Gallant

A Review: Mesenchymal Stem Cells for Treatment of Canine Osteoarthritis

August 2019

Introduction

Osteoarthritis (OA) is a systemic, chronic disease of the joints hallmarked by progressive degeneration of articular cartilage.[1] Other joint tissues may be involved to varying degrees, including the synovium, meniscus, tendons, ligaments, muscles, and subchondral bone. In dogs, the most commonly affected joints are the stifles, elbows, and hips.[2]

It is estimated that 20% of dogs over the age of one year have OA.[3] As OA is generally considered a disease of old age, this figure climbs markedly with each year of life, to reach an 80% prevalence rate among dogs older than 8 years.[2] Early symptoms may be subclinical, so time of onset can be difficult to pinpoint, and diagnoses are often not made until advanced disease is present.

OA may be driven by multiple risk factors beyond age, such as breed (genetics), obesity, cranial cruciate ligament rupture, pathological loading, repetitive stress, and hormones.[1,2] This latter factor is somewhat debated; while male dogs are more likely to develop OA than females, and this could be due to reduction of gonadal hormones, it could just as feasibly be due to increased bodyweight secondary to neutering. Granted, these explanations are not mutually exclusive, but comparatively, bodyweight is a more strongly supported risk factor, evidenced by higher rates of OA among obese and large breed dogs.

Regardless of broader drivers of disease, all dogs with OA share a common pathophysiological process that ultimately drives joint degeneration: inflammation.[1,2] It is therefore unsurprising that non-steroidal anti-inflammatory medications (NSAIDs) are the best supported, most effective form of therapy for OA.[3] Several other therapies, which may modify joint structures, have a moderate amount of evidence in their favor; these include polysulfated glycosaminoglycan (PSGAG) injections, elk velvet antler, and green-lipped mussel. Other options have weak or no evidence, including gold wire acupuncture, electrostimulated acupuncture, extracorporeal shockwave therapy, doxycycline, intra-articular hyaluronan injections, pentosan polysulfate, turmeric, and tiaprofenic acid.

In contrast with the initial hypothesis that stem cells would improve OA primarily by modifying joint structures (i.e. repairing damaged articular cartilage), it has since been shown that stem cells dampen OA predominantly through immunomodulatory and anti-inflammatory means.[4] However, as this article will discuss, exact mechanisms of action remain contested.

Over the past two decades, a number of studies have investigated the use of stem cells for OA, involving humans, dogs, rodents, horses, goats, and other species.[5,6] Although results in veterinary medicine have been encouraging, they have not been without challenges or controversy.

In a 2018 review article about stem cells in veterinary medicine, Sophie Helen Bogers, DVM, PhD, of Virginia-Maryland College of Veterinary Medicine, wrote, "Effectively, what we have learned is that it is impossible to draw finite conclusions from current data."[6] Dr. Bogers described various clinical obstacles in veterinary medicine that have delayed progress, such as limited funding, processing method challenges, differences between studies barring clear comparison, and a lack of guidelines and regulations. Despite these concerns, however, like others, Dr. Bogers concluded that stem cell therapy may still have a bright future in veterinary medicine.

The present article aims to dive deeper into this topic, focusing on the use of stem cells to treat canine OA. First, general information about stem cells will be discussed, including sources of stem cells, mechanisms of action, and doses. Next, an overview of safety will be provided, followed by a closer look at efficacy studies and associated experimental challenges. Finally, some basic laboratory considerations will be covered.

General information

Defining mesenchymal stem cells (MSCs)

Adult stem cells are classified by their differentiating potential, such as hematopoietic stem cells, which give rise to red and white blood cells, or neural stem cells, which become various brain cells.[7] Mesenchymal stem cells (MSCs) are most relevant to OA research; they are precursors to fat cells (adipocytes), cartilage cells (chondrocytes), bone cells (osteoblasts and osteocytes), and stromal cells, the latter of which help with blood formation. Initially, MSCs were defined as multipotent cells isolated from bone marrow, which was their source of discovery.[8] Since that time, however, MSCs have been isolated from virtually all tissues in the body, including adipose, synovia, periosteum, skeletal muscle, reproductive tissue, lung, teeth, and others. As such, MSCs now have a more complex definition; they are described as clonal, plastic adherent, non-hematopoietic cells, which give rise to mesodermal cell lineages. (*In vitro*, MSCs have also been shown to differentiate into endodermal and neuroectodermal cells.)

Types of MSCs

Most stem cell studies for canine OA have used adipose-derived MSCs (AD-MSCs; see **Table 1**).[9] Compared with bone-marrow derived MSCs (BM-MSCs), which are the next most commonly used type across all canine studies, AD-MSCs are easier to harvest and isolate, providing a 500-fold greater yield of MSCs from an equivalent volume of fat versus bone marrow, with better proliferation ability.[4,9] These relative differences between AD-MSCs and BM-MSCs are generally accepted, but other comparisons of MSC types remain debated. For instance, some research has suggested that BM-MSCs have greater chondrogenic potential than AD-MSCs.[9] Other studies have suggested that synovium-derived and infrapatellar fat pad-derived MSCs have greater proliferative capacity than both AD-MSCs and BM-MSCs.[9,10] However, such comparisons need to be considered in light of varying methodologies between

trials, involving cultures, growth factors, and tissue source origins.[9] Any of these elements may impact the characteristics of MSCs, complicating direct comparisons.

