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CONCISE REVIEW

STEM CELLS TRANSLATIONAL MEDICINE

Allogeneic mesenchymal stromal cells for cartilage regeneration: A review of in vitro evaluation, clinical experience, and translational opportunities

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Abstract

The paracrine signaling, immunogenic properties and possible applications of mesenchymal stromal cells (MSCs) for cartilage tissue engineering and regenerative medicine therapies have been investigated through numerous in vitro, animal model and clinical studies. The emerging knowledge largely supports the concept of MSCs as signaling and modulatory cells, exerting their influence through trophic and immune mediation rather than as a cell replacement therapy. The virtues of allogeneic cells as a ready-to-use product with well-defined characteristics of cell surface marker expression, proliferative ability, and differentiation capacity are well established. With clinical applications in mind, a greater focus on allogeneic cell sources is evident, and this review summarizes the latest published and upcoming clinical trials focused on cartilage regeneration adopting allogeneic and autologous cell sources. Moreover, we review the current understanding of immune modulatory mechanisms and the role of trophic factors in articular chondrocyte-MSC interactions that offer feasible targets for evaluating MSC activity in vivo within the intra-articular environment. Furthermore, bringing labeling and tracking techniques to the clinical setting, while inherently challenging, will be extremely informative as clinicians and researchers seek to bolster the case for the safety and efficacy of allogeneic MSCs. We therefore review multiple promising approaches for cell tracking and labeling, including both chimerism studies and imaging-based techniques, that have been widely explored in vitro and in animal models. Understanding the distribution and persistence of transplanted MSCs is necessary to fully realize their potential in cartilage regeneration techniques and tissue engineering applications.

Ellison D. Aldrich, Xiaolin Cui, and Caroline A. Murphy contributed equally to this study.

KEYWORDS
chondrogenesis, mesenchymal stromal cells, allogeneic, tissue engineering, cartilage regeneration, umbilical cord blood

Significance statement

As allogeneic mesenchymal stromal cell (MSC)-based therapies become increasingly prevalent, it is vital that we interlace the mechanistic understanding with clinical experiences, both human and veterinary. Herein, the present study analyzed the mechanisms by which MSCs exert immune modulatory and paracrine signaling functions, summarize current knowledge on the safety of allogeneic MSCs, highlight important cell labeling and tracking approaches, all within the context of overviewing the current state and outcomes of the latest human clinical trials utilizing allogeneic and autologous MSCs for articular cartilage repair.

1 | INTRODUCTION

Repair and regeneration of articular cartilage has presented a complex set of challenges for researchers and clinicians alike. Articular cartilage is an avascular tissue with poor healing potential once injured. Although small defects may fill over time without intervention, the repair tissue is comprised of fibrocartilage, which is inferior to native hyaline cartilage.[1] One of the central struggles of cartilage regenerative medicine strategies is the integration of repair tissue with surrounding native cartilage. Though many clinical applications of MSC based therapies show superior results in algofunctional indices, long-term (>1 year) follow-up is consistently challenging and limited by a lack of noninvasive modalities for fully evaluating repair tissue.[2] A robust body of in vitro and preclinical studies have sought to answer the many questions around optimal cell source, accounting for donor variability, and assessing ideal timing and method of delivery. In addition, the ability to meet the challenge of large-scale manufacturing while adhering to stringent quality control standards remains a daunting prospect.[3]

For decades researchers have struggled to recapitulate the complex biomechanical properties of this tissue with its intricate extracellular matrix, zonal structure, and unique cellular composition. Mesenchymal stromal cells (MSCs), from their first discovery, have held promise as a treatment strategy for a multitude of disease processes. In the past decade, MSCs have been fervently pursued for cartilage tissue engineering and regenerative medicine applications, with a wealth of knowledge being generated regarding the properties and possible implementation of MSCs in this specific field. However, a definitive way forward remains elusive and numerous questions remain unanswered. We have witnessed a paradigm shift from the concept of MSCs as a replacement cell to a role centered around signaling (trophic) mechanisms and immune modulation of existing cell populations, with some even calling for a shift in terminology to "Medicinal Signaling Cells."[4]

The comparative merits of allogeneic MSCs have been well described, with mounting evidence in the literature, both human and veterinary, supporting the safety of allogeneic cell sources.[5-7] The allure of allogeneic MSCs is principally in their availability as an "off-the-shelf" product that is readily available at the most appropriate time in the disease process, circumventing the delay of autologous collection and expansion. In addition, allogeneic MSC sources allow the opportunity to select cells based on appropriate cell surface marker expression for optimal proliferation, differentiation capabilities, as well as low immunogenicity.

The wealth of knowledge in cell surface expression, immune properties, cell sources, and optimization of delivery and scaffold types are a springboard for in vivo applications. Though there is an obvious linear progression from in vitro to in vivo, there must also be a continuous feedback loop as we expand the clinical use of allogeneic MSCs for cartilage repair. It is imperative to integrate data and techniques from in vitro studies and in vivo animal models, to draw on the veterinary clinical data. Likewise, the challenges and successes identified in recent clinical trials offer invaluable insight for designing in vitro studies that answer clinically applicable questions.

A vast body of research over the preceding decades now expands our understanding of the mechanistic principles of MSCs, variation between cell sources, their role in mitigating inflammation, paracrine and cell-cell signaling, as well as optimization of chondrogenic matrix production and cell scaffold interaction.[3,8,9] This narrative review will provide an overview of the mechanisms by which MSCs exert immune modulatory and paracrine signaling functions, summarize current knowledge on the safety of allogeneic MSCs, highlight cell labeling and tracking applications, and conclude with an overview update of the current state of human clinical trials utilizing culture expanded MSCs for articular cartilage repair. These sections highlight direct connections between current trends and challenges in clinical trials as well as promising techniques emerging from in vitro and animal models.

