
26 355 Ethical approval was obtained on 5 May 2018 from the central Emilia Wide Area Ethical
27
28 356 Committee of the Emilia-Romagna Region (CE-AVEC) settled at the University General Hospital
29
30 357 Sant'Orsola-Malpighi of Bologna.

35 359 *Protocol amendments*

37 360 Minor protocol amendments, for example, database production changes to facilitate monitoring
38
39
40 361 processes or improve outcome assessment by questionnaire, are fully documented. In case of major
41
42 362 amendments, for example, changes to the patient information sheet and consent form, change of a
43
44 363 local project leader or the inclusion of a new project site, they are submitted for approval by the
45
46
47 364 lead Ethics Committee as required.

51 366 *Consent or assent*

53 367 All participants will provide informed written consent in Italian and they may dropout the trial at
54
55
56 368 any time during the study course.

58 369 *Confidentiality*

1
2
3 370 Data are recorded using case report forms and processed centrally at the Rizzoli Orthopedics
4
5 371 Institute, Bologna, Italy. The hard copies of case report forms are stored in a locked area with
6
7
8 372 secured and restricted access. The electronic data are stored on password protected servers with
9
10 373 restricted access. All data collected are kept strictly confidential. Daily backups of all electronic
11
12 374 data occur to minimize any risk of lost data. After study completion, paper copies of data are
13
14
15 375 archived in secure storage. Identifiers are be removed in case follow-up of study patients is
16
17 376 necessary; however, electronic data continue to be kept in a secure electronic database. This
18
19 377 remains password protected and with access given only to the study investigators unless otherwise
20
21
22 378 authorized by the study team.

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24 379
25
26 380 *Access to data*

27
28 381 Only members of the research team who need to contact study patients, enter data, or perform data
29
30 382 quality control have access to patient information.

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33 383
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35 384 *Dissemination policy*

36
37 385 This trial is produced according to the SPIRIT (Standard Protocol Items: Recommendations for
38
39
40 386 Interventional Trials) international standards. Results are disseminated through peer-reviewed
41
42 387 publications and will be submitted for presentation at national and international conferences. The
43
44
45 388 authorship is based on International Committee of Medical Journal Editors 2018 Recommendations.

46
47 389
48
49 390 *Scientific relevance and broader impact*

50
51 391 This study provides a detailed method of treatment for knee OA and can offer clear indications on
52
53
54 392 the potential and limitations of the combined use of intra-articular and subchondral bone injections
55
56 393 of BMAC. The BMAC analysis provides characterisation of this product to shed greater light on the
57
58 394 properties ensuring its effectiveness. Baseline patient-related and disease-related factors analysis

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3 395 can allow to better define those characteristics that make different subjects more or less responsive
4
5 396 to this type of treatment.

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8 397
9
10 398 *Contributorship statement*

11
12 399 ADM is the principal investigator of this study. SS, LA, AB, DR wrote the manuscript and will
13
14 400 conduct the trial. GV and MM are responsible of imaging evaluation. CC and BG are involved in
15
16
17 401 products and patients' characterization. ADM, SZ and GF applied for funding and supervise the
18
19 402 trial. All authors read and approved the final protocol.

21
22 403
23
24 404 *Competing interest*

25
26 405 SZ reports non-financial support from personal fees from I+SRL, grants from FidiaFarmaceutici
27
28 406 S.p.A., Cartiheal Ltd, IGEA clinical biophysics, BIOMET, and Kensey Nash, outside the submitted
29
30
31 407 work. The funders had no role in the design of the study, in the collection, analyses, or
32
33 408 interpretation of data, in the writing of the manuscript, or in the decision to publish the results. The
34
35 409 principal investigator and other authors declare no financial and other competing interests.

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38 410
39
40 411 *Funding*

41
42 412 The study is funded by Italian Health Ministry in the Project "Giovane Ricercatore" (GR-2016-
43
44 413 02361990).

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47 414
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49 415 *Data sharing statement*

50
51 416 No data are available

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42 463 **FIGURE LEGEND**

43
44 464 Figure 1 - Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration
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47 465 (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and
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49 466 subchondral Bone Marrow Aspirate Concentrate injections (F).

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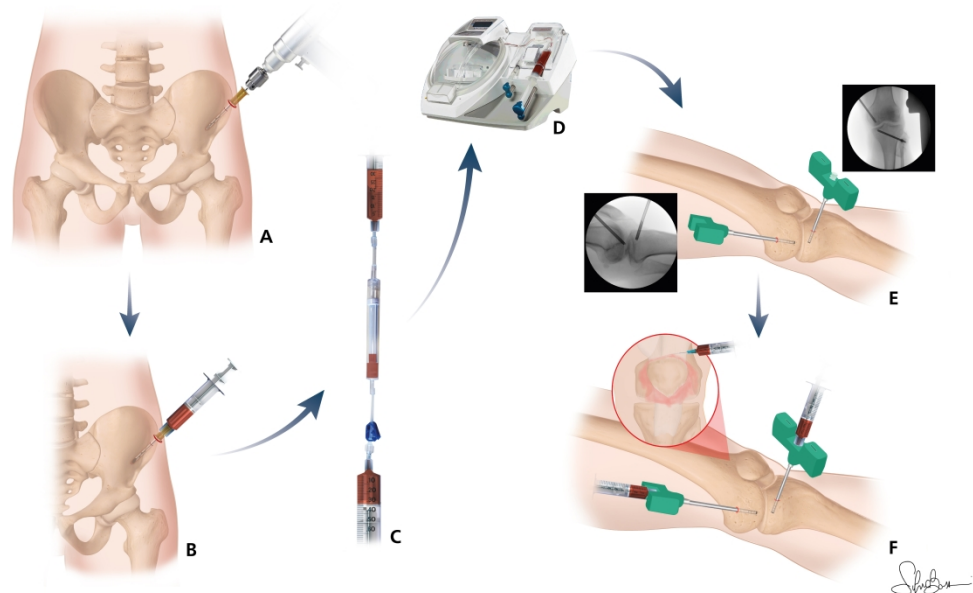


Figure 1: Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and subchondral Bone Marrow Aspirate Concentrate injections (F).

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.