Neonatal and reproductive tissue-derived MSCs

As described above, multiple types of tissues can serve as sources of MSCs. Among these, neonatal and reproductive tissue-derived MSCs have garnered particular attention, for both practical and ethical reasons. Early human studies showed that the placenta could be a viable source of multipotent stem cells, providing a relatively large yield.[11] Amnion-derived stem cells have also shown promise, owing to immunoprivileged status and broad differential potential.[12] Beyond tissue regenerative properties, stem cells can modulate immune function, and a human study by Wolbank and colleagues suggested that this capacity is equivalent between AD-MSCs, BM-MSCs, and amnion-derived cells.[12]

Safety also appears to be maintained in neonatal tissue-derived MSCs. In horses, both autologous and allogeneic placenta-derived MSCs were well tolerated after intra-articular injection.[13] More relevant to this article, a study by Saulnier and colleagues found that various neonatal tissue-derived MSCs were feasible to harvest and had favorable characteristics.[14] In contrast with the aforementioned human study by Wolbank and colleagues, Saulnier and colleagues found that canine neonatal tissue-derived MSCs were more immunomodulatory than bone marrow-derived MSCs, and more osteogenic. Further testing showed that placenta-derived MSCs had a higher proliferation rate than MSCs from amniotic or umbilical tissue.

Despite the encouraging properties of neonatal tissue-derived MSCs, they may be ethically scrutinized, and Cesarean sections are increasingly uncommon in veterinary practice.[15] A recent review by Sultana and colleagues suggested that canine reproductive tissues, namely, the testes, ovaries, and surrounding tissues, offer a less controversial and more practical source of MSCs.[15] Research in this area is minimal, partly because human studies have been limited by a lack of available tissue for research. Still, human findings suggest that the testes and ovaries are viable sources of MSCs, and a recent canine study showed that MSCs from ovarian tissue are "accessible, expandable, multipotent and [have] high plasticity, holding promise for applications in regenerative medicine."[16-18] Unlike human reproductive tissues, the supply of canine equivalents is virtually unlimited, owing to the commonality of veterinary sterilization procedures, from which tissues are currently discarded as waste.

Mechanisms of action in OA

Investigators initially hypothesized that stem cells would improve OA by engrafting within the joint, thereby regenerating articular cartilage.[4] This has been demonstrated, but it is no longer considered the main mechanism of action by which MSCs improve OA. Instead, multiple studies have shown that MSC efficacy in OA is defined by immunomodulation, both systemically and within injected joints.[4,19-21] Multiple inflammatory mediators become active in OA, including peripheral blood mononuclear cells (PBMCs), cytokines, and proteinases; it is these drivers of inflammation that MSCs suppress, thereby reducing ensuant pathology.[22,23]

Relatively few canine studies have been conducted in the area of inflammatory pathways in OA, and scant data are available for inflammatory profile changes in response MSCs; however, research points to shared processes across species.[4,24,25] It is widely agreed that TNF-α is the most prominent cytokine at the time of OA onset, IL-1 is the most highly expressed cytokine during early and late disease, and

macrophages are the predominant PBMC in osteoarthritic joints, contributing to both synovitis and fibrosis.[25,26]

Canine-specific research has found that the most highly expressed cytokines in the joint fluid of dogs with OA are TNF-α, IL-1, IL-8, and TGF-β, with lower expression of IL-6, IL-12, and IFN-γ.[24] Among proteinases, canine prostaglandin E2 (PGE 2) and matrix metalloproteinase-2 (MMP-2) have been implicated.[27]

MSCs dampen OA progression by actively suppressing both the innate and adaptive immune systems, primarily via secretion of growth factors and cytokines, such as IL-1 receptor antagonist.[4,23] These factors downregulate various cytokines, including TNF-α, COX-2, IL-1β, IFN-γ, and iNOS.[28] MSCs further modulate the proliferation and activities of various immune cells, including T and B lymphocytes, neutrophils, dendritic cells, natural killer cells, and macrophages.[23] Of particular note, MSCs polarize macrophages into an M2 subtype, which has anti-inflammatory properties.[26]

