We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

The Role of Mesenchymal Stromal Cells in the Management of Osteoarthritis of the Knee

Charan Thej and Pawan Kumar Gupta

Abstract

Osteoarthritis (OA) is one of the most common chronic, inflammatory, and degenerative diseases affecting the synovial joints, the hip, and the knee. OA is commonly managed clinically by treating pain with anti-inflammatory medicines using nonsteroidal anti-inflammatory drugs (NSAIDS) or analgesics. In severe OA patients, invasive knee replacement surgery is the last option. Treatment of OA using mesenchymal stromal cells (MSCs) has been widely explored due to their anti-inflammatory properties and chondrogenic differentiation potential. In this chapter, we comprehensively discuss in detail the in vitro OA potency development, OA preclinical studies, and clinical trials conducted using MSCs.

Keywords: osteoarthritis, pooled human bone marrow-derived mesenchymal stromal cells, potency assay, preclinical studies, clinical studies

1. Introduction

Common factors linked to osteoarthritis (OA) occurrence are increasing age (>55 years) and obesity [1]. The gender also seems to play a major role, where the majority of OA patients are women and higher prevalence has been liked to menopause. Radiological evidence suggests that about 70% of women above the age of 65 years are affected by OA [2, 3]. Other factors such as genetic predisposition, extrinsic environmental factors, nutrition, and lack of exercise are reasons for the increased prevalence of OA. It has been reported by the World Health Organization (WHO) that 10–15% of the populations aged >60 years exhibit a certain degree of OA [4]. It has been reported by the National Health Portal of India that 22–39% of the Indian population are affected by OA. As reported by the United Nations Organization (UNO), 130 million people will be affected by OA with over 40 million people with severe disability due to disease progression [3].

The etiology of OA is believed to be multifactorial. Some of the main reasons include the biomechanical disease progression due to the narrowing of space in the joints, bone hypertrophy, and formation of new osteophytes in the articular margins causing stiffness and pain in the joints. In addition, an imbalance in the synthesis and release of cytokines by chondrocytes in the disease state could be the main reason for the continual inflammatory state in the joint. During the initial stages of OA, catabolic interleukins (IL) such as IL-1α and IL-1β and tumor necrosis factor α (TNFα) increase inflammation affecting cartilage metabolism and homeostasis. TNFα is a proinflammatory cytokine implicated in the degradation of matrix proteins synthesized by

chondrocytes and synoviocytes [5]. Further, increase in the levels of interferon γ (IFNγ) in the joint worsens the inflammatory state and structure of the joint leading to degradation of proteoglycans such as sulfated glycosaminoglycans (sGAG) [5, 6].

2. Current treatment options for osteoarthritis

Currently, pain in OA is pharmacologically managed using nonsteroidal anti-inflammatory drugs (NSAIDS), opioids, and analgesics. Corticosteroid injections have also been used for relieving severe pain in OA patients. Recent attempts have been made to use TNFα blockers as recent studies have proven the significant role of TNFα in contribution to the pathogenesis of OA [7]. Research by several groups has implicated the role of nerve growth factor (NGF) and its binding to tropomyosin receptor kinase A (trk A) which leads to downstream signaling and activation of peripheral and central pain molecules causing severe pain. The therapeutic efficiency of anti-NGF antibodies to block NGF or its antagonists has been studied by several groups for relieving pain. The pain-relieving effects of anti-NGF antibodies fasinumab and fulranumab manufactured by Regeneron Pharmaceuticals and Janssen Pharmaceutica, respectively, have been evaluated in phase III clinical trials [8]. In addition to pain relief, efforts have been made to halt further cartilage damage using slow-acting symptomatic drugs such as chondroitin sulfate and glucosamine sulfate. Orally administered chondroitin and glucosamine have shown to relieve joint pain equivalently compared to NSAIDs. These molecules, intact or broken, could be absorbed into the matrix of the joint and prevent cartilage degeneration. Although glucosamine and chondroitin sulfate have been clinically proven to be safe, their therapeutic efficacy in protecting the cartilage matrix was found to the variable [9]. In grade 4 OA (Kellgren and Lawrence classifications), patients are advised to opt for total knee replacement surgery [10]. Alternatively, autologous chondrocyte implantation (ACI) has been suggested and reported to be successful. In the ACI method, the chondrocytes from patients are taken, culture-expanded in vitro, and then implanted back into the knees of patients. This procedure is invasive and has a lesser success rate than total knee replacement surgeries [11].

Apart from ACI, the efficacy of autologous platelet-rich plasma (PRP) in providing pain relief and promoting cartilage regeneration has been recently investigated by several groups [12]. The PRP is rich in platelets that secrete several growth factors and cytokines such as platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), and prostaglandin E2 (PGE-2) [13]. Several research groups have reported that intra-articular injections of PRP primarily reduced inflammation mediated by PGE-2, HGF, and IGF-1. IGF-1 synthesized and secreted by platelets is shown to prevent leukocyte infiltration into the joint space, thereby reducing the levels of IL-1β and TNFα in the synovial fluid [13]. Overall intra-articular injection of PRP has been shown to maintain joint homeostasis. However, clinical trial data suggest that the effect of PRP seems to last for only 3 weeks and thereafter reduces. The symptoms of OA were seen to relapse after a period of 1 year. Although promising results were observed using PRP in the hydrogel, chitosan, or hyaluronic acid (HA) scaffolds [14], efficacy is yet to be shown in elaborate randomized clinical trials (RCTs).

3. Mesenchymal stromal cells

The history of mesenchymal stromal/stem cells (MSCs) dates back to 1960 when seminal studies conducted by Friedenstein showed the isolation of MSCs from bone

marrow (BM) which were capable of forming ectopic bone in vivo. This was found to be a non-hematopoietic fibroblast-like, colony-forming cell which primarily supported hematopoietic stem cells in the perivascular niche [15]. Owen and Friedenstein discovered that these cells were capable of differentiating into the osteogenic lineage [16]. Subsequently, the multipotent plasticity of that bone marrow MSCs (BMMSCs) was identified and shown that they were capable of differentiating into osteocytes, chondrocytes, and adipocytes in vitro [17]. In addition to the abovementioned three lineages, Caplan and colleagues demonstrated that these cells were capable of differentiating into cells of the muscle, tendons/ligaments, and connective tissue after which he coined the term "mesenchymal stem cells" [18]. Bianco and Gehron Robey deduced that *cbfa1* gene was the master regulator for directing the osteogenic fate of MSCs. Because of the ability of MSCs to form osteocytes, they named them skeletal stem cells [19]. In 2006, the International Society for Cellular Therapy (ISCT) proposed the name multipotent mesenchymal stromal cells and defined that MSCs must adhere to the criteria of being plastic adherent; express surface markers CD105, CD73, and CD90; lack the expression of hematopoietic markers CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR; and differentiate into osteoblasts, chondrocytes, and adipocytes under suitable conditions in vitro [20]. In addition to their differentiation capacity, MSCs have been shown to elicit immunosuppressive and immunomodulatory effects on T lymphocytes, B cells, dendritic cells (DC), and natural killer (NK) cells either by cell-cell interactions or by secretion of anti-inflammatory molecules such as indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE-2), interleukin-4 (IL-4), interleukin-10 (IL-10), and transforming growth factor β (TGFβ) making them ideal cell types for treatment of diseases [21–23]. Because of their ability to differentiate into chondrocytes in vitro and with their anti-inflammatory and immunomodulatory functions, they were believed to be candidate cell type to treat diseases such as OA. MSCs have been isolated from over 18 different tissue sources. The most commonly used tissue sources for isolating MSCs apart from bone marrow are the adipose tissue, umbilical cord, placenta, and dental pulp. However, autologous or allogeneic BMMSCs are currently the most widely used cell type in clinical trials for various disease indications. They are considered the "gold standard" MSC type because of their extensive characterization that took place for over 5 decades.

4. Possible mechanism of action (MoA) of BMMSCs for treatment of osteoarthritis

The pathophysiology of OA is characterized by degradation of hyaline cartilage causing narrowing of joint space leading to subchondral sclerosis, subchondral cysts, hypertrophic chondrocytes, and formation of osteophytes. The friction caused by the rubbing of joints results in chronic pain in OA patients [24]. Degeneration of cartilage extracellular matrix (ECM) may be caused due to the increase in the levels of proteolytic enzymes such as matrix metalloproteases (MMPs) and aggrecanases mediated by IL-1β and TNFα [25]. BMMSCs express a wide range of properties that are anticipated to be beneficial for treating genetic, mechanical, and age-related degeneration in diseases such as OA. In our previous publication, we have in detail attempted to deduce the possible mechanism of action (MoA) of allogeneic pooled BMMSC population [25]. Briefly, BMMSCs are known to be immunomodulatory in nature, primarily because of their potential to significantly suppress the proliferation of inflammatory T cells, monocytes, and dendritic cells either by direct cell-to-cell contact. In addition, they secrete a wide range of anti-inflammatory molecules such as PGE-2, IDO, IL1Ra, and IL-10 [26, 27]. BMMSCs influence the local osteoarthritic microenvironment by stimulating

resident chondrogenic progenitor cells and promote their differentiation into mature chondrocytes mediated by secretion of bone morphogenetic proteins (BMPs) and TGFβ1 [28]. BMMSCs are known to differentiate into chondrocytes in vitro using differentiation cues such as BMP-7 and TGFβ1. A similar mechanism could be involved in the differentiation of BMMSCs in vivo. With the increase in the levels of BMP-7 and TGFβ1 in the local joint milieu, mediated by a change in expression of master regulatory genes such as Sox9, HoxA, HoxD, and Gli3, BMMSCs could differentiate into chondrogenic progenitor cells (CPCs) in vivo. The CPCs further differentiate into chondroblasts characterized by definitive upregulation of collagen types II B, IX, and XI. Subsequently, the CPCs differentiate into mature chondrocytes regulated by balanced expression of collagen X (Col X) and synthesize the secretion of collagen II which is made of sGAG building blocks which maintain the structural integrity of hyaline cartilage [25]. Very high expression of collagen X has been linked to hypertrophy of chondrocytes and formation of fibrous cartilage, and thus a regulated expression of Col X would likely result in deposition of hyaline cartilage [29]. From the above-described multimodal MoA, it is clear that BMMSCs are an ideal cell population which could contribute significantly for an effective treatment of OA.

5. Advantages of using a pooled human BMMSC (phBMMSC, Stempeucel®) product for treating osteoarthritis

In the current therapeutic scenario, the common practice is to screen several individual donors, isolate MSCs, and characterize them based on their key characteristics such as their surface marker expression, tri-lineage differentiation potential, and immunomodulatory and paracrine properties [30–32]. It is inevitable that a product that is manufactured using a master cell bank (MCB) made from a single donor will result in exhaustion. Successively, a product that is made using another single donor MSC bank, although presumably similar in basic characteristics qualifying the identity and safety criteria, may not have the same functional attributes which may lead to varied therapeutic outcomes. Eminent scientific groups have demonstrated donor-to-donor variability in properties of MSCs such as their clonogenicity, growth kinetics, and differentiation potential [33]. A comparative analysis of five different BMMSC populations showed significant variation in the proteomic profile of these cells. Only 13% similarity in the proteomic profile which included transcriptional and translational regulators, kinases, receptor proteins, and cytokines between the five BMMSC populations was found. A maximum of 72% similarity in the proteome was observed between two of the five analyzed cell populations [34]. Disparities in clinical trial outcomes have been reported where BMMSCs derived from single donors have been used. A steroid-refractory acute graft-versus-host disease (SR-aGvHD) clinical trial conducted in both children (n = 25) and adults (n = 30) using BMMSC products derived from 92 HLA-matched and HLA-mismatched donors resulted in only 50% overall durable complete response, while the remaining patients did not respond or partially responded to the treatment [35]. Similar variations with limited response rates were observed in a phase III GvHD trial conducted by Osiris Therapeutics using Prochymal® with only 35% complete response rate compared to 30% in the placebo arm [36]. It has been suggested that improper selection of a BM donor and making a single donor-derived cell product could lead to substantial variations in therapeutic outcomes [37]. In order to challenge this issue, some scientific groups have suggested pooling of BMMSCs from two or more donors in order to compensate for the variation and balance the properties between different donor cell populations. Samuelsson et al. showed that a two- or three-donor pooled BMMSC product could

