

Real-world evidence of mesenchymal stem cell therapy in knee osteoarthritis: a large prospective two-year case series

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Objective: To evaluate the long-term safety and efficacy of adipose-derived mesenchymal stem cell (ADMSC) therapy in the treatment of knee osteoarthritis (OA). **Methods:** 329 participants with knee OA underwent intra-articular ADMSC therapy. Participants were followed up for 24 months and were separated based on radiological OA grade. **Results:** Treatment was well tolerated with no related serious adverse events. All participant groups reported clinically and statistically significant pain improvement. Clinical outcome was not influenced by patients' age or BMI. **Conclusion:** ADMSC therapy is an effective, safe and long-lasting treatment option for knee OA with the potential to delay total joint replacement. In addition to the observed clinical benefits, ADMSC therapy promises to reduce the global economic burden of OA.

Trial registration number: ACTRN12617000638336

Plain language summary: The aim of this study was to assess the benefit of stem cell therapy in the treatment of mild to severe knee osteoarthritis. A total of 329 study participants with painful knee osteoarthritis undertook stem cell therapy and were followed up for two years.

Stem cell therapy was well tolerated and safe. Significant pain and functional improvement were observed in all of the participant groups including those with severe bone-on-bone osteoarthritis. Participants' age and weight did not influence the clinical outcome.

This study shows that stem cell therapy is an effective, safe and long-lasting treatment for knee osteoarthritis and greatly reduces knee pain and improves the function of the knee. Stem cell therapy may delay or prevent knee replacement surgery and result in significant global health and economic benefit.

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Keywords: adipose-derived mesenchymal stem cells • arthritis • mesenchymal stem cells • osteoarthritis • stem cells • total knee replacement

Arthritis is a progressive degenerative condition and a leading cause of disability worldwide [1,2]. Work-related inefficiencies and costs due to symptomatic arthritis account for up to 0.5% of international GDP and up to 2.5% of GDP in developed countries [3,4]. Osteoarthritis (OA) is recognized as the most common form of arthritis and the second most common musculoskeletal complaint seen by general practitioners with an observed 26.4 consultations for OA per 1000 general practitioner encounters [5]. Up to 10% of males and 18% of females over the age of 45 have symptomatic arthritis with the average length of time living with OA-associated poor quality of life being 30 years [6–8]. The medical cost of OA is estimated at over US\$27 billion annually in the USA, with a total economic cost of \$100 billion [9,10]. With an aging population, OA is projected to remain a significant healthcare burden with an increasing global socioeconomic impact.

As there is no cure or long-term effective treatment available for OA sufferers, OA is internationally recognized as an unmet clinical need. Current conservative management strategies for OA are aimed at symptomatic management and do not achieve disease modification. Importantly, pharmaceutical therapies including the use of simple analgesics and oral anti-inflammatories are limited in their effectiveness and may also be associated with a significant risk of adverse side effects and related long-term complications [11,12]. Education regarding weight loss remains a critical conservative measure with a 10% weight loss corresponding to a 50% reduction in pain in many individuals [13]. Furthermore, an appropriate functional and prescribed exercise program may be associated with a similar improvement in pain when compared with regular anti-inflammatory use [14].

Commonly used injectable therapies for symptomatic OA include corticosteroids and the visco-supplement, hyaluronic acid (HA). Despite corticosteroids being recommended in international clinical practice guidelines, including those published by the Osteoarthritis Research Society International and the National Institute of Health and Care Excellence, there is a growing awareness that continued and repetitive use of intra-articular (IA) corticosteroids leads to an increase in OA progression [15]. Further, systematic review of trials assessing the use of corticosteroids in the treatment of knee OA has shown limited benefit beyond 3–4 weeks [16]. Similarly, with HA use in mild to moderate knee OA, a formal systematic Cochrane review has indicated an initial reduction in weight-bearing pain at 5–13 weeks postinjection, but benefit are unpredictable beyond 6 months and of limited benefit beyond placebo [17].

In addition to corticosteroids and HA, autologous platelet-rich plasma (PRP) or platelet-rich concentrate (PRC) variants are increasingly being used by musculoskeletal clinicians in the treatment of symptomatic knee OA. While formal research indicates a reproducible improvement for 6–12 months following PRP therapy in moderate knee OA, the use of PRP remains debated due to a lack of agreement on the method of preparation and, therefore, difference in the therapeutic product between trials [18–20]. Unfortunately, no current injectable therapies are associated with disease modification or long-term sustained clinical symptom relief.

Management of OA where conservative measures have failed remains total joint replacement. In the USA, over 700,000 total knee replacements (TKRs) are performed annually. With an aging population, this incidence is anticipated to grow alarmingly by over 600% between the years 2005 and 2030 [21,22]. Of particular concern is the increasing proportion of TKRs being performed in patients under the age of 65, with these surgeries being 2.5-times more likely to require revision surgery, which carries a far greater risk and failure rate [23,24]. Despite TKR being accepted as a suitable treatment for end-stage knee OA, it remains an unpredictable operation with up to 20% of patients experiencing a persistent loss of function and/or pain at 12 months, with additional research suggesting persistent severe/extreme pain in 15% of patients at 3–4 years postsurgery [25,26]. Up to 30% of patients are dissatisfied with their outcomes following TKR [27]. Furthermore, 2% of all TKR surgeries have been reported to have major surgery-related serious complications, including myocardial infarct, stroke, infection and death, with a 90-day postoperative mortality rate of 0.6% [28,29].

In regard to the pathophysiology of OA, there exists an imbalance between anabolic and catabolic/inflammatory pathways due to mechanical or biological insult to the knee microarchitecture, with aging further exacerbating this imbalance and the cartilage matrix being even less capable of maintaining homeostasis when stressed [30–33]. The release of reactive oxygen species (free radicals) leads to an observed chondrocyte cell senescence with accompanied upregulation of inflammatory cytokines including IL-1 and TNF α ; matrix metalloproteinases such as MMP-13 and reduced anabolic growth factor response with resultant extracellular matrix degradation [33]. This ultimately leads to joint deformity with abnormal structural features including cartilage degeneration with subsequent joint space loss, synovitis, osteophyte development and subchondral bone deformity with sclerosis and cyst formation, resulting in pain and reduced mobility [34].

Mesenchymal stem cells (MSCs) display an ability to differentiate along a mesodermal lineage and develop connective tissue including, bone, muscle and cartilage leading to considerable interest in their application in orthopedic/musculoskeletal medicine. While this cellular multipotency suggests an intrinsic potential role in tissue repair and regeneration, it is now more broadly accepted that MSCs exert their reparative effects through paracrine and cell–cell interactions with the immunomodulatory and trophic effects of these interactions leading to tissue repair/homeostasis and the restoration of joint function [35].

Preclinical trials have consistently indicated the benefit of MSC therapy in OA with observations of reduced lameness and both structural stabilization and repair in induced and naturally occurring animal OA models [36–39]. These preclinical results have been supported by early phase I & II clinical trials whereby the use of culture-expanded

MSC therapies has also resulted in reproducible pain and functional improvement with additionally observed disease modification effects whereby cartilage degradation was stabilized or cartilage volume increased [40–42].