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

In your methods section, say that you used the SPIRIT reporting 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

			Page
		Reporting Item	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

1		name of intended registry	
2			
3			
4	Trial registration: data	#2b All items from the World Health Organization Trial	1
5			
6	set	Registration Data Set	
7			
8			
9	Protocol version	#3 Date and version identifier	3
10			
11			
12	Funding	#4 Sources and types of financial, material, and other support	17
13			
14			
15	Roles and	#5a Names, affiliations, and roles of protocol contributors	4
16			
17	responsibilities:		
18			
19	contributorship		
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23	Roles and	#5b Name and contact information for the trial sponsor	1
24			
25	responsibilities:		
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27	sponsor contact		
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29	information		
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33	Roles and	#5c Role of study sponsor and funders, if any, in study design;	1
34			
35	responsibilities:	collection, management, analysis, and interpretation of	
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37	sponsor and funder	data; writing of the report; and the decision to submit the	
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39		report for publication, including whether they will have	
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41		ultimate authority over any of these activities	
42			
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45	Roles and	#5d Composition, roles, and responsibilities of the coordinating	1
46			
47	responsibilities:	centre, steering committee, endpoint adjudication	
48			
49	committees	committee, data management team, and other individuals	
50			
51		or groups overseeing the trial, if applicable (see Item 21a	
52			
53		for data monitoring committee)	
54			
55			
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57	Introduction		
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1	Background and	#6a	Description of research question and justification for	5
2				
3	rationale		undertaking the trial, including summary of relevant studies	
4			(published and unpublished) examining benefits and harms	
5			for each intervention	
6				
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10				
11	Background and	#6b	Explanation for choice of comparators	5
12				
13	rationale: choice of			
14				
15	comparators			
16				
17				
18	Objectives	#7	Specific objectives or hypotheses	6
19				
20				
21				
22	Trial design	#8	Description of trial design including type of trial (eg, parallel	6
23			group, crossover, factorial, single group), allocation ratio,	
24			and framework (eg, superiority, equivalence, non-inferiority,	
25			exploratory)	
26				
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30				
31	Methods:			
32				
33	Participants,			
34				
35	interventions, and			
36				
37	outcomes			
38				
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40				
41	Study setting	#9	Description of study settings (eg, community clinic,	6
42			academic hospital) and list of countries where data will be	
43			collected. Reference to where list of study sites can be	
44			obtained	
45				
46				
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51	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	7
52			applicable, eligibility criteria for study centres and	
53			individuals who will perform the interventions (eg,	
54				
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1		surgeons, psychotherapists)	
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3			
4	Interventions:	#11a Interventions for each group with sufficient detail to allow	7
5			
6	description	replication, including how and when they will be	
7			
8		administered	
9			
10			
11	Interventions:	#11b Criteria for discontinuing or modifying allocated	7
12			
13	modifications	interventions for a given trial participant (eg, drug dose	
14			
15		change in response to harms, participant request, or	
16			
17		improving / worsening disease)	
18			
19			
20			
21	Interventions:	#11c Strategies to improve adherence to intervention protocols,	9
22			
23	adherence	and any procedures for monitoring adherence (eg, drug	
24			
25		tablet return; laboratory tests)	
26			
27			
28			
29	Interventions:	#11d Relevant concomitant care and interventions that are	9
30			
31	concomitant care	permitted or prohibited during the trial	
32			
33			
34	Outcomes	#12 Primary, secondary, and other outcomes, including the	9
35			
36		specific measurement variable (eg, systolic blood	
37			
38		pressure), analysis metric (eg, change from baseline, final	
39			
40		value, time to event), method of aggregation (eg, median,	
41			
42		proportion), and time point for each outcome. Explanation	
43			
44		of the clinical relevance of chosen efficacy and harm	
45			
46		outcomes is strongly recommended	
47			
48			
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51	Participant timeline	#13 Time schedule of enrolment, interventions (including any	10
52			
53		run-ins and washouts), assessments, and visits for	
54			
55		participants. A schematic diagram is highly recommended	
56			
57		(see Figure)	
58			
59			
60			