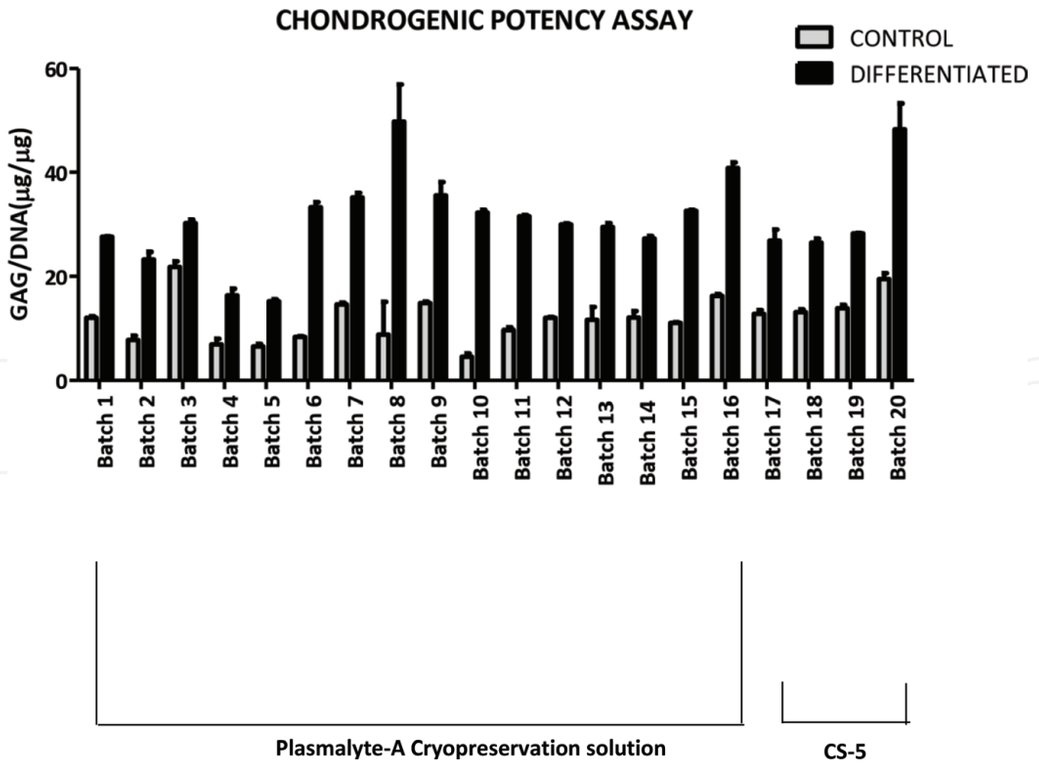


Figure 1.
Chondrogenic potency assessment using quantification of sGAG in 16 batches of Stempeucel® cryopreserved in PlasmaLyte A-based cryopreservation solution and 4 batches of Stempeucel® cryopreserved in CS 5.

optimize the immunosuppressive properties of these cells in vitro [38]. Later, Kuçi et al. showed substantial variability in the immunosuppressive properties of individual donor-derived BMMSCs (n = 8). On the contrary, a mesenchymal end product (MEP) made by pooling BMMNCs from eight donors resulted in a cell population that consistently suppressed an MLR in vitro [39]. Subsequently, they went on to conduct a multicentric SR-aGvHD clinical trial in 51 children and 18 adults using MEP/MSC Frankfurt am Main (MSC-FFM, Obnitix®) cells and observed 83% overall response (complete response, 32%; partial response, 51%) [40]. At Stempeutics Research Pvt. Ltd., we were the first group to develop an allogeneic pooled human BMMSC product called Stempeucel® using an established, robust pooling protocol and a two-tier manufacturing and banking system as previously described [41, 42]. Recently, we have published our comprehensive studies including in vitro chondrogenic properties and preclinical and clinical findings establishing the efficacy and safety of using Stempeucel® for the treatment of OA of the knee joint [43]. In this study, we found that several manufactured batches of Stempeucel®, when differentiated into the chondrocyte lineage, downregulated the expression of the gene Sox9 and upregulated the expression of collagen type 2A (Col2A) gene confirming their differentiation into the chondrogenic lineage. The same Stempeucel® batches synthesized substantial levels of sGAG (30 ± 1.8 µg/µg GAG/DNA) which were estimated using a dimethylmethylene blue-based biochemical assay kit (**Figure 1**). These properties indicate that Stempeucel® could be a potential treatment option for treating OA.

6. Development of a potency assay for Stempeucel® intended to treat osteoarthritis

The US Food and Drug Administration (USFDA) describes potency assays as "The specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the

product in the manner intended, to effect a given result" (US-FDA, 21 CFR 600). For any cell therapy product (CTP) intended to be used for a particular indication, a specific, quantifiable, potency test or array must be developed. The development of a potency assay must begin with in vitro and preclinical studies based on the MoA of the CTP. The confirmation of the assay or the identified marker must be evaluated in every large-scale manufactured batch of the CTP during the progress of the phase I and phase II clinical studies. A quantifiable range for the potency test must be defined and implemented during the course of phase III clinical trial [44]. In order to predict the efficacy of a CTP, either in vitro biochemical assays or biological assays or in vivo biological assessment could be implemented. For example, a company called TiGenix (Leuven, Belgium) has developed and adopted an assay matrix where an ex vivo polymerase chain reaction (PCR) array for autologous chondrocytes (ChondroCelect) is performed and ectopic cartilage formation is correlated to the histology sections of an orthotopic goat model where ChondroCelect is implanted [45, 46]. Jeong et al. have demonstrated that thrombospondin-2 (TSP-2) could be an effective marker to predict the chondrogenic efficiency of umbilical cord-derived MSCs (UC-MSCs). They demonstrated that UC-MSCs, through the TSP-2 secretion, can promote chondrogenesis via PKCa, ERK, P38/MAPK, and Notch signaling pathways [47]. Recently, another group estimated the levels of TSP-2 to evaluate the chondrogenic potency of a UC-MSC product (Cellistem®OA, Cells for Cells, Brazil) intended to be used in phase I/phase II RCT for knee OA [48]. Other scientific groups have shown that autologous culture-expanded chondrocytes could be embedded in collagen-1 and injected subcutaneously in nude mice to predict the potency of several bioactive molecules in promoting chondrogenesis [49]. For the first time, we have developed a chondrogenic potency assay for an allogeneic pooled bone marrow-derived MSC product (phBMMSCs, Stempeucel®). Preliminarily, we culture-expanded and differentiated several Stempeucel® batches into the chondrogenic lineage using commercially available differentiation assay kits (Thermo Fisher Scientific, USA). To confirm the differentiation, we evaluated the Col2A mRNA expression in differentiated cells and compared them with the undifferentiated control cells. After observing a significant increase in the Col2A expression of differentiated cells, we enzymatically digested both the differentiated and undifferentiated cells to quantify the levels of sGAG synthesized by these cells using a 1,9-dimethylmethylene blue (DMMB)-based assay kit (Blyscan, Biocolor, UK). We further normalized the levels of sGAG with the amount of DNA from the same number of cells. We evaluated the sGAG levels in 20 batches of Stempeucel® of which 16 batches were cryopreserved in our older formulation (10% dimethyl sulfoxide (DMSO), 5% human serum albumin (HSA) and PlasmaLyte A) and also four batches of Stempeucel® cryopreserved in a new cGMP grade CryoStor 5 solution (CS5, BioLife Solutions). We observed a significant and consistent increase in the levels of sGAG in the differentiated cells compared to the undifferentiated cells (undifferentiated, 11.9 ± 4.6 GAG/DNA (μg/μg); differentiated, 31 ± 8.6 GAG/DNA (μg/μg; $P < 0.0001$; $n = 20$)) (**Figure 1**). Based on our results, we propose that the sGAG assay is a simple, quantifiable, and robust potency assay which could also be a part of a bigger potency assay matrix to predict the chondrogenic potency of therapeutic cells intended to treat cartilage defects.

7. Preclinical efficacy studies in OA

Many studies have demonstrated that MSCs are nontoxic and non-tumorigenic when tested in various animal models [50, 51]. Prior to evaluating the efficacy of Stempeucel® in an appropriate preclinical model of OA, we had earlier evaluated the preclinical safety and toxicity of Stempeucel® in rodent and non-rodent

Author/ year	Animal	OA model	Cell type and dose	Vehicle	Study duration time points	Reference
Murphy et al. (2003)	Goat	ACLT-meniscectomy	10×10^6 Autologous (goat) BMMSC + HA	HA	12 and 26 weeks	[65]
Frisbie et al. (2009)	Horse	Arthroscopic surgery	10.5×10^6 Autologous (horse) BMMSC	Saline	10 weeks	[61]
Sato et al. (2012)	Pig	Spontaneous	7×10^6 Xenogeneic (human) BMMSC	HA/PBS	1, 3, and 5 weeks	[66]
Song et al. (2014)	Sheep	ACLT-meniscectomy	10×10^6 Autologous (sheep) BMMSC	PBS	8 weeks	[63]
Delling et al. (2015)	Sheep	Bilateral meniscectomy	20×10^6 Autologous (sheep) BMMSC	PBS	0, 1, 4, 8, and 12 weeks	[64]
Singh et al. (2014)	Rabbit	ACLT	1×10^6 Autologous (rabbit) BMMSC	Culture medium	4 and 6 weeks	[55]
Chiang et al. (2016)	Rabbit	ACLT	1×10^6 Allogeneic (rabbit) BMMSC	HA	6 and 12 weeks	[56]
Diekman et al. (2013)	Mouse	Closed tibial plateau fracture	1×10^5 Allogeneic (mice) BMMSC	Saline/mouse albumin	8 weeks	[52]
Suhaeb et al. (2012)	Rat	MIA injection	3.5×10^6 Allogeneic (rat) BMMSC	HA	3 and 9 weeks	[67]
Kim et al. (2014)	Rat	ACLT-meniscectomy	1×10^6 Allogeneic (rat) BMMSC	Culture medium	3 and 6 weeks	[53]
Yang et al. (2015)	Rat	ACLT-meniscectomy	0.5×10^6 Autologous (rat) BMMSC	PBS	3 weeks	[54]
Gupta et al. (2016)	Rat	MIA injection	0.6×10^6 or 1.3×10^6 Xenogeneic (human) pooled BMMSC	PlasmaLyte A	4, 8, and 12 weeks	[43]

Table 1.
OA preclinical studies using BMMSCs.

models. In the same study, we evaluated the feasibility of multiple routes of cell injection. Tumorigenic analysis in severe combined immunodeficient (SCID) mice showed that Stempeucel® is non-tumorigenic. In addition, the biodistribution kinetics of CM-DiI labeled Stempeucel® in the systemic circulation and also in muscle tissue were studied in both rats and mice [51].

It is important to demonstrate the efficacy of any cell therapy product in an animal model of disease before administrating the product in humans with the same disease. It is imperative to determine the suitability of using animal stem cells in animals or human stem cells in immunocompromised/immunocompetent animals. A common regulatory requirement is to have animal data for the same test product that is intended to be tested in humans. In our recently published work, we evaluated the efficacy of Stempeucel® in a monosodium iodoacetate (MIA)-induced

OA model in Wistar rats. We demonstrated the dose-dependent efficacy of two Stempeucel® doses of 0.65×10^6 (25×10^6 human equivalent dose, HED) and 1.3×10^6 (50×10^6 HED) followed by an injection of hyaluronic acid (HA). A significant dose-dependent reduction in pain scores was observed in both low and high Stempeucel® doses compared to the HA alone and disease control group. Histological evaluation of joint tissue sections in all study groups showed significant improvement in proteoglycan staining in both low and high Stempeucel® administered groups indicating significant regeneration of the cartilage in both groups compared to the HA alone and disease control groups [43].

Similar to the animal model we used, other scientific groups have created articular cartilage defects in small animals, such as mice [52], rats [43, 53, 54], and rabbits [55, 56]. Smaller animal models are cost-effective and easy to house, and rodents are available in a variety of genetically modified strains with minimal biological variability [57, 58]. However, the small joint size, thin cartilage, altered biomechanics, and increased spontaneous intrinsic healing hamper the study of the regenerative capacity of stem cells and these mechanisms of healing which cannot be fully extrapolated to human cartilage repair [59, 60]. Rodents have mainly been used to assess the chondrogenesis of cell-based therapies by subcutaneous, intramuscular, and intra-articular implantations of cells [60]. Of all small animals, the rabbit model is the most utilized model in cartilage regeneration studies because of the slightly larger knee joint size than rodents [55, 56]. Despite their limited translational capacity, small animals can be very useful as a proof-of-principle study and to assess therapy safety before moving on to preclinical studies using larger animals [60].

Large animal models play a more substantial role in translational research because of a larger joint size and thicker cartilage; however, their preclinical use is often hindered by high costs and difficulties in animal handling. A variety of large animal models have been used to investigate cartilage repair strategies, including horses [61], dogs [62], sheep [63, 64], goats [65], and pigs [66], each with their own strengths and limitations. We have listed some relevant published studies which have used autologous, allogeneic, or xenogeneic BMMSCs to treat OA induced by various methods (**Table 1**).

Based on the positive efficacy outcomes of our preclinical study, subsequently, we demonstrated the safety and optimal dose for efficacy in a phBMMSC product, Stempeucel®, in a randomized, double-blind, placebo-controlled dose-finding phase II clinical trial in Indian patients [43].