Importantly, a formal systematic review/meta-analysis of 36 published clinical trials assessing the therapeutic application of intravascularly delivered autologous or allogeneic culture-expanded MSCs (a combined total of over 1000 participants) showed no evidence of serious adverse events (AEs) [43]. Additionally, a systematic review of eight clinical trials of IA-delivered MSC therapies (assessing a combined 844 procedures with a mean follow-up of 21 months) showed a similar absence of association with serious AEs such as infection, cancer or death [44].

In a recently published randomized controlled trial (RCT), the authors of this paper assessed the use of autologous culture-expanded adipose-derived MSCs (ADMSCs) in the treatment of moderate knee OA [42]. In a dosing protocol, ADMSC therapy was well tolerated with effective clinical and statistically significant pain and functional improvements coupled with MRI-confirmed structural stabilization of OA. The purpose of this study was to complement the published RCT with real-world evidence (RWE) in a much larger patient cohort of 329 patients, effectively assessing whether the use of ADMSC therapy is both safe and effective in the management of OA in the broader community over a 2-year period.

Materials & methods

This case series represents a combined and collective outcome report from an initial trial approved by the Human Research Ethics Committee (HREC) of Monash University (Australian New Zealand Clinical Trial Registry: ACTRN12615000258550) and a later case series extension approved by the HREC of Charles Sturt University (Australian New Zealand Clinical Trials Registry: ACTRN12617000638336). The case series is a prospective single-center trial with all participants undergoing a formal screening procedure prior to inclusion in the trial, with signed informed consent provided by all participants.

Enrolled participants underwent an initial lipoharvest procedure and later IA injections of cultured-expanded ADMSCs at baseline and again at the 6-month time point. Participants were recruited over a 5-year time period with a total of 609 participants assessed for inclusion with 425 meeting the inclusion criteria and being formally enrolled in the case series. Of the enrolled participants, 39 were later excluded/withdrawn from the case series as they underwent additional therapies for symptomatic OA within the period of follow-up, which were assessed as potential positive confounding factors to the observed outcome. An additional five participants withdrew their consent. Of the 381 enrolled and consenting participants, 52 participants were lost to follow-up over 24 months with a total of 329 participants completing follow-up as part of the case series (see Figure 1).

Participant selection criteria

Eligible participants were 18 years of age or older with documented OA of the knee using conventional radiography (x-ray) or MRI. Grade I–IV OA was included to enable the assessment of ADMSC efficacy against the severity of the disease. Both primary and secondary OA were accepted. All participants had attempted other conservative management options including the use of simple analgesics, exercise and weight management programs and a trial of biomechanical/orthotic adjustment, where relevant. Formal eligibility criteria are outlined in Table 1.

Baseline eligibility assessment involved a formal musculoskeletal assessment including physical examination and review of diagnostic imaging. All eligible patients received written information regarding the trial and written information/education regarding accepted OA treatment options/alternatives. Prior to the commencement of the trial, all participants completed a formal written informed consent.

Participants were graded into categories of mild, moderate or severe OA according to internationally recognized radiological criteria (Table 2). Both conventional radiography (x-ray) and MRI were used for the assessment of OA severity. The Park Grading System was used in the assessment of MRIs, as this has been shown to correlate highly with Kellgren–Lawrence grading (Table 3) [46]. Participants with evidence of focal chondropathology were included in the mild OA category.

Autologous adipose-derived mesenchymal stem cell preparation

Lipoaspirate harvest procedure

Adipose tissue is an attractive source of autologous MSCs due to its ease of harvest and the relative abundance of MSCs in comparison to alternative sources (3% of the whole cell population within adipose-derived stromal vascular fraction, which is a 2500-fold increase compared with the MSC proportion found in bone marrow concentrate/aspirate) [49,50]. In addition, ADMSCs exhibit retention of their multipotency with reproducible

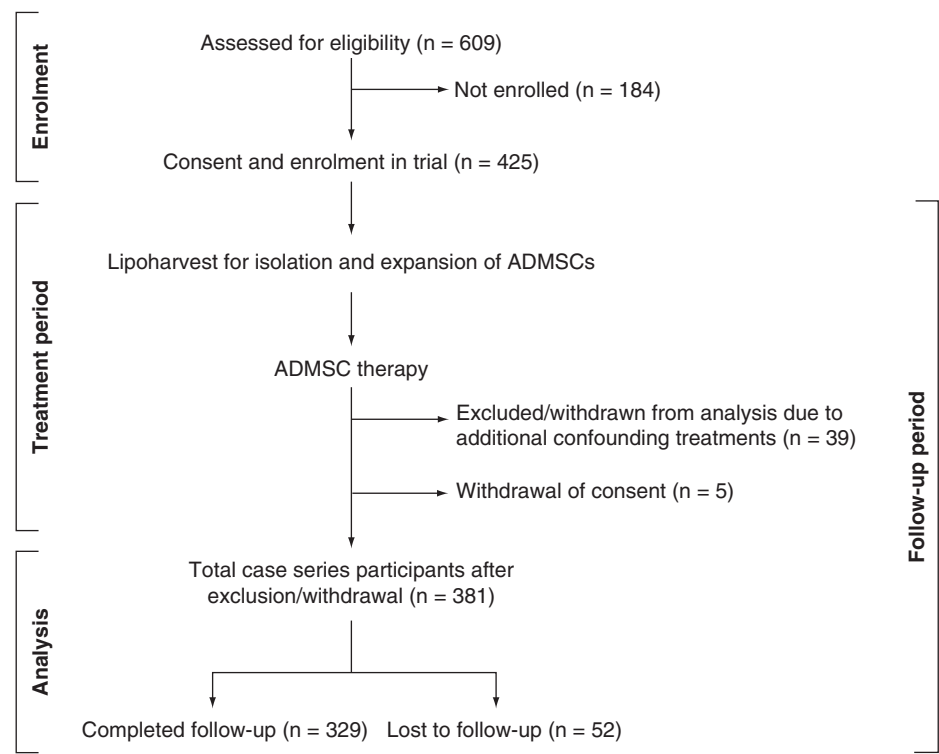


Figure 1. Participant recruitment and follow-up.

Table 1. Eligibility criteria.	
Inclusion criteria	
1. Radiological diagnosis of osteoarthritis/chondropathology of the knee.	
2. Age 18 years or over.	
3. Primary conservative treatment already undertaken including analgesia/anti-inflammatory medication, attempted prescribed exercise and weight management program and biomechanical adjustment including bracing if appropriate as prescribed by a physiotherapist, podiatrist or medical practitioner.	
4. Sufficient English skills to complete the questionnaires required for the study.	
5. Normal ECG and blood screening for acceptable biochemistry/hematology profile.	
Exclusion criteria	
1. Age <18 years.	
2. Pregnant.	
3. Breastfeeding.	
4. Have any other causes of their symptoms suspected to be due to serious pathology such as tumor or referral from the spine.	
5. History of inflammatory arthropathy.	
6. Current cancer.	
7. History of significant organ impairment/failure (i.e., renal failure).	
8. History of allergy to any substances used in ADMSC preparation and treatment.	
ADMSC: Adipose-derived mesenchymal stem cells.	
Table 1 reproduced from a previous publication with permission from Regenerative Medicine as agreed by Future Medicine Ltd. [45].	