Beyond the anti-inflammatory effects of MSCs, therapeutic properties for OA may also be attributed to stimulation of endogenous stem cell populations and production of factors that slow disease progression in the short term.[23,29,30] MSCs secrete a long list of trophic factors that are anti-apoptotic, anti-fibrotic, angiogenic, chemoattractive, and mitotic, all of which contribute to tissue repair.[31] These are extensively described in a 2011 review article by Singer and colleagues.[32] Example bioactive molecules include vascular endothelial growth factor (VEGF), which is anti-apoptotic and angiogenic; hepatocyte growth factor (HGF), which is anti-fibrotic and anti-apoptotic; and stem cell factor (SCF), which encourages mitosis of resident progenitor/stem cells.[29] In addition to the impacts of secreted molecules, direct contact between MSCs and cartilage chondrocytes appears to stimulate cartilage matrix formation and chondrogenic differentiation of endogenous stem cells.[29]

Safety and efficacy

Dose

MSC veterinary studies vary widely in methodology, including administered doses.[9] Intra-articular doses range more than 10-fold, from 200,000 to 66 million cells per joint.[20,21,33] Higher doses have been given intravenously, reaching approximately 200 million cells with large dogs.[27] Based on these broad ranges, and aforementioned difficulties in comparing studies, it is currently impossible to reach reliable conclusions about optimal dosing.[6]

Safety and biodistribution

Generally, MSCs are well tolerated and safe for treatment of OA (see **Table 1**), as they are immunopriveleged and typically go undetected by the recipient immune system.[23] Still, short-term inflammation and pain after intra-articular injections have been demonstrated.[6] Reactions upon first injection could be due to xeno-contamination of MSCs, most likely with proteins from fetal bovine serum (FBS), which is commonly used as a culture medium.[34] For subsequent injections, repeated allogeneic therapy could theoretically increase the risk of alloantibody production and related immune responses.[6] Ideally, allogeneic MSCs should not express major histocompatibility complex II (MHC II), as this allows for immune detection in unmatched recipients; however, it has been demonstrated that

certain inflammatory conditions may induce MSCs to express MHC II, thereby leading to an alloantibody response.[6]

Alloantibody responses were demonstrated by Joswig and colleagues in a randomized, controlled, open-label equine study involving 18 horses.[34] Repeated intra-articular injections of allogeneic MSCs led to increased lameness, synovial cell counts, and total protein, whereas repeated injections of autogenous MSCs did not have the same effect, suggesting that the horses' immune systems were detecting a non-self antigen in the allogeneic MSCs.

Reflecting on this study, Dr. Bogers wrote, "These findings indicate that even if [studies are conducted] in a prospective, controlled and double-blinded manner, if measurements are taken after the acute period and are based on a single injection, safety of these products may be falsely represented. Specifically, the results cannot be extrapolated to repeat use of allogeneic cell lines and missing evaluation in the initial 24–48 h would fail to detect initial transient inflammation or pain."[6]

An uncontrolled, non-randomized, open-label canine study by Cabon and colleagues observed similar inflammatory reactions to the aforementioned horse study, albeit without an autogenous MSC group for comparison.[35] Five out of 22 dogs had "mild, immediate self-limiting inflammatory joint reactions" after the first intra-articular injection of neonatal, allogeneic MSCs, while 6 out of 8 dogs injected a second time, six months later, had the same issue. According the investigators, these reactions were likely due to the injection procedure itself, instead of immune responses to MSCs, as no alloantibodies could be detected after first or second injections, either by IgG or IgM analysis, or cross-match. One dog had a positive cross-match assay 12 weeks after the second injection, but only after exacerbating MSC immunogenicity with an IFN-γ primer, which serves to increase MHC I and MHC II expression. The investigators attributed relatively low expression of MHC II to the use of neonatal stem cells, as these are considered less immunogenic than BM-MSCs, which have been used in previous evaluations of MHC expression. In the dog with a positive cross-match, the investigators concluded that a humoral response could not be ruled out. They noted that the dog had a favorable clinical evolution, but still suggested that MHC class typing (i.e. DLA typing) deserved further investigation to better understand mechanisms of action associated with possible immune responses. Incidentally, this study was one of the longest to date, with a 2-year average follow-up. No MSC-related adverse events (AEs) were revealed.

In the largest canine study involving MSCs and OA, 203 dogs with OA and other joint defects were given intra-articular or intravenous injections of allogeneic AD-MSCs.[36] Out of 203 dogs, 128 had a single intra-articular injection, 65 dogs had a single intravenous injection, and 10 dogs had both types of injections. Across all subjects, no severe adverse events were observed. Although some dogs exhibited slight discomfort after injection, as with other trials, these instances were self-limiting. Two dogs had a "mild skin allergy" (likely at injection site, but not stated) that was managed with anti-allergy medication. The investigators summarized the safety of MSCs as follows: "Due to the anti-inflammatory properties and immune modulation capabilities, MSCs are safe to use in recipients without causing any immune response and other adverse effects," they wrote. "The result from our own experiences stated in this report supports the similar findings by other workers."