8. Clinical trials in osteoarthritis of the knee joint

8.1 Safety of mesenchymal stromal cells in clinical trials

Lalu MM et al. conducted a systematic review of clinical trials that examined the use of MSCs to evaluate their safety [68]. A total of 36 studies having 1012 participants with different clinical conditions was evaluated. Eight studies were randomized control trials (RCTs) and enrolled 321 participants. Only prospective clinical trials that used the intravenous or intra-arterial route of administration in different age groups were analyzed. Meta-analysis did not detect an association with MSC administration and acute infusional toxicity, organ system complications, infection, and death. There was a significant association between MSCs and transient fever at or shortly after MSC administration which was not associated with long-term sequelae. Most importantly, the meta-analysis showed no serious adverse event due to the administration of MSCs and specifically found no association between MSCs and tumor formation. In another study, Peeters et al. [69] did a systemic review of

the safety of intra-articular administration of culture-expanded stem cells. A total of 844 procedures (mean follow-up of 21 months) was analyzed. Four SAEs were reported—one infection following bone marrow aspiration (BMA) that resolved with antibiotics, one pulmonary embolism after 2 weeks of BMA, and two adverse events not related to the therapy. Other adverse events documented were increased pain/swelling and dehydration after BMA. In another review, a recent analysis of adverse events (AEs) in 2372 orthopedic patients treated with autologous stem cell therapies and followed up for 2.2 years has been published [70]. The common AEs reported included post-procedure pain and pain due to progressive degenerative joint disease in under 4% of the population. Hence, we can conclude that the systemic administration of MSC including intra-articular administration is safe.

8.2 Efficacy of stem cells including mesenchymal stromal cells in clinical trials of osteoarthritis of the knee joint

Several clinical trials have been conducted using bone marrow mononuclear cells, adipose tissue-derived stromal vascular fraction (AD-SVF), adipose tissue-derived mesenchymal stromal cells (AD-MSCs), or bone marrow-derived mesenchymal stromal cells (BMMSCs) in OA of the knee joint. The list of the published clinical trials in chronological order is given in **Table 2**. Administration of the cells has been fairly standardized, with the cells being administered either directly intra-articularly or under ultrasound guidance. Few trials have been conducted using the arthroscopic method of administration with direct implantation of the cells alone or with a scaffold at the site of cartilage injury.

The first clinical study has been published way back in 2002 by Wakitani et al. [71]. In this study of 12 patients who underwent high tibial osteotomy, BMMSCs at a dose of 13 million cells were embedded in collagen gel and transplanted into the cartilage defect and covered with autologous periosteum. The clinical improvement was not significantly different from the control group, but the arthroscopic and histological evidence was better in the transplanted group than the control arm. Since then many studies have been published, but still many contentious issues regarding cell therapy in OA are being discussed. We will try to discuss a few burning issues in this chapter:

a. **Level of evidence regarding the use of MSC therapy in OA**: Jevotovsky et al. [106] did a systemic review of 61 studies to look at the study evidence level, MSC protocol, treatment results, and AEs. The levels of evidence were defined by Marx et al. stating the level of evidence as level 1, randomized controlled trial; level II, prospective cohort study or observational study with dramatic effects; level III, retrospective cohort study or case–control study; level IV, case series; and level V, mechanism-based reasoning [107]. These levels of evidence help physicians to come to clinical decisions. In this review, a total of 2390 patients in 61 studies was identified. Most of the studies used adipose-derived stem cells (ADSCs) (n = 29) or bone marrow-derived stem cells (BMSCs) (n = 30). The majority of the studies (57%) were level IV evidence which consists of therapeutic case series without comparative groups. Only five and nine studies were level I and level II evidence, respectively, in a total of 288 patients. Additionally, 11% were level III retrospective cohort studies, and 8% were level V single-patient case reports. The published data highlights the need for more level I and level II evidence to evaluate the role of MSC treatment in OA patients. However, the majority of the studies have reported positive results and an association between MSC therapy and symptomatic and radiologic improvement in these patients.

Author/ year	Sample size	Study design	Grade of OA	Cell type and dose	Control group	Outcome measures	Outcomes	Follow-up period	Reference
Wakitani (2002)	24	A single-arm control study	Stage I to stage II Ahlback changes	Auto BMMSC, 13 × 10^6 cells embedded in soluble collagen (n = 12)	High tibial osteotomy (n = 12)	Hospital for Special Surgery knee rating scale, arthroscopy and histology	No significant difference in clinical evaluations between the two groups, arthroscopy and histology showed partially hyaline cartilage-like tissue	16 months	[71]
Centeno et al. (2008)	1	Case study	—	Auto BMMSC, 2.4 × 10^6 cells + 1 ml NC + 1 ml PRP	Nil	VAS, ROM, and MRI	Decreased VAS pain scores; increased the range of motion; MRI, statistically significant cartilage and meniscus growth	6 months	[72]
Haleem et al. (2010)	5	Case series	Outerbridge grade III or grade IV	Auto BMMSC + PR-FG, 15 × 10^6 cells	Nil	Lysholm and RHSSK scores and X-rays and MRI	Lysholm and RHSSK scores showed statistically significant improvement, MRI of three patients revealed complete defect fill	12 months	[73]
Nejadnik et al. (2010)	72	Cohort study	Lesion grade 3 or grade 4	Auto BMMSC	n = 12 each in BMMSC and chondrocyte group	ICRS, SF-36, IKDC, Lysholm knee scale and Tegner activity level scale	SF-36 showed physical role functioning improvement in the BMMSC group, no difference in other outcome measures	24 months	[74]
Davatchi et al. (2011)	4	Single-arm study	Moderate to severe OA	Auto BMMSC, 8–9 × 10^6 cells	Nil	VAS pain score, time to walk and number of stairs to climb to produce pain, the resting time to induce the gelling pain, ROM and patellae crepitus	Walking time for the pain to appear improved for three patient, VAS pain score and number of stairs to climb improved for all patients, improvement in crepitus	6 months	[75]
Saw et al. (2011)	5	Single-arm study	ICRS grade 3 or grade 4	Auto PBPC (8 ml) + HA/ weekly injection × 5	Nil	Arthroscopy and histology	Arthroscopy showed articular cartilage regeneration and histologically showed hyaline cartilage	26 months	[76]

Author/ year	Sample size	Study design	Grade of OA	Cell type and dose	Control group	Outcome measures	Outcomes	Follow-up period	Reference
Koh et al. (2012)	25	Case–control study	KL grade 3 or grade 4	Arthroscopic debridement + Auto AD-MSC, 1.89 × 10^6 cells + PRP	Arthroscopic debridement + PRP	Lysholm score, Tegner activity scale, and VAS scores	The clinical scores preoperatively were significantly poorer than those of the control group but at the last follow-up visit were similar and not significantly different between the two groups	18 months	[77]
Orozco et al. (2013)	12	Case series	KL grade 2 to grade 4	Auto BMMSC, 40 × 10^6 cells	Nil	VAS score, Lequesne indexes, WOMAC scores, MRI T2 mapping	All clinical scores decreased significantly, MRI T2 mapping showed improvement of cartilage quality	12 months	[78]
Koh et al. (2013)	18	Case series	KL grade 3 or grade 4	Auto AD-MSC, 1.18 × 10^6 cells + PRP	Nil	WOMAC score, Lysholm score, Tegner activity scale, and VAS scores, MRI (WORMS score)	WOMAC, Lysholm, Tegner, and VAS scores improved significantly, WORMS score in MRI improved significantly	26 months	[79]
Van Pham et al. (2014)	21	Case series	KL grade 2 or grade 3	Auto SVF	Nil	VAS, Lysholm score, and MRI	VAS scores improved, Lysholm scores increased, MRI showed increased cartilage thickness	8.5 months	[80]
Koh et al. (2014)	37 knees (35 patients)	Case series	KL grade 1 or grade 2	Auto AD-MSC, 3.8 × 10^6 cells	Nil	IKDC score, Tegner activity scale, and cartilage repair assessed using ICRS grading	IKDC and Tegner activity scale scores significantly improved, ICRS grades showed 2 of the 37 lesions (5%) were grade I (normal) and 7 (19%) were grade II (near normal)	26.5 months	[81]
Koh et al. (2015)	30	Case series	KL grade 2 or grade 3	Auto SVF + PRP, 42 × 10^6 cells	Nil	Lysholm score, KOOS, VAS score, arthroscopic evaluation (n = 16)	Significant improvement in all clinical outcomes, 87.5% of patients (14/16) improved or maintained cartilage status	24 months	[82]

Author/ year	Sample size	Study design	Grade of OA	Cell type and dose	Control group	Outcome measures	Outcomes	Follow-up period	Reference
Munar et al. (2015)	50	Case series	KL grade 2 to grade 4	Auto BMMSC, 40 × 10^6 cells	Nil	VAS, Lequesne and WOMAC indices, MRI (T2 mapping)	All clinical scores improved, T2 mapping, PCI decreased significantly	12 months	[83]
Davatchi et al. (2016)	4	Case series	Moderate to severe OA	Auto BMMSC, 8–9 × 10^6 cells	Nil	VAS pain score, time to walk and number of stairs to climb to produce pain, the resting time to induce the gelling pain, ROM and patellae crepitus	All parameters still better than baseline at 5 years follow-up for three patients	60 months	[84]
Soler et al. (2016)	15	Single-arm, open-label phase I/phase II trial	KL grade 2 or grade 3	Auto BMMSC, 41 × 10^6 cells	Nil	VAS score, questionnaire, QOL SF-36 questionnaire, Lequesne functional index and WOMAC score, MRI (T2 mapping)	The clinical scores improved, SF-36 showed improvement of parameters, T2 mapping showed signs of cartilage regeneration	12 months	[85]
Sampson et al. (2016)	125	Retrospective case series	KL grade 3 or grade 4	Auto BMC + PRP (8 weeks apart)	Nil	VAS score, patient satisfaction scale	VAS score and patient satisfaction score improved in all patients	4.8 months	[86]
Fodor and Paulseth (2016)	6 patients (8 knees)	Case series	KL grade 1 to grade 3	Auto SVF, 14.1 × 10^6 cells	Nil	VAS score, WOMAC score, ROM, TUG test, and MRI	VAS and WOMAC scores significantly improved, ROM and TUG improved, MRI showed no detectable structural differences	12 months	[87]
Kim et al. (2016)	20 patients (24 knees)	Case series	KL grade 1 or grade 2	Auto AD-MSC, 4.4 × 10^6 cells	Nil	IKDC score, Tegner activity scale, MRI MOAKS and MOCART score	Clinical outcomes significantly improved, MOAKS and MOCART score significantly improved	24 months	[88]

Author/ year	Sample size	Study design	Grade of OA	Cell type and dose	Control group	Outcome measures	Outcomes	Follow-up period	Reference
Pak et al. (2016)	3	Case reports	KL grade 3	Auto AD-SVF + HA + PRP, PRP repeated weekly × 3	Nil	FRI, ROM and VAS score, MRI	All clinical scores improved in three patients, MRI showed cartilage-like tissue regeneration	4.5 months	[89]
Pers et al. (2016)	18	Open-label, phase I study	KL grade 3 or grade 4	Auto AD-MSC, low (2×10^6), medium (10×10^6), and high dose ($50x10^6$), 6 patients each	Nil	Safety, WOMAC, VAS, PGA, SAS and KOOS index, MRI, dGEMRIC in 6 patients	Safety established, low dose most effective, and all parameters improved as compared to baseline, dGEMRIC improved in three patients	6 months	[90]
Koh et al. (2016)	80	RCT	ICRS grade 3 or grade 4	Auto AD-MSC + fibrin glue + microfracture, 5×10^6 cells (group 1)	n = 40 (group 2) (microfracture)	Lysholm score, KOOS, VAS score, MRI, cartilage repair tissue scoring system, arthroscopy and histology	MRI, better signal intensity for repair tissue in group 1 (80%) as compared to 72.5% in group 2; KOOS pain and symptom subscores, significantly greater for group 1; arthroscopy and histology, no significant difference	24 months	[91]
Gupta et al. (2016)	60	Double-blind, phase II, RCT	KL grade 2 or grade 3	Allo BMMSC + HA, four doses (25, 50, 75, 150×10^6 cells)	n = 20 (placebo + HA)	Safety, VAS, ICOAP, WOMAC, MRI, WORMS score	Safety established; AE were predominant in the higher-dose groups; VAS, ICOAP, and WOMAC scores best in the lowest dose; MRI, no significant difference	24 months	[43]
Lamo-Espinosa et al. (2016)	30	Phase I/phase II, RCT	KL grade 2 to grade 4	Auto BMMSC + HA, two doses (10 and 100×10^6 cells)	n = 10, (HA)	Safety, VAS score, WOMAC, MRI, WORMS	Safety established. VAS, WOMAC, and WORMS scores significant in high-dose group at 12 months follow-up	12 months	[92]
de Windt et al. (2017)	10	Phase I/phase II single-center study	Modified Outerbridge grade 3 or grade 4	Allo BMMSCs +10 or 20% autologous chondrons	Nil	Safety, KOOS, VAS, MRI, second-look arthroscopy, histology	No SUSAR; KOOS and VAS scores improved significantly; MRI, showed complete filling of the defect; arthroscopy, effective defect fill, and integration in the surrounding tissue; histology, positive staining for both type I and type II collagen and proteoglycan	12 months	[93]