Table 2. Osteoarthritis severity.				
Imaging modality	Grading system	OA category		
		Mild	Moderate	Severe
x-ray	Kellgren-Lawrence	Grade I	Grade II/III	Grade IV
MRI	Park Grading System	Grade I	Grade II/III	Grade IV
MRI	ICRS	Focal chondropathology: ICRS grades II–IV		NA

ICRS: International Cartilage Repair Society; OA: Osteoarthritis.

Table 3. Park MRI Scoring System of osteoarthritis.

Grade	Description
0	No cartilage pathology [†] with no or minimal osteophyte (<5 mm).
I	Cartilage pathology grade I with one or more of the following: osteophytes >5 mm, bone marrow edema >10 mm, subchondral cyst >10 mm.
II	Cartilage pathology grade II with one or more of the following: osteophytes >5 mm, bone marrow edema >10 mm, subchondral cyst >10 mm.
III	Cartilage pathology grade III with one or more of the following: osteophytes >5 mm, bone marrow edema >10 mm, subchondral cyst >10 mm.
IV	Cartilage pathology grade III and meniscal pathology [‡] grade III.

[†] Cartilage pathology graded according to the Noyes classification [47].
[‡] Meniscus pathology graded according to the Stoller classification [48].

chondrogenic differentiation potential and inherent immunomodulatory properties after repeat expansion/long-term passaging [51,52]. All participants underwent a minimally invasive liposuction procedure (lipoharvest) as formally described in past publications [42,45,53–55]. The procedure was performed using local anesthetic tumescent control with an adequate lipoaspirate (typically 20–100 ml) collected using either a manual syringe suction technique or mechanical suction into a sterile single-use container (Shippert Medical, CO, USA). The lipoharvest was performed abdominally or under ultrasound assistance using the lateral thigh. At the completion of the procedure, the lipoaspirate was transferred directly (via an airlock connection) from the operating theatre to an onsite cleanroom laboratory facility (Magellan Stem Cells, Victoria, Australia).

Isolation & expansion of mesenchymal stem cells

Formal isolation and expansion protocols for ADMSCs have previously been published [42]. Briefly, lipoaspirate was rinsed with sterile phosphate-buffered saline to remove any red blood cells and underwent enzymatic digestion as per the standard operating procedures (SOPs) developed by Magellan Stem Cells. The cleanroom facility in which all ADMSC processing was performed had an air quality of ISO5 or greater. All manual tasks/tissue handling were performed by qualified laboratory staff in class II biological safety cabinets. Isolated stromal vascular fraction (SVF) cells were seeded in tissue culture flasks with basal growth media supplemented with fetal bovine serum (FBS) and incubated at 37°C until 80% confluency of the cells was observed under microscope examination. Cells underwent repeated passage until the expansion of the predetermined total treatment dose (100 million ADMSCs). Cell expansion was not performed beyond passage four.

At the completion of cell expansion, cultured ADMSCs were harvested and cryopreserved under strict cryopreservation conditions using a validated control rate freezing method and stored in liquid nitrogen until required [56,57]. Isolated cell populations were characterized using flow cytometry with fluorescence-activated cell sorting (FACS) and assessed according to standards established by the International Society of Cellular Therapy [58]. All isolated cell samples were assessed to confirm a ≥95% cell population with the expression of MSC-specific surface markers CD90, CD73 and CD105 and the absence of hematopoietic surface markers CD14, CD19, CD34 and CD45 (≤2% cell population). In addition to FACS analysis and prior to commencement of treatment, all isolated culture-expanded ADMSC samples were analyzed by an independent pathology provider for microbial contamination to ensure an aseptic technique and the absence of any microbial contamination.

Carrier media

On the day of injection, MSCs were resuspended in 4 ml of a suitable carrier media. Carrier media were either autologous conditioned serum (ACS), autologous platelet lysate (APL) or an inert sterile isotonic solution (i.e., Plasma-Lyte 148). Each participant used the same carrier media at both their baseline and 6-month injection.

ACS preparation involved the initial withdrawal of a total of 27 ml of whole blood via venipuncture using 3 × 9 ml sterile S-Monovette[®] clotting activator tubes (Sarstedt, Numbrecht, Germany). Following a period of incubation at 38°C over 24 h, the S-Monovette[®] tubes underwent centrifugation at 1000 r.p.m. for 5 min. At the completion of centrifugation, the separated serum layer was aspirated and passed through a 0.2-micron syringe filter (PALL, NY, USA) to produce a final acellular sample of ACS.

APL carrier media preparation involved the initial withdrawal of 25.5 ml of whole blood via venipuncture, collected in 3 × 8.5 ml ACD (trisodium citrate 22.0 g/l, citric acid 8.0 g/l, and dextrose 24.5 g/l) BD Vacutainers (BD, NJ, USA). The tubes were placed in a benchtop centrifuge and underwent centrifugation at 1000 r.p.m. (soft spin) for 10 min with the resultant separated plasma layer collected in a single sterile tube. The tube was

Table 4. Outcome measures.

Measure	Measurement point (months)
Primary outcome measures:	
1. Adverse events	0, 6, 12, 24
2. Numeric Pain Rating Scale	0, 6, 12, 24
Secondary outcome measures:	
3. Knee Injury and Osteoarthritis Outcome Score (KOOS)	0, 6, 12, 24
4. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)	0, 6, 12, 24
5. Patient Global Impression of Change (PGIC)	24

centrifuged at 3500 r.p.m. (hard spin) for a total of 5 min with the formation of a platelet concentrate plug and platelet-poor plasma (PPP). The excess PPP was removed leaving approximately 6 ml of PPP and a platelet plug that was reconstituted in the reduced PPP using a gentle manual agitation method, creating a PRP preparation. Following the addition of calcium chloride (0.2 ml at 10%), the PRP was incubated at 37°C for 10 min allowing the formation of a fibrin clot that was allowed to retract and resorb over time. A 0.2-micron syringe filter (PALL, NY, USA) was used to produce an acellular and sterile APL. Autologous carrier media preparations were stored at -20°C until required.

Intervention

Injection method

Participants underwent a baseline IA injection of 50 × 10⁶ ADMSCs and another injection at a planned 6-month time point (average time of second injection 6 months + 3 weeks). Five participants received a single ADMSC injection at baseline only. Cryopreserved autologous ADMSCs were removed from cryostorage on the day of injection and thawed in a prewarmed sterile water bath. The cryoprotectant media was removed by centrifugation and washed with chilled sterile phosphate-buffered saline before resuspending the washed cells in 4 ml of the appropriate carrier media. Post-thaw cell count and viability were assessed using a Muse Cell Analyzer (Merck Millipore, MA, USA). The resuspended cells were drawn into a sterile syringe for injection. In accordance with the SOPs of the clinical laboratory, Magellan Stem Cells, the minimum acceptable cell viability was 90%.

