A Prospective, Single-Blind, Placebo-Controlled Trial of Bone Marrow Aspirate Concentrate for Knee Osteoarthritis

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Investigation performed at the Mayo Clinic, Jacksonville, Florida, USA

Background: Bone marrow aspirate concentrate (BMAC) is increasingly used as a regenerative therapy for musculoskeletal pathological conditions despite limited evidence-based support.

Hypothesis: BMAC will prove feasible, safe, and efficacious for the treatment of pain due to mild to moderate degenerative joint disease of the knee.

Study Design: Randomized controlled trial; Level of evidence, 2.

Methods: In this prospective, single-blind, placebo-controlled trial, 25 patients with bilateral knee pain from bilateral osteoarthritis were randomized to receive BMAC into one knee and saline placebo into the other. Fifty-two milliliters of bone marrow was aspirated from the iliac crests and concentrated in an automated centrifuge. The resulting BMAC was combined with platelet-poor plasma for an injection into the arthritic knee and was compared with a saline injection into the contralateral knee, thereby utilizing each patient as his or her own control. Safety outcomes, pain relief, and function as measured by Osteoarthritis Research Society International (OARSI) measures and the visual analog scale (VAS) score were tracked initially at 1 week, 3 months, and 6 months after the procedure.

Results: There were no serious adverse events from the BMAC procedure. OARSI Intermittent and Constant Osteoarthritis Pain and VAS pain scores in both knees decreased significantly from baseline at 1 week, 3 months, and 6 months ($P \leq .019$ for all). Pain relief, although dramatic, did not differ significantly between treated knees ($P > .09$ for all).

Conclusion: Early results show that BMAC is safe to use and is a reliable and viable cellular product. Study patients experienced a similar relief of pain in both BMAC- and saline-treated arthritic knees. Further study is required to determine the mechanisms of action, duration of efficacy, optimal frequency of treatments, and regenerative potential.

Registration: ClinicalTrials.gov record 12-004459.

Keywords: knee osteoarthritis; degenerative joint disease; stem cells; bone marrow; BMAC; MSC

Osteoarthritis is a painful, degenerative condition of the joints that affects millions of patients, and half of all Americans will suffer from it during their lifetime.[45] Although osteoarthritis is a disease of abnormal joint biomechanics with slow deterioration of articular cartilage, its pathological changes are biochemically mediated.[42] Osteoarthritis of the knee is one of the most common and debilitating areas of joint degeneration. Efforts at disease modification from a biochemical and therapeutic perspective have been mostly unsuccessful, and there is currently no United States Food and Drug Administration (FDA)–licensed or –approved therapy, biological intervention, or procedure that prevents the progressive destruction of diseased degenerative joints. The mainstay of treatment thus falls to symptomatic relief,[39] with costly knee replacement to follow when symptomatic therapy is no longer effective.[9] The insufficiency of nonoperative therapies is reflected in

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a recent American Academy of Orthopaedic Surgeons position paper, which recommended against most conservative medical therapies for osteoarthritis.[1]

A number of recent treatment advances use cellular-based therapies such as platelet-rich plasma (PRP)[17,21,39,50,53,54] and mesenchymal stem cells (MSCs)[¶] to treat joint pain. MSCs are found in all human tissues, and their trilineage potential holds the promise of tissue regeneration, most notably for their chondrogenic potential. Additional therapeutic mechanisms of action include trophic and immunomodulatory effects of MSCs.[6] While the exact therapeutic mechanisms of MSCs are yet to be clarified, their use in clinical practice has dramatically increased,[56] and bone marrow aspirate concentrate (BMAC) may represent the safest and most feasible source of MSCs. A recent FDA draft guidance advised that a same-day concentration of bone marrow (BMAC) without additives constitutes minimal manipulation and covered under Code of Federal Regulations 361.[18,19,58] Nevertheless, while a number of studies reported the use of MSCs in arthritic disease,[11,15,30,33-38,47,48] few controlled trials of BMAC have been reported. We report the safety outcomes and short-term follow-up from a prospective, placebo-controlled, patient-blinded pilot study of BMAC for osteoarthritis of the knees.

