CONTROVERSIES IN REGENERATIVE MEDICINE: SHOULD KNEE JOINT OSTEOARTHRITIS BE TREATED WITH MESENCHYMAL STROMAL CELLS?

R. Ossendorff[1,§,*], S.G. Walter[2,§], F.A. Schildberg[1], M. Khoury[3] and G.M. Salzmann[4,5]

[1] Department for Orthopaedics and Trauma Surgery, University Hospital Bonn, Bonn, Germany
[2] Department for Orthopaedics and Trauma Surgery, University Hospital Cologne, Cologne, Germany
[3] Universidad de Los Andes Santiago, Santiago, Chile
[4] Gelenkzentrum Rhein-Main, Hochheim, Germany
[5] Schulthess Clinic, Zurich, Switzerland
[§] These authors contributed equally to this work

Abstract

Knee joint osteoarthritis is a complex immunological and degenerative disease. Current treatment strategies fail to alter its progression. Mesenchymal stromal cell (MSC) therapy for osteoarthritis has been object of research for more than 30 years. The aim of MSC therapy is intended to be holistic, with regeneration of all affected knee joint structures. The paracrine effect of the MSC secretome has been shown to be central for the regenerative capacity of MSCs. Activation of local knee-joint-specific MSCs leads to an immunomodulatory, anti-catabolic, anti-apoptotic and chondrogenic stimulus. Preclinical models have demonstrated the symptom- and disease-modifying effects of MSC therapy. At the bedside, there is evidence that autologous and allogeneic MSC therapy shows significant improvement in symptom-modifying and functional outcome. Despite this, a variety of contradictory clinical outcomes are available in the literature. The effectiveness of MSC therapy is still unclear, although there have been promising results. Regarding the diversity of cell sources, isolation, culture protocols and other factors, a comparison of different studies is difficult. Clinical translation of disease-modifying effects has not yet been shown. This narrative review presents a controversial overview of the current preclinical and clinical studies on MSC therapy in knee joint osteoarthritis.

Keywords: Mesenchymal stem cell, mesenchymal stromal cell, cartilage repair, osteoarthritis, knee joint osteoarthritis, knee joint, chondrogenesis.

***Address for correspondence**: Robert Ossendorff, Department for Orthopaedics and Trauma, University Hospital Bonn, Venusberg Campus 1, 53127 Bonn, Germany.
Telephone number: +49 022828714170 Email: robert.ossendorff@gmx.de

List of Abbreviations

ACI	autologous chondrocyte implantation
ACL	anterior cruciate ligament
ADMSC	adipose-tissue-derived MSC
ATMP	advanced therapy medicinal product
BMSC	bone-marrow-derived MSC
CD	cluster of differentiation
CPC	chondrogenic progenitor cell
DAMPs	damage-associated molecular patterns
ECM	extracellular matrix
EMA	European Medicines Agency
EV	extracellular vesicle
GAG	glycosaminoglycan
GvHD	graft-*versus*-host disease
KOOS	knee OA outcome score
HA	hyaluronic acid
IFN-γ	interferon gamma
IL	interleukin
ISCT	International Society for Cell Therapy
MMP	matrix metalloproteinase
MPC	mesenchymal precursor cell
MSC	mesenchymal stromal cell
NSAID	non-steroidal anti-inflammatory drug
OA	osteoarthritis

PMN	polymorphonuclear cell
PRP	platelet-rich plasma
PTOA	post traumatic OA
RCT	randomised controlled trial
SOP	standard operating procedure
SMSC	synovial MSC
TGF	transforming growth factor
TIMP	the tissue inhibitors of MMP
TNF	tumour necrosis factor
UCMSC	umbilical-cord-derived MSC
VAS	visual analogue scale
WOMAC	Western Ontario and McMaster Universities OA index
WORMS	whole-organ magnetic resonance imaging score

Introduction

What is knee OA?

Articular (hyaline) cartilage consists of chondrocytes and ECM. These form a structure providing optimal mechanical properties, such as low friction between joint parts and elastic features, that account for reduced compression of underlying bone during movement. OA has a high prevalence in the modern society. The lifetime risk of developing symptomatic knee OA is 13.8 % (Losina *et al.*, 2013). OA is highly heterogenous in aetiology and is influenced by biomechanical, traumatic and genetic factors. These individual differences affect the OA phenotype and impact the development of disease-modifying therapies for OA in general. Furthermore, OA is characterised by degeneration of articular cartilage – with loss of its typical structure, affecting the surrounding tissue, subchondral bone – and an inflammatory response in cartilage and synovium. OA is divided into early and advanced stage. Late stages of symptomatic OA are usually treated by total joint replacement. The implantation of knee joint prostheses is a routine surgical intervention, however it comes with significant complications, such as persistent pain, infection and joint loosening. Early stages of OA can be divided into two types: focal and diffuse (Stefanik *et al.*, 2016). In focal early OA, the cartilage defect is surrounded by degenerative tissue. In diffuse early OA, the whole knee joint is affected. The goal of any cartilage repair procedure is long-term joint preservation. In the guidelines for the management of knee OA, published by the American College of Rheumatology/Arthritis Foundation in 2020, conservative topical and oral treatments with NSAID as well as glucocorticoid injections were strongly recommended. Topical capsaicin was conditionally recommended for patients with knee OA due to small effect sizes and wide confidence intervals (Kolasinski *et al.*, 2020). HA also received a conditional recommendation – in the context of a shared decision-making that recognises the limited evidence for the benefits of this treatment – when other alternatives have been exhausted or have failed to provide satisfactory benefit (Kolasinski *et al.*, 2020). In contrast to the intra-articular therapies discussed above, PRP was strongly recommended against due to donor variability and lack of standardisation of procedure leading to the high heterogeneity of the injected product (Chou and Shih, 2021). A similar concern was raised against the use of stromal cells in general. However, MSCs have been reported as a promising therapy strategy for the whole knee joint in early and advanced OA.

