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Clinical Outcomes and Safety of a Combined Autologous Bone Marrow Concentrate Intraosseous and Intraarticular Injection for Knee Osteoarthritis At 12 Months

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Abstract

Objective: Safety and therapeutic benefit were assessed for treating knee osteoarthritis with dual intraosseous (tibial plateau) and intraarticular bone marrow concentrate injections. Study participant-reported outcomes for Knee Society Score-Knee, Knee Society Score-Function, Lower Extremity Functional Scale, and Visual Analog Scale were assessed. Outcomes at 1-year were evaluated for influence by study participant factors, including participant demographics, pre-treatment clinical outcome values and bone marrow concentrate cellular composition.

Methods: Twenty knees with Kellgren-Lawrence II-III osteoarthritis were treated prospectively at a single site/single investigator in an open label pilot study with autologous bone marrow concentrate. Each participant received 80% of their bone marrow concentrate in the tibial plateau intraosseous, and 20% intraarticular. Each bone marrow concentrate was analysed for total nucleated cells and levels of progenitor cell colony forming units by tissue culture.

Results: No serious adverse events were associated with the treatment. Meaningful improvement in mean clinical outcome metrics (P-values: 0.0001 to 0.005) from baseline to 52-weeks was observed. Mean change in Visual Analog Scale (-2.6) exceeded the published minimum clinically important difference of -2.5. The Lower Extremity Functional Scale mean change was +15.8, which exceeds a published 9-point minimum clinically important difference. Influence on 52-week outcome changes for the four metrics were limited to pre-treatment values, while the Knee Society Score-Knee and Visual Analog Scale outcomes were influenced by a 10-fold increase in the Total Nucleated Cell concentration.

Conclusion: Safety was demonstrated for the bone marrow concentrate-combined treatment via intraosseous and intraarticular routes for treating Kellgren-Lawrence II-III knee osteoarthritis. Mean changes at 52-weeks showed substantial improvement from baseline in the outcome metrics, with Visual Analog Scale and Lower Extremity Functional Score exceeding published minimal clinical important difference values. Changes in clinical metrics were influenced by pre-treatment values and Total Nucleated Cell concentration, but not other assessed patient factors

Keywords

Bone Marrow Concentrate (BMC); Intraosseous; Intraarticular; Knee OA; Visual Analog Scale (VAS); Lower Extremity Functional Scale (LEFS); Orthobiologic

Introduction

Knee osteoarthritis (OA) increasingly is considered to be a whole-joint disease, of which degeneration of the articular cartilage is a critical component of OA pathology, along with alterations to the synovial membrane and changes to the subchondral bone supporting the cartilage [1]. Compounding the treatment of OA is the slow and usually limited recovery of damaged articular cartilage [2]. Conventional therapies, including viscosupplementation, steroids, physical therapy, and non-steroidal anti-inflammatory agents, have shown some benefit in reducing OA-associated knee pain, and improving quality of life/functionality, at least for some period of time [1], but lack evidence of regenerative or long-lasting benefits [3]. Orthobiologics such as Platelet-rich Plasma (PRP) and Bone Marrow Concentrate (BMC) also have been used in treating OA, with varying degrees of success. PRP is reported to be more effective when compared to hyaluronic acid at reducing pain and improving quality of life, especially at longer milestones (e.g., 12 months) [4]. Shapiro, et al. [5] performed a randomized control trial to study the clinical outcomes of injecting bilateral knees with saline or BMC. They found that the benefit of BMC treatment was not significantly different from the placebo control (saline) used in their trial out to the most recently reported milestone of one year [6]. Although most publications concerning treatment of knee OA use an intraarticular route of injection [5,7,8], there are a few recent publications that have described an intraosseous route for injecting an Orth biologic. Sánchez, et al. [9]. combined a primary intraosseous injection of PRP with two intraarticular PRP injections in comparison with three intraarticular PRP injections, while Su, et al. [10] compared a combination of intraosseous and intraarticular PRP injections with intraarticular-only injections of PRP or hyaluronic acid. Intraosseous injection of BMC has a long history due to the clinical efforts of Dr. Philippe Hernigou, who has reported therapeutic benefit in treating patients suffering from a variety of orthopedic conditions, including non-union [11], rotator cuff repair [12], and avascular necrosis [13].

