Journal of Regenerative Medicine

Research Article

A SCITECHNOL JOURNAL

Two-Year Outcome in the Treatment of Knee Osteoarthritis with a Combination of Intraarticular and Intraosseous Autologous Bone Marrow Concentrate

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Citation: Sheinkop M, Langhenry M, Sand T(2024) Two-Year Outcome in the Treatment of Knee Osteoarthritis with a Combination of Intraarticular and Intraosseous Autologous Bone Marrow Concentrate. J Regen Med 13:2.

Received: : 05-Mar-2024, Manuscript No. JRGM-24-128805; **Editor assigned:** 07-Mar-2024, PreQC No. JRGM-24-128805 (PQ); **Reviewed:** 21-Mar-2024, QC No. JRGM-24-128805; **Revised:** 23-Mar-2024, Manuscript No. JRGM-24-128805 (R); **Published:**30-Mar-2024, DOI:10.4172/2325-9620.1000300

Abstract

Objective: Safety and therapeutic benefit were assessed for treating knee osteoarthritis with dual intraosseous and intraarticular bone marrow concentrate injections at the two-year milestone. Participant-reported outcomes for Knee Society Score-Knee, Knee Society Score-Function, Lower Extremity Functional Scale, and Visual Analog Scale were assessed, along with Range of Motion and changes in Kellgren-Lawrence Grades.

Methods: Eighteen of 22 knees injected at the start of the study survived to the two-year milestone. The study was performed prospectively at a single site with a 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.

Results: No serious adverse events were attributed to the treatment during the two-year study. Statistically meaningful improvement in mean outcomes and range of motion from baseline to 24-months was observed. Kellgren-Lawrence scores worsened by one level for five knees (29.4%), improved by one level for three knees (17.6%) and were unchanged for nine knees (52.9%). Three study participants progressed to a total knee arthroplasty with an average time to surgery of 16.3 months following treatment.

Conclusion: Safety was demonstrated for the bone marrow concentrate-combined treatment via intraosseous and intraarticular routes for treating Kellgren-Lawrence II-III knee osteoarthritis during the two-year study period. Mean changes at 24-months showed sustained improvement from baseline for range of motion, Knee Society Score-Knee, Knee Society Score-Function, and Visual Analog Scale, although the Lower Extremity Functional Score decreased by 8.8%. Most (70.5%) knees showed no change or an improvement in their Kellgren-Lawrence scores from baseline to the two-year endpoint. These results point to a potential durable benefit in pain mitigation and improved quality of life for patients with knee osteoarthritis who receive bone marrow concentrate via the intraosseous and intraarticular routes for treating knee osteoarthritis.

Keywords: Bone Marrow Concentrate, BMC, Intraosseous, Intraarticular, Knee OA, VAS, LEFS, Orthobiologic, Subchondral, ROM

Abbreviations: ACDA: Anticoagulated Citrate Dextrose Solution A; ANOVA: Analysis Of Variance; BMC: Bone Marrow Concentrate; BML: Bone Marrow Lesion; EQ-VAS: EuroQol-Visual Analogue Scale; HA: Hyaluronic Acid; IL-1β: Interleukin-1β; IA: Intra-articular; IKDC: International Knee Documentation Committee; IO: Intraosseous; KL: Kellgren-Lawrence; KOOS: Knee Injury and Osteoarthritis Outcome Score; KSS: Knee Society Score; LEFS: Lower Extremity Functional Scale; MMP-13: Matrix Metallo Proteinase-13; OA: Osteoarthritis; PPP: Platelet-Poor Plasma; PRP: Platelet-Rich Plasma; QoL: Quality Of Life; RCTs: Randomized Controlled Clinical Trials; ROM: Range Of Motion; SD: Standard Deviation; SE: Standard Error; TKA: Total Knee Arthroplasty; TNC: Total Nucleated Cell; VAS: Visual Analog Scale.

Introduction

Osteoarthritis (OA) is a degenerative disease characterized by loss of articular cartilage with accompanying structural defects, osteophyte formation, and subchondral sclerosis. It most frequently affects older patients and appears in joints with the highest weight-bearing loads [1]. Knee osteoarthritis is the most prevalent joint affected by OA and is reported to be present in approximately 17% of patients older than 45 years old [2]. In people older than 60, the prevalence is 37% [3]. Multiple tissues within and surrounding the knee joint are thought to be associated with the progression of OA including subchondral changes (sclerosis and bone marrow lesions [BML]), articular cartilage loss and synovial membrane inflammation [4]. An important manifestation of knee OA is the presence of cartilage degrading enzymes (e.g., MMP-13), along with pro-inflammatory cytokines like IL-1β in the synovial fluid of the OA knee [4, 5]. The presence of a pro-inflammatory milieu reflects the imbalance in anabolic and catabolic metabolism in the various tissues of the knee [4]. As a result, without intervention OA is a progressive disease, increasing in severity as a patient age.