What are MSCs?

MSCs are defined as precursors with the capacity for clonal cell expansion and differentiation, in defined *in vitro* conditions, into cells of connective tissue lineages, *e.g.* bone, fat, cartilage and muscle (Nancarrow-Lei *et al.*, 2017). The definition is tri-lineage (osteogenic, chondrogenic, adipogenic) differentiation but in practice the three lineages are not necessarily reached *in vivo*. These cells can be extracted from bone marrow, adipose tissue, umbilical cord, dental tissue as well as other sources and expanded in culture. MSCs were first described by Friedenstein *et al.* (1966), who isolated fibroblastic cells and reported a differentiation into osteocytes. The ISCT defined MSCs as plastic-adherent cells when maintained in standard culture conditions and by expression of surface markers CD105, CD73 and CD90 as well as absence (< 2 %) of CD45, CD34, CD14, CD19 and HLA-DR (Dominici *et al.*, 2006). These cells further retain a multipotent phenotype, with the ability to differentiate into adipocytes, osteoblasts and chondrocytes under standard differentiation conditions. However, ISCT criteria for MSCs refer to *in vitro* characteristics of the cells, which do not necessarily reflect their *in vivo* functional properties. The definition and origin of MSCs are still a matter of debate, as some report a perivascular origin (Barry, 2019). MSCs can be detected in most of the knee-joint-specific tissues as local sub-types with different functions. They were first described by De Bari *et al.* (2001) in the synovial membrane. These MSCs act as a cell reservoir for repair processes in the knee joint and play a key role in immunomodulation to reduce inflammation and finally development of OA (Kim *et al.*, 2020; Mancuso *et al.*, 2019). SMSCs have a higher chondrogenic capacity compared to human BMSCs (Jones *et al.*, 2008).

Current clinical cell-based approaches to treat OA

Stromal cell therapy for OA has been a research topic that has raised a lot of interest for over 30 years. Different sources of MSCs (in particular bone marrow, adipose tissue, umbilical cord and synovium) have been investigated. The gold standard and most described cell source of MSCs is the bone marrow. The typical location for bone marrow harvesting is the iliac crest. Comorbidity, especially pain, is reported following bone marrow harvesting. In the last decade, less invasive alternatives to human BMSCs have been developed (Shariatzadeh *et al.*,

2019). Adipose tissue as cell source was extensively investigated, with clinical improvement in the treatment of OA and symptomatic cartilage defects (Koh *et al.*, 2016; Torres-Torrillas *et al.*, 2019). The advantage of this cell origin is an easier surgical access and lower comorbidities (Torres-Torrillas *et al.*, 2019). Liposuction can be performed under local anaesthesia (Pers *et al.*, 2016). Cell sources that require the use of non-invasive procedures – such as post-birth tissue, including Wharton's jelly and umbilical cord blood – are preferred (Song *et al.*, 2020). SMSCs are also of particular interest due to their homologous phenotype (To *et al.*, 2019).

In early OA, especially in focal early OA, cartilage regenerative procedures, such as microfracture, osteochondral transplantation and ACI, are often performed. In diffuse and advanced OA, regenerative cartilage repair is not feasible. The risk of implant failure after ACI is increased in osteoarthritic surroundings and associated with elevated levels of pro-inflammatory cytokines (Angele *et al.*, 2015). This is the consequence of a limited differentiation capacity and a progress of de-differentiation following cell expansion. On the other hand, MSC implantation can be an alternative for OA cartilage regenerative therapy. Typical cell sources of MSCs are bone marrow and adipose tissue (Nancarrow-Lei *et al.*, 2017). Bone marrow is typically harvested from the iliac crest. Adipose tissue can be harvested from all regions presenting body fat and is, therefore, easier to obtain. Other cell sources such as the umbilical cord and the synovia are currently less clinically relevant (Shariatzadeh *et al.*, 2019). MSC therapy can be both autologous and allogeneic. After cell isolation, MSCs are expanded as a monolayer in specific laboratory conditions. As therapy for general knee OA, MSCs are commonly injected intra-articularly (Arshi *et al.*, 2020). A combination with an adjuvant, predominantly HA and PRP, is typically reported (Doyle *et al.*, 2020). For the treatment of cartilage defects and focal OA, MSCs can be seeded onto a scaffold, for example collagen-based, and finally transplanted into the defect. This matrix-assisted transplantation has the advantage that MSCs are not washed out and can stimulate the surrounding tissue for a long time. This procedure can be performed either arthroscopically or by mini-arthrotomy. Additionally, the combined procedures of surgical knee joint repair and MSC therapy are reported for focal cartilage defects (Koh *et al.*, 2016; Wang *et al.*, 2017). A comparison study of microfracture and autologous ADMSC application *versus* microfracture alone showed a significant improvement in clinical and radiological outcome scores for the co-treatment group (Koh *et al.*, 2016).