Building on the foundational work of Dr. Hernigou, the current study was structured to assess the safety and potential therapeutic benefit of treating patients with mild to moderate knee osteoarthritis with a dual injection of BMC, such that approximately 80% of the injectate was delivered intraosseous to the tibial plateau, and 20% was delivered intraarticular. Each BMC preparation was analyzed for Total Nucleated Cells (TNC), and culture-based Colony Forming Units-Fibroblast (CFU-F; as a biomarker of mesenchymal signaling cells, MSCs), CFU-Osteogenic (CFU-O; as a biomarker of osteogenic progenitor cells) and CFU-Chondrogenic (CFU-C; as a biomarker of chondrogenic progenitor cells). Clinical outcomes were recorded for Knee Society Score-Function (KSS-Function), Knee Society Score-

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Knee (KSS-Knee), Lower Extremity Functional Scale (LEFS) and Visual Analog Scale (VAS), and assessed for correlations with patient factors, including cellularity (TNC and CFUs) and pre-treatment clinical outcome values. The hypothesis under consideration is that the dual injection into the tibial plateau and knee capsule of KL II-III OA knees would be well-tolerated and provide therapeutic benefit in terms of pain mitigation and improved quality of life.

Materials and Methods

Study design and clinical protocol

This study is a prospective, open label, non-randomized pilot study conducted at a single site with a single investigator (MBS). The purpose of this study was to evaluate the safety and potential benefit of treating mild to moderate knee osteoarthritis with an injection of autologous BMC, which was split between an intraosseous injection (80% of the volume) into the tibial plateau and an intraarticular injection (20% of the volume, augmented with autologous concentrated Platelet-poor Plasma) into the joint space of the affected knee. Patients were recruited from the clinic of the first author (MBS). Patients with knee osteoarthritis characterized on radiological exam as Kellgren-Lawrence (KL) II-III and meeting the inclusion/ exclusion criteria outlined in Table 1 were eligible for inclusion in the study. Figure 1 shows representative X-rays of the pre-treatment Kellgren-Lawrence III scores for four study participants. Following recruitment, patients who consented to participate in the clinical study were enrolled. A total of 20 patients consented to participate in the study during the period from January 27, 2017 to April 4, 2018. Primary clinical endpoints were KSS-Knee, KSS-Function, and LEFS, with a secondary endpoint of VAS (10-point scale). The endpoints of the study were assessed prior to treatment and at 6-weeks, 13-weeks, 26-weeks, and 52-weeks post-treatment. The study was approved by an Institutional Review Board (Institute of Regenerative and Cellular Medicine, IRCM-2016-125).

Inclusion Criteria	• Voluntary signature of the IRB-approved Informed Consent • Participant is a candidate for autologous bone marrow knee injection • Ages 18 to 70 • Diagnosis of Knee Osteoarthritis • Kellgren-Lawrence Grade II-III on X-Ray • BMI <32.5 • Minimum flexion to 110 degrees ROM • Varus < 7 degrees and Valgus <12 degrees • Instability in any plane <2 mm translation • ACL intact and no past ACL reconstruction • Knee Society Score >60 (100-point score) • If bilateral, both knees will be enrolled in the study • Participant is able to follow post-treatment guidelines • Participant agrees to return for periodic assessments
Exclusion Criteria	• Participant is unable to conform to the study protocol follow-up procedures and visits • Participant's condition requires surgical repair (e.g., meniscal tear), has had a joint infection in the last five years, has been treated with intraarticular PRP, steroids or viscosupplementation in the previous three months or has had previous knee surgery within the last six months • Participant has flexion contracture >10 degrees • Participant has lower back pain with radiculopathy or with "significant" radiological changes • Participant has received immunosuppression or chemotherapy within the last five years • Participant has a systemic neurological disease, is HIV positive or has chronic hepatitis • Participant has a significant co-morbidity that in the opinion of the investigator should exclude the participant from the study • Participant is pregnant

