Editorial Commentary

Mesenchymal stromal cells for the treatment of osteoarthritis of knee joint: context and perspective

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Evidence from completed preclinical and clinical studies conducted worldwide suggests that mesenchymal stromal cells (MSCs) are a novel and promising option for the treatment of osteoarthritis (OA). However, ideal tissue sources, route of administration, dose of the cells, single or repeated injection of MSCs to achieve the desired therapeutic efficacy in a disease with complex pathophysiology such as OA are yet to be confirmed. In order to obtain maximum therapeutic efficacy, it is important to concentrate the majority of MSCs in the diseased site. Thus, for knee OA, based on evidence from numerous preclinical and clinical studies, direct intra-articular injection of MSCs into the knee synovium either blindly or ultrasound guided is the ideal method of delivering the cells in the affected joint. Dose-finding investigations are being conducted to identify the most efficacious dose to reduce inflammation and regenerate hyaline cartilage. In addition, the feasibility of repeat MSC injections is being studied to enhance their therapeutic benefit. Although most MSC treatments have resulted in a significant reduction in pain scores which is likely due to the release of anti-inflammatory molecules by MSCs, some studies have also shown a reduction of stiffness and improvement of physical function measured using Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Lysholm and other scales. Measurement of cartilage volumes using magnetic resonance imaging (MRI) and T2 relaxation time mapping has shown some improvement in cartilage quality but no change in cartilage quantity. However, more studies are needed to confirm cartilage regeneration and also measure the cartilage type and quality. With all the existing evidence it is reasonable to expect that MSCs may prove to be an important therapy for OA.

Recently, Kim SH and colleagues (1) reported the meta-analysis of randomized controlled trials (RCT) in clinical outcome and cartilage repair in OA of knee. Amongst the 5 studies (220 patients) that were shortlisted for analysis by them, the study with the lowest risk of bias based on Cochrane Collaboration's risk of bias tool was found to be Gupta *et al.* (60 patients) (2) followed by Vega *et al.* (30 patients) (3), whereas the other 3 studies were found to be associated with high risk of bias based on allocation concealment, blinding of participants and personnel, blinding of outcome measures and selective reporting criteria. All 5 studies selected for analysis used MSCs and the route of cell administration was common (intra-articular). The variation in tissue source (bone marrow and adipose tissue) from where the MSCs were derived and the variability of autologous and allogeneic cell administration could have contributed to the stark difference in clinical trial outcomes. The meta-analysis of pain scores in three studies (2-4) using the visual analog scale (VAS) showed a significant reduction of pain scores (P=0.02) at 12 months with moderate heterogeneity (I^2=41%) in 56 patients administered with MSCs compared to 58 control

patients. Similarly, pain analysis using WOMAC in 35 patients injected with MSCs also showed a reduction in pain with moderate heterogeneity (I^2=33%) after 12 months follow-up, although, the WOMAC scores were not significant compared to the control groups (P=0.26). A cumulative analysis of VAS and WOMAC scales showed significant reduction of pain (P=0.004) with cumulative moderate heterogeneity (I^2=39%) in the studies analyzed. Interestingly, bone marrow-derived MSCs (BM-MSCs) were used in all three studies described above indicating that BM-MSCs could be a preferred cell type for pain management in inflamed tissues. Even though BM-MSCs were used in the three studies, differences in autologous versus allogeneic cells, different donors, single versus multiple donor cell populations and variable culture methodologies could have contributed to the moderate heterogeneities that have been reported in pain measurement outcomes. Unlike the significant improvement in outcomes of pain scores in the analyzed studies, only marginally significant improvement in functional outcomes measured by Lysholm knee scale (P=0.05) was observed and no significant improvement was seen in MRI evaluation (P=0.20). Moreover, substantial heterogeneity was reported for Lysholm knee scores (I^2=39%) and MRI evaluation (I^2=39%). The meta-analysis is indicative that although different MSC populations at variable doses are likely protecting the articular cartilage from further degeneration. The anti-inflammatory response that has been observed in these clinical studies is likely mediated by the well-known anti-inflammatory molecules that are secreted by MSCs such as interleukin 10 (IL-10), prostaglandin E2 (PGE-2), indoleamine 2, 3-dioxygenase (IDO), transforming growth factor β (TGFβ), etc. (5,6), and they are seldom contributing to substantial regeneration of hyaline cartilage.