Data supporting the use of MSCs in knee joint OA

At the bench

Relevance of the paracrine effect upon joint homeostasis

MSCs exert their therapeutic function by recruiting joint-resident MSCs for endogenous cartilage repair (Fig. 1) (Mancuso *et al.*, 2019; Murphy *et al.*, 2020). Moreover, MSC-derived paracrine factors have a disease-modifying influence on immune system cells. While the widespread mechanism of MSC action in OA has not been completely established, their paracrine effect has taken a central role in deciphering the observed beneficial effects (Barry, 2019). In response to the inflammatory milieu priming on injected MSCs, they secrete multiple signalling molecules including growth factors, chemokines and EVs, the secretome (Herrmann *et al.*, 2020; To *et al.*, 2020). The MSC secretome has been heavily studied by utilising MSC-conditioned media to analyse its effects (D'Arrigo *et al.*, 2019). The released MSC-secretome mediates the activation, recruitment

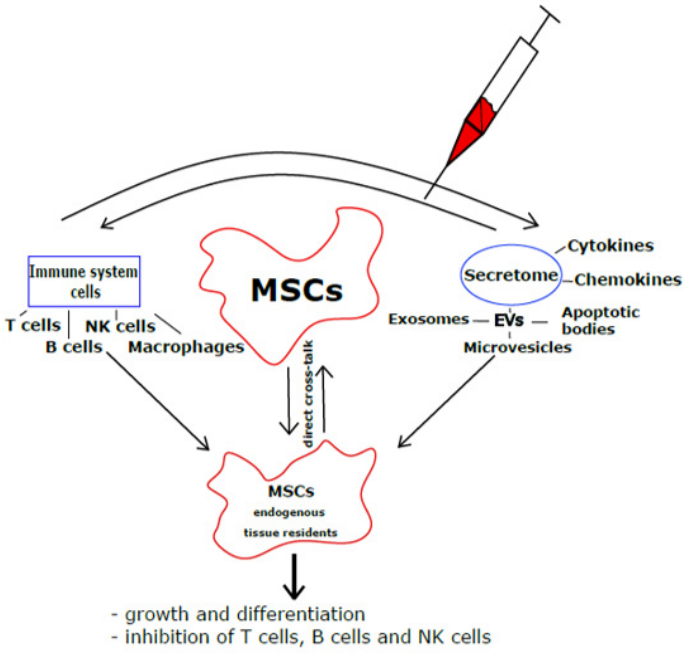

Fig. 1. Fundamental mechanisms of MSC immunomodulation with focus on the endogenous tissue-resident MSCs. Interactions with system cells can be differentiated in direct MSC cross-talk, immune system cell activation, secretome and influence of endogenous tissue-resident MSCs. The functional consequences are stromal cell growth and differentiation as well as inhibition of local immune system cells.

and migration of endogenous stem and progenitor cells (Mao *et al.*, 2018). Additionally, these signalling factors lead to an immunomodulatory, anti-catabolic, anti-apoptotic and chondrogenic stimulus on articular stromal and immune system cells, resulting into endogenous cartilage repair (Mancuso *et al.*, 2019). One set of secretome components are EVs, defined as small subcellular structures 30-5,000 nm in diameter that play an important role in cell-cell interaction and signalling pathways (Cocucci and Meldolesi, 2015). Preclinical studies of MSC-derived EVs demonstrated a chondroprotective effect on OA chondrocytes and a regenerative stimulus (Fang and Vangsness, 2020; Tan *et al.*, 2020; Wang *et al.*, 2020). This is at least partially due to the immunoregulatory capacity of MSC-based EVs. EVs can modulate immunological cell functionality of B cells, T cells, dendritic cells, natural killer cells and macrophages (Burrello *et al.*, 2016). Tofino-Vian *et al.* (2018) investigated the effect of MSC-derived EVs (allogeneic ADMSCs) in chondrocytes of OA patients. Production of inflammatory mediators and degrading enzymes was decreased, the level of the anti-inflammatory cytokine IL-10 increased. MSC-EV application is also discussed as an alternative strategy that might in fact overcome some of the drawbacks associated with MSC-based therapies. Niada *et al.* (2019) found a significant reduction in TNFα-induced hypertrophy and catabolic factors (MMP-3, MMP-13) in primary human osteoarthritic chondrocytes by supplementation of MSCs (allogeneic ADMSCs). An increased TIMP concentration correlates with a reduced MMP activity. Immunomodulation is reported due to the interaction of MSCs with immune system cells in the knee joint (T cells, B cells, macrophages, dendritic cells) (Glenn and Whartenby, 2014). For example, a polarisation of anti-inflammatory phenotype (M2) macrophages by MSCs provides a chondrogenic stimulus (Fernandes *et al.*, 2020). Moreover, MSC apoptosis leads to a paracrine immunomodulatory stimulus. Mancuso *et al.* (2019) , in a review, revealed that that a direct immunosuppressive environment by secretion of TGFβ and IL-10 reduces the macrophage secretion of proinflammatory cytokines (IL-1β, TNFα) *in vitro*. Galleu *et al.* (2017) investigated, in a murine GvHD model, the immunomodulatory potential of apoptotic MSCs (autologous BMSCs). Cytotoxic activity is reduced after MSC infusion. Murphy *et al.* (2020) showed that a resident MSC population can be induced to generate cartilage for treatment of localised chondral disease in OA. In this adult mouse model, a local MSC fraction on the cartilage surface was induced to proliferate following microfracture. However, the amount of local MSC significantly decreased in aged mouse and human samples.