2 | MSC MECHANISMS OF IMMUNOGENICITY AND IMMUNE MODULATION

The knowledge surrounding MSC immune privilege and modification of immune cell populations has been critical for understanding how

MSCs work and establishing allogeneic cell sources as a viable alternative to autologous cell sources. Since Bartholomew and colleagues found that bone marrow-derived MSCs (BM-MSCs) have the capability to modulate immunosuppression in a baboon skin allograft model in vivo,[10] a number of subsequent studies have demonstrated the ability of BM-MSCs to modulate immune system through either promoting or restraining inflammation, known as the function of "sensor and switcher of the immune system."[11] Both BM- and umbilical cord-derived MSCs (UC-MSCs) have been demonstrated to sense different danger signals through toll-like receptors (TLRs),[12,13] thus MSCs can be activated into pro-inflammatory or anti-inflammatory phenotypes to modulate the immune cells.[14] For instance, TLRs recognize the molecules from injured cells or pathogens and further activate MSCs to release anti-inflammatory factors to prevent the activation and proliferation of T- and B- cells.[15] Others have demonstrated that TLR-primed allogeneic BM-MSCs are resistant to IL-2-activated clearance by natural killer cells (NK).[16]

The mechanism of MSC immune modulation is mainly through the interaction between MSCs and different immune cell types including B cells, T cells, dendritic cells (DCs), NKs, macrophages and neutrophils, which up- or down-regulate immune cell migration, proliferation, and function (detailed in Figure 1A). For instance, pro-inflammatory MSCs (MSC2) can secrete MIP-1 (macrophage inflammatory protein-1), CCL5 (C-C motif ligand 5), CXCL9 (C-X-C motif ligand 9) and CXCL10 to active T cells[19] to promote an immune response. Meanwhile, BM-MSCs can suppress DC activation and maturation through inhibiting the expression of CD80, CD86 and IL-12 in DCs.[20,21]

In addition, soluble immune factors, such as prostaglandin E2 (PGE-2), indoleamine 2,3-dioxygenase (IDO), and nitric oxide (NO), are now thought to be heavily involved, as shown in Figure 1B. For example, PGE-2 released from MSCs can restrain DCs maturation.[22] Furthermore, extracellular vesicles secreted by MSCs can promote the generation of M2 macrophages, suppress the maturation of monocytes and inhibit the proliferation of T and B cells.[23]

In an equine LPS-induced synovitis model, Williams et al demonstrated that equine allogeneic UC-MSCs reduced the total nucleated cell count within the joint, but induced a transient inflammation as evidenced by an increase in synovial PGE-2 up to 72 hours after injection.[24] Other authors have reported variable inflammatory responses after intra-articular injection with allogeneic equine MSCs, but interestingly, these do not appear to be dose dependent and variation in the magnitude of joint effusion is variable between individuals.[25] In another equine induced osteoarthritis (OA) model, the

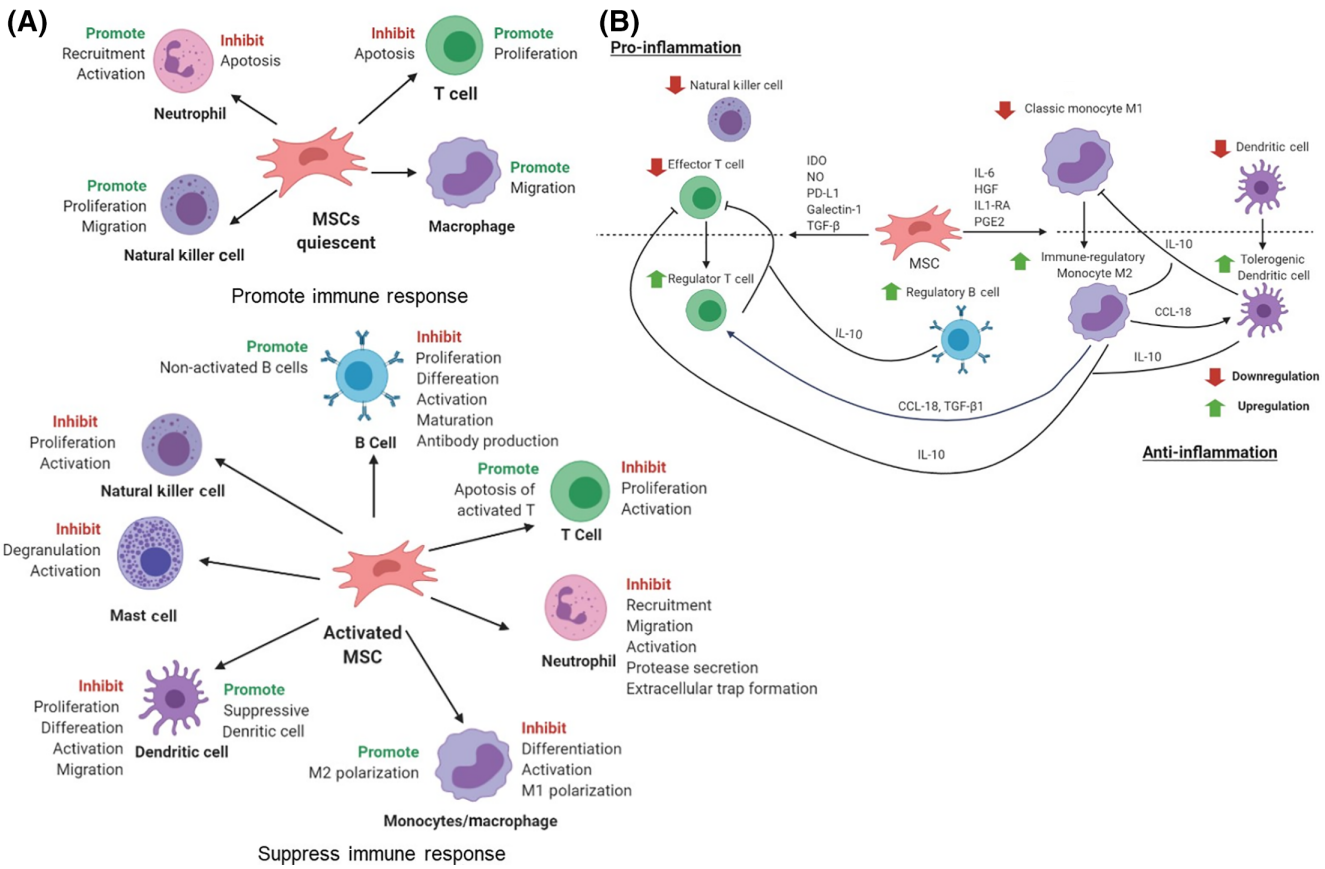

FIGURE 1 Schematic illustrating how MSCs can modulate the immune response through interaction with immune cells including natural killer cells, mast cells, dendritic cells, monocytes, neutrophil, T cells and B cells by: A, either promoting or inhibiting (supressing) immune cell migration, proliferation, activation, and function.[17] And/or B, upregulating (red arrows) or downregulating (green arrows) immune cell function through soluble immune factors modulating pro-inflammatory or anti-inflammatory pathways[18]

intra-articular injection of allogeneic BM-MSCs primed with IFN- γ and TNF-α created transient local inflammation after a second MSC injection was administered, but showed greater anti-inflammatory effect compared with unprimed cells as evidenced by synovial histopathology, and at the 2 month time point greater down-regulation of IL-1β and COX2.[26] At 6 months, the primed MSC group upregulated COL2A1, aggrecan, COMP, TIMP-2, and TGF-β1. Others have reported enhanced immune modulation in equine BM-MSCs primed with TLR3.[27]

Taken together, the immune modulation and immunosuppressive characteristics of MSCs contribute to their therapeutic effect in tissue regeneration by regulating the inflammatory response, mitigating the cytotoxic effects of pro-inflammatory cell populations, and producing an improved therapeutic outcome. Integration of this body of knowledge from the in vitro and animal model space is essential to optimize the performance of allogeneic MSC in clinical therapeutic applications. For instance, mechanistic considerations will necessarily dictate both the design of devices and cell-based therapies and the definition of clinical endpoints in randomized controlled trials.