All IA injections of ADMSCs were performed under ultrasound guidance with the site of injection prepared following standard sterility protocols. As part of standard care and prior to injection of ADMSCs, 2 ml of 1% lidocaine was injected superficially to the joint capsule with care taken to ensure no infiltration into the IA space. Using a superolateral approach to the patella, the resuspended autologous ADMSCs were injected intra-articularly into the knee joint cavity. Following ADMSC therapy, compression was applied to the knee with the use of a Tubigrip garment with additional application of an ice pack.

Outcome measures

The primary objective of this trial was to assess the safety/tolerability of ADMSCs over a 24-month period and determine long-term changes in pain as measured by the validated Numeric Pain Rating Scale (NPRS). A secondary objective was to measure additional pain and functional outcomes using the Knee Injury and Osteoarthritis Outcome Score (KOOS), the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and the Patient Global Impression of Change (PGIC; Table 4).

Questionnaires

Participants completed subjective questionnaire-based outcome measures at baseline, 6, 12 and 24 months.

- NPRS: average pain intensity over the past week was graded by participants using an 11-point scale of 0–10 [59]. A decrease of 1 point or more on an 11-point NPRS was defined as a minimal clinically important difference (MCID) [60];
- KOOS: the KOOS questionnaire is designed to assess both short- and long-term patient-relevant outcomes following a knee injury and/or OA. It consists of five subscales: pain, symptoms, function in daily living (ADL), function in sport and recreation (S & R) and knee-related quality of life (QoL). A subscale score of 100 indicates no symptoms and 0 indicates maximal symptoms [61]. An improvement of 8 points or more was defined as a MCID [62];

- WOMAC: a validated QoL score used to assess patients with symptomatic OA [63]. In this trial, it is expressed as a percentage/value to be comparable with the KOOS subscales [61]. In addition, an improvement of 8 points or more was defined as an MCID to allow comparison with KOOS;
- PGIC: a self-report measure used to indicate the participant's belief regarding the effectiveness of treatment [64]. PGIC is a 7-point scale with participants rating change as 'fully/completely recovered', 'much improved', 'slightly improved', 'no change', 'slightly worse', 'much worse' or 'very much worse'.

The software program Clinical Intelligence (Clinical Intelligence, Melbourne, Australia) was used to record outcome measures and was accessible to participants remotely via online login with an emailed reminder at time points of follow-up.

Sample size

No sample size calculation was performed as this represents an RWE case series.

Statistical analysis

Sigma Plot 10 graphical and statistical software (Systat Software, Inc., CA, USA) was used to perform all statistical analyses. One-way repeated-measures ANOVA was used to determine mean differences in time (baseline vs 6, 12 and 24 months) for each quantitative outcome measure. Subset analyses using one-way repeated-measures ANOVAs were also calculated on grade of OA, carrier media and compartment of OA analysis. The all-pairwise multiple-comparison procedure (Holm-Sidak method) was employed when statistically significant ($p < 0.05$) interactions between the different time points were apparent. The overall significance level was set at $p = 0.05$. Simple linear regression analyses were also performed to assess the predictability of functional outcomes when accounting for age and BMI. MCID was used to define clinically meaningful/significant improvement in pain and function.

Adverse events

AEs were recorded throughout the follow-up period of 2 years. Treatment-emergent AEs (TEAEs) were defined as undesirable clinical occurrences that either increased in severity after treatment or were not present prior to treatment. TEAEs were additionally defined as related or unrelated to the treatment and expected or unexpected based upon prior published MSC clinical trials. AEs were graded by severity:

- Mild: a transient/short-term symptom that resolves with conservative management including the use of simple analgesia and that does not interfere with the patient's usual activity;
- Moderate: a symptom that requires regular intervention including prescription analgesia (i.e., opiate analgesia) and which limits the patient's ability to perform ADLs for 1–2 weeks;
- Severe: a symptom that may require the use of regular prescription analgesia and that significantly limits the patient's ability to perform ADLs and extends for >2 weeks;
- Serious: defined as a significant medical event resulting in either a life-threatening condition, hospital admission and/or chronic and significant disability or death.

Results

Demographic characteristics

In this case series, 425 participants were formally enrolled after undergoing an initial screening process with 39 participants later excluded owing to interventions received outside of the trial during the follow-up period that may have improved/impacted their outcome. An additional five participants withdrew their consent to participate. Of the remaining 381 participants, 52 were lost to follow-up. The final case series was of 329 participants with an age ranging 23–85 years, with a mean age of 58.6 years and with 60.6% of participants being male (Table 5). The primary site of OA was in multiple compartments (145 knees) and was hence categorized as multicompartmental OA.

Participants had a mean BMI of 28.5 kg/m^2 with 30.7% of participants categorized as obese or greater. Participant occupation was recorded as per the International Standard Classification of Occupations [65]. Occupational physical activity was separated into high, medium and low using previously published criteria [66].

Table 5. Demographic characteristics.

Demographic characteristics	Data
Total number (n)	329
Gender % (male/female)	60.6/39.4
Age (years) (mean $\pm$ SD)	58.6 $\pm$ 11.8
BMI (kg/m ²) (mean $\pm$ SD)	28.5 $\pm$ 5.1
Site of OA:	
– Medial compartment	124
– Lateral compartment	15
– Patello-femoral compartment	45
– Multicompartment	145
Grade of OA:	
– Mild (grade I)	21
– Moderate (grade II–III)	101
– Severe (grade IV)	207
Occupation (ISCO-08):	
– Managers	51
– Professionals	78
– Technicians and associate professionals	22
– Clerical support workers	4
– Services and sales workers	24
– Skilled agricultural, forestry and fishery workers	12
– Craft and related trades workers	22
– Plant and machinery operators and assemblers	3
– Elementary occupations	15
– Armed forces occupations	1
Occupational physical activity:	
– High	66
– Medium	39
– Low	127

ISCO-08: International Standard Classification of Occupations; OA: Osteoarthritis.

Pain & function

Of the 329 participants, 32 failed therapy and after 12 months of follow-up underwent surgical TKR. Of the 32 participants who failed treatment, three participants were in the moderate OA group and 29 participants were in the severe OA group. All data beyond 12 months represents that of the remaining 297 participants. Importantly, there was no statistically significant difference in outcome data (NPRS and WOMAC) in the first 12 months when comparing results of the entire 329-participant cohort with those who did not undergo TKR after 12 months (297 participants; see [Supplementary Material](#)). Consistent clinically and statistically significant improvement in pain and function was seen across the period of follow-up in all severities of OA ([Figure 2B & 4B](#)). The most dramatic improvement occurred over the initial 12 months with improvement maintained or improved further until completion of follow-up at 24 months.

Overall, pain, as measured by NPRS, showed clinically and statistically significant improvement against baseline at all time points of follow-up, improving from a mean ($\pm$ SD) score of 5.2 ($\pm$ 2.3) at baseline to 2.4 ($\pm$ 2.2) at 24 months ([Figure 2A](#)). Participants experienced an average 54% improvement in pain over the 24 months. Maximum improvement was seen in the moderate knee OA group with a 70.2% improvement in pain scores compared with a 44.8% improvement in severe OA. There was a statistically significant difference ($p < 0.001$) in NPRS scores between moderate and severe knee OA cohorts at the 24-month time point ([Figure 2B](#)).