1	Sample size	#14	Estimated number of participants needed to achieve study	13
2			objectives and how it was determined, including clinical and	
3			statistical assumptions supporting any sample size	
4			calculations	
5				
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10				
11	Recruitment	#15	Strategies for achieving adequate participant enrolment to	11
12			reach target sample size	
13				
14				
15				
16	Methods: Assignment			
17	of interventions (for			
18	controlled trials)			
19				
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24	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	12
25	generation		computer-generated random numbers), and list of any	
26			factors for stratification. To reduce predictability of a	
27			random sequence, details of any planned restriction (eg,	
28			blocking) should be provided in a separate document that is	
29			unavailable to those who enrol participants or assign	
30			interventions	
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41	Allocation	#16b	Mechanism of implementing the allocation sequence (eg,	12
42	concealment		central telephone; sequentially numbered, opaque, sealed	
43	mechanism		envelopes), describing any steps to conceal the sequence	
44			until interventions are assigned	
45				
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51	Allocation:	#16c	Who will generate the allocation sequence, who will enrol	13
52	implementation		participants, and who will assign participants to	
53			interventions	
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1	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg,	11
2			trial participants, care providers, outcome assessors, data	
3			analysts), and how	
4				
5				
6				
7				
8	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is	11
9	emergency		permissible, and procedure for revealing a participant's	
10			allocated intervention during the trial	
11	unblinding			
12				
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16	Methods: Data			
17	collection,			
18	management, and			
19	analysis			
20				
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26	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline,	13
27			and other trial data, including any related processes to	
28			promote data quality (eg, duplicate measurements, training	
29			of assessors) and a description of study instruments (eg,	
30			questionnaires, laboratory tests) along with their reliability	
31			and validity, if known. Reference to where data collection	
32			forms can be found, if not in the protocol	
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43	Data collection plan:	#18b	Plans to promote participant retention and complete follow-	13
44	retention		up, including list of any outcome data to be collected for	
45			participants who discontinue or deviate from intervention	
46			protocols	
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53	Data management	#19	Plans for data entry, coding, security, and storage,	13
54			including any related processes to promote data quality	
55			(eg, double data entry; range checks for data values).	
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1		Reference to where details of data management	
2			
3		procedures can be found, if not in the protocol	
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5			
6	Statistics: outcomes	#20a Statistical methods for analysing primary and secondary	13
7			
8		outcomes. Reference to where other details of the	
9			
10		statistical analysis plan can be found, if not in the protocol	
11			
12			
13	Statistics: additional	#20b Methods for any additional analyses (eg, subgroup and	13
14			
15	analyses	adjusted analyses)	
16			
17			
18	Statistics: analysis	#20c Definition of analysis population relating to protocol non-	13
19			
20	population and	adherence (eg, as randomised analysis), and any statistical	
21			
22	missing data	methods to handle missing data (eg, multiple imputation)	
23			
24			
25			
26	Methods: Monitoring		
27			
28			
29	Data monitoring:	#21a Composition of data monitoring committee (DMC);	15
30			
31	formal committee	summary of its role and reporting structure; statement of	
32			
33		whether it is independent from the sponsor and competing	
34			
35		interests; and reference to where further details about its	
36			
37		charter can be found, if not in the protocol. Alternatively, an	
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39		explanation of why a DMC is not needed	
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44	Data monitoring:	#21b Description of any interim analyses and stopping	15
45			
46	interim analysis	guidelines, including who will have access to these interim	
47			
48		results and make the final decision to terminate the trial	
49			
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51	Harms	#22 Plans for collecting, assessing, reporting, and managing	12
52			
53		solicited and spontaneously reported adverse events and	
54			
55		other unintended effects of trial interventions or trial	
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1		conduct	
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4	Auditing	#23 Frequency and procedures for auditing trial conduct, if any,	14
5			
6		and whether the process will be independent from	
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8		investigators and the sponsor	
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11	Ethics and		
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13	dissemination		
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16	Research ethics	#24 Plans for seeking research ethics committee / institutional	15
17			
18	approval	review board (REC / IRB) approval	
19			
20			
21			
22	Protocol	#25 Plans for communicating important protocol modifications	15
23			
24	amendments	(eg, changes to eligibility criteria, outcomes, analyses) to	
25			
26		relevant parties (eg, investigators, REC / IRBs, trial	
27			
28		participants, trial registries, journals, regulators)	
29			
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31			
32	Consent or assent	#26a Who will obtain informed consent or assent from potential	15
33			
34		trial participants or authorised surrogates, and how (see	
35			
36		Item 32)	
37			
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39	Consent or assent:	#26b Additional consent provisions for collection and use of	n/a
40			
41	ancillary studies	participant data and biological specimens in ancillary	
42			
43		studies, if applicable	
44			
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46			
47	Confidentiality	#27 How personal information about potential and enrolled	16
48			
49		participants will be collected, shared, and maintained in	
50			
51		order to protect confidentiality before, during, and after the	
52			
53		trial	
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57	Declaration of	#28 Financial and other competing interests for principal	17
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1	interests		investigators for the overall trial and each study site	
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4	Data access	#29	Statement of who will have access to the final trial dataset,	16
5			and disclosure of contractual agreements that limit such	
6			access for investigators	
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11	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial care, and for	n/a
12			compensation to those who suffer harm from trial	
13	trial care		participation	
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19	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial	16
20			results to participants, healthcare professionals, the public,	
21	trial results		and other relevant groups (eg, via publication, reporting in	
22			results databases, or other data sharing arrangements),	
23			including any publication restrictions	
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31	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	16
32			professional writers	
33	authorship			
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36	Dissemination policy:	#31c	Plans, if any, for granting public access to the full protocol,	n/a
37			participant-level dataset, and statistical code	
38	reproducible research			
39				
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42	Appendices			
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45	Informed consent	#32	Model consent form and other related documentation given	n/a
46			to participants and authorised surrogates	
47	materials			
48				
49				
50	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of	n/a
51			biological specimens for genetic or molecular analysis in	
52			the current trial and for future use in ancillary studies, if	
53			applicable	
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2 Commons Attribution License CC-BY-NC. This checklist was completed on 04. March 2022 using
3 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
4 [Penelope.ai](#)
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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.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-062632.R2
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3 1 **A prospective double-blind randomized controlled trial protocol comparing**
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5 2 **bone marrow aspirate concentrate intra-articular injection combined with**
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7 3 **subchondral injection versus intra-articular injection alone**
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9 4 **for the treatment of symptomatic knee osteoarthritis.**
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17 7 Vara², Marco Miceli², Carola Cavallo³, Brunella Grigolo³, Stefano Zaffagnini¹, Giuseppe Filardo⁴
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49 21 *Keywords:* Osteoarthritis; Mesenchymal Stromal Cells; Subchondral injections; Bone marrow
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51 22 aspirate concentrate; BMAC
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56 24 *Word Count:* 4079 words (excluding title page, references, figures and tables)
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3 27 **ABSTRACT**
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6 28 *Introduction:* Subchondral and intra-articular injections of bone marrow aspirate concentrate
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8 29 (BMAC) showed promising results for knee OA patients. To date, there is no evidence to
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10 30 demonstrate whether the combination of these treatments provides higher benefits than the intra-
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12 31 articular injection alone.
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16 32 *Methods and analysis:* Eighty-six patients with symptomatic knee OA (aged between 40 and 70) are
17
18 33 randomized to BMAC intra-articular injection combined with subchondral BMAC injection or
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20 34 BMAC intra-articular injection alone in a ratio of 1:1. The primary outcome is the total Western
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22 35 Ontario and McMaster Universities Osteoarthritis Index (WOMAC), the secondary outcomes the
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24 36 International Knee Documentation Committee (IKDC) Subjective and Objective Knee Evaluation
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26 37 Form, the Tegner activity scale, the EuroQol-visual analogue scale (EQ-VAS), and the health
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28 38 questionnaire EQ-5D score. Additional CT and MRI evaluations are performed at the baseline
29
30 39 assessment and at the final 12-month follow-up. The hypothesis is that the combined injection
31
32 40 provides higher knee pain and function improvement compared to BMAC intra-articular injection
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34 41 alone. The primary analysis follows an intention-to-treat principle.
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40 42 *Ethics and dissemination:* The study protocol has been approved by the Emilia Wide Area Ethical
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42 43 Committee of the Emilia-Romagna Region (CE-AVEC), Bologna, Italy. Written informed consent
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44 44 is obtained from all the participants. Findings of this study will be disseminated the through peer-
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46 45 reviewed publications and conference presentations.
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3 47 *Strengths and limitations of this study:*
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8 49 • This is the first prospective, randomized, double-blind and controlled trial evaluating
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10 results of BMAC intra-articular injection combined with subchondral injection compared to
11 50
12 BMAC intra-articular injection alone in knee OA.
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15 52 • Patients are analyzed using PROMs, objective measures, MRI and CT examination,
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17 and biomarker evaluation.
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20 54 • Patient base-line characteristics and disease-related factors can help to better define
21
22 the aspects that make different individuals more or less responsive to this type of treatments.
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25 56 • The uncontrolled pain medication use by patients (although being discouraged) could
26
27 influence the primary outcome and this is a relevant limitation of the study.
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30 58 • This study can clarify the benefits, and limitations, of the newly proposed
31
32 combination of intra-articular and subchondral BMAC injections, providing clear
33 59
34 60 indications for the clinical practice.
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38 62 *Trial registration:* Clinical Trials.gov Identifier number NCT03876795
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43 64 *Protocol version:* Version 1 (14 May 2018)
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47 66 *Ethical approval:* (Prot. n. 0003132) for study protocol Interface (identifier: 207/2018/Sper/IOR)
48
49 was obtained on 5 May 2018 from the Ethical Committee Area Vasta Emilia Centro (CE-AVEC) of
50 67
51 the Emilia-Romagna Region settled at the University General Hospital Sant'Orsola-Malpighi of
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53 Bologna.
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59 71 *Roles and responsibilities:*
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94 INTRODUCTION