Table 1: Inclusion/Exclusion Criteria for Study Participant Enrolment

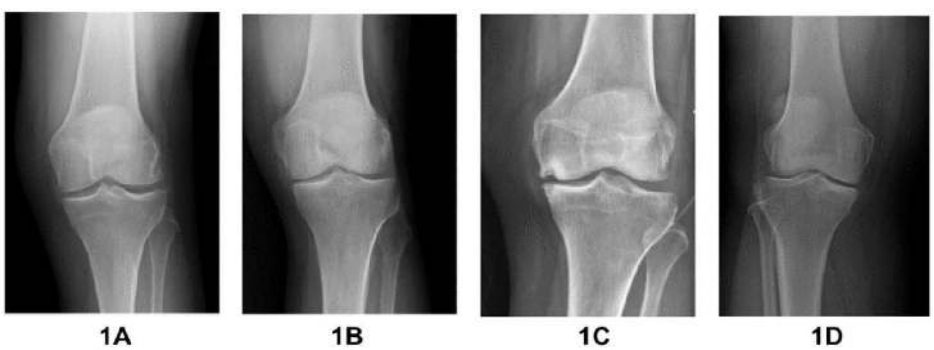

Figure 1: Figure 1A, 1B and 1D are representative images (AP of the knee) of Kellgren-Lawrence III scores for study participants. Figure 1C is a representative image (Standing AP of the knee) of a Kellgren-Lawrence III study participant.

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Bone marrow aspiration

Prior to aspiration, each study participant was given conscious sedation (intravenous Versed), administered by an anesthesiologist with the patient positioned prone. The skin and subcutaneous tissue opposite the insertional point for aspiration were numbed with lidocaine (2 mL of a 1% solution diluted 1:4 v/v with sterile saline) after sterile site preparation of the study participant's posterior iliac crest and posterior superior iliac spine. Care was taken not to inject local anesthetic deep into the subcutaneous fat. The Jamshidi needle (Ranfac Corp., Avon, MA) was rinsed and 10cc syringes were filled with 1 mL of acid citrate dextrose solution A (ACDA; Incell, San Antonio, TX). The Jamshidi needle was inserted into the posterior superior iliac spine and posterior iliac crest between the tables approximately 6-8 cm into the intramedullary compartment. A 10cc syringe was attached and the plunger was rapidly pulled back to initiate aspiration. Rotation of the needle at the same level was performed once, followed by proximal repositioning of the needle by 2 cm to repeat the cycle. A fresh 10cc syringe was used after approximately 10 mL of bone marrow aspirate was recovered. A total of 60 mL of bone marrow was collected.

Bone marrow and platelet poor plasma

The bone marrow aspirate was loaded into a device (ART BMCPlus™, Celling Biosciences, Austin, TX) and centrifuged according to the manufacturer's instructions. On average, 5 mL (range 2.5 to 6 mL) of BMC was recovered. Platelet Poor Plasma (PPP) was transferred to an on-board filter chamber prior to collecting the BMC portion and was concentrated according to the manufacturer's instructions. An aliquot (≤ 1 mL) of the recovered BMC was obtained for further analysis. The remaining BMC was divided into two portions: 80% of the volume was retained for intraosseous injection into the tibial plateau, while 20% of the BMC was mixed with concentrated PPP to produce a 10 mL-preparation for intraarticular injection.

Treatment protocol

Following appropriate site preparation, percutaneous intraosseous injection of the BMC (typically 4 mL) was performed with a Jamshidi needle into the tibial plateau (medial or lateral depending on the primary location of articular cartilage degradation and subchondral sclerosis). The remaining BMC/concentrated PPP preparation was injected intraarticular following topical numbing with Lidocaine (2 mL of 1% Lidocaine diluted 1:4 v/v with sterile saline, taking care to avoid penetrating the joint capsule). Ultrasound image guidance was used to confirm delivery within the capsule. Fluoroscopic guidance was used to ensure appropriate location of the trocar used to deliver the intraosseous BMC. The interval between bone marrow aspiration and treatment with the BMC was less than an hour.

Supplemental treatment

A number of study participants presented with synovitis with or without pain of the BMC-treated knee during the first- year post-treatment. For participants requesting treatment, a choice based on medical history was made, with 75% of the participants electing to receive a Platelet-rich Plasma (PRP) treatment and the remaining participants receiving physical therapy, corticosteroids, anesthetic, or viscosupplementation. The PRP preparation involved the drawing of 17 mL of whole blood (in two tubes: BD Vacutainer, ACD Solution A, #364606; Franklin Lakes, NJ), which were spun for 5-minutes at 500xg (Horizon Centrifuge [642VFD Plus Ca], Druker Co., Port Matilda, PA). A total of 5-mL of the plasma layer close to the interface was collected from both tubes and injected into the knee capsule with ultrasound guidance.