Disease-modifying effects in preclinical models

Numerous *in vitro* and *in vivo* studies have shown the high regenerative potential of MSCs. Knee OA animal models were established to investigate the effect of MSC transplantation. Typically implemented small animal OA models, such as ACL and meniscal transection in rats or rabbits, are surgery-induced. Chemical induction of OA is also described in different animal models, for example intra-articular sodium iodoacetate injection in guinea pigs or rabbits (Wang *et al.*, 2019). Large-animal OA models for MSC therapy, such as dog, sheep or horse, have been performed for clinical translation. For instance, the effect of MSC therapy in dogs has been extensively reviewed by Sasaki *et al.* (2019). Preclinical studies have demonstrated the safety and effectiveness of MSC transplantation (Wang *et al.*, 2019). In a rabbit model, a single intra-articular infusion of autologous SMSCs into the knee triggered cellular adherence close to the meniscal defect – a pre-OA deformity – but, most importantly, supported meniscal regeneration (Hatsushika *et al.*, 2013). Huurne *et al.* (2012) investigated the therapeutic potential of ADMSCs in an OA mouse model. Knee joint injection of collagenase induced OA. ADMSCs from mouse inguinal lymph nodes were injected 7 d after pre-treatment. Single MSC administration inhibited synovial thickening, formation of enthesophytes associated with ligaments and cartilage destruction. However, there was no significant effect in late treatment (14 d after collagenase induction). In clinical translation, the time point of administration could be important for a therapeutic effect. The potential therapeutic success might be dependent on the OA stage. Furthermore, the same group detected a significant reduction in DAMPs S100A8 and S100A9, which are major catabolic factors produced by damaged cells after single MSC injection (Schelbergen *et al.*, 2014). On top, the proinflammatory cytokine IL-1β level was significantly reduced, which highlights the immunomodulatory capacity of MSCs. Early OA stages, synovitis and increased inflammation are investigated as potential targets for the therapeutical MSC effect. Furthermore, the mechanism was analysed in a co-culture of murine PMNs and ADMSCs (van Dalen *et al.*, 2019). IL-1β-related upregulation of MSC-released chemokines enhanced the phagocytic capacity of PMN as mechanism of synovitis reduction.

Recently, a cell-free alternative to cell-based treatment has been developed by using MSC-based mediators, with a focus on EVs, to avoid the drawbacks associated with MSC-related therapies. A systematic review demonstrated the preclinical efficacy of human MSC-derived EV therapy in cartilage injury models (D'Arrigo *et al.*, 2019). Tao *et al.* (2017) showed a prevention of PTOA by application of MSC-derived EVs (autologous SMSCs) in a mouse model with transection of the medial meniscus and ACL. Wang *et al.* (2020) investigated the EV response of allogeneic CPCs in a cartilage injury mouse model. A cartilage repair response in proliferation, migration and differentiation was demonstrated. This

observation enhances the concept of modulation by MSCs.

All in all, current research demonstrates that the paracrine effect is more important than the tissue-restoring effect.

Summary and outlook

Preclinical MSC therapy in knee OA models demonstrates a symptom- and disease-modifying effect. The functional concept of MSC therapy has changed from a tissue replacement to a trophic stimulation. Investigations of the paracrine effect are a current research focus.

At the bedside

OA is an immunological disease affecting the whole knee joint. Therefore, the regenerative capacity of MSCs is an attractive characteristic for a whole-organ approach, addressing all affected structural components of the knee joint. Recently, numerous RCTs of MSC therapy have been performed with good clinical outcomes at long-term follow-ups (Ding *et al.*, 2020; Doyle *et al.*, 2020; Maheshwer *et al.*, 2020).

Frequency, dose and application method

When and how often MSCs should be implanted is object of ongoing research. Most studies have injected a single MSC dose and evaluated the clinical follow-up (Doyle *et al.*, 2020). Matas *et al.* (2019) reported no significant differences in adverse events between a single and repeated application of allogeneic UCMSCs in patients with symptomatic knee OA. Nevertheless, at the end of the study follow-up (12 months), the group with repeated UCMSC applications (every 6 months) experienced significant clinical changes in the total WOMAC, pain component and VAS when compared with HA-treated patients (Matas *et al.*, 2019).

Another important question is the application method, which currently differs between clinical studies. In most studies, MSCs are intra-articularly injected. Different media are applied in the injection suspension, predominantly HA and PRP (Kolasinski *et al.*, 2020). HA is most frequently used and has an anti-inflammatory effect. Kim YS *et al.* (2020) showed in a matched pair analysis of short-term clinical outcomes that treatment with HA improved the clinical outcome after 3 months. The combination of HA and MSCs was superior at the 1-year follow-up. Bastos *et al.* (2019) performed a RCT of MSC (autologous human BMSCs) therapy with and without addition of PRP, having a corticosteroid injection control group. At the 12-month follow-up, the KOOS was improved in all groups. MSC therapy was significantly better compared to standard therapy with corticoids but there was no difference between MSCs and MSCs + PRP.