95
96 Knee osteoarthritis (OA) is a chronic, degenerative disease leading to irreversible structural and
97 functional changes in the entire joint, including subchondral bone sclerosis and cartilage loss, and
98 progressively determines debilitating pain and loss of function.[1-2] It affects a large part of the
99 aging population with a high impact on patients and healthcare costs.[3] Total knee arthroplasty
100 represents a definitive solution to address knee OA, but it is also encumbered by several
101 complications.[4] Conservative approaches, such as physical therapy and anti-inflammatory drugs,
102 should be pursued, but their benefits are generally temporary with short-term relief, and they are not
103 able to affect the natural course of the disease progression.[5] Thus, to delay or avoid the need for
104 arthroplasty, research efforts have been made to find new minimally invasive and more effective
105 procedures to address knee OA.

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

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3 120 Moreover, the combination of subchondral and intra-articular injections of bone marrow aspirate
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5 121 concentrate (BMAC) already showed promising results in terms of safety and clinical
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8 122 outcomes.[12] However, beside promising early findings and the increasing use of this approach in
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10 123 the clinical practice, there is only limited and low-level evidence, and it would be clinically relevant
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12 124 to evaluate with a high-level study design the real benefit provided by the addition of these
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15 125 subchondral injections to improve the results of BMAC intra-articular injections for knee OA.
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17 126 18 19 127 *Objectives and trial design*

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21 128 A double-blinded randomized controlled trial (RCT) was designed to compare the efficacy of a
22
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24 129 combination of intra-articular and subchondral injections of BMAC (treatment group) versus
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26 130 BMAC intra-articular injection alone (control group) to treat knee OA, with a 1:1 allocation ratio.
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29 131 The aim of this superiority trial is to evaluate the safety and the clinical potential of this new
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31 132 treatment approach up to one year of follow-up, and to verify the hypothesis that the combination of
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33 133 subchondral and intra-articular injections provides higher knee pain and function improvement
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35 134 compared to BMAC intra-articular injection alone in knee OA.
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37 135 38 39 136 **METHODS AND ANALYSIS**

40 41 42 137 *Study setting*

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44 138 The study is a single center double-blind RCT, with all activities related to the study performed in a
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47 139 single site, the IRCCS Rizzoli Orthopaedic Institute, Bologna, Italy.

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49 140 This trial protocol is produced according to the SPIRIT (Standard Protocol Items:
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51 141 Recommendations for Interventional Trials) reporting guidelines. [13]
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53 54 142 55 56 143 *Patient and public involvement*

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58 144 Patients are not involved in planning of research questions, outcome measures or design of the
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60 145 study.

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45 147 *Eligibility criteria*6
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8 148 Patients are recruited according to the following criteria.9
10 149 Inclusion criteria:

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- 12 150 - Male or female patients, aged between 40 and 70 years old;
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- 15 151 - OA of the medial compartment of the knee (grade II or III according to the Kellgren-
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- 17 152 Lawrence classification);
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- 19 153 - Failure after at least 6 months of conservative treatment (drug therapy with NSAIDs and
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- 22 154 painkillers, hyaluronic acid infiltration, corticosteroid infiltration, PRP);
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- 24 155 - Patients' ability and consent to participate in clinical and radiological follow-up;
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- 26 156 - Signature of informed consent.

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28 157 Exclusion criteria:

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- 31 158 - Patients with trauma in the 6 months prior to surgery;
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- 33 159 - Patients with malignancy;
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- 35 160 - Patients suffering from rheumatic diseases;
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- 38 161 - Patients suffering from uncompensated diabetes;
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- 40 162 - Patients suffering from uncompensated thyroid metabolic disorders;
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- 42 163 - Patients abusing alcoholic beverages or drugs;
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- 45 164 - Patients with axial deviations
- $> 5^\circ$
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- 47 165 - Body Mass Index
- > 35
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- 49 166 - Patients treated with joint injections in the previous 6 months;
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- 51 167 - Patients treated with surgery at the same knee in the previous 12 months.

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56 169 *Intervention*57
58 170 All patients are treated by orthopedic surgeons with established experience in cartilage and
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60 171 osteoarthritis orthobiologic procedures. The procedure is performed in a single step in the operating

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172 room with patients in supine position under spinal loco-regional anaesthesia. The ipsilateral hip is
173 sterilely prepared and draped for anterior iliac crest bone marrow aspiration. The anterior superior
174 iliac spine is the anatomical landmark for a small surgical incision. A diamond tip trocar is inserted
175 in this point and then advanced into the bone marrow using a drill. Bone marrow is collected using
176 two 30 ml syringes coated with heparin for a total of 60 ml. The harvested bone marrow is filtered
177 with a heparin washed filter and then centrifuged through the Magellan® centrifuge (Arteriocyte
178 Medical Systems, MA, USA) at a rate of 3600 RPM for approximately 15 minutes, thus obtaining
179 10 ml of BMAC. The BMAC procedure involved a kit available in the clinical practice. In fact, the
180 purpose of the study was not to evaluate a new product, but rather to explore the potential of
181 applying BMAC also at the subchondral bone level, to give indications on the potential of this
182 approach for physicians considering this technique for their clinical practice.

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

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

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

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3 198 both groups of treatment receive a 3ml intra-articular injection of BMAC using a lateral
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5 199 suprapatellar approach. An elastic bandage is made after wounds medication. The whole procedure
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8 200 is presented in Figure 1.