Improvement in NPRS scores correlated with KOOS subscale changes with clinically and statistically significant improvement in all subscales at all time points. Observed improvements were essentially stable and maintained through 24 months of follow-up ([Figure 3](#)). The Quality of Life subscale improved by an average of 75.8%. Global WOMAC score expressed as an inverse percentage reflected the improvements seen in the KOOS subscales with

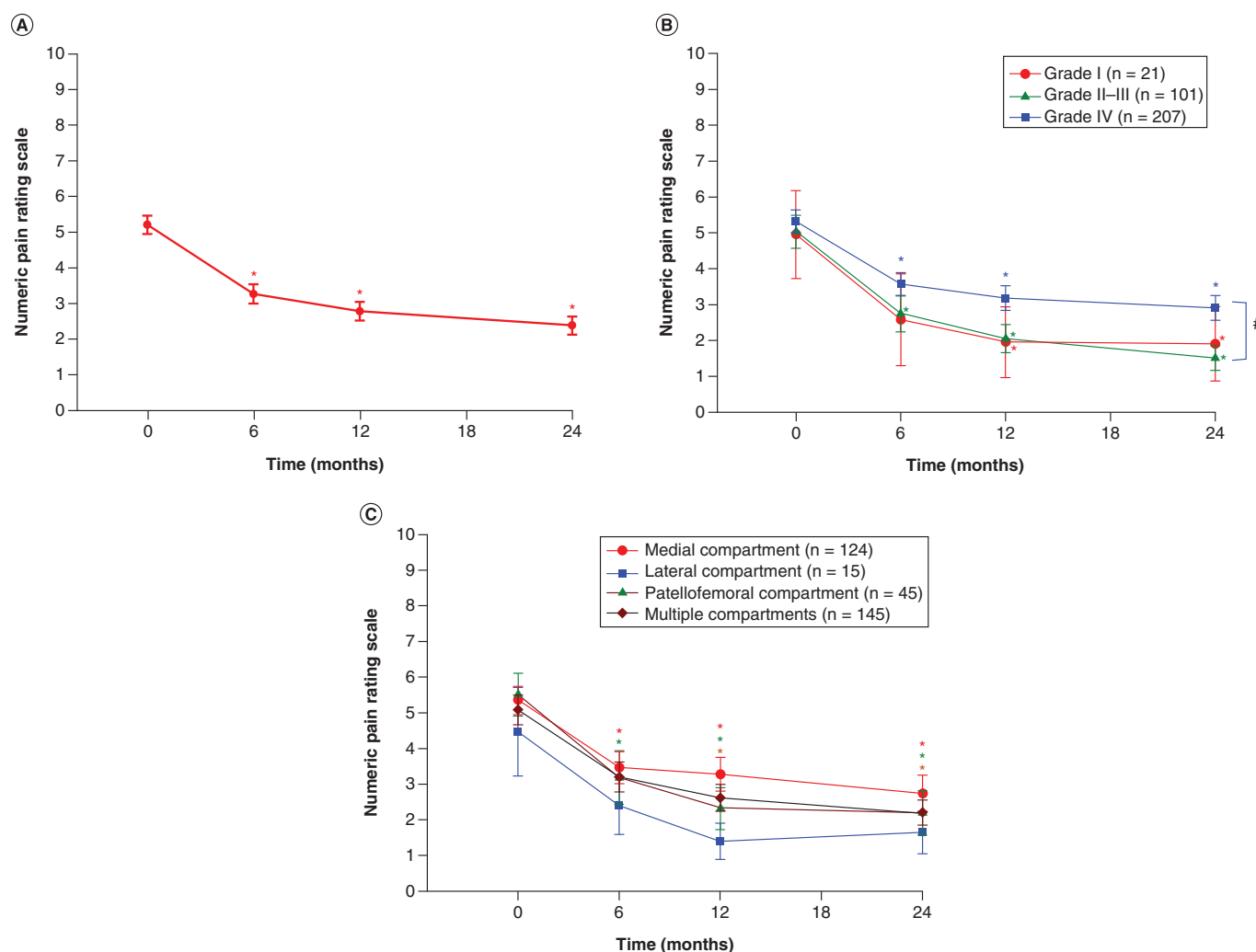


Figure 2. Numeric Pain Rating Scale (NPRS) scores at baseline, 6, 12 and 24 months. Mean data ($n = 329$ at 0, 6 and 12 months and $n = 297$ at 24 months) and 95% CIs are depicted. *Equals significant differences ($p < .05$) when compared with baseline. #indicates significant intergroup difference. Power of performed tests with alpha 0.05 = 1. **(A)** Mean NPRS of entire participant cohort. Significant difference ($p < .001$) at all follow-up time points compared with baseline. **(B)** Mean NPRS scores for each osteoarthritis grade. A significant difference between grade II-III and grade IV was observed at the 24-month time point. **(C)** Mean NPRS scores for each osteoarthritis compartment. No significant differences were apparent between compartments at any time point.

both clinically and statistically significant improvement noted across all time points with an overall improvement of 21.7% at 24 months (Figure 4A). While both moderate and severe OA groups had statistically and clinically significant improvement across the period of follow-up for global WOMAC scores, a comparison between groups showed a statistically significant difference between moderate and severe OA at 6 months. No statistically significant difference in global WOMAC was observed between groups at 24 months (Figure 4B).

In regard to grouping participants by the location (compartment) of their OA, between compartment analyses revealed no statistical differences between compartments at any time point. Within-compartment analyses, however, showed significant decreases in pain (NPRS) at all time points when compared with baseline except for lateral compartment OA where the number of participants ($n = 15$) was low (Figure 2C). Importantly, improvement in NPRS was clinically relevant for all compartments. Similarly, Global WOMAC scores showed no statistical differences in outcomes between compartments at any time point (see Supplementary Material).

Comparison of outcome against carrier media showed no statistically significant difference between carrier media at any time point when assessing against NPRS or WOMAC score (see Supplementary Material). In addition, BMI and age were shown to be poor predictors of functional outcomes with simple linear regression showing a 1% or