METHODS

Study Patients and Data Collection

This study was approved by the Institutional Review Board at the Mayo Clinic and received Investigational New Drug authorization (No. 15352) from the FDA. A total of 25 patients seen for painful bilateral knee osteoarthritis at the Mayo Clinic in Jacksonville, Florida, between November 2013 and February 2015 were included in this study. Patients were considered for inclusion if they had longstanding bilateral knee pain from mild to moderate bilateral osteoarthritis despite conventional treatments such as activity modification, weight loss, physical therapy, analgesics, nonsteroidal anti-inflammatory drugs, or injection therapy for at least 6 weeks. Exclusion criteria were severe osteoarthritis (Kellgren-Lawrence grade 4), rheumatological or other systemic disease, diabetes, malignancy, or infections (see Appendix Table A1, available online at http://ajsm.sagepub.com/supplemental). All patients were required to wait 3 months from any prior intra-articular injection before participating. Each patient received an intra-articular injection of the study treatment (BMAC) into one knee and saline placebo into the contralateral knee, thereby utilizing each patient as his or her own control and thus avoiding sham bone marrow aspirations as a control treatment. Treatments (ie, BMAC or placebo) were assigned to knees within each patient using computer-based randomization via the dynamic allocation method of Pocock and Simon.[51] According to the single-blind study protocol, patients were shielded from viewing the contents of each injection by a large curtain hung just proximal to their thighs such that they did not have knowledge of which knee was injected with BMAC, but the operating physician did.

Information was collected regarding baseline demographic characteristics, activity level, and patient-reported pain as assessed using the Osteoarthritis Research Society International (OARSI) Intermittent and Constant Osteoarthritis Pain (ICOAP) questionnaire, visual analog scale (VAS) pain score, and algometry measures. The ICOAP is a patient-reported outcome measurement (PROM) tool composed of 11 items in 2 subscales: constant pain and intermittent pain. Two separate scores are generated, but they can be combined into a total score that has been validated and endorsed by the OARSI and shown to correlate with other commonly used PROMs such as the Western Ontario and McMaster Universities Arthritis Index (WOMAC) and the Knee injury and Osteoarthritis Outcome Score (KOOS).[4,20,24,25,44] Outcomes were measured by an orthopaedic research nurse independent of the operating physician at baseline and at 1 week, 3 months, and 6 months after surgery. Adverse events were recorded and clinical examinations performed at the same intervals to document effusions, warmth, erythema, joint line tenderness, and range of motion. There was no other therapeutic intervention (bracing, physical therapy, etc). Patients were discouraged from taking any prescription or over-the-counter pain medication for as long as they were pain free and reported any rescue medication to the research nurse. This was designed as such to study the BMAC injection as the only treatment intervention for the duration of the study. Joint surveillance included bilateral radiographs and quantitative T2-mapping magnetic resonance imaging (MRI) at baseline, with follow-up MRI at 6 months and radiography at 12 months as performed by a fellowship-trained musculoskeletal radiologist. A small-volume synovial fluid aspiration of 1 to 2 mL from each knee was performed at 1 week and 6 months.

Surgical Technique

A total of 26 mL of bone marrow was harvested from each superior iliac crest in the following manner: in a Mayo Clinic procedure room, the principal investigator (S.A.S.) and a qualified hematology registered nurse-practitioner filled a 60-mL syringe with 8 mL of anticoagulant citrate dextrose solution A (ACDA; Ivex Pharmaceuticals). After appropriate local anesthesia of the skin and subcutaneous soft tissues with 1% lidocaine without epinephrine, a 1-cm stab incision with a No. 11 scalpel was performed over each iliac crest. Five to 10 mL of bone marrow was aspirated with an 11-gauge, 11-cm Jamshidi needle at each site. Effort was taken to use a parallel approach, with the needle directed parallel to the iliac wing between the inner and outer tables, according to the technique described by Hernigou et al.[27,28] The Jamshidi needle was subsequently withdrawn and repositioned along each iliac crest. For each reposition, the needle was re-stilleted to clear the Jamshidi bore of marrow debris. This process was repeated

[¶]References 6, 8, 11-15, 22, 30, 33-38, 43, 47, 48.

until approximately 26 mL of bone marrow from 3 sites on each iliac crest was harvested for a total of 52 mL of bone marrow aspirate. The aspirated marrow and ACDA were then passed through a sterile 170-μm filter (ICU Medical) into a separate 60-mL syringe to remove particulate matter. One milliliter of this filtered material was sent for laboratory analysis, and the remainder was transferred to a Magellan Autologous Platelet Separator System (Arteriocyte) for centrifugation and resulting marrow cell concentration. The concentration process yielded approximately 6 mL of cellular product. One milliliter of concentrated cells was reserved for analysis and preservation by a transfusion medicine laboratory assistant.