Post-treatment protocol

The study participant was instructed to limit weight-bearing for three days. A set of rehabilitation exercises commenced on Day 3, including stationary cycling, aquatherapy and water walking. Formal physical therapy was initiated at 3-weeks post-treatment. NSAIDS were not allowed for 1-week after treatment, but non-steroidal and non-narcotic pain management were permitted.

Bone marrow concentrate analysis

A small aliquot of the BMC preparation was transferred to a sterile cryo-vial (Costar, Corning, Tewksbury, MA), placed in an insulated box with 5°C cold packs and shipped to Celling Biosciences (Austin, TX) for analysis. Upon receipt, each BMC preparation was diluted with Phosphate Buffered Saline (PBS), and a cell count was determined with the NucleoCounter NC-100 (Chemometec, Inc, Lillerød, Denmark). The diluted BMC was processed on a Ficoll-Paque density gradient (1:1 v: v; GE Healthcare Sciences, Piscataway, NJ). Recovered cells were counted and four pre-set aliquots of the RBC-depleted BMC preparation were added (in triplicate) to each of three 12-well plates, followed by addition of 1-mL of culture medium (containing 5% Fetal Bovine Serum—FBS; Sciencell Research Laboratories, Carlsbad, CA) to each well. The plates were transferred to a 37°C, 5% CO_2 and 100% humidity incubator. Half of the volume of culture medium in each well was replaced every three days with fresh culture medium/5% FBS.

Following nine days in culture (D10), one plate was processed for assessing Colony Forming Units-Fibroblast (CFU-F), by aspirating the culture medium, washing with one mL of PBS per well— twice, followed by washing with one mL of distilled water (dH_2O), aspirating and staining the wells with one mL of 0.5% crystal violet (in 10% formalin; Sigma-Aldrich, St. Louis, MO). The plate was placed on an orbital shaker at 50 rpm for 20 minutes at room temperature. The stain was removed with a PBS rinse, followed by rinsing with water to clear all stain and allowed to air dry. Colonies were assessed using an inverted microscope, and those with 20 or more cells were scored as a CFU-F. On the 10th day after culturing, the plates for Colony Forming Units-Osteogenic (CFU-O) and Colony Forming Units-Chondrogenic (CFU-C) were converted to Osteogenic and Chondrogenic culture media (Sciencell Research Laboratories, Carlsbad, CA), respectively, by aspirating the fluid from each well and adding one mL of the appropriate medium. The plates were returned to the incubator. Fresh medium was added to the plates on Days 13 and 17. On Day 20, the CFU-O and CFU-C plates were handled as described for the CFU-F plate for washing, but after the second wash of dH_2O, 0.5 mL of 5% formalin solution (Sigma-Aldrich, St. Louis, MO) was added and incubated for 20 minutes at room temperature. Stain for the CFU-O assay was prepared by following the manufacturer's instructions for the Vector ALP-Blue kit (Vector Laboratories, Burlingame, CA). The wells were washed once with PBS and one mL of the ALP-Blue stain was added per well, after which the plate was placed in a refrigerator (4°C) for 25 minutes. Following the incubation, the wells were washed three times with two mL of dH_2O, followed by adding 0.5 mL of Alizarin Red solution (Sigma-Aldrich, St. Louis, MO) per well. The plate was placed on an orbital shaker (50 rpm) for 5 minutes. Stain was removed, and the plate was rinsed in a sink with water. Excess water was removed, and the plate

was air-dried. Colonies were assessed as described for the CFU-F assay. The CFU-C plate was stained by adding one mL of PBS after the formalin fixation step, followed by aspiration and the addition of 0.5 mL of Alcian Blue (Sigma-Aldrich, St. Louis, MO) per well. The plate was placed on an orbital shaker (50 rpm) for 20 minutes. Stain was aspirated, and the wells washed three times with one mL of dH_2O. After removing the wash fluid, 0.5 mL of Nuclear Fast Red (Sigma-Aldrich, St. Louis, MO) was added to each well, and the plate was placed on an orbital shaker (50 rpm) for 5 minutes. After the incubation, the fluid was aspirated, and the plate washed in the sink. CFU-C colonies were assessed as described for the CFU-F assay.