Author/ year	Sample size	Study design	Grade of OA	Cell type and dose	Control group	Outcome measures	Outcomes	Follow-up period	Reference
Turajane et al. (2017)	60	RCT, single-center study	KL grade 1 to grade 3	3 groups, 20 each; first group (AAPBSC + PRP + G-CSF+ HA + MCS); second group (AAPBSC + PRP + HA + MCS); third group (control) (all given weekly × 3 injections)	20 patients, IA, HA alone	Avoidance of TKA intervention and WOMAC scores	TKA done in three patients in the control group but not in the cell group; WOMAC, all groups reached statistically significant improvements within the individual (intra) groups	12 months	[94]
Shapiro et al. (2017)	25 patients, 50 knees	RCT, single-blind, placebo-controlled	Bilateral OA, KL grade 1 to grade 3	25 knees; 5 ml of Auto BMAC +10 ml of platelet-poor bone marrow plasma	25 knees; sterile saline, 15 ml	ICOAP, VAS scores, MRI, T2 mapping	No SAE, patients had a similar decrease in scores in VAS and ICOAP scores in both BMAC- and saline-treated arthritic knees	6 months	[95]
Park et al. (2017)	7	Open-label, single-arm, phase I/ phase II	KL grade 3 and ICRS grade 4	Two doses; Allo hUCB MSCs and HA hydrogel	Nil	ICRS cartilage repair, VAS, IKDC, MRI, histological findings	VAS and IKDC improved at 24 weeks and stable till 7 years; histology at 1 year showed hyaline-like cartilage; MRI at 3 years showed the persistence of regenerated cartilage	7 years	[96]
Nguyen et al. (2017)	30	Placebo-controlled trial	KL grade 2 or grade 3	15 patients; AM + Auto AD (SVF + PRP; 10^7 SVF cells/ml)	15 patients, AM	Safety, WOMAC, Lysholm, and modified VAS scores, MRI	Safety established, WOMAC scores not significant between two arms at 6 and 12 months but significant at 18 months; increased Lysholm and VAS scores in the treatment group compared with the placebo; MRI, MRI demonstrated cartilage layer was thicker in the treatment group	18 months	[97]
Pintat et al. (2017)	19	Single-arm study	Patellofemoral OA	IA AD-MSC + PRP	Nil	WOMAC, MRI, T2 mapping	WOMAC scores significantly lower in treatment arm than baseline; MRI, no change	12 months	[98]

Author/ year	Sample size	Study design	Grade of OA	Cell type and dose	Control group	Outcome measures	Outcomes	Follow-up period	Reference
Russo et al. (2017)	30	Single-arm study	KL grade 1 to grade 3, grade > II (ICRS classification)	Auto microfragmented adipose tissue	Nil	VAS, KOOS, IKCD, subjective, Tegner Lysholm knee	IKDC and KOOS, improvement of 20 points; VAS and Tegner Lysholm score, improvement in 24 and 31 points, respectively	12 months	[99]
Yokota et al. (2017)	13	Single-arm study	KL grade 3 or grade 4	IA Auto AD- SVF; 2.5 ml SVF containing 3×10^7 SVF cells/knee	Nil	VAS, JKOM, WOMAC	Scores improved by an average of 35% over baseline for JKOM, 32% improvement in WOMAC, and 40% for pain (VAS)	6 months	[100]
Jo et al. (2017)	18	Single-arm study	Knee OA	Auto AD-MSC; 3 doses (10×10^6, 50×10^6, and 100×10^6 AD-MSCs)	Nil	WOMAC, KSS, KOOS, VAS, MRI, size and depth of the cartilage defect, the signal intensity of regenerated cartilage and cartilage volume	No TEAE; WOMAC, KSS, and KOOS, improved knee function; VAS, improved pain (statistical significance in high dose); MRI, improvements in all parameters	24 months	[101]
Garay Mendoza et al. (2017)	61	Open-label, phase I/phase II controlled trial	Knee OA	Cell group, BM stimulation with subcutaneous administration of G-CSF (n = 30)	Control group, oral acetaminophen (n = 31)	VAS and WOMAC scores	BM-SC group showed significant improvement in knee pain and quality of life	6 months	[102]
Kuah et al. (2018)	20	RCT, double-blind, placebo-controlled	KL grade 1 to grade 3	Randomized 4:1; Progenza (PRG) (Allo AD-MSC + culture supernatant); 2 groups, 8 pts. each, 3.9 or 6.7 million cells	4 patients, placebo administered	Safety, WOMAC, VAS, AQoL-4D, biomarkers (urine, C2C and CTX-II; serum, MIF and CTX-I; MRI, MOAKS score)	All patients experienced at least one TEAE; VAS and WOMAC, statistically significant within-group reduction from baseline in PRG group, no statistically significant differences at any time point between placebo and PRG groups; MRI, no decrease in lateral tibial cartilage volume while the placebo group showed a statistically significant cartilage loss	12 months	[103]

Author/ year	Sample size	Study design	Grade of OA	Cell type and dose	Control group	Outcome measures	Outcomes	Follow-up period	Reference
Matas et al. (2018)	29	Phase I/phase II RCT, triple-blind trial	KL grade 1 to grade 3	Allo UC-MSC, single (20×10^6) or repeat dose (20×10^6 baseline and 6 months), 10 pts. each	9 patients, HA (baseline and 6 months)	VAS, WOMAC, MRI, WORMS score	No SAEs, repeat dose group had a significant decrease in VAS and WOMAC scores as compared to HA group, no changes in function subscale, SF36, and MRI	12 months	[48]
Emadedin et al. (2018)	43	RCT, phase I/phase II, placebo-controlled, triple-blind	KL grade 2 to grade 4	Auto BMMSC, 40×10^6 cells (n = 19)	5 ml normal saline (placebo) (n = 24)	VAS, WOMAC, walking distance, painless walking distance, standing time and knee flexion compared	WOMAC, significant improvements in total score, pain, and physical function subscales and improvement in painless walking distance compared with placebo	6 months	[104]
Khalifeh Soltani et al. (2019)	20	RCT, double-blind, placebo-controlled	KL grade 2 to grade 4	Placental-derived MSC, $50–60 \times 10^6$ cells (n = 10)	Normal saline (n = 10)	VAS, KOOS, knee flexion range of motion (ROM), MRI	No SAEs; significant knee ROM improvement at 2 and 24 weeks; VAS, no change; KOOS, improvement till 8 weeks; MRI, chondral thickness improved in about 10% of the total knee joint area AT 24 weeks	24 weeks	[105]

AAPBSC, autologous activated peripheral blood stem cells; AD-MSCs, adipose tissue-derived mesenchymal stromal cells; AEs, adverse events; Auto, autologous; Allo, allogeneic; AM, arthroscopic microfracture; AQoL-4D, assessment of quality of life 4D questionnaire; BMAC, bone marrow aspirate concentrate; BMMSCs, bone marrow-derived mesenchymal stromal cells; C2C, type II collagen C2C peptide; CTX-1, C-terminal telopeptide of type I collagen; CTX-II, C-terminal telopeptide of type II collagen; dGEMRIC, delayed gadolinium-enhanced magnetic resonance imaging of cartilage; FRI, functional rating index; HA, hyaluronic acid; G-CSF, granulocyte colony-stimulating factor; IA, intra-articular; ICOAP, Intermittent and Constant Osteoarthritis Pain; ICRS, International Cartilage Repair Society Cartilage Injury Evaluation Package; IKDC, International Knee Documentation Committee Subjective Knee Evaluation Form; JKOM, Japanese Knee Osteoarthritis Measure; KL grade, Kellgren and Lawrence grade; KOOS, Knee Injury and Osteoarthritis Outcome Scores; KSS, Knee Society clinical rating system; MIF, macrophage migration inhibitory factor; MOAKS, MRI Osteoarthritis Knee Score; MOCART, magnetic resonance observation of cartilage repair tissue; MCS, mesenchymal cell stimulation; MRI, magnetic resonance imaging; NC, nucleated cells; PCI, poor cartilage index; PBPC, peripheral blood progenitor cells; PGA, patient global assessment; PR-FG, platelet-rich fibrin glue; PRP, platelet-rich plasma; QOL, quality of life; RCT, randomized controlled trial; RHSSK, Revised Hospital for Special Surgery knee scores; ROM, range of motion; SAE, serious adverse event; SAS, Short Arthritis Assessment Scale; SF-36, Short Form-36 quality of life questionnaire; SUSAR, suspected unexpected serious adverse reaction; SVF, stromal vascular fraction; TEAE, treatment emergent adverse event; TKA, total knee arthroplasty; TUG, timed up and go; UC-MSC, umbilical cord-derived MSC; VAS, visual analog pain score; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; WORMS, whole-organ magnetic resonance imaging score.

Table 2.
Chronological list of publications of stem cell application for cartilage repair.

b. **Best source of MSC for treatment of OA**: Many studies have been published using different sources of MSCs, and there is no consensus as to which MSC type is the most effective in treating OA. Recently few studies have been published using SVF, bone marrow aspirate concentrate, and micro-fragmented adipose tissue, which further adds to the variability of this issue. The most common problem affecting the clinical outcome in OA is the tendency of MSCs to differentiate into fibrous-like tissue instead of hyaline cartilage [108]. To eliminate or reduce chondrogenesis of the injected MSCs, one school of thought is to identify new sources of MSCs for cartilage repair. Recently synovium-derived stem cells have been used for OA study as it is believed that epigenetic memory may play a role and impact the specific lineage differentiation of MSCs [109]. Hence, the use of synovium stem cells predicts a better outcome as chondrogenic differentiation is expected as it belongs to the same lineage. Fetal stem cells have higher plasticity and proliferation ability than adult stem cells. Hence, fetal tissue-derived stem cells, especially derived from the fetal cartilage, may show higher chondrogenic activity [110] and may be the ideal source of cells for OA. More controlled clinical trials are required to come to a conclusion as to which cell type may be the best choice for the effective treatment of OA.

c. **Autologous or allogeneic source of MSCs**: Most of the published trials used autologous MSCs to minimize immune response, which may lead to best clinical outcomes. Six of the studies in **Table 2** attempted to investigate the potential application of allogeneic MSCs [43, 48, 93, 96, 103, 105] in OA. Recently in the last 2–3 years, most of the studies have attempted to use an allogeneic source of MSCs due to the ease of application. Further, no observed serious adverse effects indicate the safety of allogeneic cells in OA. Around 3000 patients have been administered allogeneic MSCs for different conditions, and no immune response has been reported to date [111]. In a recently published trial using allogeneic umbilical cord-derived MSCs (UC-MSC) in knee OA, patients were randomized to receive hyaluronic acid at baseline and 6 months (HA, n = 8), single-dose (20×10^6) UC-MSC at baseline (MSC-1, n = 9), or repeated UC-MSC doses at baseline and 6 months ($20 \times 10^6 \times 2$; MSC-2, n = 9). No serious adverse events were reported. At 12 months of follow-up, MSC-2-treated group had significantly lower levels of pain [visual analog score (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), total score, and pain subscale] than HA group [48]. Hence, it can be safely concluded that the use of allogeneic MSCs is safe and may be efficacious in OA.

d. **The optimal dose of MSCs for best efficacy in OA**: MSCs have been used in different doses in several clinical trials of OA (**Table 2**). The dose varied from as low as 1.18 million cells [79] to as high as 150 million cells [43]. In a study by Koh et al. [79], 18 patients were given intra-articular injections with adipose tissue-derived MSCs in a mean dose of 1.18 million cells and platelet-rich plasma. At 26 months of follow-up, patients had significant improvement in VAS, Lysholm, and WOMAC scores. Magnetic resonance imaging (MRI) was evaluated using WORMS score and showed statistically significant improvement in the total and cartilage scores. In another dose-finding study, Pers et al. [90] recruited 18 patients who were treated with autologous AD-MSCs in three different doses: low dose (2×10^6 cells), medium dose (10×10^6 cells), and high dose (50×10^6 cells). After 6 months of follow-up, the procedure was found to be safe, and no serious adverse events were reported. Patients in the low dose had significant improvement in pain levels and functions as compared to baseline. In a dose-finding study conducted by Gupta et al. [43], four different doses (25, 50, 75 and 150 million

cells) of allogeneic BMMSCs were used in a total of 60 patients. At 1 year of follow-up, the lower doses of 25 million had shown improvement in pain levels and function as compared to placebo and baseline. However in a study by Jo et al. [101], 18 patients were injected with autologous AD-MSCs in three different doses: 10, 50, and 100 million cells. At 2 years of follow-up, significant improvement in the Knee Society clinical rating system (KSS), Knee Injury and Osteoarthritis Outcome Score (KOOS), and VAS scores was seen in the highest dose of 100 million cells. As can be seen, most of the studies are single-arm studies without any control arm. Hence, to determine the most efficacious dose in OA, more randomized controlled, dose-finding clinical trials are required.

e. **Selection of endpoints for the conduct of clinical trial**: The FDA 2018 draft guidance document for OA regarding the development of structural endpoints for the development of drugs, devices, and biological products for treatment states that approvals for OA to date have been based on patient-reported outcome measures that assess pain and function. For the development of new product in OA, the goal of treatment should be inhibition of structural damage or targeting the underlying pathophysiology associated with OA or significantly delay the complications of joint failure and the need for joint replacement and also to reduce the deterioration of function and worsening of pain. All of the above may be taken into consideration for the development of endpoints for the study in OA [112].