MSCs derived from several tissues of the body seem to be emerging as a promising therapeutic option for the treatment of different diseases affecting different tissues including OA. The increasing literature evidence that mechanism of action (MoA) by which MSCs exert a therapeutic benefit is primarily by secreting a plethora of bioactive molecules which in turn induce immunomodulatory and regenerative effects in diseased tissues (7,8). The differentiation of MSCs into the chondrogenic lineage is believed to be orchestrated by key master transcriptional factors such as Sox 9 and Runx2 whose intrinsic signaling cascade could be induced using differentiation factors such as TGFβ3 and bone morphogenic proteins (BMPs) (9,10). One of the major concerns is the excessive differentiation of MSCs leading to hypertrophy of cells and their subsequent formation of fibrous cartilage instead of the desired hyaline cartilage. In this regard, Weiss *et al.* (11) have demonstrated that the combinatorial use of parathyroid hormone-like peptide (PTHrP) and basic fibroblast growth factor (bFGF) in the chondrogenesis directed BMMSC pellet cultures could inhibit their terminal differentiation and hypertrophy by suppressing the synthesis of collagen X and promoting the synthesis of other essential matrix proteins such as collagen II. Among the different kinds of MSCs evaluated, it was found that BM-MSCs and synovium-derived MSCs exhibited enhanced differentiation into the chondrogenic lineage (12). BM-MSCs have been characterized for several decades and thus they are the most preferred MSC type for targeting a plethora of disease indication including OA.

Based on the therapeutic MoA of a cell-based therapeutics such as MSCs, development of a relevant potency assay is recommended by the United States Food and Drug Administration (US-FDA) and European Medicines Agency (EMA) for products manufactured on a large-scale. As per the guidelines of the regulatory agencies, it is mandatory that a potency assay must provide a quantifiable readout of the relevant biological function of the cell-therapy product (CTP) (13). Relevant potency assays have been developed and implemented for CTPs targeting OA. A company, Tigenix has developed and adopted a polymerase chain reaction (PCR) based array for measuring chondrogenic potency of their autologous chondrocytes product—ChondroSelect (14). For another umbilical cord MSC (UC-MSC) product Cellistem-OA, quantification of Thrombospondin-2 has been developed as a surrogate marker to measure the potency of the cells to promote chondrogenesis *in vivo* (15). In order to predict the chondrogenic potency of bioactive molecules, Thorup *et al.* developed an *in vivo* potency assay where autologous chondrocytes and collagen type I are injected locally in mice to induce the formation of hyaline cartilage (16). In fact, for our allogeneic, pooled, BM-MSC product—Stempeucel®, we initially demonstrated in six large-scale batches that *in vitro* differentiation Stempeucel® consistently downregulated Sox2 and upregulated the expression of Col2A and Runx2 (2). Additionally, for over twenty large-scale batches, we developed and selected the quantification of sulphated glycosaminoglycans (sGAG) in undifferentiated and differentiated cells as a surrogate marker to predict the chondrogenic potency. Ideally, for MSC based CTPs, their

anti-inflammatory properties must also be evaluated *in vitro* such as measuring the suppression of activated T-cells or mixed lymphocyte reactions (MLRs) and quantifying soluble anti-inflammatory molecules such as IL-10, PGE-2 and IDO. An attempt to also be made to correlate the suppression of T-cells or MLRs and the quantities of anti-inflammatory molecules and include it as a part of the potency assay matrix. In this manner, the efficacy response of intra-articularly injected MSCs in reducing the pain and regenerating cartilage can be predicted using appropriate measurements of the potency assay matrix.

In their study, Kim *et al.* (1) reported limited pain relief and functional improvement in knee OA patients who are intra-articularly injected with MSCs. In a similar meta-analysis published earlier which included 582 OA patients in a total of 11 randomized controlled trials using various efficacy questionnaires showed that treatment with MSCs significantly improved VAS, International Knee Documentation Committee (IKDC) scores post 24 months follow up in comparison to the control groups (17). In addition, it was shown that MSC treatment significantly improved WOMAC scores, Tegner activity scale (Tegner), Lysholm knee scale (Lysholm) and Lequesne algofunctional indices (Lequesne) at follow up time points of 12 or 24 months. Hence, it is clear that MSCs do have a role in the improvement in pain and function of the joint as seen in different clinical trials.

The meta-analysis conducted by Kim *et al.* (1) suggests that intra-articular injection of MSCs may not be the only ideal strategy for promoting regeneration of cartilage in knee OA. Multiparametric MRI is currently being used clinically for measuring cartilage regeneration. Most commonly, the volume of cartilage in the knee joint, cartilage thickness which is measured at different points of the joint compartment, whole-organ magnetic resonance imaging score (WORMS), T2 relaxation time mapping, Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scoring and MRI Osteoarthritis Knee Score (MOAKS) score are measured using MRI. More recently, MRI contrast chemical elements such as gadolinium are being used in order to improve the quality of the MRI image (dGEMRIC) (18). From existing literature, it seems that the sensitivity of T2 time mapping and WORMS are higher and these qualitative measurements are used to measure subtle changes in collagen fibril orientation and hydration of articular cartilage. Orozco *et al.* (19) demonstrated a significant decrease in areas with poor cartilage quality (average of 27%) and further observed areas with improved cartilage quality as determined by T2 mapping. In a study conducted by Soler Rich *et al.* (20), T2 mapping was used to evaluate 50 patients administered with autologous BM-MSCs. They observed no change in cartilage quality in 10 patients (20%) and only 3 (6%) worsened. Interestingly, the poor cartilage index (PCI) reduced significantly in 37 out of 50 patients (74%). Conclusively, T2 mapping by MRI seems to be a reliable method to determine the degradation of the extracellular matrix and evaluate cartilage quality.