A complete blood count (CBC) analysis and white blood cell (WBC) differential were performed on both preconcentration and postconcentration samples using the XE-5000 Automated Hematology System (Sysmex Corp). Red blood cell, WBC, platelet, and hematocrit values were reported from the CBC analysis. Percentages of mononuclear cells (MNCs) were derived from the WBC differential by totaling the percentages of lymphocytes, atypical lymphocytes, monocytes, and blast cells. Absolute MNC counts were derived by multiplying the percentage of MNCs by the absolute WBC count. Hematopoietic stem cell (HSC) and MSC contents of the preconcentration and postconcentration bone marrow samples were determined by flow cytometric analysis using a BD Accuri C6 Flow Cytometer (BD Biosciences). HSCs were enumerated by staining for coexpression of CD45 and CD34 surface markers. MSCs were enumerated using the BD Stemflow Human MSC Analysis Kit (Cat No. 562245; BD Biosciences). Cells were analyzed for positive coexpression of CD90, CD105, and CD73 surface markers, concurrently with negative expression of CD45, CD34, CD19, CD11b, and HLA-DR, in accordance with the minimal criteria for defining MSCs set forth by the International Society for Cellular Therapy. Cell viability was determined for all samples by flow cytometry using 7AAD staining.

Five milliliters of treatment cells were combined with 10 mL of previously separated platelet-poor bone marrow plasma to increase the volume of injectate within the knee. An intra-articular injection was performed into the randomly assigned knee through a superolateral approach under continuous ultrasound guidance with an in-plane needle by the principal investigator, who had 8 years of experience performing ultrasound-guided injections at the time of the study. The contralateral knee was injected with 15 mL of sterile saline. Both knees were aspirated before the injection. The injection procedure took place in the same procedure room as the concentration process.

Statistical Analysis

As this was a pilot study, no power analysis was performed. All analyses were performed on the basis of the intention-to-treat principle. Continuous variables were summarized using the sample median and range. Comparisons between ICOAP and VAS pain scores at baseline between BMAC- and placebo-treated knees were made using a paired Wilcoxon signed-rank test. Categorical variables were summarized using the number and percentage of patients. For each outcome that was examined, comparisons between baseline measures and measures obtained at each follow-up time point in the separate BMAC and placebo knee groups were made using a paired Wilcoxon signed-rank test. Similarly, changes in outcome measures from baseline to each follow-up time point were compared between BMAC and placebo knee groups using a paired Wilcoxon signed-rank test. The Spearman test of correlation was used to evaluate the correlation of total MSCs injected with the change from baseline in pain as measured by the ICOAP pain questionnaire, VAS pain score, and algometry measures; Spearman correlation coefficient rs and 95% CIs were estimated. $P \leq .05$ was considered as statistically significant, with no adjustment for multiple testing made in this exploratory pilot study. All statistical analyses were performed using SAS (version 9.2; SAS Institute Inc) and R Statistical Software (version 2.14.0; R Foundation for Statistical Computing).

RESULTS

A total of 279 patients were screened to include 25 study patients (see the CONSORT flow diagram in Appendix Figure A1, available online). Baseline demographic information for the 25 study patients is summarized in Table 1. The median patient age was 60 years (range, 42-68 years), and 7 patients (28%) were male. The majority of patients (80%) were white, and the median body mass index was 27.1 kg/m^2 (range, 18.4-37.5 kg/m^2). Of the 50 knees treated, 27 were radiographically graded Kellgren-Lawrence 2 (11 BMAC, 16 placebo), 19 were graded Kellgren-Lawrence 3 (12 BMAC, 7 placebo), and 4 were graded Kellgren-Lawrence 1 (2 BMAC, 2 placebo). Thirteen patients (52%) received BMAC in their right knee and placebo in their left knee, while the remaining 12 patients (48%) received BMAC in their left knee and placebo in their right knee. The cellular product is characterized in Table 2. The concentration process yielded a BMAC product containing a median of 34,400 MSCs with 97% cellular viability, consistent with previously published results for 60 mL of bone marrow.[26,61]