3 | ALLOGENEIC MSC SAFETY DATA

As the immune modulatory mechanisms of MSCs come into sharper focus and evidence for the safety of allogeneic applications has emerged, both autologous and allogeneic MSCs are now being considered as safe cell sources in cellular therapies, as reflected in current clinical trends.[3,28] For example, Togel et al demonstrated that allogeneic BM-MSCs were just as effective as autologous cells for treating acute kidney injury in a rat model, with no significant MSC-induced adverse effects observed.[29] Another study which used pooled allogeneic MSCs in a rat and a rabbit model demonstrated that the injection of cells did not induce any toxicity nor tumor formation.[30] This finding is particularly important, because tumorigenicity is one of the key concerns in stem cell based therapy.[31] In a rabbit osteochondral defect model, magnetically labeled allogeneic BM-MSCs did not enhance the hyper-response of T cells to donor antigens, supporting the conclusion that labeled allogeneic cells are likely a safe alternative to autologous MSCs for osteochondral defect repair.[32] The merits of equine allogeneic vs autologous equine BM-MSCs were evaluated in vitro and demonstrated equivalent immune modulatory capabilities, primarily through PGE2-mediated T cell suppression.[33]

The clinical use of MSCs administered intra-articularly and within tendon and ligament lesions has become common practice over the last 15 years in equine veterinary medicine, with increasing clinical application of allogeneic cell sources. As such, several retrospective analyses and clinical trials in the equine space can provide perspective on the safety of these practices. For instance, in a retrospective evaluation of 230 injections of allogeneic BM-MSCs from a single donor in 168 different horses, only 10 adverse reactions were reported with no impact on long-term positive outcomes.[34] In a recent prospective study comparing single vs repeated intra-articular injection of equine allogeneic MSCs in horses with naturally occurring OA, there was only one significant adverse event and three instances of mild joint effusion. All horses had positive outcomes as defined by clinical improvement in subjective gait analysis and return to athletic function, including those with transient inflammation postinjection.[35] While the absence of placebo or a "no injection" control group makes it difficult to distinguish the benefit of MSCs from rehabilitation, the low rate of adverse events even after repeated injection is encouraging for clinical safety.

Apart from the animal trials, several human clinical trials have been conducted using allogeneic MSC to evaluate their safety and therapeutic effect. In a multicenter phase I-II trials (NCT01586312), allogeneic BM-MSCs were injected into 15 patients to treat osteoarthritis and followed up after 1 year.[36] Compared with the control group, patients in the treatment group showed substantial improvement in algofunctional indices. The further T2 image mapping demonstrated notable reduction of cartilage defect area in the treatment group. In another Phase I-II human trial (NCT01297413), allogeneic BM-MSCs were injected intravenously into 36 patients ($n = 15$ for phase I, $n = 21$ for phase II) to treat chronic stroke.[37] Two mild adverse events (urinary tract infection and intravenous site irritation) were identified that may be potentially related to the study treatment. That being said, the treatment was considered safe based on the results from electrocardiograms, laboratory tests, and CT scans of chest/abdomen/pelvis.

Although many trials have established the relative safety and extolled the clinical benefits of using allogeneic MSCs, further evidence indicates that allogeneic MSCs may be not immune privileged. Zangi et al demonstrated that the survival rate of allogeneic MSCs was significantly shorter compared with syngeneic MSCs.[38] Similar results were found when allogeneic BM-MSCs were rapidly rejected by MHC class I and class II mismatched recipient mice.[39] In addition, allogeneic MSCs may also promote the production of T cells with a memory phenotype.

The current understanding of immune modulatory mechanisms, gleaned through animal models and equine clinical experience, provides an increasingly robust case for the safety of allogeneic MSCs for human clinical applications, but a more nuanced understanding of the role of MHC mismatch and T cell modulation is still essential. This is a pivotal step in creating a clinically feasible, affordable off the shelf products that achieve the optimal cell characteristics (chondrogenic and proliferative potential, low immunogenicity) and avoid the delays of autologous culture expansion. Therefore, the immunogenic potential of allogeneic MSCs must be considered and fully understood in order to ensure consistent success of clinical trials.

4 | MSC PARACRINE SIGNALING AND POTENTIAL IN CHONDROGENIC APPLICATIONS

The delivery of MSCs in vivo has shown promising results in stimulating the regeneration of articular cartilage and as a treatment of osteoarthritis.[40-42] While it is evident that MSCs have the capacity to

differentiate along a chosen cell lineage, which represents great promise in the area of cartilage regenerative medicine, it is also proposed that they possess a therapeutic effect achieved through a paracrine mechanism, known as trophic activity.[43,44] There is accumulating evidence that the potential mechanisms of MSCs in vivo may not only be due to the direct differentiation of MSCs into chondrocytes, but also paracrine effects though the release and delivery of MSC secretomes to the host tissue, an array of repair mediators, growth factors, cytokines, and other molecules that stimulate a host response. For cartilage regeneration, cell signaling between MSCs and chondrocytes play a vital role in understanding the regeneration of new tissue.

In vitro studies have shown that paracrine factors released by chondrocytes have a positive effect on chondrogenic differentiation of BM-MSCs and human Wharton's jelly MSCs (hWJ-MSCs) by regulating matrix remodeling, cell proliferation, and synthesis of extracellular matrix (ECM) components.[45,46] For example, Bian et al showed that mixed cell populations of human MSCs and human chondrocytes encapsulated into a hydrogel exhibited significantly higher glycosaminoglycan and collagen content, and higher mechanical properties, than in constructs with MSCs or chondrocytes alone.[46] Furthermore, differentiation of equine BM-MSCs can be enhanced by coculturing with mature articular chondrocytes, to produce a more homogeneous ECM within the newly formed cartilage, improving the expression of col2a1, aggrecan, and sox9.[47] When adipose tissue-derived MSCs (AD-MSCs) are in close contact with chondrocytes, paracrine mechanics increase the secretion of important cytokines such as epidermal growth factor (EGF), transforming growth factor beta (TGFβ), vascular endothelial growth factor (VEGF), bone morphogenetic protein 2 (BMP-2) and fibroblast growth factor-2 (FGF-2) of the MSC, which in turn enhances the synthesis of cartilage ECM.[9,48]