The ultimate treatment for knee OA has been surgery, but there is a diversity of non-surgical options available prior to knee replacement. Physical therapy and exercise, especially in early-stage OA, have been reported to reduce pain and improve Quality of Life (QoL) [6]. Other conservative interventions including NSAIDs, corticosteroid injections, and hyaluronic acid (HA) injections have

been used to provide short term pain relief but lack a durable or longer-term benefit and have no regenerative potential [7,8]. Use of autologous Platelet-rich Plasma (PRP) intraarticular (IA) injections for knee OA have been reported as safe, are well-tolerated and have a longer lasting therapeutic benefit, including pain mitigation and improved function, compared to HA as reported in a meta-analysis of 20 Randomized controlled clinical trials (RCTs) [9]. On the other hand, there are publications of RCT studies in which a single IA injection of PRP was compared to a placebo injection that failed to demonstrate a superior outcome for PRP versus the placebo at the final milestone [10, 11]. The platelets in the injectates used in these studies were obtained from 20 mL of whole blood, which would result in a platelet "dose" that is substantially less than the 10 billion platelets recently cited as the minimum dose needed to achieve a durable and statistically meaningful difference between PRP treatment and HA at the 12-month follow-up [12]. A similar durable benefit was demonstrated in a RCT of a series of three PRP injections with an average of more than 13 billion platelets per injectate [13]. While in the majority of clinical assessments of PRP in treating knee OA, delivery of the therapy was via the intraarticular route, another option is to deliver PRP via the intraosseous (IO) route. For example, in a recently reported clinical study, a combination of IA and IO PRP injections was performed at the first treatment, with two subsequent treatments of IA PRP at 7 and 14 days following the first treatment. Study participants reported a statistically meaningful improvement in QoL and pain scores at the 26-week endpoint of the study compared to baseline [14]. In another report [4], the potential therapeutic benefit of a combined IO/IA PRP treatment was compared to an IA-only PRP treatment for Kellgren-Lawrence (KL) Grade III knee OA. Both treatment groups showed substantial improvement in multiple outcomes (VAS, KOOS, etc.) at 6-months compared to baseline. The IO/IA PRP group showed a greater reduction in VAS compared to the IA-only group at 6-months, but the difference was not statistically meaningful. In fact, just one of the five KOOS parameters ("sport and recreation function") showed a statistically meaningful difference in favor of the IO/IA group over the IA-only group at the 6-month study endpoint [4]. One limitation in the use of PRP to treat knee OA is a lack of a long-term therapeutic benefit. For example, in the study [13] in which three injections of high platelet dose PRP were used, the authors reported a statistically meaningful improvement in outcome scores through the 1-year milestone, which was not maintained at the 2-year milestone.

Extending the therapeutic benefit of a biologic treatment for knee OA past the 1-year milestone has been elusive. The use of BMC delivered via the IA or IO routes recently was reported to have multi-year durable therapeutic benefit. In a recent, seminal publication [15], a prospective, randomized controlled clinical trial was performed to assess the therapeutic outcomes of 60 patients with equivalent bilateral knee OA who received a BMC injection via the IA route, while the contralateral knee received an IO (subchondral) injection of the BMC in the femoral condyle and the tibial plateau. Bone marrow was aspirated bilaterally from a study participant's iliac crests and concentrated. The BMC obtained from each study participant was divided in half, so the intraosseous compartments received one-quarter of the total BMC. Patients were followed for an average of 15 years, during which 70% of the IA-injected knees progressed to a Total Knee Arthroplasty (TKA) with an average time to TKA of 7 years. In contrast, only 20% of the IO-injected knees progressed to a TKA with an average time of 14 years. These definitive clinical results suggest that it is possible to substantially delay the progression of knee OA (as measured by the objective clinical benchmark of TKA) when treating patients with BMC either by the IA or IO routes, with a substantial differential therapeutic benefit associated with the IO route [15].

A clinical study in which a combination of IO and IA delivery of BMC to treat knee OA during the treatment was first published in 2020 [16]. Study participants received 80% of the BMC via the IO route, while the remaining 20% of the BMC was combined with concentrated Platelet-poor Plasma (PPP) and injected via the IA route. Durable and substantial improvements in participant-reported outcomes (Knee Society Score Knee [KSS-Knee], Knee Society Score Function [KSS-Function], Lower Extremity Functional Scale [LEFS], and Visual Analog Scale [VAS]) were reported out to the 1-year milestone [16]. Our updated report extends outcomes of those initial study participants treated with a combined IO/IA BMC treatment for knee OA to the 2-year follow-up.

Materials & Methods

Study Design and Clinical Protocol

This pilot study was designed as a prospective, open label, non-randomized study conducted at a single site with a single investigator (MBS). The purpose of the pilot study was to evaluate the safety and benefitoftreatingmildtomoderatekneeosteoarthritiswithaninjection of autologous BMC, divided between an intraosseous injection (80% of the volume) into the tibial plateau and an intraarticular injection (20% of the volume), augmented with autologous concentrated PPP. Patients were recruited from the clinic of the first author (MBS). Those with knee osteoarthritis characterized on radiological exam as Kellgren-Lawrence (KL) II-III and meeting the inclusion/exclusion criteria outlined as previously published [16] were eligible. 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) and Range of Motion (ROM). The endpoints of the study were assessed pretreatment and at 6-weeks, 13-weeks, 26-weeks, 1-year and 2-years post-treatment. Routine X-ray views (AP, lateral, skyline and weight-bearing when possible) of the treatment knee(s) were obtained prior to enrollment and again at the 2-year milestone. The study was approved by an Institutional Review Board (Institute of Regenerative and Cellular Medicine, approval number IRCM-2016-125).