A summary of patient-reported pain at baseline and follow-up time points, as measured by the ICOAP pain questionnaire and VAS, is provided in Table 3. At baseline, there were no significant differences between the BMAC- and placebo-treated knees regarding ICOAP or VAS pain scores ($P \geq .41$ for all). There was a significant improvement in the ICOAP constant pain score, intermittent pain score, and total pain score from baseline to 1 week, 3 months, and 6 months for knees treated with BMAC ($P \leq .012$ for all). However, there was also a significant improvement in each of these pain scores in placebo-treated knees ($P \leq .009$ for all). When comparing changes from baseline in all pain score measures between BMAC- and placebo-treated knees, no differences were evident ($P \geq .09$ for all). VAS pain scores were also significantly

TABLE 1
Baseline Demographic Information (N = 25 Patients)[a]

Age, y	60 (42-68)
Male sex	7 (28)
Height, cm	165 (152-192)
Weight, kg	73 (53-118)
Body mass index, kg/m^2	27.1 (18.4-37.5)
Randomization group	
BMAC (right knee), placebo (left knee)	13 (52)
BMAC (left knee), placebo (right knee)	12 (48)
White race	20 (80)
Prior knee surgery[b]	11 (44)
On BMAC-treated knee	9 (75)
On placebo-treated knee	3 (25)

[a]Data are reported as median (range) for continuous variables and n (%) for discrete variables. BMAC, bone marrow aspirate concentrate.
[b]One patient had undergone prior bilateral knee surgery.

TABLE 2
Cellular Characterization[a]

	n	Median (Range)
Pre-spin measures		
Viability, %	24	97.8 (75.2-99.4)
MNCs, %	25	38.5 (26.0-57.5)
Total MNCs/μL	25	6100 (1950-27,000)
HSCs, %	25	3.2 (0.04-21.0)
MSCs, %	25	0.03 (0.00-0.60)
Total MNCs × MSCs, %	25	198 (0-2673)
WBCs, 1000/μL	25	13.0 (3.9-62.8)
RBCs, Mil/μL	25	3.33 (0.17-4.44)
HCTs, %	25	32.0 (1.6-38.2)
Platelets, 1000/μL	25	95 (7-399)
Post-spin measures		
Viability, %	22	97.0 (85.4-99.6)
MNCs, %	23	56.2 (25.8-87.9)
Total MNCs/μL	23	16,000 (2900-210,000)
HSCs, %	23	4.4 (1.2-14.0)
MSCs, %	23	0.05 (0.0-0.9)
Total MNCs × MSCs, %	23	688 (8.7-28,980)
WBCs, 1000/μL	23	31.4 (5.6-97.2)
RBCs, Mil/μL	23	0.96 (0.63-3.65)
HCTs, %	23	8.5 (3.5-34.0)
Platelets, 1000/μL	22	422 (52-1515)
Total HSCs injected	23	4,620,000 (174,000-130,200,000)
Total MSCs injected	23	34,400 (435-1,449,000)

[a]HCT, hematocrit; HSC, hematopoietic stem cell; Mil, million; MNC, mononuclear cell; MSC, mesenchymal stem cell; n, number of patient samples analyzed; RBC, red blood cell; WBC, white blood cell.

improved from baseline at 1 week, 3 months, and 6 months for both BMAC- and placebo-treated knees ($P \leq .019$ for all). There was no evidence of a difference in the degree of change in VAS pain scores from baseline to these follow-up time points between the 2 treatment groups ($P \geq .44$ for all). The ICOAP constant pain score, intermittent pain score, and total pain score at follow-up points are shown for the 2 treatment groups in Figures 1 to 3. VAS pain scores are displayed in Figure 4 separately for BMAC- and placebo-treated knees. No significant correlations were observed at any time points between PROMs and the number of MSCs injected ($P > .29$ for all) at 6 months, and this is presented in Appendix Table A2 (available online). Summaries of each item of the ICOAP pain questionnaire at each time point are displayed in Appendix Tables A3 (BMAC) and A4 (placebo) (available online). Of note, the degree of improvement in ICOAP and VAS pain scores from baseline did not differ significantly between Kellgren-Lawrence grades 1 to 2 and grade 3 knees within each treatment group for any of the follow-up time points ($P \geq .11$ for all).