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10 201 Postoperatively, patients are discharged on the same day of the procedure or the day after, based on
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12 202 patient condition. Pain control is prescribed as needed with analgesics only in the immediate period
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14 203 after treatment, and thromboembolic prophylaxis is prescribed for two weeks. During the same
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17 204 time, patients are taught to walk with the support of two crutches to allow a partial weight-bearing
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19 205 on the operated limb. Cryotherapy is started within the first 24 hours. Passive mobilisation and
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21 206 quadriceps isometric exercises are started at the second post-operative day. Patients are permitted to
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24 207 return to most of their daily activities as tolerated once they reach full weight-bearing. No other
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26 208 conservative treatments are prescribed during the study period. Joint impacting sport activities are
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28 209 discouraged within the first month after treatment.

30 210 31 210 32 33 211 *Outcomes*

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35 212 The primary outcome is the total Western Ontario and McMaster Universities Osteoarthritis Index
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37 213 (WOMAC) at 12 month, a 24 items self-administered questionnaire taking into account articular
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40 214 pain and stiffness and physical function limitations due to knee OA. It ranges from 0 to 96 points
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42 215 and higher WOMAC scores indicate worse pain, stiffness, and functional limitations. The total
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44 216 WOMAC score was chosen as primary outcome aiming at capturing a more comprehensive
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47 217 assessment of symptoms and function benefits offered by the treatments.

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49 218 The secondary outcomes include the total WOMAC score at other follow-ups, the WOMAC
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51 219 subscales (pain, stiffness, and physical function), as well as the International Knee Documentation
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54 220 Committee (IKDC) Subjective and Objective Knee Evaluation Form (a patient-completed tool
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56 221 taking into account knee symptoms, knee function, and sport activity), the Tegner activity scale (a
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58 222 one-item score based on work and sports activities), the EuroQol-visual analogue scale (EQ-VAS)

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3 223 that provides an assessment of patients global health, and the health questionnaire EQ-5D score (a 5
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5 224 level self-assessed, health related, quality of life questionnaire).
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8 225 Patients also undergo MRI and CT assessments. MRI scans are obtained with a high-resolution 3
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10 226 Tesla MRI scanner with PD-weighted Turbo Spin Echo 3D sequences with and without fat
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12 227 saturation (FS), 3D T2* Gradient Echo (MERGE) with FS, axial PD-weighted Fast Spin Echo
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14 228 sequences with FS, and Multi-Echo T2 Mapping on the sagittal plane with 8 different Echo Times.
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17 229 The Whole-Organ Magnetic Resonance Imaging Score (WORMS) is used to assess seven features
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19 230 of the treated knees: articular cartilage morphology, bone marrow oedema, subchondral cysts,
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21 231 articular profile, marginal osteophytes, meniscal integrity, and synovitis.
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23
24 232 Articular cartilage morphology is examined with the 3D MERGE and the T2 Mapping; bone
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26 233 marrow oedema and synovitis with the PD fat sat sequences, the articular profiles with the PD and
27
28 234 MERGE sequences, and the meniscal integrity with the DP sequences.
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31 235 CT knee scans are obtained with a 64-channels CT scanner to better assess the structural resolution
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33 236 of bone trabeculae as well as to assess the presence of osteophytes, calcifications, and cancellous
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35 237 bone microcysts. The images are acquired using a slice thickness of 1.25 mm and an interval of
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37 238 0.625 mm at 120kV with 250 mA, post-processed with the “Bone” filter, and reformatted in the
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40 239 coronal and sagittal plane.
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42 240 Blood samples are obtained from participants before treatment and at 2, 6, and 12 months of follow-
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44 241 up. Samples are analysed for inflammatory (IL-1 β , TNF α) and OA progression markers (Cleavage
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46 242 of Type II Collagen, Serum C-telopeptide fragments of type II collagen).
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51 244 *Participant timeline*
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54 245 Research assistants first conduct a screening of potential candidates over the telephone. If early
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56 246 checks of study eligibility are favourable, participants are booked in for a face-to-face screening
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58 247 visit with an orthopaedic specialist to confirm eligibility and explain the study protocol. After the
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60 248 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

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3 267 This is a double-blind RCT with both participants and physicians assessing outcomes being blinded
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5 268 to treatment allocation. Only after the evaluation at the 12-month follow-up the blinding is opened
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8 269 and it is revealed to the patient which one of the two treatments was administered.

9
10 270 The blindness of treated patients is further guaranteed by the same number of surgical access and by
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12 271 the same length of the surgical incision for both treatments. For ethical reason, no bone puncturing
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14
15 272 and injection was performed in the control group. This, however, did not compromise blinding
16
17 273 since patients presented the same number and type of surgical incisions. Early unblinding occurs in
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19 274 case of premature patients drop-out. The level of blinding prevents from the enhanced placebo
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21
22 275 effect that a subchondral injection could add to the placebo effect of the intra-articular injection
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24 276 alone.[14]

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26 277 Imaging evaluation is provided by experienced radiologists which are blinded as well to the type of
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28 278 treatment that the patients have received and evaluation time.

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32 33 280 *Allocation*

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35 281 A total of 86 eligible patients are allocated to receive either a combination of intra articular and
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37 282 subchondral BMAC injection or BMAC intra articular injection alone, in a 1:1 ratio (43 patients for
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39
40 283 each group of treatment) based on a computer-generated random numbers randomisation. This is
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42 284 conducted by research staff members dedicated to study organization and monitoring with no direct
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44
45 285 involvement in the study procedures. The randomization list is covered by password and accessible
46
47 286 only by staff members with no direct involvement in the study.

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50 51 288 *Adverse events and assessment process*

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53 289 Adverse events are monitored throughout the study, intraoperatively and at clinical follow-up
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56 290 evaluations. Standard safety and efficacy monitoring is performed through regular face-to-face
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58 291 visits and phone calls between visits. The patients are also requested to report any adverse events to
59
60 292 the research staff spontaneously. Every adverse event is recorded in the patient Case Report Form

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3 293 (CRF). Serious adverse events are considered those resulting in death or being life-threatening,
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5 294 requiring hospitalization or intervention to prevent permanent impairment or damage; they are
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8 295 reported in accordance with the requirements of the Ethical Committee. Use of rescue pain
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10 296 medication is recorded at all visits without a diary and without homogenizing the type of
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12 297 medication, which is decided by patients autonomously (although discouraged for study purposes).
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15 298 To ensure high-quality execution of the trial in accordance with the protocol, all trial staff is trained
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17 299 by the chief investigators and provided with a standard protocol book which contains details of
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19 300 standard operating procedures, trial contacts, visits, measurements, monitoring, and case report
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21 301 forms.
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24 302 25 26 303 *Data collection methods*