Paracrine signals originate from the chondrocyte and cause an increase in synovial derived MSCs (S-MSC) chondrogenic differentiation potential though the secretion of cytokines.[49] Other studies have found that the increased effects in coculture models are due to the trophic role of MSCs which promote proliferation and matrix deposition by the host chondrocyte, rather than MSCs actively undergoing chondrogenic differentiation.[50,51] By identifying species-specific gene expression in a xenogenic system, Wu et al demonstrated that cartilage matrix gene expression was derived from the chondrocyte species of origin, indicating an overgrowth of chondrocytes or loss of MSCs.[50] This was also found to be irrespective of the MSC origin or culture condition.[51] Given that chondrocytes remain metabolically active in the area of OA and continue to synthesize cartilage ECM, it is hypothesized that with implantation of MSCs to the defect site, trophic factors and direct cell to cell contact between MSCs and chondrocytes may be able to assist the native chondrocytes in depositing cartilage ECM.[50,52] Studies have demonstrated that articular chondrocytes secrete parathyroid hormone-related protein (PTHrP) which inhibit hypertrophy of MSC and chondrocytes.[53-55] Therefore MSCs and chondrocytes may have a symbiotic paracrine activity wherein growth factors and cytokines nourish native cartilage and chondrocytes promote MSC differentiation in a feedback loop.[56]

Trophic activities have been reported to inhibit ischemia-caused apoptosis and scarring.[43,57] It has also been suggested that adipose derived stem cells could aid neurogenic pain through the release of cytokines and proteins, thus may be used for the treatment of OA pain.[9,58] In this context, the MSCs serve as "drugstores" to promote and support the natural regeneration of the injured tissue.[57] Therefore, these paracrine mechanisms may be used to manipulate the disease process in vivo, in turn, alleviating OA. However, more in vivo studies and basic science research, must be carried out to better understand overall paracrine signaling and molecular mechanisms between implanted cells and the host tissue to prevent the progression of OA in humans.

5 | TRACKING AND LABELING OF MSCs

Although in vitro studies are often most suitable for answering mechanistic research questions, in vivo animal studies and randomized controlled trials are essential to develop a more complete view of the roles and interactions of these cells. The nuances of persistence and distribution of MSCs after injection or implantation is inextricable from their mechanistic functions. Therefore, means to track the cells in vivo or histologically postmortem in terminal animal studies is critically important. Ideal methods for evaluating MSCs used for cartilage regeneration would be a noninvasive method to provide an understanding of the localization of cells over time. Answering questions about the persistence and spatial distribution of these cells will be pivotal in determining appropriate dosages and optimal routes and vehicles of cell delivery.

5.1 | Molecular and genetic methods of cell tracking

One of the key strategies in tracking and monitoring transplanted MSCs are chimerism studies, whereby genetic and phenotypic differences between donor and recipient cells are analyzed using techniques such as cellular phenotyping, sex chromosome markers, fluorescent in situ hybridization (FISH), quantitative PCR (qPCR), digital droplet PCR (ddPCR), and next-generation sequencing (NGS), which have been previously well reviewed.[62,63] More recently, clustered regularly interspaced short palindromic repeats (CRISPR) technique, specifically CRISPR-Cas9, which utilizes an enzyme Cas9, has been used in conjunction with FISH. This method tracks fluorescent genome loci labeled with CRISPR-Cas9 complex probes (CASFISH). The technique is nondestructive, more rapid, and offers excellent spatial resolution.[64,65] Although these techniques are used in a clinical setting when tracking hematopoietic stem cell transplantation, more invasive procedures, are often necessary for cartilage regeneration advancements such as performing chimerism analysis on a biopsy. For example, de Windt et al, reported on a clinical study using allogeneic MSCs and recycled autologous chondrons analyzing cell chimerism, using DNA short tandem repeat (STR) on biopsies, from the center of

the repair tissue, 1 year post transplantation.[66] The study revealed novel results whereby the regenerated tissue contained patient-DNA only, giving great insight and supporting the hypothesis that allogeneic MSCs stimulate a regenerative host response. Furthermore, in an allograft procedure filling a large osteochondral defects, chimerism analysis using STR revealed 32% of the allograft donor cells had been replaced by the patient's own cells at 1 year follow-up.[67] In an effort to advance cartilage regeneration techniques, chimerism studies have also been performed frequently in preclinical studies utilizing flow cytometry to identify the role of circulating cells or immunohistochemistry evaluation of the repair tissue itself.[68,69] Immunostaining using human-specific vimentin (hVIM) antibody in xenogeneic preclinical models have revealed human cell chimerism after human embryonic stem cells (hESCs) and human BM-MSCs (hBM-MSCs) were transplanted into osteochondral defects in rat knees, with transplanted cell numbers reducing over time.[70-72] At 12 weeks follow up, regenerated cartilage was found to be primarily composed of the host rat chondrocytes populating the defect site, further supporting the role of paracrine functions of transplanted cells in cartilage repair.[72]

Chimerism studies provide valuable information in understanding the fate of the transplanted cell and provides necessary knowledge for developing cartilage regeneration techniques, such as proving the efficiency of using allogeneic MSCs. However, for cartilage regeneration methodologies in a long-term clinical setting, noninvasive imaging techniques determining the biodistribution of transplanted cells would be more relevant and preferred. When interpreting the results of chimerism studies, researchers must also take into account the delivery mechanism (eg, scaffold, gel, cell-sheet), security of graft anchoring techniques (eg, suturing, fibrin glue, flaps) and the role of local immune response in potential clearance of implanted cells, as these factors may greatly influence persistence within repair tissue.[73,74]

5.2 | Cell labeling and imaging techniques

Various cell labeling techniques have been employed to track the distribution of stem cells in vivo. For instance, the use of fluorescent nanoparticles, quantum dots (QDs) have been reported to track labeled equine autologous MSCs in osteoarthritic joints to determine distribution and settling of cells postinjection whereby MCSs were found more densely in the synovial membrane vs cartilage, as shown in Figure 2C.[61] Although this technique gives valuable information regarding the distribution of cells, it is an invasive procedure requiring a biopsy in a clinical setting. With the use of noninvasive molecular imaging, insight into the cell trafficking, homing, and retention of transplanted MSCs in vivo has been possible and can be used as a critical outcome in clinical studies.