Recently a meta-analysis was done to evaluate the different endpoints used to see the therapeutic efficacy and safety of MSCs for the treatment of patients with knee osteoarthritis [113]. Five hundred eighty-two patients in 11 randomized controlled trials were included in this meta-analysis. It showed that MSC treatment significantly improved VAS and International Knee Documentation Committee (IKDC) scores after 24 months of follow-up compared to controls. MSC therapy also showed significant improvement in the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Lequesne algofunctional indices (Lequesne), Lysholm knee scale (Lysholm), and Tegner activity scale (Tegner) at 12 or 24 months of follow-up. Hence, all the endpoints used currently for evaluation of efficacy in OA have shown significant improvement in different clinical trials:

f. **MRI to evaluate cartilage regeneration**: MRI has emerged as the leading method of imaging soft tissue structures around joints. An ideal MRI study for the cartilage should provide an accurate assessment of cartilage thickness and volume, show morphologic changes of the cartilage surface, show internal cartilage signal changes, and allow evaluation of the subchondral bone for signal abnormalities. Also, it would be desirable for MRI to provide an evaluation of the underlying cartilage physiology, including providing information about the status of the glycosaminoglycan (GAG) and collagen matrices [114]. But, in actual, there is an absence of a standard system by MRI to evaluate cartilage regeneration. Many studies as given in **Table 2** that have used MRI to evaluate cartilage regeneration are only qualitative. It is recommended to use validated imaging outcomes for cartilage regeneration for scientifically validating cell-based therapies, thus advancing the field. The most common parameters used for evaluation of cartilage regeneration by MRI are cartilage thickness in different points in all the compartments of the joint [97], cartilage volume [101], whole-organ magnetic resonance imaging score (WORMS) [43, 48], T2 relaxation time mapping [78, 83, 85, 95, 98], MRI Osteoarthritis Knee Score (MOAKS) score [88, 103], magnetic resonance observation of cartilage repair tissue (MOCART) score [88],

and contrast-enhanced imaging technique known as delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) [90]. Among all the parameters, T2 mapping and WORMS seem to be the most commonly used qualitative parameters used for evaluation of cartilage regeneration as it is sensitive to both changes in cartilage hydration and collagen fibril orientation. In a study by Orozco et al. [78], T2 relaxation measurements demonstrated a highly significant decrease of poor cartilage areas (on average, 27%), with the improvement of cartilage quality in 11 of the 12 patients. In another study by Rich et al. [83], a total of 50 patients was evaluated by T2 mapping at 12 months of follow-up after administration of autologous BMMSCs. The mean poor cartilage index (PCI) significantly decreased in 37 of 50 patients (74%), 10 remained the same (20%), and 3 worsened between 7 and 10% (6%). Hence, cartilage T2 mapping may be a sensitive marker for monitoring cartilage quality in subjects with knee OA as it allows us to accurately determine the grade of disorganization of the extracellular matrix.

g. **Use of MSC alone or MSC with a scaffold for intra-articular injection in OA**: When MSCs are injected intra-articularly alone, MSCs scatter widely in the joint, making it impossible to obtain consistent local concentration at the site of cartilage defect. Hence, with a hope to enhance their efficacy in cartilage regeneration, MSC implantation using scaffolds is being attempted in different clinical trials so that the cells are delivered to the site of interest. Compared to direct intra-articular injection, MSC delivery via a scaffold affords more control of proliferation, matrix production, and self-renewal which may help in the regeneration/repair of degenerated or damaged articular cartilage. Different scaffolds have been designed as the delivery system for the repair of articular cartilage. The different scaffolds which can be used are either made of poly-lactic-co-glycolic acids (PLGA) [115], collagen [116], gelatin [117], tricalcium (TCP) [118], poly-lactic acid (PLA) [115], hyaluronic acid (HA) [119], poly-glycolic acid (PGA), or fibrin glue [120]. HA has been used frequently for implantation of MSCs into the joint. Many clinical studies (**Table 2**) have used HA as scaffold along with MSCs for implantation of the cells. Cartistem®, an approved drug by the Korean FDA for knee OA, is a combination of human umbilical cord blood-derived MSCs and sodium hyaluronate which is directly implanted at the site of cartilage injury into the joint by arthroscopy [96, 121]. Hence, cells with scaffold are the ideal combination for intra-articular delivery for cartilage degeneration. However, further studies are necessary to find optimal implantation vehicles that can result in the regeneration of articular cartilage.

8.3 Clinical trials in India

Few clinical trials using autologous or allogeneic MSCs or mononuclear stem cells in OA have been conducted in India. The trials registered in the Clinical Trials Registry of India are the two trials done by Stempeutics (one phase II trial completed and the other phase III trial ongoing). However, one published trial by Bansal et al. [122] for the single-arm study was done in India in which a total of 10 patients were treated with AD-MSCs. The patients were evaluated for safety, WOMAC, 6-minute walk test (6MWT), and MRI for cartilage thickness. The patients were followed up for 2 years. The total WOMAC and its subscale scores and 6MWT were significantly improved at all-time points till 2 years of follow-up. Cartilage thickness as determined by MRI improved by at least 0.2 mm in six patients, was unchanged in two patients, and decreased by at least 0.2 mm in two patients. The authors concluded that the procedure demonstrated a strong safety profile with no severe adverse events or complications reported.

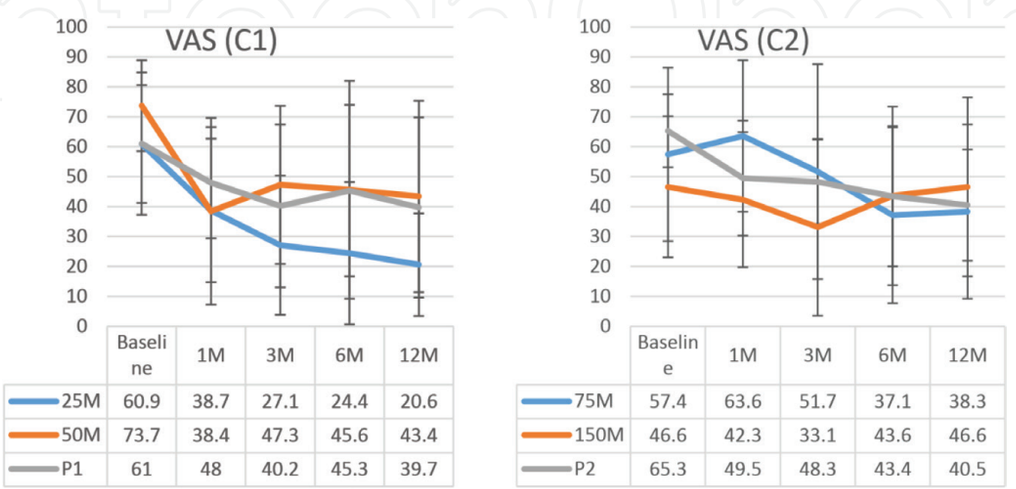
8.4 Stempeutics Research experience in osteoarthritis of the knee joint

The off-the-shelf allogeneic, pooled BMMSC product developed by Stempeutics has completed one phase II clinical trial [43] and currently ongoing phase III trial in knee OA. In our completed phase II trial, we included patients of idiopathic OA in grade 2 or 3 of Kellgren and Lawrence radiographic criteria; patients who had self-reported difficulty in at least one of the following activities attributed to knee pain, lifting and carrying groceries, walking 400 m, getting in and out of a chair, or going up and down stairs; and patients who had been on stable medication, including nonsteroidal anti-inflammatory drugs/opioid analgesics for the past 3 months and in the age group of 40–70 years. All the criteria have to be present before being included in the study [43].

8.4.1 Phase II study in patients with osteoarthritis of the knee joint

The phase II results of Stempeucel® in OA patients have been published [43]. Briefly, it was a double-blind, randomized, placebo-controlled, dose-finding study. In this study, 60 OA patients were randomized to receive different doses of Stempeucel®, 25, 50, 75, and 150 million cells or placebo. Stempeucel® was administered intra-articularly (IA) to the knee joint followed by 2 ml of hyaluronic acid (20 mg). The subjects were followed up for 2 years and were evaluated for safety parameters including AEs, and for efficacy parameters, VAS for pain, Intermittent and Constant Osteoarthritis Pain (ICOAP), WOMAC (total score and its subscales), and MRI were done to evaluate the WORMS score. The intra-articular administration of Stempeucel® was safe with knee pain and swelling as the most common AEs. Clinically relevant improvement in a persistent manner was seen in 25 million dose group in all subjective parameters (VAS, ICOAP, and WOMAC scores) (**Figures 2–4**). WORMS of MRI knee did not reveal any difference from the baseline and placebo group. It was concluded that intra-articular administration of Stempeucel® is safe and 25 million dose may be the most effective among the doses tested.

Currently, we are conducting a phase III trial in OA of the knee joint. This is a randomized, double-blind, multicentric, placebo-controlled study assessing the efficacy and safety of intra-articular administration of Stempeucel® in patients with osteoarthritis of the knee joint. One hundred and forty-six patients will be



	Baseline	1M	3M	6M	12M
25M	60.9	38.7	27.1	24.4	20.6
50M	73.7	38.4	47.3	45.6	43.4
P1	61	48	40.2	45.3	39.7

	Baseline	1M	3M	6M	12M
75M	57.4	63.6	51.7	37.1	38.3
150M	46.6	42.3	33.1	43.6	46.6
P2	65.3	49.5	48.3	43.4	40.5

Figure 2.
Visual analog scale values. Data presented as mean value ± SD; C1 = cohort 1; C2 = cohort 2; 25M, 50M, 75M, 150M = 25, 50, 75, 150 million cells, respectively; 1M, 3M, 6M, 12M = 1, 3, 6, 12 months, respectively.

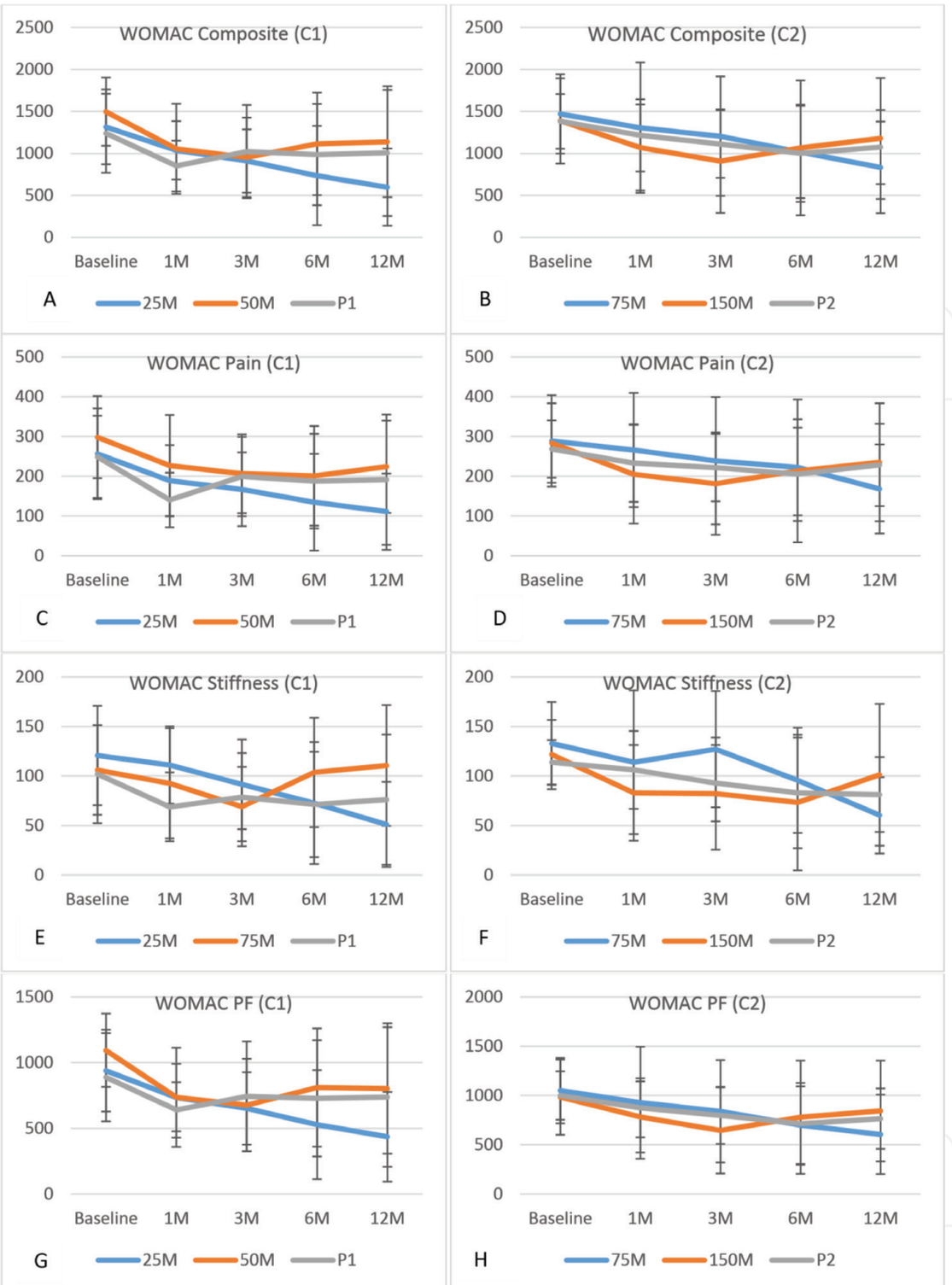


Figure 3.
WOMAC results. WOMAC: (A, B) composite; (C, D) pain; (E, F) stiffness; and (G, H) physical function. Data presented as mean value ± SD; C1 = cohort 1; C2 = cohort 2; 25M, 50M, 75M, 150M = 25, 50, 75, 150 million cells, respectively; 1M, 3M, 6M, 12M = 1, 3, 6, 12 months, respectively; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index.