Autologous or allogeneic?

Typically, MSC therapy in OA is considered to be an autologous approach. Autologous MSCs are safe; however, a disadvantage of this procedure is a donor site morbidity due to the harvesting. Especially-persistent pain is reported after bone marrow aspiration and liposuction. Moreover, there is an infection risk and high variability in terms of MSC content and quality (Ding *et al.*, 2020). Allogeneic MSCs provide an implemented alternative and are a current research focus (Zhang *et al.*, 2019). Allogeneic MSCs have been reported to be safe, effective and provide a therapy without the need for an additional surgical procedure (Gupta *et al.*, 2016; Vega *et al.*, 2015). Two allogeneic MSC products are currently approved by the EMA as ATMPs for treatment of advanced knee OA but have not yet been submitted for marketing authorisation (Shariatzadeh *et al.*, 2019). Cartistem® is an umbilical cord blood-derived MSC product. Park *et al.* (2017) presented good clinical long-term results after a single injection of Cartistem® in patients suffering symptomatic knee OA. Stempeucel® is an *ex vivo*-cultured human BMSC product. Gupta *et al.* (2016) investigated the efficacy and safety of this product. The dose of 25×10^6 cells showed the best therapeutic effect. Higher doses increased the risk of adverse events. Nevertheless, clinical scores did not show significant differences compared to the placebo control. Allogeneic MSCs are an attractive alternative but there is a potential risk of disease transmission and host-graft interactions due to their immunological response.

Rehabilitation strategy

Mechanical stimulation is crucial for a chondrogenic stimulus. The positive effect on cartilage homeostasis is also reported for MSCs (human BMSCs), which undergo chondrogenic differentiation (Fahy *et al.*, 2018). MSC chondrogenesis and GAG production can be enhanced by dynamic compressive loading and shear compared to an unloaded control (Kisiday *et al.*, 2009; Mauck *et al.*, 2007). Gardner *et al.* (2017) showed that mechanical multiaxial stimulation of MSCs (human BMSCs) in polyurethane scaffolds results in a release of TGF-β1, which is an important factor for chondrogenic differentiation.

In clinical translation, Iijima *et al.* (2018) (Table 1) reported in a review about rehabilitation after MSC therapy that functional scores (self-reporting assessment) are significantly improved in patients performing structured professional rehabilitation compared to no rehabilitation protocol.

Safety

Intra-articular MSC therapy is a non-homologous approach and is performed using both autologous and allogeneic sources. Therefore, concerns about safety of the therapy and risk of complications are a great issue. Yubo *et al.* (2017) demonstrated in a meta-analysis that MSC treatment for OA is a safe treatment strategy. Typically reported adverse events after treatment with MSCs are pain and swelling (50 %) (Lamo-Espinosa *et al.*, 2018; Peeters *et al.*, 2013). Usually, these complications disappear after 24-48 h.

Table 1. Meta-analysis of MSC therapy in knee OA indicating different study types, follow-up period, cell sources and ways of application.

Meta-analysis (year)	Studies (number and type)	Follow-up (months)	Patient numbers	Cell source (bone marrow/adipose tissue/other)	Type (autologous/ allogenic)	Application (intra-articular injection/ implantation)	Includes only cultured MSCs (no aspirates)	Main outcome
Cui *et al.* (2016)	18 (10 single arm, 4 quasi experiment, 4 RCT)	3-24	565	10/6/2	17/1	13/5	No	Improved pain and function No dose responsive association
Yubo *et al.* (2017)	11 (RCT)	12-24	582	7/2/2	Not available	11/0	No	Improved pain and function
Iijima *et al.* (2018)	35 (21 single arm, 7 quasi experiment, 7 RCT)	3-60	2385	16/15/4	33/2	27/8	No	Improved pain and function Effect autologous > allogenic Rehabilitation beneficial for clinical outcome
Awad e*t al.* (2019)	33 (4 RCT, 11 cases series, 4 quasi experiment, 14 observational cohorts)	3-75	724	33/0/0	33/0	9/24	No	Improved pain and function
Kim *et al.* (2019)	6 (RCT)	6-12	203	4/1/1	2/4	6/0	Yes	Improved pain
Ding *et al.* (2020)	13 (RCT)	6-12	NA	5/4/4	6/7	13/0	Yes	Improved pain and function
Tan *et al.* (2021)	19 (RCT and non RCT)	6	440	9/10/0	17/2	17/2	No	Improved pain and function Significantly better outcomes with the use of BMSCs as compared with ADMSCs

Gupta *et al.* (2016) reported, for a phase II trial of an allogeneic UDMSC product (Stempeucel®), that the cell dose is important to reduce the risk of adverse events. Doses higher than 25×10^6 cells correlate with predominantly higher risk of pain and knee swelling. All in all, MSC therapy is safe and adverse events are rarely reported (Peeters *et al.*, 2013).