There are two primary methods of molecular imaging; stem cell labeling and reporter-gene imaging. Stem cell labeling uses contrast agent such as radionuclides to directly label the cells whereas reporter-gene imaging genetically alters the cell to express a reporter protein. With regards to stem cell labeling, several modalities including positron emission tomography (PET),[75] nuclear scintigraphy/single-photon emission computed tomography (SPECT)[76-79] and magnetic resonance imaging (MRI)[59,80,81] have been touted for their promise of tracking of MSCs. PET uses radioisotopes as tracers to track transplanted cells in patients, most commonly fluorine-18 (^{18}F)[82] or copper-64 (^{64}Cu). ^{18}F can be combined with organic molecules such as fluorodeoxyglucose (^{18}F-FDG) or fluorothymidine (^{18}F-FLT) to monitor metabolic activity of transplanted cells.[75,82] With SPECT, transplanted stem cells are most commonly labeled with Technetium-99 m (^{99m}Tc)[39] and Indium-111 (^{111}In).[83] While ^{111}In provides a longer time window for cell imaging, ^{99m}Tc can be used in higher doses to improve short-term imaging resolution.[84] Although ^{18}F, ^{111}In and ^{99m}Tc have been found to be successful in tracking stem cells in vivo, their tracking only remains for a limited time due to the short half-life of the radionuclides. Therefore, for tracking the differentiation and long-term fate of MSCs in clinical setting, for example, in cartilage regeneration, other noninvasive methods should be employed such as MRI (Figure 2A-B). MRI can be controlled by four different labeling methods: (a) a positive contrast agent such as gadolinium (Gd^{3+}), (b) a negative contrast agent such as superparamagnetic iron oxide (SPIO), (c) by a molecular probe that produces chemical exchange saturation transfer (CEST), and (d) by molecular probes containing ^{19}F.[85] SPIO has been widely used to track MSCs in long-term in vivo studies.[60,86] Using an equine large animal model, Burk et al tracked the biodistribution of autologous MSC by labeling MSCs with SPIO particles conjugated to Rhodamine B to allow detection of cells by MRI and Prussian blue staining as well as fluorescence-based microscopy and flow cytometry. Using this technique labeled cells could be traced at their injection site by MRI as well as histology for the full follow-up period of 24 weeks, indicating the injected cells appeared to remain viable and integrated within the injured tissue.[80] More recently, to track stem cells in cartilage defects, ferumoxytol labeling of MSCs accelerated the diagnosis of successful and failed matrix-associated stem cell implants using MRI in a large-animal model. Loss in signal can be used to avoid further follow-up studies of lost transplants and to refer patients with failing implants to alternative treatment options.[47] Although MRI offers great sensitivity for long-term studies, this technique, however, has some draw backs such as quantification of labeled cells can be difficult and false positives may also be observed due to contrast agents being transferred from dead cells to macrophages.[63]

Genetic labeling of MSCs with a reporter gene coding proteins able to generate contrast is another viable means of tracking cell fate utilizing imaging modalities.[87] The key advantage of using this technique is that it allows stem cell tracking over time as the reporter gene is transmitted to its progeny cells, whereas signals resulting from other contrast agents would become weaker with every cell division. Furthermore, the expression of a reporter gene can be made contingent upon the differentiation status of a cell. For instance, a ferritin heavy chain 1 (FTH1) MRI reporter gene was recently utilized to detect neural differentiation of MSCs in vitro.[88] Therefore, the detection of a reporter gene can correlate with stem cell viability and differentiation ability, which would be highly beneficial in tracking cartilage regeneration.[89] FTH1 has also been used to label exosomes, which were detected with in vivo MRI after intramuscular injection in mice. Although FTH1 labeling produces