Bone Marrow Aspiration

As previously summarized [16], each study participant was given conscious sedation (intravenous Versed) prior to bone marrow aspiration, administered by an anesthesiologist with the patient positioned prone. The skin and subcutaneous tissue opposite the insertional point for aspiration injected with lidocaine (2 mL of a 1% solution diluted 1:4 v/v with sterile saline) after sterile site preparation of the ipsilateral 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, USA) was rinsed with ACDA and 10cc syringes were filled with 1 mL of ACDA (Incell, San Antonio, TX. USA). The Jamshidi needle was inserted into the posterior superior iliac spine and posterior iliac crest approximately 3-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 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.

Concentration of Bone Marrow and Platelet-poor Plasma

The procedure for concentrating the bone marrow aspirate and the associated platelet-poor plasma (PPP) has been published [16]. In brief, bone marrow aspirate was concentrated in a sterile device (ART BMC Plus™, Celling Biosciences, Austin, TX, USA) and centrifuged according to the manufacturer's instructions. On average, 5 mL (range 2.5 to 6 mL) of BMC was recovered. PPP was transferred to an integrated filter chamber on the device without additional sterile breaks 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

Study participants were treated with their BMC as previously described [16]. In brief, percutaneous intraosseous injection of the BMC (typically 4 mL) was performed with a modified Jamshidi needle into the tibial plateau (medial or lateral depending on the primary location of articular cartilage degradation and subchondral sclerosis). The remaining BMC was mixed with the associated concentrated PPP preparation and injected intraarticular following subcutaneous injection with Lidocaine (2 mL of 1% Lidocaine diluted 1:4 v/v with sterile saline), taking care to avoid intraarticular placement of local anesthesia. During the period of observation and monitoring following the initial BMC treatment, several study participants presented with recurrent synovitis of the BMC-treated knee during the first year of treatment, which was treated based on medical history, with 75% of the subjects electing to receive a PRP treatment and the remaining study participants receiving physical therapy, corticosteroids, anesthetic, or viscosupplementation [16]. Supplemental treatments were requested by study participants during the second year of the study, during which four participants received 7 PRP treatments, while one participant received a steroid injection (data not shown). The PRP preparation was obtained as previously described [16], which involved the drawing of 17 mL of whole blood (in two tubes: BD Vacutainer, ACD Solution A, #364606; Franklin Lakes, NJ, USA). The tubes subsequently were spun for 5-minutes at 500xg (Horizon Centrifuge, Druker Co., Port Matilda, PA, USA). A total of 5-mL of the plasma layer close to the interface was collected from both tubes, and injected into the knee, intraarticular with ultrasound guidance [16].

Post-treatment Protocol

Study participants were instructed to limit weight-bearing for three days. A set of home-based rehabilitation exercises commenced on Day 3, including stationary cycling, aqua therapy 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.

Analysis of Bone Marrow Concentrate

An aliquot of each study participant's BMC preparation was analyzed as previously published [16], which included assessing the BMC for Total Nucleated Cell (TNC) number.

Range of Motion

To assure objectivity in assessing Range of Motion (ROM) at each study milestone, the subject's ROM was determined with an orthopedic goniometer (Pro Healthcare Products.com, Rexburg, ID, USA). The study participant was seated and actively and maximally, extended and flexed the knee following instruction with the goniometric documentation respectively recorded. This methodology was applied during the pretreatment evaluation and repeated at each follow-up visit. The resultant ROM represents an objective clinical measurement related to the study participants' response to the BMC treatment.

Statistics

Means and variance of all outcome measures were calculated at pretreatment (baseline) and at each post-treatment interval. The statistical significance of changes in study participant-reported outcomes (VAS, KSS-Function, KSS-Knee and LEFS) was determined by non-parametric Repeated Measures (RM) Analysis Of Variance (ANOVA) on ranks (Friedman Test, [17]) using commercially available software (SigmaStat, Inpixon, Palo Alto, CA, USA). The significance of changes in range of motion, a continuous variable, was determined by standard RM ANOVA. To determine if the results were correlated with cell numbers, improvement, expressed as % change vs baseline, was calculated for each outcome variable at each post-treatment time point and plotted against viable cell concentrations and total viable cell numbers. The statistical significance of each comparison was determined by linear regression.

Results

A total of 16 study participants with 18 treated knees reached the 2-year milestone. Over the course of the study, three study participants received a total knee replacement (12, 14 and 23 months), while one study participant suffered from an acute trauma during weightlifting and underwent a uni-compartmental knee arthroplasty at 6-months. Demographics for the study participants who reached the 2-year milestone were shown in **(Table 1)**. Kellgren-Lawrence Grades were obtained at the 2-year milestone and compared to the pretreatment KL grades as shown in (**Figure 1**).