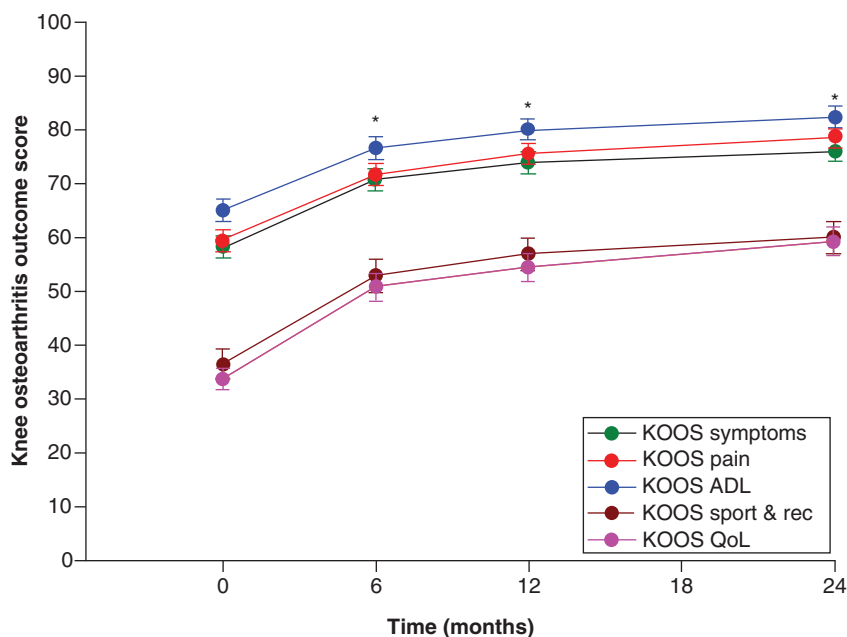


Figure 3. Knee Osteoarthritis Outcome Scores for all five KOOS subscales at baseline, 6, 12 and 24 months. Group mean data (n = 329 at 0, 6 and 12 months and n = 297 at 24 months) and 95% CIs are depicted. All KOOS subscales were significantly different.

* $p < 0.001$ at 6, 12 and 24 months when compared with their individual baseline scores. Power of performed tests with alpha 0.05 = 1.

KOOS: Knee Osteoarthritis Outcome Scores.

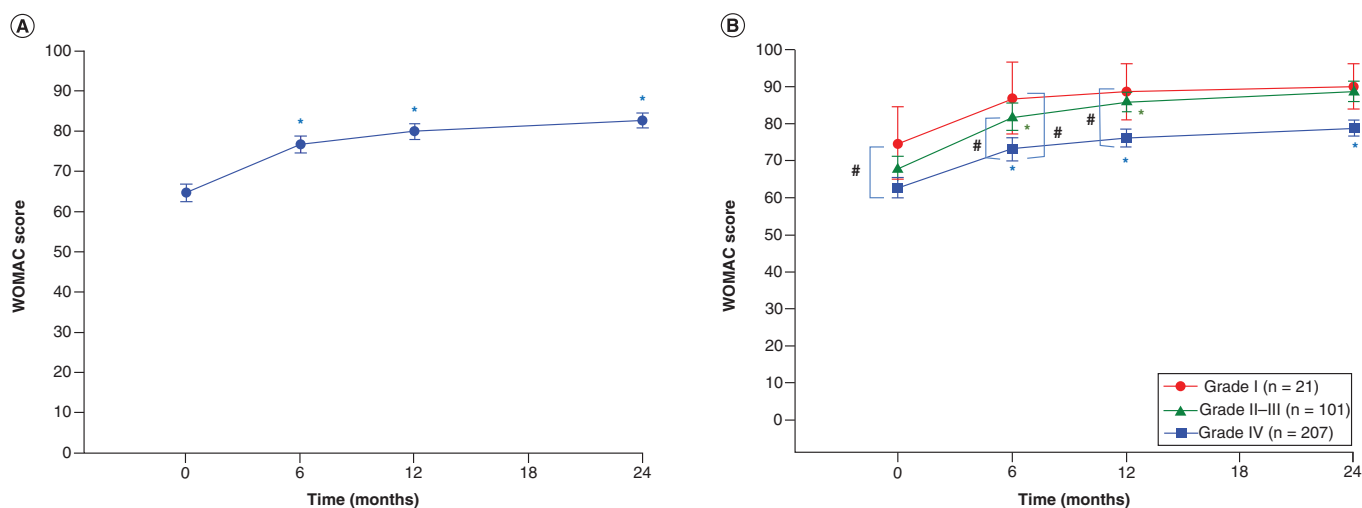


Figure 4. Global WOMAC at baseline, 6, 12 and 24 months. Expressed as a percentage/value to allow for direct comparison with KOOS. Mean data (n = 329 at 0, 6 and 12 months and n = 297 at 24 months) and 95% CIs are depicted. (A) Mean Global WOMAC score for entire participant cohort. Significant differences ($p < 0.0001$) at all time points of follow-up compared with baseline. (B) Mean Global WOMAC score for each osteoarthritis grade. Significant differences within grades II-III and IV were apparent at 6, 12 and 24 months when compared with baseline with significant differences (#) also found between grades I and IV at baseline, 6- and 12-month time points and grade II-III and grade IV at 6 months.

*Equals significant differences ($p < .05$) when compared with baseline; #Indicates significant intergroup difference. Power of performed tests with alpha 0.05 = 1.

KOOS: Knee Osteoarthritis Outcome Scores; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

Table 6. Patient global impression of change at 24 months (n = 297).

PGIC at 24 months	Patients (%)	Grade I OA patients (%)	Grades II–III OA patients (%)	Grade IV OA patients (%)
Fully recovered	10.8	28.6	20.8	3.9
Much improved	56.2	28.5	61.5	56.4
Slightly improved	20.9	38.1	9.3	25.1
No change	5.4	4.8	4.2	5.6
Slightly worse	4.0	0	2.1	5.6
Much worse	2.4	0	0	0
Very much worse	0.3	0	2.1	3.4

OA: Osteoarthritis; PGIC: Patient Global Impression of Change.

Table 7. Minimal Clinically Important Difference at follow-up time points in comparison to baseline data.

Outcome measure	Percentage achieving MCID		
	6 months (n = 329)	12 months (n = 329)	24 months (n = 297)
NPRS	71.5	76.6	80.1
WOMAC	55.3	65.1	66.8
KOOS (pain improvement)	57.3	66.5	71.7
KOOS (symptom improvement)	53.8	61.7	67.9
KOOS (ADL)	54.5	63.2	66.1
KOOS (S & R)	60	68	69.7
KOOS (QoL)	58.6	68.8	71.1
Average	58.7%	67.1%	70.5%

All data are shown in percentages and expressed as the percentage of subjects who registered an MCID when compared with their baseline value. Of the 329 participants, 32 had TKRs after 12 months with assessment performed on the remaining 297 participants at 24 months.

ADL: Function in daily living; MCID: Minimal Clinically Important Difference; NPRS: Numeric Pain Rating Scale; PGIC: Patient Global Impression of Change; QoL: Quality of Life; S & R: Function in sports and recreation; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

0.3% prediction power, respectively, in the ability of these two independent variables to predict the variability in a change in WOMAC score (at 24 months) from baseline (see [Supplementary Material](#)).

Of the 297 participants who completed 24 months of follow-up, satisfaction with treatment, as represented by PGIC, indicated that 87.9% of participants improved, with 10.8% stating they were fully/completely recovered and 56.2% indicating that they were much improved. Only 12.1% of participants felt that they were no better or had gotten worse over the time period of follow-up (Table 6).

Importantly, improvements observed in pain and function were both statistically significant and clinically relevant/significant with 70.5% of participants achieving an MCID at 24 months when averaging all quantitative questionnaire-based outcome measures (Table 7). This was even more impressive when the analysis was segregated by OA grade, with 75.2% of mild/moderate OA patients achieving an MCID at 24 months with other grades being comparable (Table 8).