Statistics

Changes in clinical outcome metrics were assessed with a 1-sample permutation test, while demographics, cell/colony concentrations and numbers were compared using a 2-sample permutation test [14, 15]. Colony and cell concentrations and numbers represent back-transformed values from the means of log10(y) and ranges. Table values shown are means with standard deviations (SD) and ranges. A general linear model was used to assess the impact of demographics (sex, age, height, and weight), cellularity (CFUs, TNC concentrations and numbers) and pre-procedure clinical outcome values on changes observed at the 1-yr milestone; residual plots demonstrated that our general linear model was appropriate. The SAS/STAT software package, Version 9.3 of the SAS System for Windows (copyright © 2002–2010 SAS Institute Inc.), and R (https://www.R-project.org/) were used for all analyses.

Results

Some study participants reported discomfort immediately following the combined intraosseous/intraarticular procedure, which resolved within 1-3 days following treatment. One study participant experienced an acute, traumatic synovitis in the treated knee at 9-weeks, which was thought to be unrelated to the treatment. Six study participants reported a single instance of synovitis, with or without pain, in the treated knee: one report at 6-months, one at 7-months, one at 9-months and three at 1-year post-treatment. Four study participants reported at least two adverse events during the first year of follow-up, with four reports of synovitis (with or without pain) at 3-months, two reports at 6-months, one report at 8-months, one report at 9-months and two reports at 12-months. One study participant reported four adverse events with the treated knee at 3-, 6-, 8- and 12-months. Participants presenting with synovitis/pain were given a choice of treatment, based on previous medical history, including a "PRP booster", anesthetic, corticosteroid, physical therapy or viscosupplementation, with 75% of the study participants electing to receive a PRP booster. All study participants reporting adverse events have remained in the study through the first-year milestone. Two patients enrolled in the study but cancelled their procedure prior to treatment. Another participant had returned to full activity but suffered a severe injury in the treated knee while weightlifting (reported at 6-month follow-up), which required surgical resolution (a uni-compartmental knee arthroplasty). Another participant complained of pain in the contralateral, untreated knee at 6-month follow-up, but elected to undergo a total knee arthroplasty on the study knee.

A total of 18 study participants with 20-treated knees remained in the study through the 1-yr milestone. The demographics [mean (standard deviations), range] of the study participants are shown in Table 2, along with the means and ranges for the group's CFU-F, CFU-C, CFU-O, and total nucleated cells (TNC) determined for the volume of BMC that was recovered. Table 3 shows the means (standard deviations) and ranges for the pre-treatment values (baseline) for KSS-Knee, KSS-Function, LEFS and VAS, along with the mean and range of the changes at 52-weeks. The values of the clinical outcomes at 52-weeks reflect statistically meaningful differences compared to the pre-treatment values. Figure 2A shows the mean (solid line), 25-75% range of data values (box), and the remaining data values (lines and individual points) of the clinical outcome metrics at pre-treatment, 6-weeks, 13-weeks, 26-weeks and 52-weeks post-treatment. Figure 2B shows the distribution and mean of all clinical outcome values at 52-weeks for each of the

Characteristic	
Participants	18
Knees Treated	20
Age, years	60.1 (8.2) 41–70
Height, in	67.7 (4.4) 61–75
Weight, lb	180 (37.8) 121–245
Sex, F:M	10:8
Side of treatment, L: R: B**	10:6:2
Kellgren-Lawrence score	2.9 (0.3) 2–3
Total CFU-F, x 10^4	3.4 0–80
Total CFU-O, x 10^4	9.8 0.5–107
Total CFU-C, x 10^4	8.5 0.2–178
Nucleated cells, x 10^6	1932 230–11697

Table 2: Study participant demographics and cellular analysis of bone marrow concentrate preparations. Group characteristics where age, height, weight, and Kellgren-Lawrence scores represent mean (SD) and range. For sex and side of treatment, table entries represent counts. Table entries for total Colony Forming Unit- Fibroblast (CFU-F), CFU-O (osteogenic), CFU-C (chondrogenic), and nucleated cells in the Bone Marrow Concentrate preparation represent back-transformed values of the means for log10 (y) and ranges. B**: bilateral

Table 3: Changes in outcome scores with table entries represented by mean (SD) and range for the 20 knees from pre-treatment (baseline) to 52-weeks. All outcomes were analyzed using 100,000 replications of a 1-sample permutation test.