One of the important research questions is what could be the optimal dose of MSCs to achieve maximum efficacy. A wide range of MSC doses varying from 1.18×10^6 (21) to 150×10^6 (2) have been used so far in global clinical trials. In a clinical study conducted by Koh *et al.* (21), 1.18×10^6 adipose tissue-derived MSCs (AD-MSCs) were intra-articularly injected in 18 patients in addition to platelet-rich plasma. A significant improvement was observed in WOMAC, Lysholm and VAS scores after 26 months follow-up. Additionally, significant cartilage improvement was observed which was determined using WORMS score using MRI. Pers *et al.* (22) reported that injecting a low dose of 2×10^6 autologous AD-MSCs into knee joints of OA patients brought about an improvement in pain and function compared to baseline, medium dose (10×10^6 cells) and high dose (50×10^6 cells). They also did not report any serious adverse events and found the procedure to be safe. Similar to these results, Gupta *et al.* (2), in a total of 60 randomized patients, found that their lowest dose of 25×10^6 allogeneic pooled BM-MSCs (Stempeucel®) showed improvement in pain scores and function compared to the placebo, baseline and other doses of 50, 75 and 150×10^6 cells after one year follow-up. In contrast, a study conducted by Jo *et al.* (23) found significant improvement in pain and function in the highest dose of 100×10^6 cells compared to lower doses of 10 and 50×10^6 cells measure using Knee injury and osteoarthritis outcome score (KOOS), VAS and Knee Society clinical rating system (KSS) scoring after two years of treatment. Although most studies show that an MSC dose of ranging from $25–50\times10^6$ cells, more randomized, controlled, dose-finding clinical studies are needed to identify the optimal therapeutic dose.

The next point of discussion is to analyse whether single dose or repeat dose of MSC product would be most efficacious for knee OA patients. Most of the studies in OA use a single dose of MSC product and the patients are followed up for a variable period of time (12 to 24 months). The efficacy of single and repeat administration of UC-MSCs was assessed in patients with knee OA (15).

The symptomatic patients were divided into three groups where the first group of eight patients were administered with hyaluronic acid (HA) alone at the baseline time-point and also after 6 months. The second group (MSC-1, N=9) received a single dose of UC-MSCs (20×10^6) at baseline and the third group received UC-MSCs (20×10^6) (MSC-2, N=9) at baseline and also at the 6-month time-point. The patients were evaluated for 12 months for clinical scores and MRI. There were no serious adverse events reported in any group. Significant improvement in pain and function scores were observed only in the groups treated with UC-MSCs compared to the baseline (P=0.001). A follow up of 12 months, using the WOMAC scale, showed significant reduction of pain levels in the group with repeat UC-MSC dose (MSC-2) (1.1±1.3) compared to the group of patients injected with HA (2.4±2.1 *vs.* 22.1±9.8, P=0.03). In addition, at 12 months, VAS was significantly lower in the MSC-2 group compared to the HA group (2.4±2.1 *vs.* 22.1±9.8, P=0.03). At the end of the study, total WOMAC scores were observed to be lower in the MSC-2 group compared to the HA group of patients at 12 months (4.2±3.9 *vs.* 15.2±11, P=0.05). No difference between the groups was observed by MRI measurements using the WORMS scoring system at any time point. It may be concluded that repeat injection of MSCs may be superior as compared to a single dose of MSCs. However, more studies are required to come to a definitive conclusion.