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28 304 Data are firstly collected on paper-based case report forms, with the help of research trained
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31 305 orthopaedic residents blinded to treatment allocation, and subsequently trained data analysts process
32
33 306 data into electronic form for statistical analysis. Baseline and final MRI and CT knee scans are
34
35 307 coded and stored at the Rizzoli Orthopaedic Institute to ensure data quality control. Operative data
36
37
38 308 are collected electronically by the respective surgeons shortly after surgery.
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40 309 41 42 310 *Data management*

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44 311 Study data are stored in a password-protected spreadsheet on a server that is hosted at the Rizzoli
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46
47 312 Orthopaedical Institute. Data transfer is encrypted with all data de-identified. Only trained research
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49 313 personnel specifically dedicated to the data handling can access the database and ensures the
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51 314 correspondence of the electronic data with the original paper-based questionnaires and medical
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53 315 charts.
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55 56 316 57 58 317 *Statistical methods*

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3 318 A power analysis (G*Power 3.1.9.2) was conducted using assumptions of 90% of power and 5% of
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5 319 probability of type 1 error ($\alpha = 0.05$), with a SD of 18.2 points based on a pilot study and a
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7
8 320 hypothesized 10-point difference in total WOMAC score at 12 months between treatments.
9
10 321 Accordingly, 76 participants are needed. This leads to a moderate size-effect (0.55) as per the
11
12 322 Cohen convention (effects: small $\geq .20$, medium $\geq .50$, large $\geq .80$), and is in line with other effect
13
14 323 sizes and SD reported in the literature. We increased the number of participants to a total of 86
15
16
17 324 patients (43 in each arm) to account for a possible 10% loss to follow-up. The primary analyses are
18
19 325 intention-to-treat analyses of primary and secondary outcomes. Per protocol analyses will be
20
21 326 performed as the secondary analyses. All those who have started the treatment are considered part
22
23
24 327 of the research, regardless of whether they will complete it. For the missing data, they will be
25
26 328 analyzed using the multiple imputation analysis, performed by filling the missing data with random
27
28 329 values from the distribution of the variable.
29
30
31 330 Continuous variables are be expressed as means and standard deviations if normally distributed,
32
33 331 as medians and range if not. Categorical variables are be expressed as frequencies and percentage.
34
35 332 Normality of the distribution is be assessed using the Shapiro Wilks test. The Levene test is be used
36
37
38 333 to assess the homoscedasticity of the data. The Repeated Measures ANOVA, followed by the post
39
40 334 hoc Sidak pairwise test is performed to compare the scores at different follow-up times. The
41
42 335 OneWay ANOVA test is performed to assess the between group differences of continuous and
43
44 336 normally distributed and homoscedastic data; the Mann Whitney test is used otherwise. The
45
46
47 337 ANOVA test, followed by the Scheffè post hoc pairwise comparison, is used also to assess the
48
49 338 among groups differences of continuous, normally distributed and homoscedastic data; the Kruskal
50
51 339 Wallis, test followed by the Mann Whitney test with the Bonferroni correction for multiple
52
53 340 comparison, is used otherwise. The Monte Carlo method is used to evaluate the non-parametric tests
54
55
56 341 in case of small size of the sub-groups. Pearson chi square exact test is performed to investigate
57
58 342 relationships between grouping variables. The Spearman rank Correlation is used to assess
59
60 343 correlations between the numerical scores and continuous data. The General linear model, or the

1
2
3 344 Generalized linear model in case of not normal distribution, is used as multivariate analysis to
4
5 345 compare the group's outcomes corrected by the influencing factors. The Kaplan Meyer analysis is
6
7
8 346 performed to assess survival to major adverse events. For all tests $p < 0.05$ is considered significant.
9
10 347 SPSS version 19.0 (IBM Corp., Armonk, NY, USA) is applied for the analyses.
11

12 348 13 14 15 349 *Data monitoring*

16
17 350 A central project data manager is tasked to perform data quality control on all collected data. An
18
19 351 interim report and a final report are foreseen, to be submitted to the Ministry of Health who funded
20
21
22 352 the project. The monitoring personnel belongs to a research structure of the Scientific Direction of
23
24 353 the Institution, the Applied and Translational Research Center, and it is independent from the Clinic
25
26 354 and the medical personnel performing the study procedures. A further project auditing is performed
27
28
29 355 by another independent entity of the Institution, the Clinical Trial Center. The final study report is
30
31 356 also sent to the Ethic Committee.
32

33 357 34 35 358 **ETHICS AND DISSEMINATION**

36 37 359 *Research ethics approval*

38
39
40 360 Ethical approval was obtained on 5 May 2018 from the central Emilia Wide Area Ethical
41
42 361 Committee of the Emilia-Romagna Region (CE-AVEC) settled at the University General Hospital
43
44 362 Sant'Orsola-Malpighi of Bologna.
45

46 47 363 48 49 364 *Protocol amendments*

50
51 365 Minor protocol amendments, for example, database production changes to facilitate monitoring
52
53 366 processes or improve outcome assessment by questionnaire, are fully documented. In case of major
54
55
56 367 amendments, for example, changes to the patient information sheet and consent form, change of a
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58 368 local project leader or the inclusion of a new project site, they are submitted for approval by the
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60 369 lead Ethics Committee as required.

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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 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.

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3 396 *Scientific relevance and broader impact*
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5 397 This study provides a detailed method of treatment for knee OA and can offer clear indications on
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7
8 398 the potential and limitations of the combined use of intra-articular and subchondral bone injections
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10 399 of BMAC. The BMAC analysis provides characterisation of this product to shed greater light on the
11
12 400 properties ensuring its effectiveness. Baseline patient-related and disease-related factors analysis
13
14 401 can allow to better define those characteristics that make different subjects more or less responsive
15
16
17 402 to this type of treatment.
18

19 403

20
21 404 *Contributorship statement*
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23
24 405 ADM is the principal investigator of this study. SS, LA, AB, DR wrote the manuscript and will
25
26 406 conduct the trial. GV and MM are responsible of imaging evaluation. CC and BG are involved in
27
28 407 products and patients' characterization. ADM, SZ and GF applied for funding and supervise the
29
30 408 trial. All authors read and approved the final protocol.
31
32

33 409

34
35 410 *Competing interest*
36

37
38 411 SZ reports non-financial support from personal fees from I+SRL, grants from FidiaFarmaceutici
39
40 412 S.p.A., Cartiheal Ltd, IGEA clinical biophysics, BIOMET, and Kensey Nash, outside the submitted
41
42 413 work. The funders had no role in the design of the study, in the collection, analyses, or
43
44 414 interpretation of data, in the writing of the manuscript, or in the decision to publish the results. The
45
46 415 principal investigator and other authors declare no financial and other competing interests.
47
48