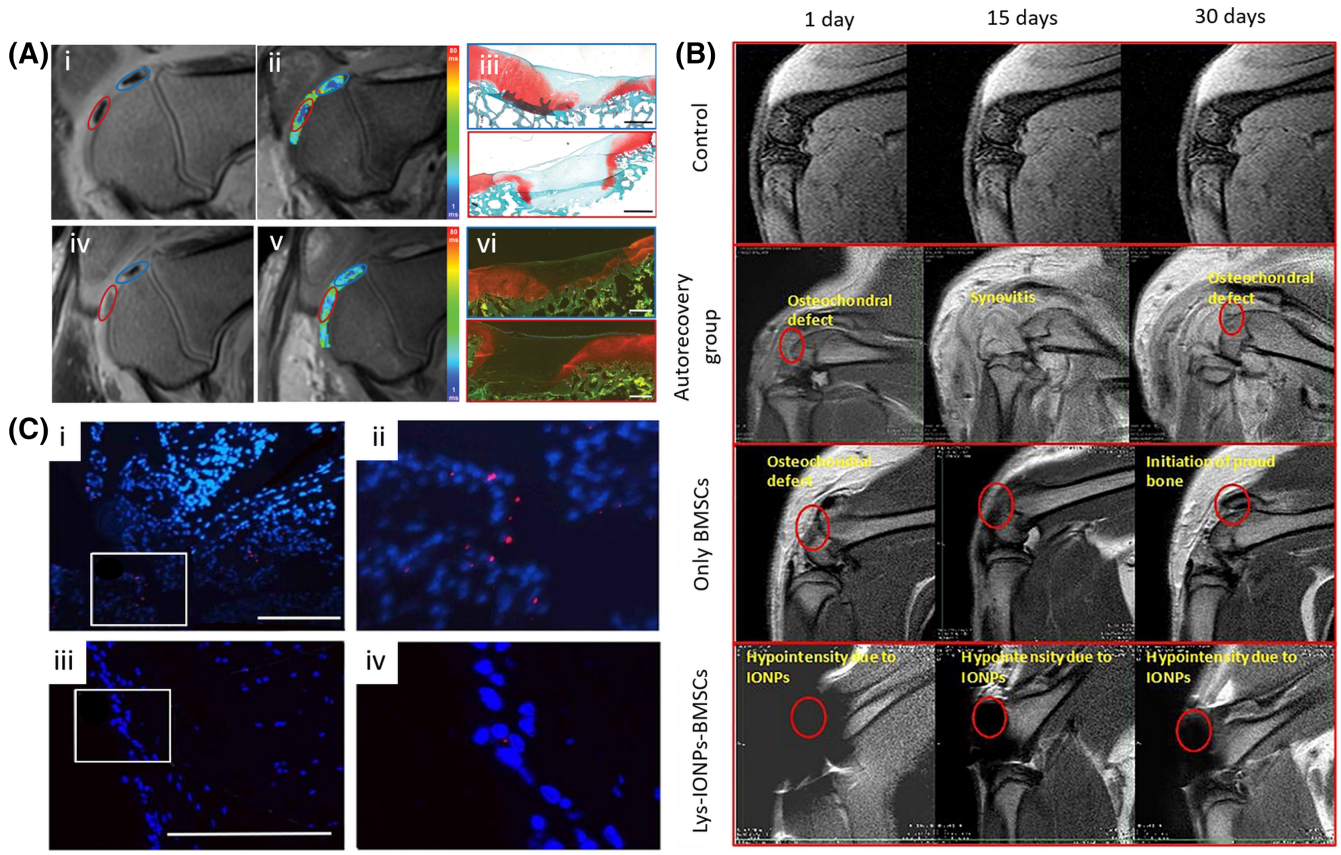

FIGURE 2 Imaging techniques tracking stem cells in vivo. A, MRI and histologic assessment of labeled viable and apoptotic matrix-associated stem cell implants (MASIs) in cartilage defects of Göttingen minipigs. (i) Sagittal image from proton density-weighted MRI and (ii) corresponding color-encoded T2 map overlaid on a T2-weighted spin-echo image obtained 1 week after implantation show similar iron signals and T2 relaxation times for ferumoxytol-labeled viable (blue circle) and apoptotic (red circle) MASIs. (iii) Safranin-O staining 3 months after the MASI procedure shows better regeneration of the cartilage defect that was implanted with a viable MASI (blue frame) compared with the cartilage defect that was implanted with an apoptotic MASI (red frame). Scale bar = 1000 μm. (iv) Sagittal image from proton density–weighted MRI and (v) corresponding color-encoded T2 map overlaid on an image from T2-weighted spin-echo MRI performed at week 2 after implantation show loss of iron signal and increased T2 relaxation time in the apoptotic implant (red circle) but not in the viable transplant (blue circle). (vi) Immunofluorescent staining of collagen1 (green) and collagen 2 (red) 3 months after the MASI procedure show better regeneration of the cartilage defect that was implanted with a viable MASI (blue frame) compared with the cartilage defect that was implanted with an apoptotic MASI (red frame). Scale bar = 1000 μm. Reprinted with permission from reference 59. B, In vivo MRI of BMSCs labeled iron oxide nanoparticles (IONPs) in a rat model, sagittal T2-weighted MRI of control and treatment groups in an osteochondral defect. Reprinted with permission from reference 60. C, Distribution of quantum dot (QD) labeled mesenchymal stem cells (MSCs; red) to synovial membrane (i and ii) and articular cartilage (iii, iv). (iii) and (iv) are expanded images from (i) and (ii) denoted by the white boxes. Scale bar = 200 μm. Reprinted with permission from reference 61

daughter cells that express the gene, proliferation of MSCs may be significantly decreased by FTH1 expression.[90]

In addition to proliferation, effects on cytotoxicity and MSC differentiation must be considered when selecting a labeling strategy. For instance, the use of iron-containing particles can lead to the formation of reactive oxygen species (ROS); however, the addition of surface coating polymers has been shown to protect the iron core while mitigating toxic effects.[91] Some nanoparticles ($Gd@C_{82}[OH]_{22}$) appear to be protective with regard to macrophage-induced damage and at low concentrations may facilitate osteogenic differentiation within an inflammatory microenvironment.[92]

Information regarding the persistence and distribution of cells in vivo is vital to our understanding of how they exert the many functions we have identified in vitro. In addition, valuable clues regarding dose and optimal route of administration are lost by not investigating spatial distribution. Through animal studies and a small number of clinical trials, it is evident that each technique has limitations; therefore, it may be advantageous to employ multiple strategies to elucidate the activity of cells both through an early time point evaluation of spatial distribution, survival and proliferation, and later using MRI evaluation through well-established imaging protocols.

6 | EFFICACY OF RECENT CLINICAL TRIALS UTILIZING CULTURE EXPANDED ALLOGENEIC AND AUTOLOGOUS MSCs

Several recent meta-analyses offer a valuable birds-eye view of the current status of clinical trial data on the efficacy and safety of MSC therapies for the treatment of OA, whether by intra-articular injection

TABLE 1 Summary of the methods and key findings of latest clinical studies utilizing allogeneic and autologous mesenchymal stromal cells (MSCs) for cartilage tissue engineering and regenerative medicine applications

Cell source	Cell carrier/ adjunctive therapy	Intervention	Follow up	Standard outcome	Advanced outcome	Results	Reference
Allogeneic UC-MSCs							
UC-MSC	HA hydrogel (CARTISTEM)	HTO and administration of cell-hydrogel composite (500 mL/cm^2 of defect at concentration 0.5 × 10^7 cells/mL)	Mean 1.7 y (range 1.0-3.5)	IKDC, WOMAC, KSS (all patients) ICRS CRA, Koshino regeneration staging (49 patients with second arthroscopy)		Improved IKDC, WOMAC, KSS pain and function scores; improved ICRS grades	Chung et al[93]
		7 OA patients; UC-MSC + HA hydrogel implantation via mini arthrotomy Group A- mean defect size = 4.9 cm^2; dose = 1.15-1.25 × 10^7 UC-MSC Group B- mean defect size = 7.3 cm^2; dose = 1.65-2.0 × 10^7 UC-MSC 7 OA patients; UC-MSC + HA hydrogel implantation via mini arthrotomy	7 y	VAS, Kellgren-Lawrence grade, WOMAC, IKDC	MRI dGEMRIC (n = 5 at 3 y), 12 wks second look arthroscopy (ICRS grade), histology (n = 2 1 y)	No osteogenesis or tumorogenesis, high GAG content in regenerated tissue (dGEMRIC), histology (n = 2) similar to native cartilage, VAS, IKDC scores improved at 24 wks, stable clinical outcomes over 7 y	Park et al[94]
		41 patients, HTO and administration of cell-hydrogel composite (500 mL/cm^2 of defect at concentration 0.5 × 10^7 cells/mL)	2 y	WOMAC, VAS, IKDC	ICRS score (14 patients)	Improved VAS and IKDC for patients <65 y; larger size of defect also associated with greater improvement of IKDC, VAS and WOMAC; ICRS grade I (6 patients) or grade II (8 patients)	Song et al[95]
		Arthroscopic debridement (128 cases), 4 mm drill subchondral bone, injection of (500 mL/cm^2 of defect at concentration 0.5 × 10^7 cells/mL)	2 y minimum	VAS, WOMAC, IKDC, MRI		Improved VAS, WOMAC, IKDC post-op	Song et al[96]
		Arthroscopic OCD debridement (2 cases), 4 mm drill subchondral bone, injection of (500 mL/cm^2 of defect at concentration 0.5 × 10^7 cells/mL)	33-35 mo	VAS, IKDC, ICRS, M-MOCART, Tegner score		IKDC, VAS, modified 2D MOCART, ICRS and Tegner scores improved	Song et al[97]

(Continues)

TABLE 1 (Continued)