randomized to stem cell and placebo arm in a ratio of 1:1. Seventy-three patients will receive Stempeucel® (25 million) followed by 2 ml of hyaluronan, and 73 patients will receive only intra-articular injection of 2 ml of placebo followed by 2 ml of hyaluronan. The patients will be followed up for a total of 2 years after IMP administration. The details of the study are found in the Clinical Trials Registry of India (CTRI/2018/09/015785).

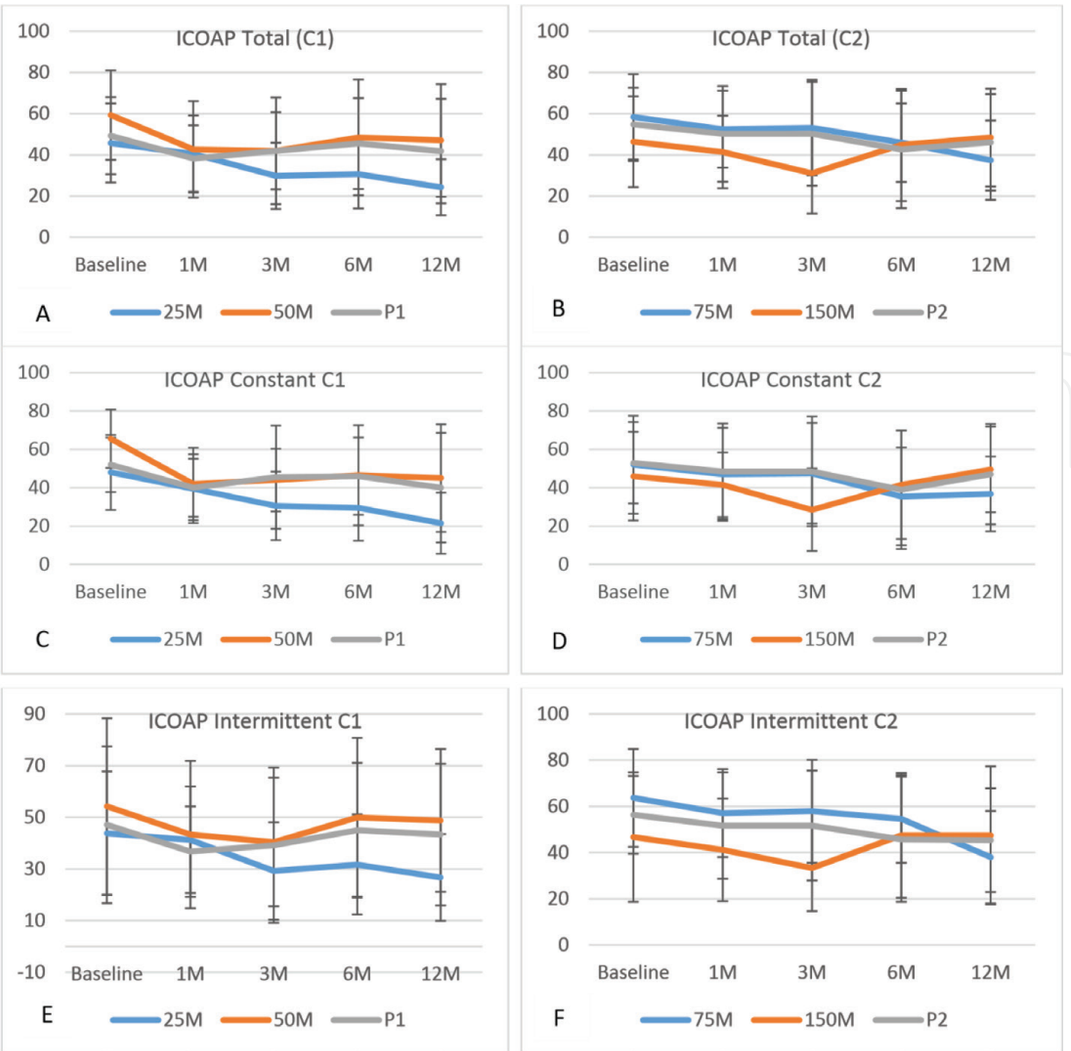


Figure 4.
ICOAP results. ICOAP: (A, B) total; (C, D) continuous pain; and (E, F) intermittent pain. Data presented as mean value ± SD; C1 = cohort 1; C2 = cohort 2; 25M, 50M, 75M, 150M = 25, 50, 75, 150 million cells, respectively; 1M, 3M, 6M, 12M = 1, 3, 6, 12 months, respectively; ICOAP = Intermittent and Constant Osteoarthritis Pain.

9. Conclusion

Osteoarthritis is a common disorder involving damage to synovial joint tissues particularly the cartilage and bone. Current treatments are mostly targeted at end-stage disease, but biological therapies including stem cell therapy show a 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. Pooled BMMSCs with their enhanced anti-inflammatory potential, immunomodulatory properties, and secretion of paracrine factors create the optimum environment for a controlled reparative pathway in the affected joint. Pooled BMMSC treatment, perhaps combined with other modalities like a scaffold, would be advantageous in providing treatment in early OA to slow disease progression, thus delaying or avoiding total joint replacement.

Acknowledgements

We thank Stempeutics Research Pvt. Ltd. and Mr. BN Manohar for proving the required funding for the relevant studies. We would like to thank

Dr. Anish S Majumdar and Dr. Sudha Balasubramanian for their critical scientific inputs for potency assay development, preclinical studies, and clinical studies conducted by Stempeutics Research. We also thank Dr. Anoop Chullikana for his valuable inputs in the clinical studies.

Conflict of interest

The authors declare no conflict of interests.

Author details

Charan Thej and Pawan Kumar Gupta*
Stempeutics Research Pvt. Ltd., Manipal Hospitals Whitefield Pvt. Ltd., Bengaluru, India

*Address all correspondence to: pawan.gupta@stempeutics.com

References

[1] Yucesoy B, Charles LE, Baker B, Burchfiel CM. Occupational and genetic risk factors for osteoarthritis: A review. Work [Internet]. 2015;**50**(2):261-273. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24004806

[2] Hame SL, Alexander RA. Knee osteoarthritis in women. Current Reviews in Musculoskeletal Medicine [Internet]. 2013;**6**(2):182-187. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23471773

[3] Pal CP, Singh P, Chaturvedi S, Pruthi KK, Vij A. Epidemiology of knee osteoarthritis in India and related factors. Indian Journal of Orthopaedics [Internet]. 2016;**50**(5):518-522. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27746495

[4] Musumeci G, Aiello FC, Szychlinska MA, Di Rosa M, Castrogiovanni P, Mobasheri A. Osteoarthritis in the XXIst century: Risk factors and behaviours that influence disease onset and progression. International Journal of Molecular Sciences [Internet]. 2015;**16**(3):6093-6112. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25785564

[5] Martel-Pelletier J, Barr AJ, Cicuttini FM, Conaghan PG, Cooper C, Goldring MB, et al. Osteoarthritis. Nature Reviews Disease Primers [Internet]. 2016;**2**:16072. Available from: http://www.nature.com/articles/nrdp201672

[6] Stubendorff JJ, Lammentausta E, Struglics A, Lindberg L, Heinegård D, Dahlberg LE. Is cartilage sGAG content related to early changes in cartilage disease? Implications for interpretation of dGEMRIC. Osteoarthritis and Cartilage [Internet]. 2012;**20**(5):396-404. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1063458412000398

[7] Hermann W, Lambova S, Müller-Ladner U. Current treatment options for osteoarthritis. Current Rheumatology Reviews [Internet]. 2018;**14**(2):108-116. Available from: http://www.eurekaselect.com/155279/article

[8] Chang DS, Hsu E, Hottinger DG, Cohen SP. Anti-nerve growth factor in pain management: Current evidence. Journal of Pain Research [Internet]. 2016;**9**:373-383. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27354823

[9] Vasiliadis HS, Tsikopoulos K. Glucosamine and chondroitin for the treatment of osteoarthritis. World Journal of Orthopedics [Internet]. 2017;**8**(1):1-11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28144573

[10] Kohn MD, Sassoon AA, Fernando ND. Classifications in brief: Kellgren-Lawrence classification of osteoarthritis. Clinical Orthopaedics and Related Research [Internet]. 2016;**474**(8):1886-1893. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26872913

[11] Zellner J, Krutsch W, Pfeifer C, Koch M, Nerlich M, Angele P. Autologous chondrocyte implantation for cartilage repair: Current perspectives. Orthopedic Research and Reviews [Internet]. 11 Nov 2015;**7**:149-158. Available from: https://www.dovepress.com/autologous-chondrocyte-implantation-for-cartilage-repair-current-persp-peer-reviewed-article-ORR

[12] Dhillon MS, Patel S, John R. PRP in OA knee—Update, current confusions and future options. SICOT-J [Internet]. 2017;**3**:27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28322719

[13] Jungbluth P, Grassmann J-P, Thelen S, Wild M, Sager M, Windolf J, et al. Concentration of platelets and growth factors in platelet-rich plasma from Goettingen minipigs. GMS Interdisciplinary Plastic and Reconstructive Surgery DGPW.

2014;**3**:Doc11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26504722

[14] Glynn LG, Mustafa A, Casey M, Krawczyk J, Blom J, Galvin R, et al. Platelet-rich plasma (PRP) therapy for knee arthritis: A feasibility study in primary care. Pilot and Feasibility Studies [Internet]. 2018;**4**:93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29997901

[15] Friedenstein AJ. Precursor cells of mechanocytes. International Review of Cytology [Internet]. 1976;**47**:327-359. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11195

[16] Owen M, Friedenstein AJ. Stromal stem cells: Marrow-derived osteogenic precursors. Ciba Foundation Symposium [Internet]. 1988;**136**:42-60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3068016

[17] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science [Internet]. 1999;**284**(5411):143-147. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10102814

[18] Caplan AI. Mesenchymal stem cells. Journal of Orthopaedic Research [Internet]. 1991;**9**(5):641-650. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1870029

[19] Bianco P, Gehron RP. Marrow stromal stem cells. Journal of Clinical Investigation [Internet]. 2000;**105**(12):1663-1668. Available from: http://www.jci.org/articles/view/10413

[20] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy [Internet]. 2006;**8**(4):315-317. Available from: http://www.sciencedirect.com/science/article/pii/S1465324906708817

[21] Le Blanc K, Ringdén O. Immunomodulation by mesenchymal stem cells and clinical experience. Journal of Internal Medicine. 2007;**262**(5):509-525

[22] Abdi R, Fiorina P, Adra CN, Atkinson M, Sayegh MH. Immunomodulation by mesenchymal stem cells: A potential therapeutic strategy for type 1 diabetes. Diabetes. 2008;**57**(7):1759-1767

[23] Mattar P, Bieback K. Comparing the immunomodulatory properties of bone marrow, adipose tissue, and birth-associated tissue mesenchymal stromal cells. Frontiers in Immunology. 2015;**6**(Nov):1-8

[24] Bijlsma JW, Berenbaum F, Lafeber FP. Osteoarthritis: An update with relevance for clinical practice. Lancet [Internet]. 2011;**377**(9783):2115-2126. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673611602432

[25] Gupta PK, Das AK, Chullikana A, Majumdar AS. Mesenchymal stem cells for cartilage repair in osteoarthritis. Stem Cell Research & Therapy [Internet]. 2012;**3**(4):25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22776206

[26] Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. Blood. 2007;**110**(10):3499-3506