Occupational activity level

Of the 329 participants, 232 were employed with 105 participants in occupations requiring regular moderate to high load-bearing activity. Of those participants in moderate- to high-load employment, 70 were clinically suitable for TKR (age of 65 years or greater or grade IV OA). Of these 70 participants, 63 had not required TKR at 24 months of follow-up and remained active in the workforce.

Complications & adverse events

No unexpected or serious TEAEs were observed. Pain, stiffness and swelling were commonly reported post-ADMSC treatment (62%, 52% and 66%, respectively, after baseline therapy). This reproducible but self-limiting flare has been previously observed and was documented as an expected AE. Importantly, this responded to conservative measures including ice and oral analgesics including oral nonsteroidal anti-inflammatories. An observed increase in moderate TEAEs was noted with an increased incidence of moderate pain and swelling AEs post-second MSC therapy. A single participant was diagnosed with prostate cancer during the time of follow-up, with four participants

Table 8. Minimal clinically important difference at 24 months of follow-up in comparison to baseline data for each grade of osteoarthritis.

Outcome measure at 24 months	Percentage achieving MCID at 24 months			
	All patients (n = 297)	Grade I (n = 21)	Grades II–III (n = 101)	Grade IV (n = 175)
NPRS	80.1	85.7	87.4	75.4
WOMAC	66.8	52.4	71.6	66.7
KOOS (pain improvement)	71.7	76.2	72.6	69.5
KOOS (symptom improvement)	67.9	52.4	68.4	67.8
KOOS (ADL)	66.1	47.6	71.6	66.1
KOOS (S & R)	69.7	66.7	82.1	64.4
KOOS (QoL)	71.1	90.5	72.6	69.5
Averages	70.5	67.4	75.2	68.5

All data are in percentages and expressed as the percentage of subjects who registered an MCID when compared with their baseline value.

ADL: Function in daily living; MCID: Minimal Clinically Important Difference; NPRS: Numeric Pain Rating Scale; PGIC: Patient Global Impression of Change; QoL: Quality of Life; S & R: Function in sports and recreation; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

diagnosed with polymyalgia rheumatica over the 24-month period. See [Supplementary Tables 1–3](#) for a complete list of AEs.

Discussion

The present authors previously published a RCT assessing the benefit of autologous ADMSC therapy for the treatment of moderate knee OA [42]. This current trial represents a long-term RWE case series assessing the effectiveness of ADMSCs in the treatment of symptomatic knee OA in a large patient cohort (329 subjects) that is representative of a usual clinical setting. The results of this case series indicate that ADMSCs are a safe and effective treatment for mild to severe symptomatic OA with the potential to delay and/or prevent the need for TKR.

TKR remains the accepted treatment for OA nonresponsive to conservative measures. Successful return to work (RTW) is recognized as a critical outcome marker for patients after TKR and is influenced by factors including the level of occupational activity and, therefore, work-related loading of the knee [67–70]. High physical occupational demands have been reported to be associated with poor RTW outcomes with an odds ratio of 3.3 in regard to inability to RTW with additional research indicating a high rate of retirement post-TKR in patients under the age of 65 [71,72]. In contrast, patients in nonmanual work have an observed 1.5–2.6-fold increased probability of successful RTW following TKR [73].

Of those participants in moderate- to high-load employment who are clinically suitable for TKR (age of 65 years or greater or grade IV OA), 90% had not required TKR at 24 months of follow-up and remained active in the workforce. This suggests that MSC therapy promises to have an impact on not only the direct medical costs of OA, but also the significant indirect costs, by delaying or preventing the need for TKR and, therefore, reducing costs associated with sick leave, early retirement and disability payments. In addition, TKR rates after 12 months for participants in moderate to high load-bearing occupations were less than that observed for the entire participant cohort confirming that increased workload did not adversely impact response to treatment. The observed delay in progression to TKR and continued employment of participants within high-load occupations indicates that ADMSC therapy may considerably reduce the health economic impact of OA.

In this case series, 329 participants aged 23–85 years (mean: 58.6) underwent IA ADMSC therapy. Treatment was typically well tolerated with no observed serious TEAEs during the period of 24-month follow-up. No unexpected or significant AEs were associated with the lipoharvest procedure. Participants experienced a period of discomfort with observed swelling, pain and stiffness following ADMSC therapy that was self-limiting and typically responded to the use of oral analgesia and application of ice. A small percentage of participants experienced self-limiting severe related TEAEs with pain and swelling that significantly impacted their daily activities and required the use of opiate analgesia. Due to an observed self-limiting period of reduced mobility in participants with more significant TEAEs, the authors of this trial recommend formal deep venous thrombosis (DVT) risk assessment with the commencement of low-dose aspirin in patients with moderate to high DVT risk until mobility returns to baseline post-MSC therapy. All reported AEs were managed in the outpatient setting with no requirement for inpatient care. A trend of increased severity of TEAEs was documented at the 6-month IA ADMSC injection. All related TEAEs have been well documented in past research and were recorded as expected AEs [40–42,45,53–55].

Pain and functional improvement were seen consistently with retention of improvement to 24 months. Despite 32 participants failing therapy and undergoing TKR after 12 months, a comparison of outcomes over the first 12 months between all 329 participants and those who did not undergo TKR showed neither statistically nor clinically significant differences. Across all patients, pain, as measured by NPRS, improved by 46.5% at 12 months, with this improvement being maintained until final follow-up at 24 months (54.5%). The improvement observed in NPRS was reflected in both statistically and clinically significant improvement in function as measured by KOOS (all subscales) and WOMAC assessment. Statistically significant improvements were observed in all measures across all time points of follow-up. Of significant clinical relevance, QoL (KOOS_{QoL}) improved by 61.2% at 12 months with these improvements maintained until follow-up at 24 months. Overall, 87.9% of participants noted an improvement in symptoms as measured by PGIC with 10.8% of patients noting complete recovery.

Assessment of outcomes against the grade of OA showed reproducible and clinically significant improvement in all grades. Moderate knee arthritis had the largest improvement in pain, suggesting a greater clinical response than severe knee OA. A greater clinical improvement than mild OA was considered to be a result of lower pain and higher functionality scores at baseline in the mild OA cohort. For participants with severe OA, 75.4% achieved a minimal clinically important improvement in their pain levels with 87.4% of participants with moderate knee OA achieving a minimally clinically important improvement. While the difference in pain as measured by NPRS between moderate and severe OA was statistically significant at 24 months, no statistically significant difference between grades of OA was observed in WOMAC outcome results. Additionally, the site of OA did not affect patient response to treatment, with no statistically significant differences in outcome scores apparent when comparing the location of OA (medial compartment, lateral compartment, patella-femoral or multicompartmental).