ROM was assessed during office visits out to 2-years, and the mean and Standard Error (SE) values of the ROM in degrees were shown in (**Figure 2**). **(Table 2)** showed the mean and Standard Deviation (SD) for the participant-reported outcomes (VAS, KSS-Knee, KSS-Function, LEFS, and ROM) for each of the office visits, with the mean at each milestone compared to the corresponding baseline value. Statistically meaningful differences were shown for the individual milestone results compared to baseline, while the 2-year means were all statistically meaningful compared to baseline. The progression of the change in participant-reported VAS (n = 18 knees) through the 2-year milestone was shown in (**Figure 3**) and revealed a slight increase at the 2-year milestone from a low VAS score reached at 6-months through 1-year. (**Figure 4**) showed the change in participant-reported KSS-Knee (n = 18 knees) through the 2-year milestone with a slight decrease in median and mean outcomes from the 1-year to the 2-year milestones. Participant-reported KSS-Function (n = 18 knees) was shown in (**Figure 5**) through the 2-year milestone with slight decreases in the median and medium outcomes from the 1-year to the 2-year milestones. The progression of the change in participant-reported LEFS (n = 18) through the 2-year milestone was shown in (**Figure 6**) and showed a moderate decrease in median and mean outcomes from the 1-year to the 2-year milestones. Change

Table 1: Group characteristics of study participants who reached the 2-year milestone, where age, height, weight, and Kellgren-Lawrence scores represent mean (SD) and range. For sex and side of treatment, table entries represent counts. Nucleated cells in the BMC preparation represent mean (SD) and range.

Characteristic	
Patients	16
Knees Treated	18
Age, years	60.8 (7.4) 41–70
Height, in	67.7 (4.4) 47-70
Weight, lb	181.4 (35.3) 121–223
Sex, F:M	8:8
Side of treatment, L:R:B*	9:5:2
Kellgren-Lawrence score	2.9 (0.3) 2–3
Nucleated cells, x 10^6	214 30–745

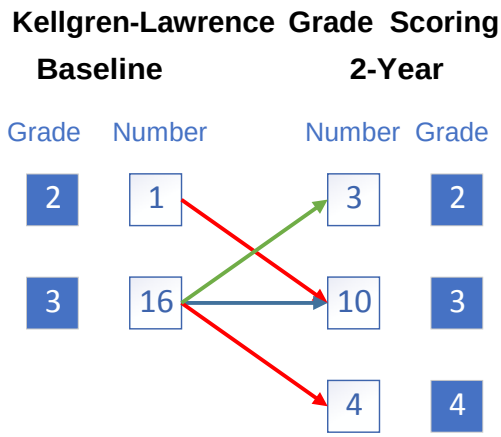

Figure 1: Change in Kellgren-Lawrence (KL) grades for the 17 knees (one KL grade wasn't obtained at 2-years) from Baseline to the 2-year milestone. Worsened KL grades are shown in red, improved KL grades are shown in green, while knees with no change in KL grade are shown in blue. The KL scores were assessed on AP, lateral, skyline and weight-bearing (when possible) X-rays of the knee at baseline and at the 2-year milestone.

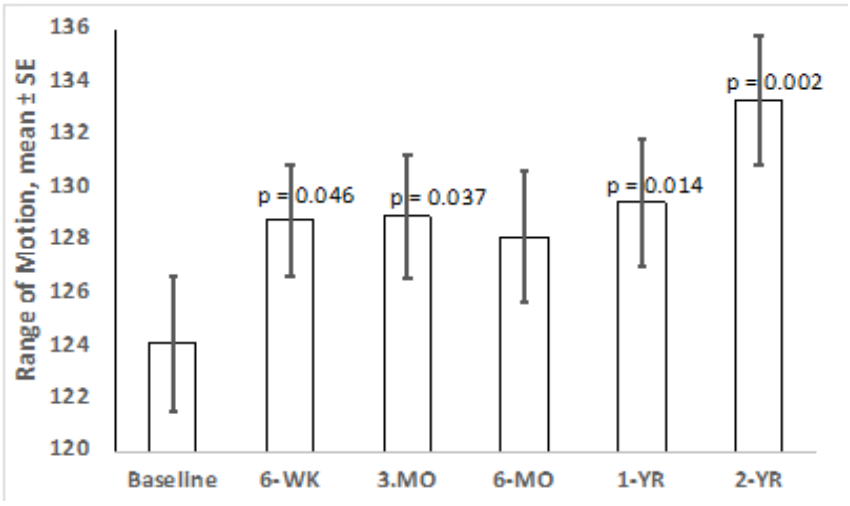

Figure 2: Range of motion of participants' knees at baseline and over 2 years following treatment in all available study participants. Statistical significance determined by RM ANOVA.

Table 2: Outcome measures expressed as mean ± SD obtained from all available study participants (18 at 2-year, 20 at 1-year and 22 at earlier time points) at baseline and over 2 years following treatment. ROM° is range of motion in degrees. The statistical significance of changes in patient-reported scores during the study period was determined by non-parametric RM ANOVA on ranks.

	Baseline (22)	6 weeks (22)	13 weeks (22)	6 months (22)	1 year (20)	2 years (18)	Significance*
VAS	5.1 + 2.0	2.8 + 1.9	2.5 + 2.0	2.6 + 2.3	2.3 + 2.4	2.6 + 2.4	p< 0.001
KSS-FXN	75.0 + 15.4	82.3 + 16.3	88,7 + 15.2	80.9 + 25.2	88.5 + 15.0	88.1 + 16.6	p < 0.001
KSS-Knee	74.4 + 11.3	87.7 + 7.6	88.5 + 8.1	86.0 + 12.0	89.7 + 10.8	89.9 + 11.6	p < 0.001 .
LEFS	45.8 + 14.1	52.7 + 14.8	58.6 + 16.1	57.6 + 17.0	62.8 + 14.6	57.3 + 15.4	p< 0.001
ROM	124.1 + 11.9	128.8 + 10.0	128.9 + 11.1	128.1 + 11.7	129.5 + 10.9	133.3 + 9.8	p = 0.003