Characteristic	Baseline	52-Week Change	P value
Visual Analog Score	5.1 (2.0) 0–7.9	–2.6 –6.7 to +2.1	0.0002
Knee Society Score-Knee	74.4 (11.3) 60–95	14.8 –3 to +32	0.0001
Knee Society Score-Function	75 (15.4) 50–100	11.8 –20 to +40	0.005
Lower Extremity Functional Score	45.8 (14.1) 25–74	15.8 –22 to +41	0.0004

Table 4: General linear modelling of participant factors affecting changes at 1-year. Table values reflect the change in 1-yr outcomes based on an increase of 1-point for the pre-treatment value or a 10-fold increase in Total Nucleated Cells/mL (TNC/mL) in BMC.

Factor	LEFS	KSS-Knee	KSS-Function	VAS
Pre-treatment Value	1 point higher	1 point higher	1 point higher	1 point higher
Change at 1-yr	0.6-point decrease	0.7-point decrease	0.6-point decrease	0.7-point decrease
P-value	0.03	0.001	0.02	0.002
TNC/mL in BMC	10x increase	10x increase	10x increase	10x increase
Change at 1-yr	No effect	12-point increase	No effect	3-point decrease
P-value	NA	0.01	NA	0.002

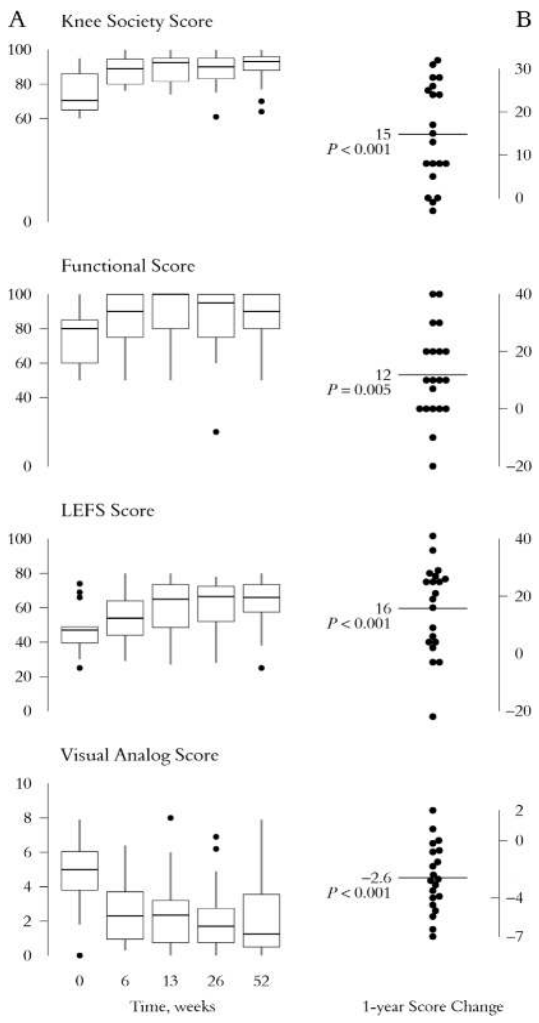

Figure 2: Figure 2A Means of the outcome values (solid line), 25-75% range (box), and remaining data values (vertical lines, with points) starting with pre-treatment and ending with 52-week milestone. Figure 2B Distribution of the clinical outcome 52-week changes for all knees, with the mean (solid line) and data values for each of the outcome metrics.

clinical outcomes. Table 4 shows the results of a general linear model assessment of factors (sex, age, height, weight, CFU-F/ CFU-C/CFU-O number or concentration, and TNC number or concentration, and pre-treatment values of clinical outcomes) that were evaluated for potential influence on the final change in clinical outcome values at the 1-yr milestone. Only TNC/mL of BMC and the pre-treatment values of the clinical outcomes of the patient-related factors assessed showed a significant correlation to the change in clinical outcome values at 1-yr. A reduction in the value reported for the clinical outcomes at 1-yr resulted from a higher value of the pre- treatment scores. In contrast, a substantial positive increase in the KSS-Knee score correlated with a 10-fold increase in the nucleated cell concentration of the BMC, while VAS decreased to a lower value for a 10-fold increase in nucleated cell concentration.