[27] Liu Q, Zheng H, Chen X, Peng Y, Huang W, Li X, et al. Human mesenchymal stromal cells enhance the immunomodulatory function of CD8(+)CD28(−) regulatory T cells. Cellular & Molecular Immunology [Internet]. Nov 2015;**12**(6):708-718. Available from: http://www.nature.com/doifinder/10.1038/cmi.2014.118%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/25482073

[28] Freitag J, Bates D, Boyd R, Shah K, Barnard A, Huguenin L, et al. Mesenchymal stem cell therapy in the treatment of osteoarthritis: Reparative pathways, safety and efficacy—A review. BMC Musculoskeletal Disorders [Internet]. 2016;**17**:230. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27229856

[29] Wang M, Shen J, Jin H, Im H-J, Sandy J, Chen D. Recent progress in understanding molecular mechanisms of cartilage degeneration during osteoarthritis. Annals of the New York Academy of Sciences [Internet]. 2011;**1240**:61-69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22172041

[30] Nauta AJ. Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. Blood [Internet]. 2006;**108**(6):2114-2120. Available from: http://www.bloodjournal.org/cgi/doi/10.1182/blood-2005-11-011650

[31] Vaes B, Van't Hof W, Deans R, Pinxteren J. Application of MultiStem(®) allogeneic cells for immunomodulatory therapy: Clinical progress and pre-clinical challenges in prophylaxis for graft versus host disease. Frontiers in Immunology [Internet]. 2012;**3**:345. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23205020

[32] Ketterl N, Brachtl G, Schuh C, Bieback K, Schallmoser K, Reinisch A, et al. A robust potency assay highlights significant donor variation of human mesenchymal stem/progenitor cell immune modulatory capacity and extended radio-resistance. Stem Cell Research & Therapy [Internet]. 2015;**6**(1):236. Available from: http://stemcellres.com/content/6/1/236

[33] Phinney DG, Kopen G, Righter W, Webster S, Tremain N, Prockop DJ. Donor variation in the growth properties and osteogenic potential of human marrow stromal cells. Journal of Cellular Biochemistry [Internet]. 1999;**75**(3):424-436. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10536366

[34] Mindaye ST, Ra M, Lo Surdo JL, Bauer SR, Alterman MA. Global proteomic signature of undifferentiated human bone marrow stromal cells: Evidence for donor-to-donor proteome heterogeneity. Stem Cell Research [Internet]. 2013;**11**(2):793-805. Available from: http://linkinghub.elsevier.com/retrieve/pii/S187350611300055X

[35] Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: A phase II study. Lancet [Internet]. 2008;**371**(9624):1579-1586. Available from: http://linkinghub.elsevier.com/retrieve/pii/S014067360860690X

[36] Martin PJ, Uberti JP, Soiffer RJ, Klingemann H, Waller EK, Daly AS, et al. Prochymal improves response rates in patients with steroid-refractory acute graft versus host disease (SR-GVHD) involving the liver and gut: Results of a randomized, placebo-controlled, multicenter phase III trial in GVHD. Biology of Blood and Marrow Transplantation [Internet]. 2010;**16**(2):S169-S170. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1083879109006533

[37] Galipeau J. The mesenchymal stromal cells dilemma-does a negative phase III trial of random donor mesenchymal stromal cells in steroid-resistant graft-versus-host disease represent a death knell or a bump in the road? Cytotherapy [Internet]. 2013;**15**(1):2-8. DOI: 10.1016/j.jcyt.2012.10.002

[38] Samuelsson H, Ringdén O, Lönnies H, Le Blanc K. Optimizing in vitro

conditions for immunomodulation and expansion of mesenchymal stromal cells. Cytotherapy. 2009;**11**(2):129-136

[39] Kuçi Z, Bönig H, Kreyenberg H, Bunos M, Jauch A, Janssen JWG, et al. Mesenchymal stromal cells from pooled mononuclear cells of multiple bone marrow donors as rescue therapy in pediatric severe steroid-refractory graft-versus-host disease: A multicenter survey. Haematologica [Internet]. 2016;**101**(8):985-994. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27175026

[40] Bader P, Kuçi Z, Bakhtiar S, Basu O, Bug G, Dennis M, et al. Effective treatment of steroid and therapy-refractory acute graft-versus-host disease with a novel mesenchymal stromal cell product (MSC-FFM). Bone Marrow Transplantation [Internet]. Jul 2018;**53**(7):852-862. Available from: http://www.nature.com/articles/s41409-018-0102-z

[41] Mamidi MK, Nathan KG, Singh G, Thrichelvam ST, Mohd Yusof NAN, Fakharuzi NA, et al. Comparative cellular and molecular analyses of pooled bone marrow multipotent mesenchymal stromal cells during continuous passaging and after successive cryopreservation. Journal of Cellular Biochemistry. 2012;**113**(10):3153-3164

[42] Chullikana A, Majumdar AS, Gottipamula S, Krishnamurthy S, Kumar AS, Prakash VS, et al. Randomized, double-blind, phase I/II study of intravenous allogeneic mesenchymal stromal cells in acute myocardial infarction. Cytotherapy. 2015;**17**:250-261

[43] Gupta PK, Chullikana A, Rengasamy M, Shetty N, Pandey V, Agarwal V, 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 Research & Therapy. 2016;**18**(1):301. Available from: http://arthritis-research.biomedcentral.com/articles/10.1186/s13075-016-1195-7

[44] Carmen J, Burger SR, McCaman M, Rowley JA. Developing assays to address identity, potency, purity and safety: cell characterization in cell therapy process development. Regenerative Medicine [Internet]. 2012;**7**(1):85-100. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22168500

[45] Dell'Accio F, Vanlauwe J, Bellemans J, Neys J, De Bari C, Luyten FP. 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. Journal of Orthopaedic Research [Internet]. 2003;**21**(1):123-131. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12507589

[46] Bravery CA, Carmen J, Fong T, Oprea W, Hoogendoorn KH, Woda J, et al. Potency assay development for cellular therapy products: An ISCT* review of the requirements and experiences in the industry. Cytotherapy [Internet]. 2013;**15**(1):9-19. DOI: 10.1016/j.jcyt.2012.10.008

[47] Jeong SY, Kim DH, Ha J, Jin HJ, Kwon S-J, Chang JW, et al. Thrombospondin-2 secreted by human umbilical cord blood-derived mesenchymal stem cells promotes chondrogenic differentiation. Stem Cells [Internet]. 2013;**31**(10):2136-2148. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23843355

[48] Matas J, Orrego M, Amenabar D, Infante C, Tapia-Limonchi R, Cadiz MI, 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 Translational Medicine. Mar 2019;**8**(3):215-224

[49] Thorup A-S, Eldridge SE, Caxaria S, Nalesso G, Thomas BL, Pitzalis C, et al. A potency assay for assessing the chondrogenic efficiency of bioactive molecules in human cartilage in vivo. Osteoarthritis and Cartilage [Internet]. 2017;**25**:S273. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1063458417305113

[50] Wang Y, Han Z, Song Y, Han ZC. Safety of mesenchymal stem cells for clinical application. Stem Cells International [Internet]. 2012;**2012**:1-4. Available from: http://www.hindawi.com/journals/sci/2012/652034/

[51] Rengasamy M, Gupta PK, Kolkundkar U, Singh G, Balasubramanian S, SundarRaj S, et al. Preclinical safety & toxicity evaluation of pooled, allogeneic human bone marrow-derived mesenchymal stromal cells. The Indian Journal of Medical Research [Internet]. 2016;**144**(6):852-864. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28474622

[52] Diekman BO, Wu C-L, Louer CR, Furman BD, Huebner JL, Kraus VB, et al. Intra-articular delivery of purified mesenchymal stem cells from C57BL/6 or MRL/MpJ superhealer mice prevents posttraumatic arthritis. Cell Transplantation [Internet]. 2013;**22**(8):1395-1408. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22889498

[53] Kim JE, Lee SM, Kim SH, Tatman P, Gee AO, Kim D-H, et al. Effect of self-assembled peptide-mesenchymal stem cell complex on the progression of osteoarthritis in a rat model. International Journal of Nanomedicine [Internet]. 2014;**9**(Suppl 1):141-157. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24872709

[54] Yang X, Zhu T-Y, Wen L-C, Cao Y-P, Liu C, Cui Y-P, et al. Intraarticular injection of allogenic mesenchymal stem cells has a protective role for the osteoarthritis. Chinese Medical Journal (English) [Internet]. 2015;**128**(18):2516-2523. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26365972

[55] Singh A, Goel SC, Gupta KK, Kumar M, Arun GR, Patil H, et al. The role of stem cells in osteoarthritis: An experimental study in rabbits. Bone & Joint Research [Internet]. 2014;**3**(2):32-37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24526748

[56] Chiang E-R, Ma H-L, Wang J-P, Liu C-L, Chen T-H, Hung S-C. allogeneic mesenchymal stem cells in combination with hyaluronic acid for the treatment of osteoarthritis in rabbits. PLoS One [Internet]. 2016;**11**(2):e0149835. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26915044

[57] Xing D, Kwong J, Yang Z, Hou Y, Zhang W, Ma B, et al. Intra-articular injection of mesenchymal stem cells in treating knee osteoarthritis: A systematic review of animal studies. Osteoarthritis and Cartilage [Internet]. 2018;**26**(4):445-461. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1063458418300487

[58] Fahey JR, Katoh H, Malcolm R, Perez AV. The case for genetic monitoring of mice and rats used in biomedical research. Mammalian Genome [Internet]. 2013;**24**(3-4):89-94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23314661

[59] Wang X, Omar O, Vazirisani F, Thomsen P, Ekström K. Mesenchymal stem cell-derived exosomes have altered microRNA profiles and induce osteogenic differentiation depending on the stage of differentiation. PLoS One [Internet]. 2018;**13**(2):e0193059. DOI: 10.1371/journal.pone.0193059. Shi X-M, editor

[60] Lo Monaco M, Merckx G, Ratajczak J, Gervois P, Hilkens P, Clegg P, et al. Stem cells for cartilage repair: Preclinical studies and insights in translational animal models and outcome measures. Stem Cells International [Internet]. 2018;**2018**:9079538. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29535784

[61] Frisbie DD, Kisiday JD, Kawcak CE, Werpy NM, McIlwraith CW. Evaluation of adipose-derived stromal vascular fraction or bone marrow-derived mesenchymal stem cells for treatment of osteoarthritis. Journal of Orthopaedic Research [Internet]. 2009;**27**(12):1675-1680. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19544397

[62] McCarthy D, Pandey S, Cekanova M, Biskup J, Drum M, Millis D. The effects of autologous mesenchymal stem cell therapy on elbow osteoarthritis in dogs: A pilot study. Veterinary and Comparative Orthopaedics and Traumatology [Internet]. 2018;**31**(S 02):A1-A25. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0038-1668216

[63] Song F, Tang J, Geng R, Hu H, Zhu C, Cui W, et al. Comparison of the efficacy of bone marrow mononuclear cells and bone mesenchymal stem cells in the treatment of osteoarthritis in a sheep model. International Journal of Clinical and Experimental Pathology [Internet]. 2014;**7**(4):1415-1426. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24817937

[64] Delling U, Brehm W, Ludewig E, Winter K, Jülke H. Longitudinal evaluation of effects of intra-articular mesenchymal stromal cell administration for the treatment of osteoarthritis in an ovine model. Cell Transplantation [Internet]. 2015;**24**(11):2391-2407. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25581789

[65] Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. Arthritis and Rheumatism [Internet]. 2003;**48**(12):3464-3474. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14673997

[66] Sato M, Uchida K, Nakajima H, Miyazaki T, Guerrero AR, Watanabe S, et al. Direct transplantation of mesenchymal stem cells into the knee joints of Hartley strain guinea pigs with spontaneous osteoarthritis. Arthritis Research & Therapy [Internet]. 2012;**14**(1):R31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22314040

[67] Suhaeb AM, Naveen S, Mansor A, Kamarul T. Hyaluronic acid with or without bone marrow derived-mesenchymal stem cells improves osteoarthritic knee changes in rat model: a preliminary report. Indian Journal of Experimental Biology [Internet]. 2012;**50**(6):383-390. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22734248

[68] Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): A systematic review and meta-analysis of clinical trials. PLoS One [Internet]. 2012;**7**(10):e47559. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23133515

[69] Peeters CMM, Leijs MJC, Reijman M, van Osch GJVM, Bos PK. Safety of intra-articular cell-therapy with culture-expanded stem cells in humans: A systematic literature review. Osteoarthritis and Cartilage. 2013;**21**(10):1465-1473

[70] Centeno CJ, Al-Sayegh H, Freeman MD, Smith J, Murrell WD, Bubnov R. A multi-center analysis of adverse events among two thousand,

three hundred and seventy two adult patients undergoing adult autologous stem cell therapy for orthopaedic conditions. International Orthopaedics. 2016;**40**(8):1755-1765

[71] Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis and Cartilage. 2002;**10**(3):199-206

[72] Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. Pain Physician. 2008;**11**(3):343-353