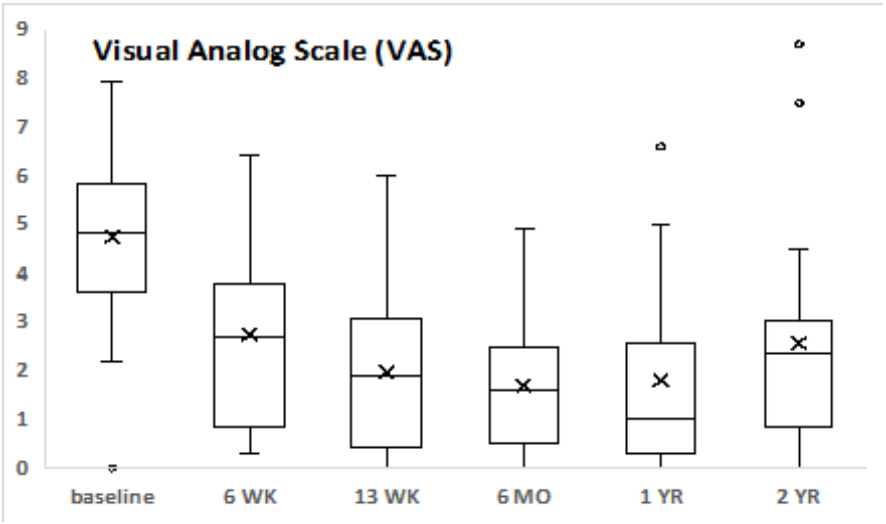

Figure 3: Reported VAS scores for study participants' knees who reached the 2-year milestone (n = 18). Results are illustrated as mean (X), median (solid line), 25-75% range (box) and minimum/maximum (vertical lines, with outliers as open circles).

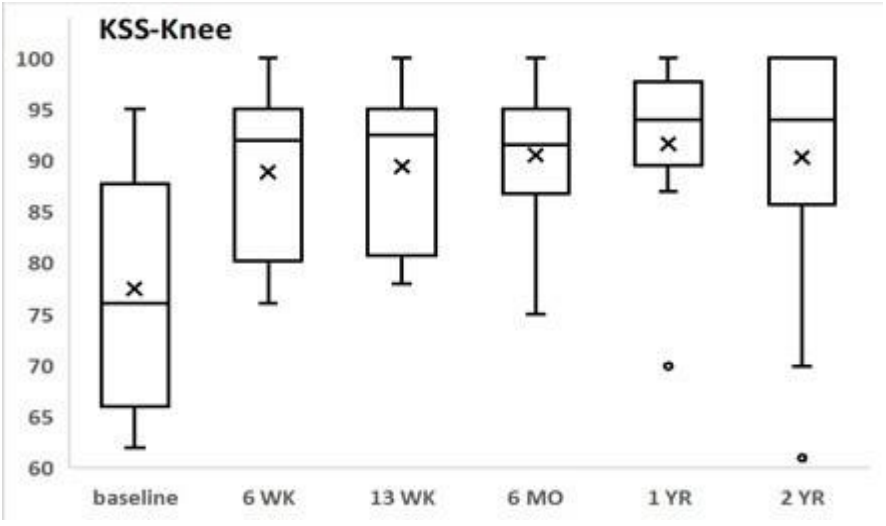

Figure 4: Reported KSS-Knee scores for study participants' knees who reached the 2 year milestone (n = 18). Results are illustrated as mean (X), median (solid line), 25-75% range (box) and minimum/maximum (vertical lines, with outliers as open circles).

in the 1- and 2-year milestones for the participant-reported outcomes for VAS, KSS-Knee, KSS-Function and LEFS were compared side-by-side with the baseline mean, median and range of outcomes as shown in (**Figure 7**). Comparison of the participant-reported outcomes with Total Nucleated Cell Number were weakly positive, but none of the comparisons was statistically meaningful ($p > 0.05$) (data not shown).

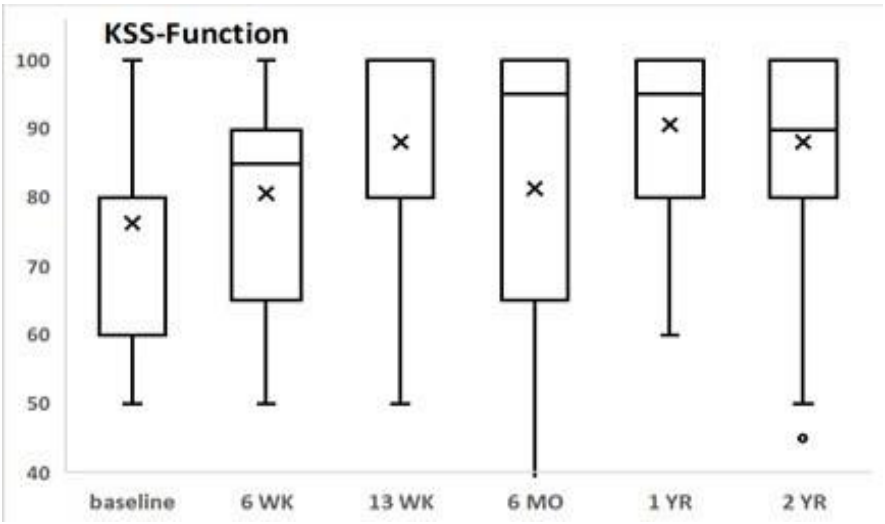

Figure 5: Reported KSS-Function scores for study participants' knees who reached the 2-year milestone (n = 18). Results are illustrated as mean (X), median (solid line), 25-75% range (box) and minimum/maximum (vertical lines, with outliers as open circles).