Another study by principal author Christopher J. Murphy, DVM, PhD, reported slightly different results.[20] Six laboratory Beagles were divided into two groups of three. In the first group, dogs received intra-articular injections of allogeneic AD-MSCs in their right stifles on weeks 1, 3, and 5, followed by euthanasia, necropsy, and histology on week 9. The second group of three dogs underwent the same

regimen, with one addition; they were also injected with 66 million MSCs labeled with fluorescent dye (Vybrant DiD) on week 6. Following the administrations of 5 million MSCs, no dogs showed any signs of lameness or pain; however, after the dose of 66 million MSCs in the second group, all 3 dogs had lameness and pain for 1 to 2 days, with 2 dogs exhibiting non-weight-bearing lameness. These issues resolved in all 3 dogs by the third day after transplantation. Although one might conclude that the higher dose was responsible for these inflammatory responses, unlike the lower doses, the higher dose was given with a fluorescent dye (Vybrant DiD), which complicated interpretation. “We could not determine if the lameness and inflammation were caused by the high cell number or the DiD label because no animal received the same larger number of unlabeled cells,” the investigators concluded.

In an earlier study by Dr. Murphy, an identical protocol was used to evaluate biodistribution of allogeneic AD-MSCs.[21] Again, DiD labeling was used for intra-articular injections of stifles in 3 research Beagles in one group, using the 66 million-cell dose. *In vivo* fluorescent imaging showed MSCs in both the lateral and medial aspects of the stifle for up to 2 weeks after injection, with peak fluorescence at 1 week. Post-mortem, *ex vivo* fluorescent imaging showed that MSCs persisted in the stifle for up to 4 weeks, with engraftment of the cartilage and joint capsule. To assess for migration, further imaging of 61 other tissues revealed significant fluorescence in the thymus and gastrointestinal tract (stomach, duodenum, jejunum, colon), but not in the lung, which has been observed after intravenous injections with humans. The level of migratory fluorescence directly correlated with the total amount of MSCs injected. The investigators also noted that imaging showed mild edema and inflammation around labeled cells, suggesting “a mild inflammatory response after deposition.” As with the other study, the relationship between inflammation and MSCs versus the fluorescent dye could not be determined.

Of note, a recent study found that repeated intravenous injection of allogeneic AD-MSCs (3 infusions, 2 weeks apart), was safe and well tolerated in 13 animals.[27] However, objective improvement was not observed, and MSCs were only detected in joint fluid of 4 animals, which the investigators suggested could be due to pulmonary entrapment.

Table 1. Studies assessing biodistribution and/or safety of mesenchymal stem cells for canine osteoarthritis									
Lead author, year	**Source**	**Initial sample size**	**Method**	**Cell number (million)**	**Target joint**	**Study Length (days)**	**Outcome Measures**	**Controlled**	**Biodistribution and/or safety outcomes**
Black, 2007	Adipose	21	Intra-articular, autologous	4.2-5	Hip	90	Lameness, pain, ROM, functional disability, owner questionnaire	Yes	2 placebo-controlled dogs exhibited biting and scratching at injection site that resolved within 48 hours, likely due to overextension during injection
Black, 2008	Adipose	14	Intra-articular, autologous	3-5	Elbow	180	Lameness, pain, ROM, functional disability, owner questionnaire	Yes	No adverse events reported
Guercio, 2012	Adipose	4	Intra-articular with platelet-rich plasma or hyaluronic acid, autologous	3-5	Elbow	30	Pain, lameness, functional disability, owner questionnaire	No	No adverse events reported
Wood, 2012	Adipose	6	Intra-articular, allogeneic	5-66	Stifle	70	Magnetic resonance and fluorescent imaging, necropsy, histology	No	Migration to the lateral and medial aspects of stifle, thymus, and gastrointestinal tract (stomach, duodenum, jejunum, colon), but not in the lung; Mild edema and inflammation around labeled cells; No adverse events reported
Park, 2013	Adipose	6	Intra-articular, allogeneic	5-66	Stifle	63	Pain, lameness, mixed leukocyte reactions, necropsy, histology	No	All 3 dogs receiving 66-million MSCs with DiD label had lameness and pain for 1 to 2 days; 2/3 dogs exhibited non-weight-bearing lameness; issues resolved in all 3 dogs by the third day after transplantation
Vilar, 2013	Adipose	13	Intra-articular with platelet-rich plasma, autologous	>30	Hip	180	Gait analysis	Yes	No adverse events reported
Cuervo, 2014	Adipose	39	Intra-articular, autologous	30	Hip	180	Pain, ROM, functional disability, owner questionnaire	No	No adverse events reported
Vilar, 2014	Adipose	15	Intra-articular, autologous	15	Hip	180	Gait analysis	Yes	1 dog in treatment group had transitory worsening after injection, likely due to poor technique (multiple attempts to find articular space)
Marx, 2014	Adipose	9	Acupoint injection of autologous stromal vascular fraction or allogeneic MSCs	0.2-0.8	Hip	30	Pain, ROM, functional disability	No	No adverse events reported
Vilar, 2016	Adipose	15	Intra-articular, autologous	15	Hip	180	Pain assessment versus gait analysis	Yes	No adverse events reported
Harman, 2016	Adipose	93	Intra-articular, allogeneic	12	Hip, elbow, stifle, shoulder	60	Pain, global score, owner questionnaire	Yes	15 adverse events reported; 6 in test group (1 lipoma, 1 neurological signs, 2 aggressiveness, 1 bacterial skin infection, 1 weight loss); 9 in control group (2 lipoma, 3 joint pain, 1 neurological signs, 1 glaucoma, 1 resp. infection, 1 vomiting)
Yun, 2016	Adipose	24	Intra-articular with platelet-rich plasma, autologous	10	Stifle	90	Lameness	Yes	No adverse events reported
Kriston-Pál, 2017	Adipose	30	Intra-articular with hyaluronic acid, allogeneic	12	Elbow	365	Owner questionnaire, arthroscopy, histology	No	2/39 joints had swelling for “several days”
Shah, 2018	Adipose	203	Intra-articular and/or intravenous, allogeneic	N/A	Various	70	Lameness, pain, ROM, functional disability	No	2 dogs had a “mild skin allergy” (likely at injection site, but not stated) that was managed with anti-allergy medication; unreported number of dogs had “slight discomfort”
Cabon, 2019	Neonatal	22	Intra-articular, allogeneic, x 2	10	Various	365	Pain, lameness, owner questionnaire	No	5/22 dogs had immediate, self-limiting inflammatory joint reactions; 6/8 dogs had same reaction after second injection
Olsen, 2019	Adipose	13	Intravenous, allogeneic, x 3	1-2 million per kilogram	Elbow	180	Synovial fluid analysis, gait analysis, owner questionnaire	No	No adverse events reported