While moderate knee OA was seen to be more predictable than severe OA, the observed reproducible and statistically and clinically significant improvement in pain seen in severe bone-on-bone grade IV OA confirms a mechanism of action (MOA) beyond structural benefit. While mediation of the local inflammatory environment associated with OA and upregulation of trophic pathways are recognized pathways of MSC action, a less considered and equally important MOA is the direct interaction of MSCs in the nociceptive/neuropathic pain seen in OA. While joint deformity and loss of function are commonly associated with advanced OA, pain remains the leading cause of disability in OA and has been documented as the primary reason for patients to seek healthcare [74–76]. As Liang has succinctly concluded, “*x-rays don’t weep*” [77].

The joint inflammation seen in OA has long been understood to be associated with hyperalgesia leading to the commonly described rest pain seen in more advanced OA [78–80]. This heightened pain response is considered to occur as a result of peripheral sensitization of nociceptive primary afferent neurons in addition to central sensitization [81]. Numerous inflammatory mediators seen in OA, including bradykinin, prostaglandins as well as cytokines IL-1, IL-6 and TNF α , are associated with sensitized afferents that exist within the joint capsule, ligaments, menisci, joint adipose tissue (i.e., Hoffa’s fat pad), periosteum and subchondral bone [81–88]. In addition, NGF is found in increased concentration within synovial fluid from patients with OA and rheumatoid arthritis and is upregulated by cytokines including IL-1 and TNF α [85]. NGF has been shown to influence nociceptive activity with upregulation of expression of the neuropeptide substance P and vasodilator mediator CGRP, which is released from peripheral sensory nerves and associated with both joint inflammation, heat and pain sensation [89–91].

ADMSCs have been shown to reduce the levels of proinflammatory cytokines IL-1 and IL-6 seen in nociceptive hypersensitization [92]. Preclinical studies have demonstrated this effect through MSC-mediated polarization of macrophages to an anti-inflammatory M2 phenotype [93]. In addition to their downregulation of the proinflammatory state, MSCs have also been found to express soluble TNF receptor type 1 (sTNFR1), which acts as an inhibitor of TNF α with high affinity [94]. It is proposed that this reduction and/or inhibition of inflammatory cytokines leads to reduced expression of NGF and thus inhibition of nociceptive sensitization. The effect of systemic administration of MSCs on central sensitization seen in OA may be a further important consideration in the management of chronic musculoskeletal pain with associated hyperalgesia.

Perhaps surprisingly, age did not correlate with functional outcome, with it only predicting 0.3% of the variability in change in WOMAC score at 24 months using linear regression analyses. Past research has indicated MSCs exhibit both a reduced potency with age in that they exhibit reduced proliferative and differentiation capacity [95,96]. In this case series, the eldest patient was 85 years old and exhibited a 100% improvement in pain with an NPRS of 5 at baseline and 0 at 24 months and a ‘complete recovery’ according to her PGIC grading. The lack of influence of age and potential reduced potency of ADMSCs in older patients may be counterbalanced by the significant total dose of ADMSCs over the course of treatment (100×10^6 ADMSCs). An equally surprising observation in this case series

was a lack of association between increasing BMI scores and lower WOMAC improvement, with regression analysis only able to predict 1% of the variability in WOMAC scores. Nevertheless, the pain and functional benefit observed with appropriate weight loss (decreased joint loading) should not be discredited and remains a key consideration in the conservative management and prevention of OA [13].

In this case series, ADMCSs were suspended in an excipient comprising three different types: autologous carrier media of either ACS or PRC or an isotonic solution. Past publications have shown an improved expression of anti-inflammatory cytokines including IL-1ra and trophic growth factors TGF- β by MSCs in the presence of a blood-derived carrier media suggesting a potential advantageous influence on patient response/outcome [97,98]. This is in contrast to a clinical publication by Bastos and colleagues where the assessment of MSC therapy in combination with PRP versus MSC therapy in isolation showed no observed difference in outcome [99]. Consistent with this earlier publication, analysis of results achieved with different carrier media showed no statistically significant difference confirming that the suspension of MSCs in a biological carrier media offered no observed additional benefit.

The results of this case series must be assessed against the potential limitation and reporting bias introduced through loss to follow-up, which remains a significant concern in long-term outcome studies. Of the 381 participants, a total of 52 were lost to follow-up over the 24-month time period, a rate of 14%. Previous research has suggested that in long-term trials, a loss to follow-up of <20% is an acceptable margin, with >20% indicating a significant risk of bias in outcome results [100]. A retention rate of 80% has been used to define high versus low quality levels of evidence in formal trials [101].

While this case series may be limited by a lack of a formal control group, it does provide further insights into the RWE comparison with the authors' previously published RCT on ADMSCs in the treatment of knee OA. RWE provides valuable complementary outcome data to that obtained in RCTs in that it more accurately reflects routine clinical practice settings and presentations of OA in the community [102]. In this case series, patients with mild to severe OA were recruited with no limitation on age (the only requirement being age of 18 years or older) resulting in a study patient demographic equivalent to what is seen by clinicians in the community. Importantly, the broad nature of recruitment has also allowed for assessment of the broader societal economic impact of ADMSC therapy, with improved pain and function in patient cohorts with high occupational activity/knee load effectively delaying consideration of TKR. This results in significant direct and indirect economic benefits with continued employment, reduced sick leave and reduced medical expense most commonly associated with TKR surgery and associated rehabilitation costs.

Conclusion

OA is a major unmet clinical need and is a leading cause of disability associated with a significant economic burden. The authors previously published an RCT assessing the use of autologous ADMSC therapy in the successful treatment of moderate knee OA. This case series of 329 participants further adds supportive RWE with long-term outcome data confirming ADMSC therapy to be well-tolerated, safe and result in clinically significant improvements in pain and functional clinical end points. Importantly, results showed reproducible positive outcomes in the treatment of mild, moderate and severe knee OA with long-term sustained improvements at 24 months follow-up. This long-term safety and effectiveness data strengthens MSC therapy as an evidence-based, effective treatment modality that provides clinicians an alternative and innovative treatment option for OA.

Translational perspective

Systematic reviews of previously published RCTs on the use of MSC therapies in OA have been supportive in observed safety and efficacy end points [103,104]. The results of this case series confirm that ADMSC therapy has translational potential in the treatment of symptomatic OA in the broader population with reproducible clinical improvements and importantly evidence indicating delay/prevention of total joint replacement.

Summary points

- Osteoarthritis (OA) is associated with a significant economic burden, accounting for up to 2.5% of GDP in developed nations.
- OA is the second most common musculoskeletal complaint seen by general practitioners and a leading cause of disability.
- Current conservative therapies are aimed at symptomatic control, have limited efficacy and may be associated with serious side effects.
- Total joint replacement is associated with a 2% serious complication rate in addition to up to 20% of patients experiencing persistent pain and loss of function at 12 months.
- Previous research has indicated the benefit of MSC therapy in the treatment of moderate knee OA.
- This case series provides real-world evidence for the effectiveness of ADMSCs in the management of mild to severe knee OA with reproducible long-term pain and functional improvement.
- ADMSC therapy was well tolerated and safe over a 24-month period of follow-up.
- ADMSC therapy may significantly reduce the global socioeconomic burden of OA, with successful delay or prevention of total joint replacement surgery.
- ADMSC therapy represents an exciting advancement in the active management of OA, a recognized condition of unmet clinical need.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/rme-2022-0002

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