In conclusion, current treatments for OA are mostly targeted at the end-stage of disease but biological therapies including stem cell therapy show promise for earlier intervention with a more prolonged benefit. With all the published clinical trial data, it is reasonable to expect that MSCs may prove to be an important therapy for OA. BM-MSCs with their enhanced anti-inflammatory potential, immuno-modulatory properties and secretion of paracrine factors create the optimum environment for a controlled reparative pathway in the affected joint.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Kim SH, Ha CW, Park YB, et al. Intra-articular injection of mesenchymal stem cells for clinical outcomes and cartilage repair in osteoarthritis of the knee: a meta-analysis of randomized controlled trials. Arch Orthop Trauma Surg 2019;139:971-80.
2. Gupta PK, Chullikana A, Rengasamy M, et al. Efficacy and safety of adult human bone marrow-derived, cultured, pooled, allogeneic mesenchymal stromal cells (Stempeucel®): Preclinical and clinical trial in osteoarthritis of the knee joint. Arthritis Res Ther 2016;18:301.
3. Vega A, Martín-Ferrero MA, Del Canto F, et al. Treatment of Knee Osteoarthritis With Allogeneic Bone Marrow Mesenchymal Stem Cells. Transplantation 2015;99:1681-90.
4. Lamo-Espinosa JM, Mora G, Blanco JF, et al. Intra-articular injection of two different doses of autologous bone marrow mesenchymal stem cells versus hyaluronic acid in the treatment of knee osteoarthritis: multicenter randomized controlled clinical trial (phase I/II). J Transl Med 2016;14:246.
5. Gupta PK, Das AK, Chullikana A, Majumdar AS. Mesenchymal stem cells for cartilage repair in osteoarthritis. Stem Cell Res Ther 2012;3:25.
6. Zachar L, Bačenková D, Rosocha J. Activation, homing, and role of the mesenchymal stem cells in the inflammatory environment. J Inflamm Res 2016;9:231-40.
7. Caplan AI, Correa D. The MSC: An injury drugstore. Cell Stem Cell 2011;9:11-5.
8. Ranganath SH, Levy O, Inamdar MS, et al. Harnessing the mesenchymal stem cell secretome for the treatment of cardiovascular disease. Cell Stem Cell 2012;10:244-58.
9. Barry F, Boynton RE, Liu B, et al. Chondrogenic Differentiation of Mesenchymal Stem Cells from Bone Marrow: Differentiation-Dependent Gene Expression of Matrix Components. Exp Cell Res 2001;268:189-200.
10. Sekiya I, Larson BL, Vuoristo JT, et al. Comparison of effect of BMP-2, -4, and -6 on in vitro cartilage formation of human adult stem cells from bone marrow stroma. Cell Tissue Res 2005;320:269-76.
11. Weiss S, Hennig T, Bock R, et al. Impact of growth factors and PTHrP on early and late chondrogenic differentiation

of human mesenchymal stem cells. J Cell Physiol 2010;223:84-93.
12. Sakaguchi Y, Sekiya I, Yagishita K, et al. Comparison of human stem cells derived from various mesenchymal tissues: Superiority of synovium as a cell source. Arthritis Rheum 2005;52:2521-9.
13. Bravery CA, Carmen J, Fong T, et al. Potency assay development for cellular therapy products: An ISCT* review of the requirements and experiences in the industry. Cytotherapy 2013;15:9-19.
14. Dell'Accio F, Vanlauwe J, Bellemans J, et al. Expanded phenotypically stable chondrocytes persist in the repair tissue and contribute to cartilage matrix formation and structural integration in a goat model of autologous chondrocyte implantation. J Orthop Res 2003;21:123-31.
15. Matas J, Orrego M, Amenabar D, et al. Umbilical Cord-Derived Mesenchymal Stromal Cells (MSCs) for Knee Osteoarthritis: Repeated MSC Dosing Is Superior to a Single MSC Dose and to Hyaluronic Acid in a Controlled Randomized Phase I/II Trial. Stem Cells Transl Med 2019;8:215-24.
16. Thorup AS, Eldridge SE, Caxaria S, et al. A Potency Assay for Assessing the Chondrogenic Efficiency of Bioactive Molecules in Human Cartilage in vivo. Osteoarthr Cartil 2017;25:S273.
17. Yubo M, Yanyan L, Li L, et al. Clinical efficacy and safety of mesenchymal stem cell transplantation for osteoarthritis treatment: A meta-analysis. PLoS One 2017;12:e0175449.
18. Thej C, Kumar Gupta P. The Role of Mesenchymal Stromal Cells in the Management of Osteoarthritis of the Knee. In: Mesenchymal Stem Cells. IntechOpen, 2019.
19. Orozco L, Munar A, Soler R, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. Transplantation 2013;95:1535-41.
20. Soler Rich R, Munar A, Soler Romagosa F, et al. Treatment of Knee Osteoarthritis with Autologous Expanded Bone Marrow Mesenchymal Stem Cells: 50 Cases Clinical and MRI Results at One Year Follow-Up. J Stem Cell Res Ther 2015;5:285.
21. Koh YG, Jo SB, Kwon OR, et al. Mesenchymal stem cell injections improve symptoms of knee osteoarthritis. Arthroscopy 2013;29:748-55.
22. Pers YM, Rackwitz L, Ferreira R, et al. Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: a phase i dose-escalation trial. Stem Cells Transl Med 2016;5:847-56.
23. Jo CH, Chai JW, Jeong EC, et al. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a 2-year follow-up study. Am J Sports Med 2017;45:2774-83.

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