ROM = range of motion

Efficacy

Most studies to date have concluded that intra-articular injection of MSCs leads to clinically significant improvements in OA (see **Table 2**); however, such findings have not been unilateral, and remain a point of debate amongst investigators. At the root of this debate is methodology itself, as most investigators have graded clinical OA severity with subjective assessments of pain and mobility, instead of with purely objective measures. Vilar and colleagues have led an effort to make efficacy evaluations more objective by using force platform analysis, which can provide numerical measurements of gait. Their controlled trials offer more neutral results, providing limited support for MSCs in patients with OA, suggesting that improvements may be transient, waning after 30 days.[37,38]

The most recent controlled trial by Vilar and colleagues delved further into this issue by demonstrating a lack of concordance between pain/lameness scores derived from clinical assessment versus force platform analysis.[39] Specifically, they reported that subjective measures showed clinical improvements after 6 months, whereas force plate analysis suggested that patients had actually returned to initial levels of lameness, casting doubt on many other MSC studies, which have generally relied upon clinical assessment.

Still, the majority of studies have reported favorable responses to MSCs in dogs with OA. The first two controlled studies of this kind, by Black and colleagues, involved 35 dogs with hip and elbow OA.[4,19] Dogs were intra-articularly injected with 3-5 million allogeneic AD-MSCs per joint. Multiple outcome measures were assessed for up to 180 days, including lameness, pain, range of motion, functional disability, and an owner questionnaire. Results showed significant improvements across all measures, and the investigators noted that MSCs were actually a life-saving treatment for 3 enrolled dogs. Prior to the trial, their owners were considering euthanasia due to pain and functional disability, but after the trial, the dogs were living “relatively pain free.” The investigators also highlighted the importance of subjective clinical scoring, as it is more practical in a clinical setting than force plate analysis, particularly in multicenter trials.

These early findings have since been supported by a number of studies, although not all have been controlled. The largest study to date, an uncontrolled trial by Shah and colleagues, involved 203 dogs.[36] Intra-articular and/or intravenous injections of allogeneic AD-MSCs were given to dogs with OA in various joints. Out of 203 dogs, 128 had a single intra-articular injection, 65 dogs had a single intravenous injection, and 10 dogs had both types of injections Results showed that 85% of all dogs had significantly improved pain and lameness, especially young dogs (less than 5 years). Approximately 90% of dogs treated with intra-articular injection, with or without intravenous injection, had good to excellent improvements, whereas 76% of dogs treated with intravenous injection alone had good to excellent improvements, suggesting that intra-articular injection is more effective than intravenous administration.

The largest controlled study to date, by Harman and colleagues, which involved 74 dogs, also reached the conclusion that intra-articular injection of allogeneic AD-MSCs led to clinically significant improvements.[40] In 3 out of 4 measures, a greater proportion of treated animals had significantly better outcomes than control subjects; the 3 categories client-specific outcome measure (CSOM; 79.2% vs

55.4%), veterinary pain upon manipulation (92.8% vs 50.2%), and veterinary global score (86.9% vs 30.8%).