Clinical data

In the last decade, numerous clinical trials have focused on the clinical outcome of MSC therapy in OA. Ding *et al.* (2020) investigated, in a systematic review and network meta-analysis, the efficacy and safety of intra-articular MSC therapy in knee OA. They reported a clinical improvement at the 12 month follow-up in KOOS and pain reduction (VAS pain). Nevertheless, disease-modifying effects were insignificant. Similar findings were reported by Kim *et al.* (2019) in a systematic review and meta-analysis of intra-articular MSC therapy without adjuvant surgery in knee OA. Recently, Tan *et al.* (2021) have reported in a systematic review and meta-analysis of intra-articular MSC injections without adjuvant therapies that single MSC application in symptomatic knee OA results in good clinical outcomes with pain reduction and knee function. Furthermore, cultured MSCs are superior to conditioned medium and therapy with BMSCs is significantly more effective compared to ADMSCs. Table 1 provides an overview of the main outcome in current meta-analysis of randomised and non-RCTs.

The combination of surgery and MSC therapy has been reported with increased frequency to prevent or alter the progress of OA. Wang *et al.* (2017) reported, in a double blinded RCT after ACL reconstruction, a significantly better clinical outcome with addition of allogeneic MPCs compared to HA alone at the 24-month follow-up. Tibiofemoral joint space narrowing as a sign of osteoarthritic progression was significantly lower in the MPC group. In contrast, a combination of MSC injection and microfracture, compared to microfracture treatment alone, for treatment of cartilage defects did not result in significant differences in clinical outcomes, radiological evaluation and histological analysis in a prospective RCT at the 2-year follow-up (Koh *et al.*, 2016). In August 2021, 98 clinical trials of MSC therapy were listed in clinicaltrial.gov, among them 10 were phase III studies and promising for providing evidence for therapeutical efficacy. A recent overview of current MSC product approvals for OA and rheumatoid arthritis is provided by Hwang *et al.* (2021).

Summary and outlook

Clinical data on MSC therapy demonstrates that the treatment is safe and effective to decrease pain and improve knee functional scores. Currently, numerous clinical phase III trials are performed and are promising to implement new MSC products on the market. Data suggest that further research and standardisation are needed for the use of MSCs in knee joint OA.

Data suggesting that further research and standardization is needed for the use of MSCs in knee joint OA

At the bench

The concept of cartilage regeneration through application of MSCs is very appealing for both scientists and clinicians as it promises to resolve the problem of OA. Thus, cartilage regeneration *ad integrum* represents the holy grail of orthopaedics as it would make (endo-)prosthetic surgery and its complications obsolete and restore the joint function to its physiological state.

In January 2021, the Boolean search string: "cartilage AND ((stem cell) OR MSC))" delivered 8.654 results in PubMed. By year, there is a large and growing scientific interest in MSC therapies for cartilage regeneration. Preclinical *in vivo* studies and *in vitro* models showed promising results. Several treatment concepts have advanced into clinical trials; however, so far there is no established practical and satisfying clinical solution. Research gaps and obstacles as well as possible strategies to tackle this problem are outlined below.

No standardised cell culture protocols

Major obstacles to current research are undefined standards for cell harvesting and cell processing. As a result, comparability of studies and study protocols are limited. A recent review on MSC preparation protocols demonstrated an inadequate reporting of cell preparation (Robinson *et al.*, 2019). There is evidence that the molecular and functional phenotypes of human BMSCs are dependent on the harvesting techniques (Walter *et al.*, 2020). Walter *et al.* (2020) observed, in a comparison analysis of outgrowth and aspirate techniques, a significant difference in functional cell phenotype as a result of using such different harvesting techniques. This resulted in a higher osteogenic differentiation potential of human BMSCs from aspirated cultures. In fact, certain markers, such as CD146, were expressed differently in aspirated and bone-chip cultures. A significantly higher osteogenic differentiation potential (and consequently lower cartilaginous differentiation potential) was found in the aspirate group. This finding is in line with other studies that reported differences in MSC phenotype when using different cell isolation procedures (Bara *et al.*, 2014; Haddouti *et al.*, 2020; Herrmann *et al.*, 2019). This further underlines the diversity of cell phenotypes and emphasises how important it is to standardise cell isolation and culture procedure. Overall, great variations in selection criteria and culture expansion conditions of MSCs, the lack of substantial diversity and consistency in the assessment of clinical outcome make the comparison of different MSC origins very complicated (Shariatzadeh *et al.*, 2019). The number

of passages in MSC expansion is crucial for cell functionality, vitality and plasticity (Moravcikova *et al.*, 2018). Such that, early passaged human MSCs show a more vital immunomodulation than late passaged MSCs (Kim H *et al.*, 2020). In fact, there is a lack of correlation between *in vitro* behaviour and *in vivo* performance and also donor age and co-morbidities can influence the functional characteristics of MSCs (Hwang *et al.*, 2021).