Discussion

The knee capsule is a common target for treating knee OA [5, 7, 8]. However, some reports [9, 10] have indicated clinical benefit in patients receiving an intraosseous injection of PRP to treat knee OA. Intraosseous injection of autologous bone marrow concentrate also has shown clinical benefit in a variety of orthopedic pathologies, including rotator cuff repair [12], long bone non-union [11] and avascular necrosis of the femoral head [13]. Consequently, the current pilot study examined the safety and potential benefit of treating mild-moderate knee OA with BMC being split between intraarticular and intraosseous routes of administration during the same treatment, with 80% of the BMC injected intraosseous, and the remaining 20% mixed with concentrated PPP and subsequently injected intraarticular.

An important finding of the study was that the protocol involving a combination of both intraosseous and intraarticular injections of BMC was well tolerated. Discomfort experienced at the site of bone marrow aspiration and/or at treatment sites resolved within 24- 72 hours for those participants reporting discomfort. No durable complications from either the intraosseous or intraarticular injections were reported by the study participants. This finding parallels the lack of adverse events observed in treatment groups receiving an intraosseous PRP treatment for knee OA [9,10]. Furthermore, in a meta-study of six publications Sundaram, et al. [16] concluded that delivery of PRP or BMC via the intraosseous route was safe and well tolerated. Taken together, an intraosseous delivery of orthobiologics in the treatment of knee OA appears to be a safe procedure.

The study participants' demographics and data on the cellularity of the BMC preparations are shown in Table 2. The mean KL Grade was 2.9, since all but two of the enrolled knees had KL III-level knee OA. X-rays showing representative knees with Kellgren-Lawrence III scores of four study participants are shown in Figure 1A-1D. As shown in Figure 2A, marked improvement in mean outcome values occurred by the 6-week or 13-week milestone, with continued averaged improvement out to the 52-week milestone. Figure 2B shows the mean change (solid line) from pre-treatment to 52-week milestone, along with the wide distributions of all reported values for each outcome metric. Outcomes for VAS, LEFS, KSS-Knee and KSS-Function all showed statistically meaningful improvements for the study participants from pre-treatment values to the 52-week milestone Table 3, although there is substantial variation observed in the range of the 52-week changes reported. For example, the study group showed a mean change in VAS at baseline to 52-weeks of -2.6 (10-point scale), with a range of -6.7 to 2.1. The minimum clinically important difference (MCID) for VAS has been reported as -2.5 [17] under a variety of conditions, so the mean VAS change of -2.6 found in this study is suggestive of a clinically relevant improvement in pain. For comparison Shapiro, et al. [6] reported a -1.9 (10-point scale) change in VAS at 1-yr for study participants receiving a single BMC intraarticular treatment Centeno, et al. [18] reported a change in VAS of -1.5 (10-point scale) at the 1-yr milestone for study participants receiving BMC along with PRP and Plasma Lysate injected into the knee capsule Sánchez, et al. [9] reported a change in VAS of -1.1 at 1-yr for study participants who received a PRP injection intraosseous with two subsequent PRP injections intraarticular. A VAS change of -4.6 at 1-yr was reported by Su, et al. [10] in study participants receiving two separate intraosseous PRP treatments (two weeks apart), along with PRP delivered intraarticular. Clearly, mitigation of pain due to knee OA can be achieved with injections of PRP and BMC via intraosseous and intraarticular routes of treatment, but the magnitude of pain relief reported varies widely.