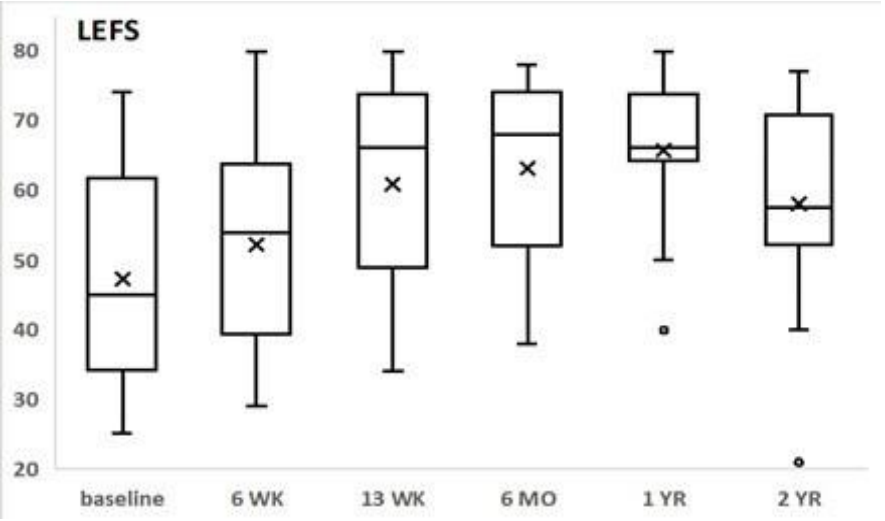

Figure 6: Reported LEFS scores for study participants' knees who reached the 2-year milestone (n = 18). Results are illustrated as mean (X), median (solid line), 25-75% range (box) and minimum/maximum (vertical lines, with outliers as open circles).

Discussion

This report shows the outcomes of a prospective, open-label clinical study of study participants out to the 2-year milestone who had received autologous BMC via both the IO and IA routes to treat knee OA. ROM was assessed during each office visit, along with participant-reported outcomes (VAS, KSS-Function, KSS-Knee and LEFS). The surviving participant cohort at the 2-year milestone showed durable statistically meaningful improvements in all metrics compared to the study participants at baseline, as shown in **(Table 2)**. However, as is evident in (**Figures 3-6**), there is increasing variability in the participant-reported outcomes at later milestones. (**Figure 7**) shows a box plot of each of the participant-reported outcomes for the 1-year and 2-year milestones in a side-by-side comparison with baseline outcomes, which shows that in three out of the four metrics the mean and median changed very little from the 1-year to the 2-year milestones. However, LEFS showed a more obvious decrease (i.e., less favorable) of approximately 8.8% over this interval. Overall, there was a statistically meaningful improvement in the participant-reported outcomes at 2-years compared to baseline. Additional support for a therapeutic benefit of the BMC treatment is shown in (**Figure 2**) for ROM. ROM showed a sustained positive outcome out to the 2-year milestone, which was statistically meaningfully different from baseline at the 1-year and 2-year milestones. In addition to ROM, another objective clinical assessment performed during the study was the change in KL grades from baseline to the 2-year milestone, which