Cell source	Cell carrier/ adjunctive therapy	Intervention	Follow up	Standard outcome	Advanced outcome	Results	Reference
	HA	15 KOA patients (28 knees) randomized into three intra-articular injection protocol groups: A: 1×10^6 UC-MSC + 2 mL HA, two weekly HA injections B: 1×10^6 UC-MSC + 2 mL HA + 8 IU somatotropin, two weekly HA + somatotropin injections C: control	12 mo	VAS, IKDC, WOMAC, MRI		Improved WOMAC scores, no change in VAS or IKDC; Medial T2 MRI improvement in group A at 12 mo	Fiolin et al[98]
	HA	29 OA patients randomized to intra-articular injection of: HA alone (baseline +6 mo) 2×10^7 UC-MSC (baseline only) 2×10^7 UC-MSC (baseline and 6 mo)	12 mo	VAS, Kellgren-Lawrence grade, WOMAC, MRI- WORMS		No severe adverse events, UC-MSC injection x2 group had improved WOMAC scores at 12 mo, no differences in MRI scores between groups	Matas et al[99]
BMAC or UC-MSC	HA membrane + fibrin glue	Arthroscopic management of various surgical lesions, microfracture, implantation of BMAC (60 mL marrow SmartPrep2) or UC-MSC (0.5×10^7 cells/mL)	2 years	VAS, IKDC, KOOS M-MOCART		There were too many differences between groups of patients to discern differences between BMAC and hUC-MSC	Ryu et al[100]
Other allogeneic MSCs							
AD-MSC		18 patients given single intra-articular allogeneic AD-MSC injections into the knee, randomized into three groups: low dose = 1×10^7 cells mid dose = 2×10^7 cells high dose = 5×10^7 cells	48 wks		Composite MRI	All dose groups improved clinically, some MRI sequences (T1rho, T2, T2star, R2star, ADC) suggest change in cartilage composition, T1rho mapping was most sensitive to delineate differences between dose groups	Zhao et al[101]
BM-MSC	Recycled autologous chondrons	35 KOA patients with chondral lesions (2.0-8.0 cm^2) received implantation of 0.9 mL per cm^2 defect of a 10:90-20:80 chondron: MSC ratio mixture in fibrin glue (1.5-2 million cells/mL)	12 mo		MRI, second look arthroscopy, histology and STR analysis of repair tissue biopsy	Histology of repair tissue similar to hyaline cartilage; STR revealed only patient DNA in center of repair tissue	de Windt et al[66]

TABLE 1 (Continued)

Cell source	Cell carrier/ adjunctive therapy	Intervention	Follow up	Standard outcome	Advanced outcome	Results	Reference
Autologous MSCs							
BM-MSC		43 KOA patients randomized to receive intra-articular implantation in the knee with: 4×10^7 BM-MSC (n = 19) or 5 mL saline (n = 24)	6 mo	VAS, WOMAC		No serious adverse events, significant improvement in WOMAC scores for MSC group	Emadedin et al[102]
	Microfracture	11 patients receiving arthroscopy for OCD or traumatic cartilage lesions in the knee randomized to: MFX alone (n = 4) MFX + BM-MSC (n = 7)	48 wks	IKDC, KOOS	MRI- T2 mapping, MOCART	No significant difference in IKDC or KOOS between pre- and post-op, no significant difference in T2 mapping, mean MOCART score significantly higher in MFX + MSC group vs MFX alone	Hashimoto et al[103]
	HA	30 KOA patients randomized into three groups: single dose = 1×10^8 cells two doses 6 mo apart = 1×10^8 cells conservative management	12 mo	NPRS, KOOS, WOMAC, MRI (MOAKS)		No serious adverse events; significant improvement in KOOS, WOMAC, NPRS scores in both AD-MSC groups; significant difference in cartilage pathology progression parameter (MOAKS)	Lamo-Espinosa et al[41]
AD-MSC		24 OA patients randomized to receive intra-articular injection in the knee with: 1×10^8 AD-MSC in 3 mL saline (n = 6) or 3 mL saline (n = 6)	6 mo	WOMAC, MRI		No adverse events, significantly improved WOMAC score in MSC vs saline group at 6 mo, no difference between groups in MRI at 6 mo	Lee et al[104]
		24 OA patients randomized to receive intra-articular injection in the knee with: 1×10^8 AD-MSC in 3 mL saline (n = 6) or 3 mL saline (n = 6)	6 mo	WOMAC, MRI		No adverse events, significantly improved WOMAC score in MSC vs saline group at 6 mo, no difference between groups in MRI at 6 mo	Lee et al[104]

(Continues)

TABLE 1 (Continued)

Cell source	Cell carrier / adjunctive therapy	Intervention	Follow up	Standard outcome	Advanced outcome	Results	Reference
Synovial-MSC cultured TEC		5 KOA patients with chondral lesions (1.5-3.0 cm^2)	24 mo	VAS, Tegner, Lysholm, KOOS, IKDC	MRI (MOCART, T2 mapping), ICRS scoring	No serious adverse events; Defect filling in all patients on second look arthroscopy and MRI; histology of repair tissue biopsy similar to hyaline cartilage	Shimomura et al[105]

Notes: Many recent studies have evaluated allogeneic UC-MSCs,[94-100] although there are many variations in dose, mode of application, the use in conjunction with surgical intervention, and addition of adjunctive therapeutic agents such as hyaluronic acid and somatotropin. Other cell sources, AD-MSCs, BM-MSCs, and synovial derived MSCs, continue to deliver promising results.[25,66,101-105] Of the 16 studies described in Table 1, only six report advanced outcome parameters such as second look arthroscopy, histology, or enhanced MRI protocols.

Abbreviations: BMAC, bone barrow aspirate concentrate; dGEMRIC, delayed gadolinium enhanced MRI of cartilage; HTO, high tibial osteotomy; IKDC, International Knee Documentation Committee; KOOS, Knee Injury and Osteoarthritis Outcome Score; M-MOCART, Magnetic Resonance Observation of Cartilage Repair Tissue; TEC, tissue-engineered construct; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; WORMS, Whole Organ Magnetic Resonance Imaging Score.

or surgical implantation. Herein, we describe a summary of the methods and key findings of latest clinical studies utilizing allogeneic and autologous MSCs for cartilage tissue engineering and regenerative medicine applications in Table 1. A common theme throughout these latest trials is the improvement of subjective measures of pain relief and functional improvement (VAS, IKDC, KOOS, TAS, and others), but a lack of statistically significant objective outcome parameters to definitively indicate improvement in the quality of repair tissue.[2,106-109] For instance, in a comprehensive evaluation index, Cui et al noted no therapeutic effect of MSCs, but when unilateral evaluation indices were parsed out, there was significant improvement in clinical symptoms and cartilage morphology in the 11 clinical trials examined.[106] Even in studies with quantitative MRI-based cartilage assessment through dGEMRIC or T2 mapping it is important to consider that MSC treatment may have little effect on cartilage volume, but this is not necessarily an adequate proxy measurement of quality, particularly as cartilage volume is not correlated with pain reduction.[107] Many call for "more elegant"[2] clinical trials that are "methodologically rigorous"[108] and include follow-up of a year or longer, in order to overcome the inherent heterogeneity of patient groups and other limitations of clinical trials. Others have noted the significant modifier effect of rehabilitation on algofunctional indices and concluded this must also be addressed as a confounding variable.[108]