The other outcomes of KSS-Knee, KSS-Function and LEFS assessed in the current study all showed significant improvements i n mean values at the 52-week milestone over baseline Table 3. In particular, the mean change in LEFS was +15.8, which is approximately 1.75 times the reported MCID for LEFS of 9 points [19], suggesting that study participants experienced a meaningful return to functional use of the treated knee. Dependency of clinical outcomes on the cellularity of the BMC preparations (CFU-F, CFU-C, CFU-O and Total Nucleated Cells; means and ranges shown in Table 2) was evaluated. As shown in Table 4, only the total nucleated cell concentration in the BMC preparations showed a correlation with KSS-Knee and VAS, but not LEFS or KSS-Function Centeno, et al. [20] indicated that there was a correlation between outcomes and total nucleated cells injected in a BMC treatment for knee OA. However, it isn't clear in the current study why just two of the four clinical outcomes were correlated with total nucleated cell concentration in the BMC injectate, and none with total number of cells. Participant factors (sex, age, height, weight) also showed no correlation with 52-week changes in clinical outcomes (data not shown). Surprisingly, the pre-treatment value of the clinical outcomes was found to influence the degree of change observed at the 52-week milestone Table 4: for every one- point higher value of the pre-treatment starting values, the final mean change for those outcomes was lower by 0.6 points (LEFS, KSS- Function) or 0.7 points (VAS, KSS-Knee). This observation implies that if a patient has a higher pre-treatment value there is less room for improvement following treatment for LEFS, KSS-Function and KSS- Knee. The implication being that there is only so much improvement possible, which will depend in part on the pre-treatment value. On the other hand, as shown in Table 4, the higher the pre-treatment VAS value (greater pain), the larger the decrease in the 52-week value. The influence of pre-treatment values on post-treatment changes for clinical outcomes observed in this study doesn't appear to have been reported previously in assessing BMC treatment of knee OA. A dependency of this sort suggests that a variety of clinical metrics should be employed when evaluating therapeutic modalities. There are several limitations of the study. Since the BMC preparation was split between intraosseous and intraarticular routes of administration, the contribution of the two treatment sites to observed improvements in the clinical outcomes can't be separated. Successfully enrolled study participants presented with a heterogenous profile of pre-treatment clinical outcome values, potentially leading to greater variability in changes observed at the 1-yr milestone. For example, one of the study participants had a pre-treatment VAS score of 0. Study participants having values of 0 for pre-treatment pain submetrics has been reported previously [5]. Finally, some study participants asked for and

received supplemental treatment for synovitis, with or without pain, following their primary BMC treatment. An assessment of those participants receiving a supplemental treatment prior to their 12-month milestones versus those who didn't receive a supplemental treatment or received it at 12-months didn't show any meaningful differences in the parameters shown in Tables 2 and 3 (data not shown). However, without activity logs it is difficult to know if the participants required supplemental treatment as a result of increased physical activity or if their request was due to a less beneficial outcome. In some cases, the adverse event was related to more strenuous activities (e.g., weightlifting).

Conclusion

The results reported in this study support the safety of using a dual injection of BMC via intraosseous and intraarticular routes to treat mild-moderate knee OA. Equally important, study participants reported a mean change in VAS at the 1-year milestone of -2.6, which is slightly larger than the reported VAS MCID of -2.5, suggesting that the treatment protocol resulted in a meaningful decrease in pain out to 1-year post-treatment. The mean change at 1-year of the LEFS outcome was +15.8 points, which is 1.75x larger than the published MCID for LEFS of 9 points. Marked improvements in KSS-Knee and KSS-Function also were observed out to the 1-year milestone. An influence of pre-treatment clinical values was observed on 1-year values for the clinical outcomes, which hadn't been reported previously in treating knee OA. Total Nucleated Cell concentration was shown to influence 1-year clinical outcome values, but not total number of nucleated cells or other values of cellularity (CFU-F, CFU-O, CFU-C). No correlations with 1-year outcome values were observed for sex, age, height, or weight. Some study participants reported no adverse events out to 1-year, while others reported from one to four starting around 3-months post-treatment, with study participants receiving supplemental treatment when requested. There were no meaningful differences for participant demographics, change in outcomes or cellularity between the subgroup who received a supplemental treatment compared to the subgroup who didn't receive a supplemental treatment out to 1-yr. Obviously, a one-size-fits-all treatment protocol for patients with knee OA seeking pain relief and a return to normal activities remains elusive, but intraosseous delivery of BMC in treating knee OA clearly merits additional study.

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