Variations upon a single intra-articular injection of MSCs have also been performed. For example, injections have been given in combination with platelet rich plasma or hyaluronic acid, both of which were found to be safe and effective.[38,41,42] In fact, a protocol involving the addition of platelet rich plasma led to the most supportive clinical data for MSC therapy out of the three relevant controlled trials by Vilar and colleagues, who have been the strongest advocates of force platform analysis. They found that, at day 180, mean values of peak vertical force and vertical impulse were significantly more improved in treated animals than control dogs.[38]

In contrast with the general consensus that MSCs improve OA primarily via immunomodulatory mechanisms, at least one recent study has shown that MSCs may, after all, be improving OA by regenerating hyaline cartilage. In an uncontrolled study by Kriston-Pál and colleagues, both arthroscopy and histology revealed significant hyaline cartilage repair in 1 dog at 1-year follow-up.[42] They concluded: "Our results strongly indicate that the significant improvement in lameness is attributable to the formation of long-lasting (sustained), hyaline-like cartilage repair tissue due to [AD-MSC] transplantation."

Long-term effects have also been reported by Cabon and colleagues in an uncontrolled study—the only efficacy research to date involving allogeneic, neonatal-derived MSCs.[35] After 1 year, the investigators observed significant improvements in pain, lameness, and owner-reported measures. Also worthy of mention, from the same study, 8 dogs showed statistically greater clinical improvement when given a second intra-articular injection of MSCs 6 months after the first, suggesting that repeated therapy could lead to optimal clinical results.

As previously described, intravenous injections have been less promising. The most recent study of this kind, an uncontrolled trial involving allogeneic AD-MSCs by Olsen and colleagues, found subjective, but not objective improvement after 180 days of observation.[27]

Table 2. Studies assessing efficacy of mesenchymal stem cells for canine osteoarthritis									
Lead author, year	**Source**	**Initial sample size**	**Method**	**Cell number (million)**	**Target joint**	**Study Length (days)**	**Outcome Measures**	**Controlled**	**Efficacy outcomes**
Black, 2007	Adipose	21	Intra-articular, autologous	4.2-5	Hip	90	Lameness, pain, ROM, functional disability, owner questionnaire	Yes	Significant improvements across all measures
Black, 2008	Adipose	14	Intra-articular, autologous	3-5	Elbow	180	Lameness, pain, ROM, functional disability, owner questionnaire	No	Significant improvements across all measures, most prominently in lameness and disability
Guercio, 2012	Adipose	4	Intra-articular with platelet-rich plasma or hyaluronic acid, autologous	3-5	Elbow	30	Pain, lameness, functional disability, owner questionnaire	No	Significant improvements across all measures for both treatments
Vilar, 2013	Adipose	13	Intra-articular with platelet-rich plasma, autologous	>30	Hip	180	Gait analysis	Yes	Significantly improved peak vertical force and vertical impulse
Cuervo, 2014	Adipose	39	Intra-articular, autologous	30	Hip	180	Pain, ROM, functional disability, owner questionnaire	No	Significant improvements across all measures
Vilar, 2014	Adipose	15	Intra-articular, autologous	15	Hip	180	Gait analysis	Yes	Significantly improved peak vertical force and vertical impulse, but only lasting 30 days
Marx, 2014	Adipose	9	Acupoint injection of autologous stromal vascular fraction or allogeneic MSCs	0.2-0.8	Hip	30	Pain, ROM, functional disability	No	After 2 weeks, 4/5 dogs that received MSCs showed significant improvements across all measures; after 1 week, 4/4 dogs receiving stromal vascular fraction showed significant improvements across all measures
Vilar, 2016	Adipose	15	Intra-articular, autologous	15	Hip	180	Pain assessment versus gait analysis	Yes	Subjective pain assessment showed improvement after 6 months, but force platform analysis showed a return to initial lameness level
Harman, 2016	Adipose	93	Intra-articular, allogeneic	12	Hip, elbow, stifle, shoulder	60	Pain, global score, owner questionnaire	Yes	Significant improvements across all measures
Yun, 2016	Adipose	24	Intra-articular with platelet-rich plasma, autologous	10	Stifle	90	Lameness	Yes	Numerical, but not statistically significant improvement
Kriston-Pál, 2017	Adipose	30	Intra-articular with hyaluronic acid, allogeneic	12	Elbow	365	Owner questionnaire, arthroscopy, histology	No	31 out of 39 joints had significant improvement in lameness according to owners, hyaline cartilage regeneration on arthroscopy and histology
Shah, 2018	Adipose	203	Intra-articular and/or intravenous, allogeneic	N/A	Various	70	Lameness, pain, ROM, functional disability	No	85% of dogs had significant improvements in lameness and pain; IA only ~90% had good-excellent improvement; IV only: 76% had good-excellent improvement; IA+IV: 90% had good-excellent improvement
Cabon, 2019	Neonatal	22	Intra-articular, allogeneic, x 2	10	Various	365	Pain, lameness, owner questionnaire	No	Significant improvements across all measures, most prominently for lameness and disability; 8 dogs re-injected at 6 months had significantly higher clinical scores than dogs not injected a second time
Olsen, 2019	Adipose	13	Intravenous, allogeneic, x 3	1-2 million per kilogram	Elbow	180	Synovial fluid analysis, gait analysis, owner questionnaire	No	Significant improvements across all measures for both treatments

ROM = range of motion

Summary of safety and efficacy

As Dr. Bogers wrote in her 2018 review, it may not be possible to draw definitive conclusions from existing data, due to differences in study design and related subjectivity; however, it is undeniable that almost all studies to date, including at least one uncontrolled trial with more than 200 dogs, and a controlled trial with more than 70 dogs, have provided evidence in favor of the efficacy of MSCs for OA, in some cases for up to 1 year, and no studies have uncovered significant safety concerns.