An improved cell biological characterisation of MSCs is required to design reliable studies to better assess the effectiveness of MSC products. It stands to reason that the current inhomogeneous protocols and a lack of standardisation lead to insufficient reproducibility of results from clinical studies. To change this, many parameters would have to be better standardised during isolation, expansion and use of MSCs. For example, MSCs can have different phenotypes which are not only dependent on the isolation methodology but also the tissue of origin (Haddouti *et al.*, 2020). Pre-existing diseases and predispositions for certain pathologies of the tissue donors must be included. The immune status, in particular, plays a special role: increased levels of IFN-γ strengthen immunomodulatory mechanisms in MSCs (Kim *et al.*, 2018). In this respect, a better characterisation of the current immune environment is of central relevance to determine the *in vivo* priming of the MSCs to be isolated. However, these aspects must also be taken into account for the *in vitro* culture. Questions such as how to actively or passively modify MSCs *in vitro* are essential and should be addressed in defined SOPs for all pre-clinical and clinical studies to allow for better comparability. Furthermore, the number of dose repetitions is unclear. In a preclinical rat model of ACL transection, repeated application of human synovial MSCs inhibited OA progression compared with a single dose (Ozeki *et al.*, 2016). However, Joswig *et al.* (2017) reported, in an equine model of repeated allogeneic BMSC application, an elevated risk of adverse events. Schu *et al.* (2012) investigated the therapeutic effect of allogenic MSCs in co-culture. The allogenic co-culture resulted in a loss of protection against cytotoxic lysis under inflammatory condition inducing complement-activating antibodies. The immunomodulatory efficacy was highly impacted.

Preconditioning of MSCs during the culture process (*e.g.* anabolic factors, nutrients, cytokines, oxygen tension) was reported in different studies to enhance their regenerative capacity (Peck *et al.*, 2019). It remains questionable whether application of preconditioned MSCs yields superior regenerative effects compared to non-preconditioned cells. Preconditioning and *in vitro* differentiation of MSCs into cartilaginous progenitor cells prior to implantation might support cellular directed differentiation into chondrocytes and, thus, reduce rates of failed differentiation into other cell lineages (Endo *et al.*, 2019; Peck *et al.*, 2019). Nevertheless, there is a risk of adverse effects of preconditioning (Kato *et al.*, 2014).

Insufficient cartilage repair technique

Murphy *et al.* (2003) observed in a caprine model of OA that the retention rate of infiltrated autologous BMSCs was low, especially in cartilage. Most MSCs (97 %) disappeared after a few days and, interestingly, they were predominantly located in the synovium and, rarely, in the articular cartilage. Nevertheless, induction of OA by resection of the ACL and medial meniscus was reduced and regeneration of the meniscus observed. This study demonstrated that regeneration was not achieved by introducing tissue-specific progenitor cells. Furthermore, this disease-modifying effect has not been reproduced in a clinical setting. In the context of clinical application of intra-articular cell therapy, it remains questionable how cells are supposed to migrate to predisposed locations. Hyaline cartilage has a particular anatomy, with certain histological features that have never been reproduced in detail neither in *in vitro* nor in *in vivo* experiments (Sophia Fox *et al.*, 2009; Weizel *et al.*, 2020). Especially the U-formed collagen fibres with a tangential course parallel to the cartilage's surface layer are important for biomechanical stability and shear stress resistance. Cartilage composition and its embryological development are well described; yet, so far no experimental MSC-based approach has tried to reproduce these mechanisms (Hall, 2015; Streeter, 1949). Nevertheless, restoring the functional ECM of hyaline cartilage is crucial for functionality and protection against a cartilaginous decay as a result of biomechanical impaction. Bioprinting may be a promising tool to reach a cartilage-like tissue in the future.

Disregard of other cellular therapies

From an immunological point of view, hyaline cartilage is (just like cornea) an immune-privileged tissue. While allogeneic corneal transplantation is well described and established, only few clinical trials have reported on allogeneic cartilage transplantation. None of these osteochondral allograft trials, however, has described immunological rejections of the grafts and significant complication rates (El-Rashidy *et al.*, 2011; Gross *et al.*, 2008; Hunt *et al.*, 2014; Oakeshott *et al.*, 1988). Thus, cartilage researchers have to evaluate whether this strategy may be more effective and promising in the mid- and long-term.

At the bedside

Insufficient translational models

Despite a plethora of positive *in vivo* animal studies, few randomised controlled clinical trials have demonstrated meaningful clinical benefits under any condition of knee OA. Moreover, clinical studies do not reproduce positive cartilage regenerative effects. Considering that at the time of inclusion into a study, animals are young and do not have

Table 2. Overview of essential advantages and disadvantages of MSC therapy in knee OA.

Advantages	Disadvantages
Regenerative capacity is preclinically investigated in detail, with the concept of the paracrine MSC effect	High research interest but low output in clinical implementation
Preclinical models show disease-modifying effects of MSCs	MSC therapy demonstrates only symptom-modifying effects but no disease modification
Clinical studies demonstrate significant pain reduction and improved function (symptom-modifying effects)	MSC preparation protocols significantly differ and hinder a comparison between studies
Autologous and allogenic products are available and have been reported to be safe	Allogenic MSC products may transmit diseases, alloreactivity
Easy access to different MSC sources	High therapy costs due to strong regulations, extensive cell culture

co-morbidities, it remains matter of debate whether current animal models for OA are comparable with this complex multifactorial degenerative disease. Furthermore, there is a lack of correlation between *in vitro* behaviour of MSCs and *in vivo* performance as well as donor age and co-morbidities.