[73] Haleem AM, Singergy AA, Sabry D, Atta HM, Rashed LA, Chu CR, et al. The clinical use of human culture-expanded autologous bone marrow mesenchymal stem cells transplanted on platelet-rich fibrin glue in the treatment of articular cartilage defects: A pilot study and preliminary results. Cartilage. 2010;**1**(4):253-261

[74] Nejadnik H, Hui JH, Feng Choong EP, Tai B-C, Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: An observational cohort study. The American Journal of Sports Medicine. 2010;**38**(6):1110-1116

[75] Davatchi F, Abdollahi BS, Mohyeddin M, Shahram F, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. International Journal of Rheumatic Diseases. 2011;**14**(2):211-215

[76] Saw K-Y, Anz A, Merican S, Tay Y-G, Ragavanaidu K, Jee CSY, et al. Articular cartilage regeneration with autologous peripheral blood progenitor cells and hyaluronic acid after arthroscopic subchondral drilling: A report of 5 cases with histology. Arthroscopy. 2011;**27**(4):493-506

[77] Koh Y-G, Choi Y-J. Infrapatellar fat pad-derived mesenchymal stem cell therapy for knee osteoarthritis. The Knee. 2012;**19**(6):902-907

[78] Orozco L, Munar A, Soler R, Alberca M, Soler F, Huguet M, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: A pilot study. Transplantation. 2013;**95**(12):1535-1541

[79] Koh Y-G, Jo S-B, Kwon O-R, Suh D-S, Lee S-W, Park S-H, et al. Mesenchymal stem cell injections improve symptoms of knee osteoarthritis. Arthroscopy. 2013;**29**(4):748-755

[80] Van Pham P, Bui KH-T, Duong TD, Nguyen NT, Nguyen TD, Le VT, et al. Symptomatic knee osteoarthritis treatment using autologous adipose derived stem cells and platelet-rich plasma: A clinical study. Biomedical Research and Therapy. 2014;**1**(1):2

[81] Koh YG, Choi YJ, Kwon OR, Kim YS. Second-look arthroscopic evaluation of cartilage lesions after mesenchymal stem cell implantation in osteoarthritic knees. The American Journal of Sports Medicine. 2014;**42**(7):1628-1637

[82] Koh Y-G, Choi Y-J, Kwon S-K, Kim Y-S, Yeo J-E. Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis. Knee Surgery, Sports Traumatology, Arthroscopy. 2015;**23**(5):1308-1316

[83] Soler Rich R, Munar A, Soler Romagosa F, Peirau X, Huguet M, 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. Journal of Stem Cell Research and Therapy. 2015;**5**:285

[84] Davatchi F, Sadeghi Abdollahi B, Mohyeddin M, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis: 5 years follow-up of three patients. International Journal of Rheumatic Diseases. 2016;**19**(3):219-225

[85] Soler R, Orozco L, Munar A, Huguet M, López R, Vives J, et al. Final results of a phase I-II trial using ex vivo expanded autologous mesenchymal stromal cells for the treatment of osteoarthritis of the knee confirming safety and suggesting cartilage regeneration. The Knee. 2016;**23**(4):647-654

[86] Sampson S, Smith J, Vincent H, Aufiero D, Zall M, Botto-van-Bemden A. Intra-articular bone marrow concentrate injection protocol: Short-term efficacy in osteoarthritis. Regenerative Medicine. 2016;**11**(6):511-520

[87] Fodor PB, Paulseth SG. Adipose derived stromal cell (ADSC) injections for pain management of osteoarthritis in the human knee joint. Aesthetic Surgery Journal. 2016;**36**(2):229-236

[88] Kim YS, Choi YJ, Lee SW, Kwon OR, Suh DS, Heo DB, et al. Assessment of clinical and MRI outcomes after mesenchymal stem cell implantation in patients with knee osteoarthritis: A prospective study. Osteoarthritis and Cartilage. 2016;**24**(2):237-245

[89] Pak J, Lee JH, Park KS, Jeong BC, Lee SH. Regeneration of cartilage in human knee osteoarthritis with autologous adipose tissue-derived stem cells and autologous extracellular matrix. BioResearch Open Access. 2016;**5**(1):192-200

[90] Pers Y-M, Rackwitz L, Ferreira R, Pullig O, Delfour C, Barry F, et al. Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: A Phase I dose-escalation trial. Stem Cells Translational Medicine. 2016;**5**(7):847-856

[91] Koh Y-G, Kwon O-R, Kim Y-S, Choi Y-J, Tak D-H. Adipose-derived mesenchymal stem cells with microfracture versus microfracture alone: 2-year follow-up of a prospective randomized trial. Arthroscopy. 2016;**32**(1):97-109

[92] Lamo-Espinosa JM, Mora G, Blanco JF, Granero-Moltó F, Nuñez-Córdoba JM, Sánchez-Echenique C, 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). Journal of Translational Medicine. 2016;**14**(1):246

[93] de Windt TS, Vonk LA, Slaper-Cortenbach ICM, van den Broek MPH, Nizak R, van Rijen MHP, et al. Allogeneic mesenchymal stem cells stimulate cartilage regeneration and are safe for single-stage cartilage repair in humans upon mixture with recycled autologous chondrons. Stem Cells. 2017;**35**(1):256-264

[94] Turajane T, Chaveewanakorn U, Fongsarun W, Aojanepong J, Papadopoulos KI. Avoidance of total knee arthroplasty in early osteoarthritis of the knee with intra-articular implantation of autologous activated peripheral blood stem cells versus hyaluronic acid: A randomized controlled trial with differential effects of growth factor. Stem Cells International. 2017;**2017**:8925132

[95] Shapiro SA, Kazmerchak SE, Heckman MG, Zubair AC, O'Connor MI. A prospective, single-blind, placebo-controlled trial of bone marrow aspirate concentrate for knee osteoarthritis. The

American Journal of Sports Medicine. 2017;**45**(1):82-90

[96] Park Y-B, Ha C-W, Lee C-H, Yoon YC, Park Y-G. Cartilage regeneration in osteoarthritic patients by a composite of allogeneic umbilical cord blood-derived mesenchymal stem cells and hyaluronate hydrogel: Results from a clinical trial for safety and proof-of-concept with 7 years of extended follow-up. Stem Cells Translational Medicine. 2017;**6**(2):613-621

[97] Nguyen PD, Tran TD-X, Nguyen HT-N, Vu HT, Le PT-B, Phan NL-C, et al. Comparative clinical observation of arthroscopic microfracture in the presence and absence of a stromal vascular fraction injection for osteoarthritis. Stem Cells Translational Medicine. 2017;**6**(1):187-195

[98] Pintat J, Silvestre A, Magalon G, Gadeau AP, Pesquer L, Perozziello A, et al. Intra-articular injection of mesenchymal stem cells and platelet-rich plasma to treat patellofemoral osteoarthritis: Preliminary results of a long-term pilot study. Journal of Vascular and Interventional Radiology. 2017;**28**(12):1708-1713

[99] Russo A, Condello V, Madonna V, Guerriero M, Zorzi C. Autologous and micro-fragmented adipose tissue for the treatment of diffuse degenerative knee osteoarthritis. Journal of Experimental Orthopaedics. 2017;**4**(1):33

[100] Yokota N, Yamakawa M, Shirata T, Kimura T, Kaneshima H. Clinical results following intra-articular injection of adipose-derived stromal vascular fraction cells in patients with osteoarthritis of the knee. Regenerative Therapy. 2017;**6**:108-112

[101] Jo CH, Chai JW, Jeong EC, Oh S, Shin JS, Shim H, et al. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: A 2-year follow-up study. The American Journal of Sports Medicine. 2017;**45**(12):2774-2783

[102] Garay-Mendoza D, Villarreal-Martínez L, Garza-Bedolla A, Pérez-Garza DM, Acosta-Olivo C, Vilchez-Cavazos F, et al. The effect of intra-articular injection of autologous bone marrow stem cells on pain and knee function in patients with osteoarthritis. International Journal of Rheumatic Diseases. 2018;**21**(1):140-147

[103] Kuah D, Sivell S, Longworth T, James K, Guermazi A, Cicuttini F, et al. Safety, tolerability and efficacy of intra-articular Progenza in knee osteoarthritis: A randomized double-blind placebo-controlled single ascending dose study. Journal of Translational Medicine. 2018;**16**(1):49

[104] Emadedin M, Labibzadeh N, Liastani MG, Karimi A, Jaroughi N, Bolurieh T, et al. Intra-articular implantation of autologous bone marrow-derived mesenchymal stromal cells to treat knee osteoarthritis: A randomized, triple-blind, placebo-controlled phase 1/2 clinical trial. Cytotherapy. 2018;**20**(10):1238-1246

[105] Khalifeh Soltani S, Forogh B, Ahmadbeigi N, Hadizadeh Kharazi H, Fallahzadeh K, Kashani L, et al. Safety and efficacy of allogenic placental mesenchymal stem cells for treating knee osteoarthritis: A pilot study. Cytotherapy. 2019;**21**(1):54-63

[106] Jevotovsky DS, Alfonso AR, Einhorn TA, Chiu ES. Osteoarthritis and stem cell therapy in humans: A systematic review. Osteoarthritis and Cartilage. 2018;**26**(6):711-729

[107] Marx RG, Wilson SM, Swiontkowski MF. Updating the assignment of levels of evidence. The

Journal of Bone and Joint Surgery. American Volume. 2015;**97**(1):1-2

[108] Vinardell T, Sheehy EJ, Buckley CT, Kelly DJ. A comparison of the functionality and in vivo phenotypic stability of cartilaginous tissues engineered from different stem cell sources. Tissue Engineering. Part A. 2012;**18**(11-12):1161-1170

[109] De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis and Rheumatism. 2001;**44**(8):1928-1942

[110] Choi WH, Kim HR, Lee SJ, Jeong N, Park SR, Choi BH, et al. Fetal cartilage-derived cells have stem cell properties and are a highly potent cell source for cartilage regeneration. Cell Transplantation. 2016;**25**(3):449-461

[111] Lohan P, Treacy O, Griffin MD, Ritter T, Ryan AE. Anti-donor immune responses elicited by allogeneic mesenchymal stem cells and their extracellular vesicles: Are we still learning? Frontiers in Immunology. 2017;**8**:1626

[112] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/osteoarthritis-structural-endpoints-development-drugs

[113] Yubo M, Yanyan L, Li L, Tao S, Bo L, Lin C. Clinical efficacy and safety of mesenchymal stem cell transplantation for osteoarthritis treatment: A meta-analysis. PLoS One. 2017;**12**(4):e0175449. Hills RK, editor

[114] Gold GE, Chen CA, Koo S, Hargreaves BA, Bangerter NK. Recent advances in MRI of articular cartilage. American Journal of Roentgenology. 2009;**193**(3):628-638

[115] Gentile P, Chiono V, Carmagnola I, Hatton P. An overview of poly(lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering. International Journal of Molecular Sciences [Internet]. 2014;**15**(3):3640-3659. Available from: http://www.mdpi.com/1422-0067/15/3/3640

[116] Tseng WJ, Huang S-W, Fang C-H, Hsu L-T, Chen C-Y, Shen H-H, et al. Treatment of osteoarthritis with collagen-based scaffold: A porcine animal model with xenograft mesenchymal stem cells. Histology and Histopathology [Internet]. 2018;**33**(12):1271-1286. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29905361

[117] Wang B, Liu W, Xing D, Li R, Lv C, Li Y, et al. Injectable nanohydroxyapatite-chitosan-gelatin micro-scaffolds induce regeneration of knee subchondral bone lesions. Scientific Reports [Internet]. 2017;**7**(1):16709. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29196647

[118] Deng C, Zhu H, Li J, Feng C, Yao Q, Wang L, et al. Bioactive scaffolds for regeneration of cartilage and subchondral bone interface. Theranostics [Internet]. 2018;**8**(7):1940-1955. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29556366

[119] Mohammadi F, Mohammadi Samani S, Tanideh N, Ahmadi F. Hybrid scaffolds of hyaluronic acid and collagen loaded with prednisolone: An interesting system for osteoarthritis. Advanced Pharmaceutical Bulletin [Internet]. 2018;**8**(1):11-19. Availablee from: http://www.ncbi.nlm.nih.gov/pubmed/29670834

[120] de Barros CN, Miluzzi Yamada AL, Junior RSF, Barraviera B, Hussni CA, de Souza JB, et al. A new heterologous fibrin sealant as a scaffold to cartilage repair—Experimental study and

preliminary results. Experimental Biology and Medicine (Maywood, N.J.) [Internet]. 2016;**241**(13):1410-1415. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26264444

[121] https://clinicaltrials.gov/ct2/show/NCT03800810

[122] Bansal H, Comella K, Leon J, Verma P, Agrawal D, Koka P, et al. Intra-articular injection in the knee of adipose derived stromal cells (stromal vascular fraction) and platelet rich plasma for osteoarthritis. Journal of Translational Medicine. 2017;**15**(1):141