Strengthening the evidence for MSCs in OA, studies in other species, including horses and humans, have reported favorable efficacy and a high margin of safety. Similarly to canine trials, such studies have varied in their findings, particularly with respect to actual disease modification (regrowth of hyaline cartilage) versus improvements in function and pain.[6,29]

References

1. Cucchiarini M, de Girolamo L, Filardo G, et al. Basic science of osteoarthritis. *J Exp Orthop.* 2016;3(1):22.
2. Anderson KL, O'Neill DG, Brodbelt DC, et al. Prevalence, duration and risk factors for appendicular osteoarthritis in a uk dog population under primary veterinary care. *Sci Rep.* 2018;8(1):5641.
3. Sanderson RO, Beata C, Flipo RM, et al. Systematic review of the management of canine osteoarthritis. *Vet Rec.* 2009;164(14):418-424.
4. Black L, Gaynor J, Gahring D, et al. Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: A randomized, double-blinded, multicenter, controlled trial. *Vet Ther.* 2007;8(4):272-284.
5. Weissman IL. Translating stem and progenitor cell biology to the clinic: Barriers and opportunities. *Science.* 2000;287(5457):1442-1446.
6. Bogers SH. Cell-based therapies for joint disease in veterinary medicine: What we have learned and what we need to know. *Front Vet Sci.* 2018;5:70.
7. NIH. National institutes of health stem cell information home page. https://stemcells.nih.gov/info.htm. Accessed August 14, 2019.
8. Arnhold S, Wenisch S. Adipose tissue derived mesenchymal stem cells for musculoskeletal repair in veterinary medicine. *Am J Stem Cells.* 2015;4(1):1-12.
9. Sasaki A, Mizuno M, Mochizuki M, Sekiya I. Mesenchymal stem cells for cartilage regeneration in dogs. *World J Stem Cells.* 2019;11(5):254-269.
10. Luyten FP. Mesenchymal stem cells in osteoarthritis. *Current Opinion in Rheumatology.* 2004;16(5):599-603.
11. Fukuchi Y, Nakajima H, Sugiyama D, Hirose I, Kitamura T, Tsuji K. Human placenta-derived cells have mesenchymal stem/progenitor cell potential. *Stem Cells.* 2004;22(5):649-658.
12. Wolbank S, Peterbauer A, Fahrner M, et al. Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: A comparison with human mesenchymal stem cells from adipose tissue. *Tissue Eng.* 2007;13(6):1173-1183.
13. Carrade DD, Owens SD, Galuppo LD, et al. Clinicopathologic findings following intra-articular injection of autologous and allogeneic placentally derived equine mesenchymal stem cells in horses. *Cytotherapy.* 2011;13(4):419-430.