There was a significant improvement in the activity level compared with baseline for each of the 1-week, 3-month, and 6-month follow-up periods ($P \leq .024$ for all). There was no difference in the degree of improvement from baseline between the 2 treatments at any of the follow-up periods ($P > .99$, $P > .99$, and $P = .51$, respectively). Self-reported pain medication usage decreased as well. Before the study, 100% of patients were using over-the-counter or prescription medications for pain, which decreased at the 3- and 6-month time points, to 24% and 36%, respectively. A summary of the activity level at baseline and follow-up is provided in Table 4 for BMAC- and placebo-treated knees.

There were no serious adverse events. Effusions were often seen for several days after the procedure, occurring in 58% of knees treated with BMAC and 25% of knees treated with placebo; however, these percentages decreased to 12% and 8%, respectively, by the 6-month follow-up. These were anticipated findings, and much of the initial effusions seen, especially in BMAC knees, were likely to be residual 15-mL product at the 1-week mark rather than inflammatory. Warmth occurred in only 1 knee at 3 days, resolving by 1 week, and erythema did not occur in any knee at any time point.

DISCUSSION

Cellular-based therapies for osteoarthritis are rapidly evolving; however, much remains to be understood regarding their efficacy and mechanisms of action. Our pilot and feasibility study of BMAC, the first patient-blinded, placebo-controlled, and FDA-monitored trial, shows our BMAC technique to be safe and well tolerated by patients. The intervention produced robust pain relief of sustained duration, while improving activity and decreasing reliance on pain medications, but did not differ significantly from comparable outcomes in saline-treated knees.

Our safety results are similar to previously published reports of BMAC use in osteoarthritic knees as they were with pain relief. Despite this, it is difficult to compare the overall outcomes of previous studies with the results of the current one given that this is the first to compare this injection procedure to placebo.[10] The only 2 previously reported BMAC studies for knee osteoarthritis showed improvement in pain and function but without a comparative

TABLE 3
ICOAP and VAS Pain Scores at Baseline and Follow-up Time Points[a]

	BMAC-Treated Knees (n = 25)	Placebo-Treated Knees (n = 25)	*P* Value (Change From Baseline: BMAC vs Placebo)
ICOAP constant pain score			
Baseline	25 (0 to 80)	25 (0 to 70)	
1-wk follow-up	15 (0 to 70)	10 (0 to 50)	
Change from baseline to 1 wk	–10 (–55 to 25)	–10 (–45 to 25)	.67
P value (vs baseline)	.012	.009	
3-mo follow-up	5 (0 to 70)	0 (0 to 65)	
Change from baseline to 3 mo	–15 (–60 to 25)	–10 (–70 to 40)	.53
P value (vs baseline)	.005	.002	
6-mo follow-up	0 (0 to 65)	0 (0 to 65)	
Change from baseline to 6 mo	–10 (–80 to 35)	–10 (–70 to 30)	.89
P value (vs baseline)	.003	.001	
ICOAP intermittent pain score			
Baseline	42 (21 to 100)	42 (21 to 75)	
1-wk follow-up	25 (0 to 75)	21 (0 to 58)	
Change from baseline to 1 wk	–17 (–79 to 8)	–21 (–50 to 29)	.41
P value (vs baseline)	<.0001	.0004	
3-mo follow-up	21 (0 to 75)	17 (0 to 75)	
Change from baseline to 3 mo	–21 (–83 to 21)	–25 (–50 to 46)	.09
P value (vs baseline)	<.0001	.001	
6-mo follow-up	21 (0 to 83)	17 (0 to 67)	
Change from baseline to 6 mo	–17 (–88 to 38)	–21 (–58 to 46)	.49
P value (vs baseline)	.0004	.001	
ICOAP total pain score			
Baseline	32 (18 to 91)	32 (0 to 73)	
1-wk follow-up	16 (0 to 73)	18 (0 to 55)	
Change from baseline to 1 wk	–16 (–68 to 16)	–16 (–39 to 27)	.57
P value (vs baseline)	.0001	.0003	
3-mo follow-up	18 (0 to 73)	11 (0 to 70)	
Change from baseline to 3 mo	–21 (–71 to 21)	–18 (–59 to 43)	.24
P value (vs baseline)	<.0001	.0001	
6-mo follow-up	16 (0 to 75)	9 (0 to 66)	
Change from baseline to 6 mo	–14 (–77 to 34)	–11 (–64 to 39)	.54
P value (vs baseline)	.0005	.0003	
VAS pain score			
Baseline	3.1 (0 to 8.1)	2.9 (0 to 7.0)	
1-wk follow-up	1.3 (0 to 7.4)	0.9 (0 to 7.7)	
Change from baseline to 1 wk	–1.2 (–6.3 to 3.9)	–1.5 (–6.5 to 5.2)	.47
P value (vs baseline)	.019	.0004	
3-mo follow-up	0.9 (0 to 8.3)	1.0 (0 to 8.2)	
Change from baseline to 3 mo	–1.5 (–6.9 to 2.9)	–1.5 (–6.8 to 5.7)	.88
P value (vs baseline)	.001	.001	
6-mo follow-up	1.5 (0 to 6.8)	0.8 (0 to 9.2)	
Change from baseline to 6 mo	–1.1 (–5.4 to 5.3)	–1.3 (–6.8 to 6.4)	.44
P value (vs baseline)	.001	.001	