49 416

50
51 417 *Funding*
52

53
54 418 The study is funded by Italian Health Ministry in the Project "Giovane Ricercatore" (GR-2016-
55
56 419 02361990).
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58 420

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60 421 *Data sharing statement*

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

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46 466 **FIGURE LEGEND**

47 467 Figure 1 - Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration
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49 468 (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and
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51 469 subchondral Bone Marrow Aspirate Concentrate injections (F).
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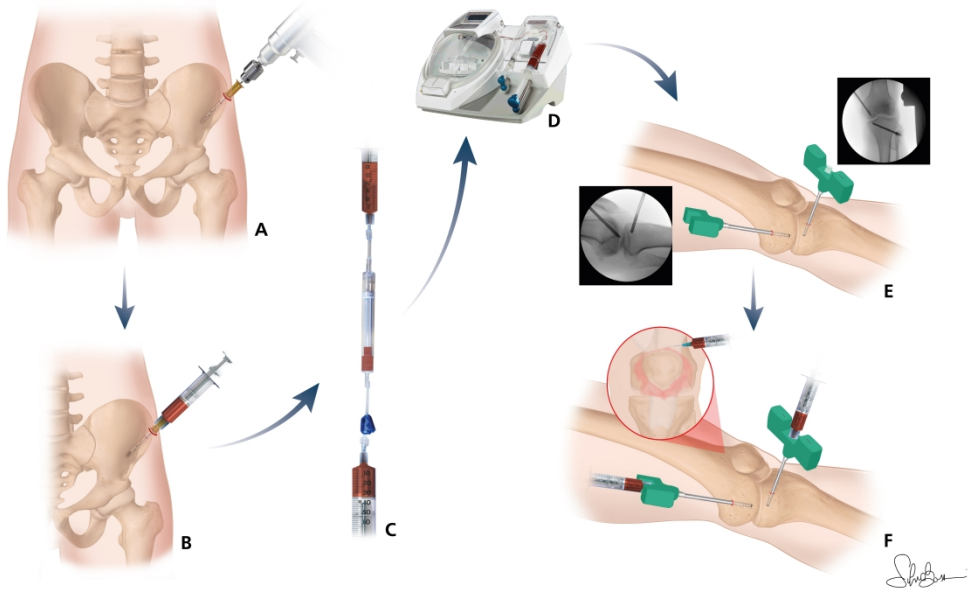


Figure 1: Anterior iliac crest trocar insertion (A); Bone Marrow (BM) harvesting (B); BM filtration (C); BM concentration (D); Trocar positioning under fluoroscopic control (E); Intra-articular and subchondral Bone Marrow Aspirate Concentrate injections (F).

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.

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

In your methods section, say that you used the SPIRIT reporting 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

			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

1		name of intended registry	
2			
3			
4	Trial registration: data	#2b All items from the World Health Organization Trial	1
5			
6	set	Registration Data Set	
7			
8			
9	Protocol version	#3 Date and version identifier	3
10			
11			
12	Funding	#4 Sources and types of financial, material, and other support	17
13			
14			
15	Roles and	#5a Names, affiliations, and roles of protocol contributors	4
16			
17	responsibilities:		
18			
19	contributorship		
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22			
23	Roles and	#5b Name and contact information for the trial sponsor	1
24			
25	responsibilities:		
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27	sponsor contact		
28			
29	information		
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33	Roles and	#5c Role of study sponsor and funders, if any, in study design;	1
34			
35	responsibilities:	collection, management, analysis, and interpretation of	
36			
37	sponsor and funder	data; writing of the report; and the decision to submit the	
38			
39		report for publication, including whether they will have	
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41		ultimate authority over any of these activities	
42			
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44			
45	Roles and	#5d Composition, roles, and responsibilities of the coordinating	1
46			
47	responsibilities:	centre, steering committee, endpoint adjudication	
48			
49	committees	committee, data management team, and other individuals	
50			
51		or groups overseeing the trial, if applicable (see Item 21a	
52			
53		for data monitoring committee)	
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56			
57	Introduction		
58			
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60			

1	Background and	#6a	Description of research question and justification for	5
2				
3	rationale		undertaking the trial, including summary of relevant studies	
4			(published and unpublished) examining benefits and harms	
5			for each intervention	
6				
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11	Background and	#6b	Explanation for choice of comparators	5
12				
13	rationale: choice of			
14				
15	comparators			
16				
17				
18	Objectives	#7	Specific objectives or hypotheses	6
19				
20				
21				
22	Trial design	#8	Description of trial design including type of trial (eg, parallel	6
23			group, crossover, factorial, single group), allocation ratio,	
24			and framework (eg, superiority, equivalence, non-inferiority,	
25			exploratory)	
26				
27				
28				
29				
30				
31	Methods:			
32				
33	Participants,			
34				
35	interventions, and			
36				
37	outcomes			
38				
39				
40				
41	Study setting	#9	Description of study settings (eg, community clinic,	6
42			academic hospital) and list of countries where data will be	
43			collected. Reference to where list of study sites can be	
44			obtained	
45				
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51	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	7
52			applicable, eligibility criteria for study centres and	
53			individuals who will perform the interventions (eg,	
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surgeons, psychotherapists)

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4	Interventions:	#11a	Interventions for each group with sufficient detail to allow
5			
6	description		replication, including how and when they will be
7			
8			administered
9			
10			
11	Interventions:	#11b	Criteria for discontinuing or modifying allocated
12			
13	modifications		interventions for a given trial participant (eg, drug dose
14			
15			change in response to harms, participant request, or
16			improving / worsening disease)
17			
18			
19			
20			
21	Interventions:	#11c	Strategies to improve adherence to intervention protocols,
22			
23	adherence		and any procedures for monitoring adherence (eg, drug
24			
25			tablet return; laboratory tests)
26			
27			
28			
29	Interventions:	#11d	Relevant concomitant care and interventions that are
30			
31	concomitant care		permitted or prohibited during the trial
32			
33			
34	Outcomes	#12	Primary, secondary, and other outcomes, including the
35			
36			specific measurement variable (eg, systolic blood
37			
38			pressure), analysis metric (eg, change from baseline, final
39			
40			value, time to event), method of aggregation (eg, median,
41			
42			proportion), and time point for each outcome. Explanation
43			
44			of the clinical relevance of chosen efficacy and harm
45			
46			outcomes is strongly recommended
47			
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51	Participant timeline	#13	Time schedule of enrolment, interventions (including any
52			
53			run-ins and washouts), assessments, and visits for
54			
55			participants. A schematic diagram is highly recommended
56			
57			(see Figure)
58			
59			
60			