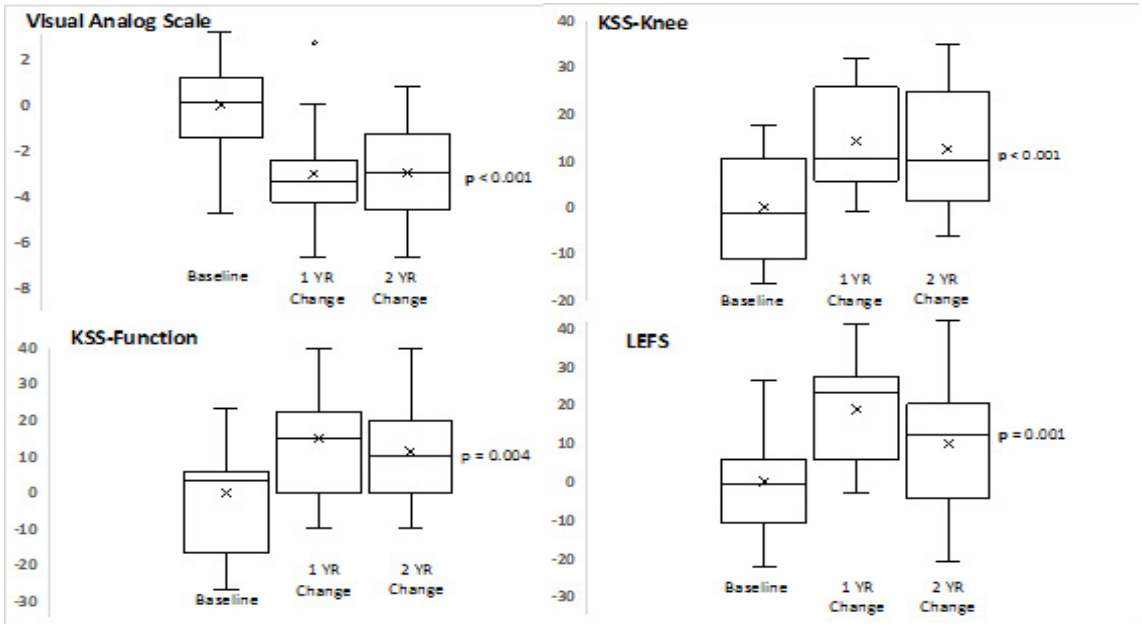

Figure 7: Change from baseline to the 1-year and 2-year milestones for participant-reported outcomes for study participants' knees who had reached the 2-year milestone (n = 18 knees). Results are illustrated as mean (X), median (solid line), 25-75% range (box) and minimum/maximum (vertical lines, with outliers as open circles).

is shown in (**Figure 1**) . Five of the study participants' knees had a KL grade that increased by one level, with four knees moving from KL 3 to KL 4 and one knee that shifted from KL 2 to KL 3. On the other hand, three KL 3 grade knees improved by one level to KL 2 at the 2-year milestone, while the majority of knees showed no change—remaining as KL 3 over the 2-year study period. Generally, knee OA is considered to be a progressively debilitating disease, so the absence of a worsening KL grade shift in most of the knees (70.5%) treated in the study points to the possibility of a stabilization of the study participants' knee OA. In fact, three knees showed a decrease in the KL scores at the 2-year milestone, which further supports the potential for stabilization of knee OA, as reflected in positive changes in the cartilage in these study participants' knees.

The potential for slowing the progression of knee OA has been reported in several recent publications. For example, in a study comparing IO versus IA treatment of patients with bilateral knee OA, both routes of BMC delivery showed a multi-year delay in the progression to TKA [15]. Assessment of the treated knees by MRI at the 2-year milestone showed that the volume of BMLs present in the femoral and tibial compartments at baseline had decreased for knees receiving IO-delivered BMC but had not decreased in the knees receiving IA-delivered BMC. The authors also reported that VAS remained statistically meaningfully higher at the 2-year milestone compared to baseline for patients receiving BMC via the IO route, but not via the IA route [15]. The same group reported in a separate study of BMC delivered via the IO route compared to TKA in patients with equivalent bilateral knee OA [18] that larger volume BMLs at baseline were positively associated with increased pain. Furthermore, patients showed a reduction in the BML volume from baseline to the 2-year milestone, while also observing an increase in cartilage volume of 2.3%. VAS remained lower for the patients receiving BMC treatment at all milestones after 6-months and remained lower out to the 15-year milestone, but the BMC-treated patients didn't show an improvement in ROM during the study [18]. Only one other clinical study has been published so far in which the BMC was split between delivery via the IO and IA routes in treating knee OA without an injection-based pre-treatment of the knee just prior to BMC treatment. In a summary of 1-year outcomes [19], the authors reported that there was meaningful improvement in IKDC (International Knee Documentation Committee) scores, all KOOS subscales and VAS scores and a meaningful decrease in bone marrow edema (assessed by MRI) through the 1-year milestone compared to baseline. However, the EQ-VAS (EuroQol-Visual Analogue Scale—a measure of a patient's generic health status) failed to show improvement [19]. In a subsequent publication of the IO/IA combined BMC treatment [20], IKDC and KOOS metrics remained elevated out to the 2-year milestone, while there was no meaningful improvement in the EQ-VAS at 2-years compared to baseline. VAS increased by 1.4 points from the 1-year to the 2-year milestones, which was a statistically meaningful worsening of the VAS metric, but the 2-year VAS score remained statistically meaningfully improved compared to baseline. No data was presented on the status of ROM in the patients for any of the clinical milestones in the two related publications [19, 20]. In the current study, ROM improved throughout the 2-year study period and VAS only increased from the 1-year to the 2-year milestone by 0.3 points, as shown in **(Table 2)**.

Treating knee OA with BMC via either the IO or IA routes only [15, 18] or with a combination of IO and IA delivery showed consistent reduction in pain and higher QoL outcomes when compared to baseline at 2-years [20 and this study], while sustained therapeutic benefit also was demonstrated out to the 15-year milestone [15, 18]. However, there are differences among the four studies. For example, ROM didn't improve in one of the studies with IO delivery only [18], whereas in the current study ROM improved over the 2-year study period. All of the studies reported on changes in KL grade scores of study participants, but the majority of KL grade scores showed no