[a]Data are reported as median (range) unless otherwise indicated. *P* values result from a paired Wilcoxon signed-rank test. BMAC, bone marrow aspirate concentrate; ICOAP, Intermittent and Constant Osteoarthritis Pain; VAS, visual analog scale.

intervention.[8,32] Additionally, the analysis of BMAC efficacy in both studies is complicated by the addition of adipose-derived product in both of the studies as well as PRP in one of these studies. Thus, although these studies showed promising outcomes, given that they are case series, lack controls, and utilize a multitherapeutic approach, the contribution of the concentrated marrow cells, as in our findings, remains unsubstantiated.

That a similar degree of pain relief was observed for both BMAC- and placebo-treated knees calls into question the mechanism that produced these improvements. Several possible explanations are to be considered. It could be postulated that the BMAC treatment did improve pain in the treated knee and consequently improved the saline-treated knee only because of easier maneuverability and the elimination of "sympathy pain" from not having to favor the BMAC-treated knee. This seems unlikely given that pain improved noticeably after only 1 week, and such a scenario would seemingly need longer to occur. A second theory is that the injection process alone, be it

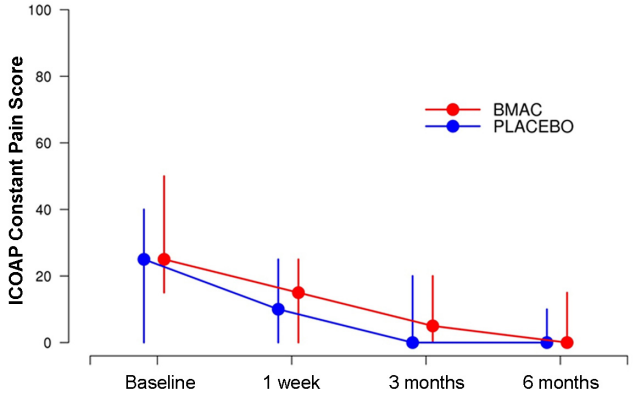

Figure 1. Intermittent and Constant Osteoarthritis Pain (ICOAP) constant pain score for bone marrow aspirate concentrate (BMAC) and placebo knee groups at each time point.

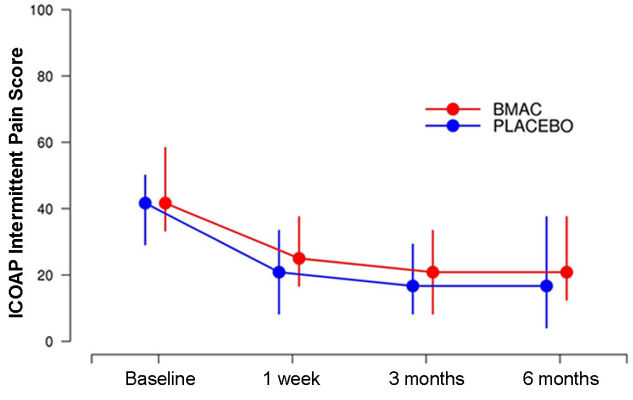

Figure 2. Intermittent and Constant Osteoarthritis Pain (ICOAP) intermittent pain score for bone marrow aspirate concentrate (BMAC) and placebo knee groups at each time point.