1	Sample size	#14	Estimated number of participants needed to achieve study	13
2			objectives and how it was determined, including clinical and	
3			statistical assumptions supporting any sample size	
4			calculations	
5				
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11	Recruitment	#15	Strategies for achieving adequate participant enrolment to	11
12			reach target sample size	
13				
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15				
16	Methods: Assignment			
17	of interventions (for			
18	controlled trials)			
19				
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24	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	12
25	generation		computer-generated random numbers), and list of any	
26			factors for stratification. To reduce predictability of a	
27			random sequence, details of any planned restriction (eg,	
28			blocking) should be provided in a separate document that is	
29			unavailable to those who enrol participants or assign	
30			interventions	
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41	Allocation	#16b	Mechanism of implementing the allocation sequence (eg,	12
42	concealment		central telephone; sequentially numbered, opaque, sealed	
43	mechanism		envelopes), describing any steps to conceal the sequence	
44			until interventions are assigned	
45				
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51	Allocation:	#16c	Who will generate the allocation sequence, who will enrol	13
52	implementation		participants, and who will assign participants to	
53			interventions	
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1	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg,	11
2			trial participants, care providers, outcome assessors, data	
3			analysts), and how	
4				
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7				
8	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is	11
9	emergency		permissible, and procedure for revealing a participant's	
10			allocated intervention during the trial	
11	unblinding			
12				
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16	Methods: Data			
17	collection,			
18	management, and			
19	analysis			
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26	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline,	13
27			and other trial data, including any related processes to	
28			promote data quality (eg, duplicate measurements, training	
29			of assessors) and a description of study instruments (eg,	
30			questionnaires, laboratory tests) along with their reliability	
31			and validity, if known. Reference to where data collection	
32			forms can be found, if not in the protocol	
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43	Data collection plan:	#18b	Plans to promote participant retention and complete follow-	13
44	retention		up, including list of any outcome data to be collected for	
45			participants who discontinue or deviate from intervention	
46			protocols	
47				
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53	Data management	#19	Plans for data entry, coding, security, and storage,	13
54			including any related processes to promote data quality	
55			(eg, double data entry; range checks for data values).	
56				
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1		Reference to where details of data management	
2			
3		procedures can be found, if not in the protocol	
4			
5			
6	Statistics: outcomes	#20a Statistical methods for analysing primary and secondary	13
7			
8		outcomes. Reference to where other details of the	
9			
10		statistical analysis plan can be found, if not in the protocol	
11			
12			
13	Statistics: additional	#20b Methods for any additional analyses (eg, subgroup and	13
14			
15	analyses	adjusted analyses)	
16			
17			
18	Statistics: analysis	#20c Definition of analysis population relating to protocol non-	13
19			
20	population and	adherence (eg, as randomised analysis), and any statistical	
21			
22	missing data	methods to handle missing data (eg, multiple imputation)	
23			
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25			
26	Methods: Monitoring		
27			
28			
29	Data monitoring:	#21a Composition of data monitoring committee (DMC);	15
30			
31	formal committee	summary of its role and reporting structure; statement of	
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33		whether it is independent from the sponsor and competing	
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35		interests; and reference to where further details about its	
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37		charter can be found, if not in the protocol. Alternatively, an	
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39		explanation of why a DMC is not needed	
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44	Data monitoring:	#21b Description of any interim analyses and stopping	15
45			
46	interim analysis	guidelines, including who will have access to these interim	
47			
48		results and make the final decision to terminate the trial	
49			
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51	Harms	#22 Plans for collecting, assessing, reporting, and managing	12
52			
53		solicited and spontaneously reported adverse events and	
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55		other unintended effects of trial interventions or trial	
56			
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1		conduct	
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4	Auditing	#23 Frequency and procedures for auditing trial conduct, if any,	14
5			
6		and whether the process will be independent from	
7			
8		investigators and the sponsor	
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11	Ethics and		
12			
13	dissemination		
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16	Research ethics	#24 Plans for seeking research ethics committee / institutional	15
17			
18	approval	review board (REC / IRB) approval	
19			
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21	Protocol	#25 Plans for communicating important protocol modifications	15
22			
23	amendments	(eg, changes to eligibility criteria, outcomes, analyses) to	
24		relevant parties (eg, investigators, REC / IRBs, trial	
25		participants, trial registries, journals, regulators)	
26			
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31	Consent or assent	#26a Who will obtain informed consent or assent from potential	15
32			
33		trial participants or authorised surrogates, and how (see	
34			
35		Item 32)	
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39	Consent or assent:	#26b Additional consent provisions for collection and use of	n/a
40			
41	ancillary studies	participant data and biological specimens in ancillary	
42			
43		studies, if applicable	
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46			
47	Confidentiality	#27 How personal information about potential and enrolled	16
48			
49		participants will be collected, shared, and maintained in	
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51		order to protect confidentiality before, during, and after the	
52			
53		trial	
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57	Declaration of	#28 Financial and other competing interests for principal	17
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1	interests		investigators for the overall trial and each study site	
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4	Data access	#29	Statement of who will have access to the final trial dataset,	16
5			and disclosure of contractual agreements that limit such	
6			access for investigators	
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11	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial care, and for	n/a
12			compensation to those who suffer harm from trial	
13	trial care		participation	
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19	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial	16
20			results to participants, healthcare professionals, the public,	
21	trial results		and other relevant groups (eg, via publication, reporting in	
22			results databases, or other data sharing arrangements),	
23			including any publication restrictions	
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31	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	16
32			professional writers	
33	authorship			
34				
35				
36	Dissemination policy:	#31c	Plans, if any, for granting public access to the full protocol,	n/a
37			participant-level dataset, and statistical code	
38	reproducible research			
39				
40				
41				
42	Appendices			
43				
44				
45	Informed consent	#32	Model consent form and other related documentation given	n/a
46			to participants and authorised surrogates	
47	materials			
48				
49				
50	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of	n/a
51			biological specimens for genetic or molecular analysis in	
52			the current trial and for future use in ancillary studies, if	
53			applicable	
54				
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2 Commons Attribution License CC-BY-NC. This checklist was completed on 04. March 2022 using
3 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
4 [Penelope.ai](#)
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