14. Saulnier N, Loriau J, Febre M, et al. Canine placenta: A promising potential source of highly proliferative and immunomodulatory mesenchymal stromal cells? *Vet Immunol Immunopathol.* 2016;171:47-55.
15. Sultana T, Lee S, Yoon HY, Lee JI. Current status of canine umbilical cord blood-derived mesenchymal stem cells in veterinary medicine. *Stem Cells Int.* 2018;2018:8329174.
16. Trindade Hill AB, Therrien J, Garcia JM, Smith LC. Mesenchymal-like stem cells in canine ovary show high differentiation potential. *Cell Prolif.* 2017;50(6).
17. Virant-Klun I, Stimpfel M, Skutella T. Stem cells in adult human ovaries: From female fertility to ovarian cancer. *Curr Pharm Des.* 2012;18(3):283-292.
18. De Chiara L, Famulari ES, Fagoonee S, et al. Characterization of human mesenchymal stem cells isolated from the testis. *Stem Cells Int.* 2018;2018:4910304.
19. Black L, Gaynor J, Gahring D, et al. Effect of intraarticular injection of autologous adipose-derived mesenchymal stem and regenerative cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs. *Vet Ther.* 2008;9(3):192-200.
20. Park SA, Reilly CM, Wood JA, et al. Safety and immunomodulatory effects of allogeneic canine adipose-derived mesenchymal stromal cells transplanted into the region of the lacrimal gland, the gland of the third eyelid and the knee joint. *Cytotherapy.* 2013;15(12):1498-1510.
21. Wood JA, Chung DJ, Park SA, et al. Periocular and intra-articular injection of canine adipose-derived mesenchymal stem cells: An in vivo imaging and migration study. *J Ocul Pharmacol Ther.* 2012;28(3):307-317.
22. 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. *J Orthop Res.* 2009;27(12):1675-1680.
23. Griffin MD, Ritter T, Mahon BP. Immunological aspects of allogeneic mesenchymal stem cell therapies. *Hum Gene Ther.* 2010;21(12):1641-1655.
24. Hegemann N, Wondimu A, Kohn B, Brunnberg L, Schmidt MFG. Cytokine profile in canine immune-mediated polyarthritis and osteoarthritis. *Veterinary and Comparative Orthopaedics and Traumatology.* 2018;18(02):67-72.
25. Goldring MB. The role of cytokines as inflammatory mediators in osteoarthritis: Lessons from animal models. *Connective Tissue Research.* 2009;40(1):1-11.
26. Chahal J, Gomez-Aristizabal A, Shestopaloff K, et al. Bone marrow mesenchymal stromal cell treatment in patients with osteoarthritis results in overall improvement in pain and symptoms and reduces synovial inflammation. *Stem Cells Transl Med.* 2019;8(8):746-757.
27. Olsen A, Johnson V, Webb T, Santangelo KS, Dow S, Duerr FM. Evaluation of intravenously delivered allogeneic mesenchymal stem cells for treatment of elbow osteoarthritis in dogs: A pilot study. *Vet Comp Orthop Traumatol.* 2019;32(3):173-181.
28. Yun S, Ku SK, Kwon YS. Adipose-derived mesenchymal stem cells and platelet-rich plasma synergistically ameliorate the surgical-induced osteoarthritis in beagle dogs. *J Orthop Surg Res.* 2016;11:9.
29. Freitag J, Bates D, Boyd R, et al. Mesenchymal stem cell therapy in the treatment of osteoarthritis: Reparative pathways, safety and efficacy - a review. *BMC Musculoskelet Disord.* 2016;17:230.
30. Whitworth DJ, Banks TA. Stem cell therapies for treating osteoarthritis: Prescient or premature? *Vet J.* 2014;202(3):416-424.
31. Caplan AI, Correa D. The msc: An injury drugstore. *Cell Stem Cell.* 2011;9(1):11-15.
32. Singer NG, Caplan AI. Mesenchymal stem cells: Mechanisms of inflammation. *Annu Rev Pathol.* 2011;6:457-478.

33. Marx C, Silveira MD, Selbach I, et al. Acupoint injection of autologous stromal vascular fraction and allogeneic adipose-derived stem cells to treat hip dysplasia in dogs. *Stem Cells Int.* 2014;2014:391274.
34. Joswig AJ, Mitchell A, Cummings KJ, et al. Repeated intra-articular injection of allogeneic mesenchymal stem cells causes an adverse response compared to autologous cells in the equine model. *Stem Cell Res Ther.* 2017;8(1):42.
35. Cabon Q, Febre M, Gomez N, et al. Long-term safety and efficacy of single or repeated intra-articular injection of allogeneic neonatal mesenchymal stromal cells for managing pain and lameness in moderate to severe canine osteoarthritis without anti-inflammatory pharmacological support: Pilot clinical study. *Front Vet Sci.* 2019;6:10.
36. Shah K, Drury T, Roic I, et al. Outcome of allogeneic adult stem cell therapy in dogs suffering from osteoarthritis and other joint defects. *Stem Cells Int.* 2018;2018:7309201.
37. Vilar JM, Batista M, Morales M, et al. Assessment of the effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells in osteoarthritic dogs using a double blinded force platform analysis. *BMC Vet Res.* 2014;10:143.
38. Vilar JM, Morales M, Santana A, et al. Controlled, blinded force platform analysis of the effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells associated to prgf-endoret in osteoarthritic dogs. *BMC Vet Res.* 2013;9:131.
39. Vilar JM, Cuervo B, Rubio M, et al. Effect of intraarticular inoculation of mesenchymal stem cells in dogs with hip osteoarthritis by means of objective force platform gait analysis: Concordance with numeric subjective scoring scales. *BMC Vet Res.* 2016;12(1):223.
40. Harman R, Carlson K, Gaynor J, et al. A prospective, randomized, masked, and placebo-controlled efficacy study of intraarticular allogeneic adipose stem cells for the treatment of osteoarthritis in dogs. *Front Vet Sci.* 2016;3:81.
41. Guercio A, Di Marco P, Casella S, et al. Production of canine mesenchymal stem cells from adipose tissue and their application in dogs with chronic osteoarthritis of the humeroradial joints. *Cell Biol Int.* 2012;36(2):189-194.
42. Kriston-Pál É, Czibula Á, Gyuris Z, et al. Characterization and therapeutic application of canine adipose mesenchymal stem cells to treat elbow osteoarthritis. *Can J Vet Res.* 2017;81(1):73-78.