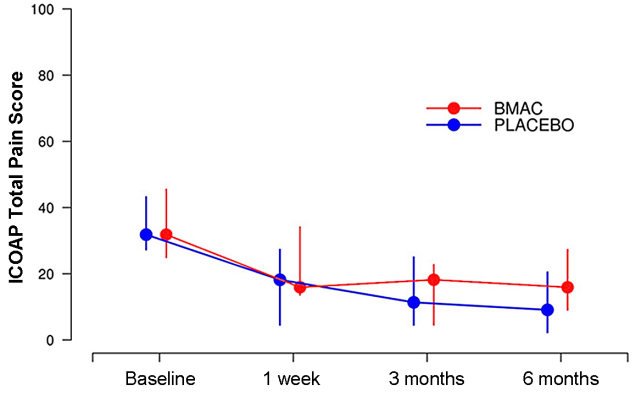

Figure 3. Intermittent and Constant Osteoarthritis Pain (ICOAP) total pain score for bone marrow aspirate concentrate (BMAC) and placebo knee groups at each time point.

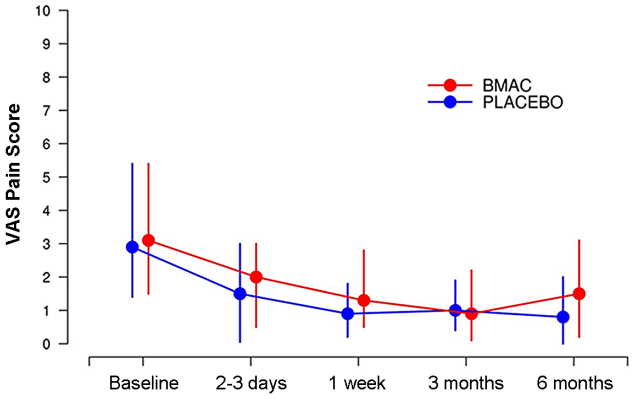

Figure 4. Visual analog scale (VAS) pain score for bone marrow aspirate concentrate (BMAC) and placebo knee groups at each time point.

BMAC or saline, caused improvements in pain. In other words, nonharmful injections into the knee have been shown to improve knee pain in osteoarthritis regardless of the injectate. However, the resulting pain relief that did not diminish over the documented 6-month follow-up seems inconsistent with this explanation. A third possibility is a dramatic placebo effect given that strong placebo responses have previously been documented for a number of different intra-articular injection types.[2,3] Despite this plausible justification, the fact that patients were aware that one knee would receive BMAC and the other would receive placebo (ie, patients would not be expecting both knees to improve) must be considered in this explanation.

A final consideration is that BMAC does have a pain-relieving effect, but rather than solely relieving pain in the injected knee, its effect is more systemically mediated, allowing for improvement in the saline-treated knee as well. The systemic effects of MSCs and their stochastic capabilities have been well demonstrated in animal models and in human cell therapy for other disease conditions.[6,16,29,40,46,57] Studies show tagged MSCs do travel to sites of inflammation even when delivered intravascularly and adhere to sites of injury when delivered intra-articularly.[16,23,31] Given the degenerative disease in both knees, cells administered in any manner could theoretically improve pain and function if explained through MSC homing capabilities, whereby a fraction of the injected stem cells enter circulation and provide similar benefit in the contralateral joint. Studies demonstrating MSCs depleted from the bone marrow of osteoarthritic knees and increased in the synovial fluid lend support to MSCs functioning not as actual building blocks of cartilage but rather as "medicinal signaling cells."[7,55]

Our cell counts raise additional questions regarding the effects of MSC dosing on theoretical efficacy. The stem cell concentration in marrow aspirates is known to be quite low, ranging from only 0.001% to 0.01% of nucleated cells, influenced by age,[7,27,28,58,61] and demonstrably lower than what can be produced utilizing culture-expanded autologous cells or allogeneic cellular products.[47,48,59] In the

TABLE 4
Activity Level at Baseline and Follow-up Time Points for BMAC- and Placebo-Treated Knees[a]

Does Your Knee Pain Limit Your Activity Level?	BMAC-Treated Knees (n = 25)	Placebo-Treated Knees (n = 25)	*P* Value (Change From Baseline: BMAC vs Placebo)
Baseline			
Not at all/mildly	6 (24.0)	8 (32.0)	
Moderately	13 (52.0)	11 (44.0)	
Severely/extremely	6 (24.0)	6 (24.0)	
1-wk follow-up			
Not at all/mildly	15 (60.0)	18 (72.0)	
Moderately	9 (36.0)	5 (20.0)	
Severely/extremely	1 (4.0)	2 (8.0)	
Improvement from baseline to 1 wk	15 (60.0)	14 (56.0)	>.99
P value (vs baseline)	.004	.024	
3-mo follow-up			
Not at all/mildly	15 (60.0)	19 (76.0)	
Moderately	8 (32.0)	4 (16.0)	
Severely/extremely	2 (8.0)	2 (8.0)	
Improvement from baseline to 3 mo	14 (56.0)	15 (60.0)	>.99
P value (vs baseline)	.003	.005	
6-mo follow-up			
Not at all/mildly	15 (60.0)	17 (68.0)	
Moderately	9 (36.0)	5 (20.0)	
Severely/extremely	1 (4.0)	3 (12.0)	
Improvement from baseline to 6 mo	17 (68.0)	15 (60.0)	.51
P value (vs baseline)	.0003	.003	