Patient selection

Alloreactivity for allogeneic MSC transplantation is rarely described in the clinical context and it remains debatable whether donor-recipient matching is necessary for allogeneic transplantation (Ding *et al.*, 2020; Gupta *et al.*, 2016). Furthermore, eligibility criteria for donors and recipients are yet to be defined. Donor characteristics such as age, sex and co-morbidities have a high impact upon MSC phenotype and function (Benisch *et al.*, 2012; Dexheimer *et al.*, 2011). For example, patients with diabetes mellitus type 2 have a reduced MSC count compared to non-diabetic patients (Cassidy *et al.*, 2020).

Insufficient clinical outcome measures

A recent meta-analysis of RCTs of MSC therapy in OA without adjuvant surgery found a significant improvement in clinical symptom-modifying scores (VAS, WOMAC) but no statistical significance in structural magnetic resonance imaging (WORMS) at 6-12 month follow-up (Kim *et al.*, 2019). However, none of these scores explains the outcomes at the cellular and molecular level (*e.g.* sufficient cell differentiation and integration, apoptosis rate of injected MSCs, *etc.*) and, overall, turns the intra-articular processes after cell transplantation into a black box. To overcome this hurdle, a histological evaluation would be necessary but is ethically critical. Magnetic resonance evaluation of ECM composition (*e.g.* T2 mapping) and rising marker evaluation in synovial fluid can contribute to a better understanding.

Intra-articular conditions

When injecting MSCs into an OA knee joint, MSCs will encounter conditions different from a healthy knee joint and data on cellular reactions in this different milieu are scarce (Akhbari *et al.*, 2020). It is questionable whether a changed composition of OA synovial fluid contributes to decreased MSC differentiation and migration to the damaged cartilage (Mancuso *et al.*, 2019).

Unclear treatment protocol

Comparability of clinical studies is limited by different study protocols (Kim *et al.*, 2019). There is currently no gold-standard and no state-of-the-art concept that yields best clinical outcomes. Therapeutic effects of carriers, predominately PRP and HA, are not proven. In contrast, application of PRP was not superior to pure autologous BMSC injection in a RCT (Bastos *et al.*, 2019). Natural products such as PRP also add another source of variation as PRP lacks standardisation as well (Collins *et al.*, 2021). As for the injected dose, the mean cell count in MSC therapy is 8.7×10^7 cells, with a large variation from 8.5×10^6 to 10×10^8 cells (Robinson *et al.*, 2019). Lamo-Espinosa *et al.* (2018) demonstrated, in an RCT of autologous BMSC therapy with two different doses, no different clinical outcome between the groups. In some studies, higher doses of MSCs resulted in a better clinical outcome but also in increased adverse events (Peeters *et al.*, 2013). However, this is far from being a closed case, as a dose escalation study (low dose 2×10^6 cells, medium dose 10×10^6 cells and high dose 50×10^6 cells) showed that patients treated with low-dose autologous ADMSCs experienced significant improvements in pain levels and function compared with baseline (Pers *et al.*, 2016). Consequently, the appropriate dose for MSC therapy in OA is still undefined.

Cost and minimal translation

MSC therapy is highly regulated in many countries. Due to its non-homologous approach, official authorisation is difficult to obtain. Moreover, the mean price of a single MSC injection is ~ 5,000 US dollars (Piuzzi *et al.*, 2018). For a single application, with no proven disease-modifying effect, the therapy costs are high. Although research efforts are tremendous, only a few phase III clinical studies have been conducted and only few products are available.

Summary

Treatment of OA by implantation of MSCs is currently associated with high hopes and expectations. A more detailed view on current data reveals that MSC-based treatment approaches lack efficacy, precision and molecular understanding. Besides the issue of the correct application of MSCs into the knee joint, the number of MSCs to be applied remains questionable. Ultimately, identification of the right patients for the MSC treatment will remain a further challenge. Routine usage of MSCs for treatment of knee OA cannot be currently recommended.

Final conclusion

As hyaline cartilage is a natural and common tissue, growing knowledge on this tissue, its development and degeneration will ultimately allow for cartilage restoration or *de novo* synthesis, possibly through treatment with MSCs. Clinical data are interesting but there is a need for more RCTs to investigate the therapeutic potential of MSCs. MSC therapy in OA is a controversially discussed topic (Table 2) and needs further investigations to reach clinical translation in the future.

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Discussion with Reviewer

Martijn van Griensven: Which additional preclinical studies would help clinical translation?
Authors: We are convinced that all preclinical and clinical studies should utilise highly standardised MSCs. This includes the clinical history of the donor organism, health status, inflammation status, isolation method, *in vitro* culture, extensive cell surface characterisation, RNAseq data, *etc.*. From our perspective, such highly standardised studies are still missing and they would significantly improve the understanding of the variability in study outcomes.

Martijn van Griensven: How would you design a clinical study to correctly investigate the possible effectivity of MSC treatment in OA? Could markers be used to define patients? Are MSC markers suitable and/or of high quality for *ex vivo* tests?
Authors: In line with the previous point, we would invest in extensive standardisation protocols, then correlate clinical outcome with functional and molecular profile of the MSCs and try to narrow down how they relate to the superior clinical outcome.

Editor's note: The Scientific Editor responsible for this paper was Mauro Alini.