Some have heeded the call for long-term follow up, but often this comes at the cost of a large sample size. One study with a notable follow-up period of 7 years demonstrated no osteogenesis or tumorogenesis in any of its seven participants, who received surgical implantation of a UC-MSCs in a hyaluronic acid hydrogel.[94] All displayed stable clinical outcomes over the 7-year period. In addition to improved VAS and IKDC scores, histology ($n = 2$) was similar to native cartilage and dGEMRIC showed high GAG content in the repair tissue.[94] In another recent phase I/IIa clinical trial of 18 KOA patient randomly assigned to three different dose groups (1×10^7; 2×10^7; 5×0^7) for intra-articular allogeneic adipose derived MSCs and assessed clinically and via compositional MRI over a 48 week period. All dose groups improved clinically as assessed by WOMAC scores compared with baseline, but perhaps of greater significance the group identified some MRI sequences (T1rho, T2, T2star, R2star, ADC) that suggest change in cartilage composition, with T1rho mapping demonstrating the most sensitivity to delineate differences between dose groups.[101]

The introduction and FDA approval of several commercial products which utilize allogeneic UC-MSCs has resulted in an increasing number of clinical trials that have moved in this direction. It is notable that across multiple clinical trials, utilizing various MSC sources and preparations, the reported number of adverse events is very low, but to our knowledge there are no studies that have directly compared allogeneic and autologous MSC therapies.[6] Thus, assertions on the safety of allogeneic MSC therapies for osteoarthritis and cartilage regeneration are still reliant on a relatively small number of clinical trials bolstered by some promising in vitro, animal model and equine clinical data.[5,99]

MSCs are not the only viable cells in novel cartilage repair therapies. The use of chondrocyte-based therapies, such as matrix guided autologous chondrocyte implantation (MACI) and particulated cartilage allografts (PCA), have been applied clinically for repair of chondral defects.[110-113] These techniques present challenges including limited availability (especially for older patients), donor site morbidity (autologous) and potential immunogenicity (allogeneic). MACI while initially promising in preclinical and clinical trials has shown some disappointing results in a 10 years' follow-up, which revealed graft softening, deterioration and fibrocartilage formation.[110] Allogeneic PCAs have been used successfully, particularly for osteochondral lesions of the talus.[113] This technique utilizes juvenile chondrocytes, which have higher metabolic activity and chondrogenic differentiation potential, compared with their adult counterparts. However, these regenerative approaches are outside the scope of the present review, and the argument could be made that the successful implantation of allograft material into osteochondral defects is an indirect vindication for the use of MSCs, which are undifferentiated.

The immune modulatory properties of MSCs, particularly their inhibition of DCs and PGE-2 based mechanism, likely account for some of the statistically significant data indicating positive VAS and WOMAC scores in human clinical trials to date, in spite of less convincing results in objective measures.[96,99,103] Understanding the mechanistic principles behind immune modulation is essential for devising clinically relevant means of quantitatively assessing the success of these therapies in clinical patients. With a more nuanced understanding of the immune modulatory characteristics of MSCs that has emerged over recent decades, there is opportunity to integrate analysis of immune cell types. Identification of known mediators of immune modulation and suppression, which can be accomplished through synovial fluid arthrocentesis, may help generate objective endpoints for clinical trials in order to fully capture the successes and shortcomings of novel therapies. Increased utilization of biomarker analysis may also be valuable in order to ascertain if the mechanisms identified in vitro are recapitulated in the in vivo ecosystem of the joint. Such quantitative measures offer the opportunity for comparison to internal or external controls.

The advantage of clinical trials as a holistic representation of the MSC immune modulatory and regenerative capacity can be overshadowed by their inherent limitations in patient number and heterogeneity, lack of control groups and other confounding variables. Additionally, all means of follow-up must be suitably noninvasive to achieve ethical approval and patient consent. Therefore, many gold standard methods of evaluation within in vitro and animal model studies are impossible to implement. In order to overcome these challenges as we press forward in our implementation of allogeneic MSC-based therapies it is vital that clinical studies adapt and integrate methods that are well established within the in vitro and animal model space.

In trials where surgical intervention is undertaken, histologic evaluation and second-look arthroscopy are invaluable when possible. These are, of course, invasive means of evaluating repair tissue; thus, every effort should be made to capture maximal data from these samples through genetic MSC tracking mechanisms such as identification of STRs as previously discussed in Section 5.1. Immunostaining for hVIM or CASFISH, in addition to standard histologic analysis techniques, may become more prevalent in future studies. Some success has been demonstrated through the use of MRI for evaluation of regenerative tissue, especially select sequences, T2 mapping, dGEMRIC, but other studies report no discernable difference in MRI scores pre- and posttreatment or between experimental groups.[101] The addition of MSC labeling techniques, such as SPIOs and ferumoxytol, designed to enhance the value of MRI as a means of tracking MSC biodistribution may soon provide a new layer of information to our assessment of clinical patients receiving MSC-based therapies. Currently, very few published clinical trials utilize these advanced diagnostic techniques for generating outcome measures (Table 1).

7 | CONCLUSION

As allogeneic MSC based therapies become increasingly prevalent it is vital that we interlace the mechanistic understanding with clinical experiences, both human and veterinary. The in vitro and in vivo literature must act as a continuous feedback loop for pioneering new and innovative means of cartilage repair and regeneration. Clinical trials may consider incorporating assays that provide information regarding immune modulatory and trophic activities of MSCs in vivo, which may serve the dual purpose of aiding in validating these in vitro findings, while also increasing objective outcome measures within these studies. In addition, numerous molecular and imaging-based strategies have been devised for tracking of MSCs; however, these technologies and methods have not transitioned seamlessly from the lab to the clinic. Promising new means of cell labeling and tracking should continue to be evaluated in animal models and eventually utilized with greater frequency in human and veterinary clinical trials to expand our understanding of MSC biodistribution, adherence, and persistence. MRI-based protocols for tracking labeled cells are particularly attractive because they are noninvasive, offer high spatial resolution, and are a cornerstone of cartilage evaluation in nearly all randomized controlled trials evaluating novel therapies and intervention for the treatment of osteoarthritis.[89] Future strategies for tracking of MSCs should be aimed at both short- (weeks) and long-term (months-years) follow-up.

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CONFLICT OF INTEREST

W.C.M. declared leadership position for Equine Orthopaedic Surgeon for clients in Southern California; Co-Chair of Executive Advisory Board of C. Wayne McIlwraith Translational Medicine Institute at Colorado State University; PI on two grants totaling $418 047.00 and Co-I on two other grants totaling $909 836.00 to Colorado State University in 2019-20; Shareholder in Advanced Regenerative Therapies (ART) in Fort Collins, CO 80537. The other authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

E.A.: conception and design, manuscript writing; X.C., C.A.M., K.L.: manuscript writing; G.H.: final approval of manuscript; C.W.M.: conception and design, final approval of manuscript; T.B.F.W.: conception and design, manuscript writing, final approval of manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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