[a]Data are reported as n (%) unless otherwise indicated. *P* values result from a paired Wilcoxon signed-rank test. BMAC, bone marrow aspirate concentrate.

current study, our concentration process produced an MSC count of 0.05% of all MNCs, but we found no statistical correlation between total MSC cell counts on any PROM or other measurement tools. Therefore, with many BMAC studies demonstrating symptomatic pain relief,[5,13,32,41,52,60] despite low cell numbers compared with culture-expanded techniques, theories of MSC paracrine signaling mechanisms to modulate joint homeostasis have further support.[6,7,22,40] Regarding the chondrogenic potential of the injected MSCs, we plan to conduct quantitative T2-mapping MRI analysis at later term follow-up of these same study patients.

Finally, other cell types within BMAC may play a therapeutic role including platelets, which were elevated in our BMAC product. PRP is known to possess biologically active growth factors inside alpha granules that contain the potential to diminish joint inflammation, decrease cartilage breakdown, and enhance tissue repair.[49] With a number of conflicting trials, the efficacy of PRP in treating knee osteoarthritis has been difficult to assess. Nevertheless, considering some previously demonstrated therapeutic effects of PRP in the treatment of knee osteoarthritis,[17,21,39,50,53,54] it is possible that PRP-released factors significantly contributed to the observed beneficial effect of BMAC. PRP is the cell therapy predecessor to BMAC, with over 10 years of clinical use in musculoskeletal disease. Bone marrow concentration techniques followed from similar density gradient centrifugation algorithms with the notion that the addition of progenitor cells such as MSCs and HSCs would have a more profound regenerative capacity than the growth factors contained within PRP alone. Much like BMAC, the exact cellular quantities, cell composition, and dosing frequencies remain unconfirmed despite years of PRP clinical use and scientific trials. It should be noted, however, that these applications of PRP are typically administered in a series of injections, and in our study, the BMAC treatment was only performed once. Direct comparisons of BMAC and PRP will be needed to determine if the proposed efficacy correlates with specific cell types and numbers.

BMAC is being used to treat osteoarthritis with increased frequency, driven by great expectations from patients and their clinicians alike because of the promise of the MSCs that they contain. Unfortunately, these expectations outpace what is known about clinical efficacy and the cellularity of the BMAC product. Although not conclusive, the results presented here provide very useful information for future studies. Patients tolerated the BMAC procedure well with no serious adverse events, similar to previous studies' demonstrated safety profiles, and the cellular characterization via flow cytometry improves our understanding of the BMAC composition. The hope is that human cell therapy will ultimately lead to regenerative mechanisms for osteoarthritis and other orthopaedic conditions. However, with few comparative trials, and the results presented here showing similar pain relief in saline-treated knees, it remains to be seen if the cellular composition of BMAC alone will be enough to treat osteoarthritic knees or if perhaps larger stem cell numbers such as those generated by culture-expanded autologous or

allogeneic products will be required to achieve superior results. As a pilot study, our sample size is small, and we note a selection bias toward normal-weight and active patients. With greater participant numbers, a more diverse patient population can be studied. Forthcoming studies will also need to include unilateral disease groups with a separate placebo control group to help better address efficacy as well as possible mechanisms of action.

Acknowledging these study limitations, we find early insight into this cell therapy technique promising in terms of its feasibility as well as its ability to concentrate MSCs for safe intra-articular use. Nevertheless, given the similar pain relief to placebo at 6 months, BMAC injection therapy requires additional study with longer term follow-up as well as further investigation into the effects of cell counts, cell types, and frequency of treatments. Until such time as these and other such comparative studies are concluded, BMAC cannot be recommended for the regular treatment of osteoarthritis of the knees.

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