change from baseline to final milestone in the current study as well as in the other reports [15, 18, 20]. The rate of progression to surgery was 1.3% knee-year for IO delivery [15], 4.6% knee-year for IA delivery [15], 6.7% knee-year for IO/IA delivery [20] and 7.5% knee-year for IO/IA delivery in this current study. The number of patients is relatively low for the two IO/IA combination studies, with just 22 knees [16] or 30 knees [19] at enrollment, whereas the other two studies had 60 knees [15] or 140 knees [18]. None of the publications in which patients were treated with IO BMC, including this study, reported on stratification of the enrolled patients according to the type or subtype of OA present. Patient stratification for OA pathology has been suggested as a confounding source of variability affecting efficacy of OA treatments [7, 21]. Furthermore, there are several differences in the study participants' baseline demographics reported in the 1-year publication of this study [16] and the other IO/IA combination study [19], including average KL grade (2.6 in [19] and 2.9 in [16]), average age (56.4 years in [19] and 60.1 years in [16]) and Body Mass Index (25.5 in [20] and 27.6 in [16]). In this study 20% of the BMC was delivered via the IA route and 80% of the BMC was injected into the tibial plateau via the IO route, compared to 33% of the BMC delivered via the IA route, with 67% of the BMC injected in both the femoral condyle and tibial plateau in the other IO/IA study [19]. A potentially key difference between the two IO/IA studies is that in this study 100% of the bone marrow aspirate was obtained from the iliac crest, whereas tibial plateau-aspirated bone marrow was concentrated and delivered via the IA route in the other study [19]. These methodological differences might have contributed to the improvement of the KL grades for three study participants' knees in the current study, while no study participants were reported to have shown an improved KL grade for the other IO/IA study at the 2-year milestone [20]. Of course, one of the significant variables among clinical studies in which BMC is evaluated for therapeutic benefit is how the BMC is obtained, which will depend on the bone marrow aspiration technique, volume of aspiration and the method of concentrating the bone marrow aspirate. Previous publications have shown that the composition of the BMC obtained will vary and depends on the commercial technology used [22, 23].

In contrast to the results obtained in this study and other recent publications [15, 18, 20], a meta-study [24] of clinical studies in which BMC was used to treat knee OA concluded that "…(BMC) has not demonstrated clinical superiority in relation to other biological therapies commonly used in the treatment of OA…". The conclusion is puzzling but might be accounted for by the cutoff date for study inclusion (July 2020) or other exclusionary actions taken by the authors [24]. However, while the analysis considered clinical studies from Level I through Level IV, it isn't clear why one publication [18] with a very positive outcome for treating knee OA with BMC out to a 15-year milestone wasn't included in the meta-analysis. On the other hand, the authors of a more recent meta-analysis [2] of Level 1 clinical studies on the treatment of knee OA with PRP or BMC compared to Hyaluronic Acid (HA), with a study inclusion cutoff of August 2022, concluded that patients receiving PRP or BMC to treat knee OA "… can be expected to experience improved clinical outcomes when compared with patients who receive HA." [2]. Neither of these meta-analyses included studies in which the BMC was delivered via the IO route, which suggests that the durable outcomes of IO-delivered BMC reported for treating knee OA in this study and others [15, 18, 20] might contribute in the future to a more uniform consensus on the beneficial use of BMC in treating knee OA.

There are several limitations of the study. The study period extended out to just 2-years but was sufficiently long enough to confirm that the therapy was safe and provided a durable therapeutic benefit for treating knee OA patients with BMC delivered via a combination of IO/IA routes. Objective clinical measures were limited to ROM and X-ray imaging, which were routinely relied upon by the first author (MBS) in managing patients treated with BMC for knee OA. Finally, the pilot study enrollment was limited to 20 participants (22 knees) but was affected by patients deciding to leave the study, while others required surgical intervention. An initially larger number of participants would have been a better strategy to balance the loss of a few study participants during the 2-year period of the study.

Conclusion

The results demonstrated in this study clearly support the benefit of treating mild to moderate knee OA with BMC delivered via a combination of IO and IA routes during the same treatment. The extension of the study period to the 2-year milestone provides additional support for the safety of the combination treatment. While the number of treated knees is low, clear therapeutic benefit was demonstrated with a majority of knees (70.5%) not progressing to a worsened KL grade, while for three study participants there was a 1-grade improvement in the KL scores. ROM also showed a durable and statistically meaningful improvement over the 2-year study period compared to baseline. Participant-reported outcomes of KSS-Function, KSS-Knee and VAS showed little to no change in the means or medians from the 1-year to the 2-year milestone. However, the LEFS mean decreased by 5.5 points (8.8%), indicating a worsened outcome from the 1-year to the 2-year milestone. The results of this study are largely in agreement with the therapeutic benefit reported in the one other clinical study in which IO/IA delivery of BMC was evaluated. There also is support for the positive therapeutic outcomes obtained at the 2-year milestone of this study in the 15-year follow-up reported in two publications that demonstrated a 3.5-fold reduction in the rate of OA progression when OA knees were treated with BMC via the IO route compared to the IA route. Overall, the use of a combination of IO and IA delivery of BMC was shown to be well tolerated and safe, while also demonstrating a durable therapeutic benefit in treating mild to moderate knee OA over a 2-year study period.

Author Contributions

MBS, ML and TTS contributed to the conception and design of the study. MBS and ML participated in patient recruitment and treatment. MBS, ML and TTS participated in the acquisition and management of the study data. TTS organized the data analysis performed by a consultant (Daniel Gingerich, Turtle Creek Biostatistical Services). All authors contributed to the drafting or revision of the manuscript and have approved the submitted version of the manuscript.

Role of the Funding Source

Celling Biosciences sponsored the clinical study by supporting the treatment of study subjects with device technology, as well as performing laboratory analysis of the BMC preparations. They also supported TTS to coordinate the data analysis and drafting of the manuscript and supported the statistical analysis.

Competing Interests

TTS was employed by Celling Biosciences when he participated in the conception and design of the study. Subsequently, TTS was

reimbursed by Celling Biosciences for contracted work on the manuscript. MBS and ML report no conflicts of interest.

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