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# Mesenchymal stem/stromal cell-based therapy for the treatment of rheumatoid arthritis: An update on preclinical studies

Mercedes Lopez-Santalla[a,b,*], Juan A. Bueren[a,b], Marina I. Garin[a,b,*]

[a] Division of Hematopoietic Innovative Therapies, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT) and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBER-ER), Madrid; Spain
[b] Advanced Therapy Unit, Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD/UAM)

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by synovial inflammation and progressive joint destruction and is a primary cause of disability worldwide. Despite the existence of numerous anti-rheumatic drugs, a significant number of patients with RA do not respond or are intolerant to current treatments. Mesenchymal stem/stromal cell (MSCs) therapy represents a promising therapeutic tool to treat RA, mainly attributable to the immunomodulatory effects of these cells. This review comprises a comprehensive analysis of the scientific literature related to preclinical studies of MSC-based therapy in RA to analyse key aspects of current protocols as well as novel approaches which aim to improve the efficacy of MSC-based therapy.



*Abbreviations:* A, allogeneic; AAV, adeno-associated virus; AD, adipose tissue; AD-MSCs, MSCs from adipose tissue; AhR, aryl hydrocarbon receptor; ALP, alkaline phosphatase; ANA, Antinuclear antibodies; APCP, $\alpha$, $\beta$ methylene selective A2A adenosine receptor competitive antagonist (CD73 inhibitor); AT, antithrombin; AS, arthritis score; AxLNs, axillar lymph nodes; BMZ, betamethasone; BM, bone marrow; BM-MSCs, MSCs from bone marrow; Bregs, regulatory B cells; Bzb, bortezomib; CAIA, collagen-antibody induced arthritis; CD, cluster of differentiation; CFA, Freund's complete adjuvant; CIA, collagen-induced arthritis; CII, collagen II; CM, conditioned medium; COMP, cartilage oligomeric matrix protein; Consec., consecutive; CRP, C reactive protein; CTLA4, cytotoxic T lymphocyte antigen; CXCR3, chemokine receptor 3-alternative; D, days; DAB, 3,3′-diaminobenzidine; DCs, dendritic cells; dLNs, draining lymph nodes; DMARDs, antirheumatic drugs; DM, dexamethasone; EMA, European medicine agency; ERDF, European Regional Development Fund; ESR, erythrocyte sedimentation rate; ETN, etanercept; EVs, extracellular vesicles; Exos, small vesicles/Exosomes; F, female; Flk, tyrosine kinase receptor for vascular endothelial growth factor; FOXP3, forkhead box P3; G, gingiva tissue; Gilz, glucocorticoid-induced leucine zipper; GIP, gastric inhibitory polypeptide; GITR, glucocorticoid-induced tumour necrosis factor receptor; GLP-1, glucagon-like peptide 1; G-MSCs, MSCs from gingiva tissue; GSH, glutathione; GSH-Px, glutathione peroxidase; HDL, high density lipoprotein; H/E, haematoxylin/eosin staining; HLA-II, human leukocyte antigen-II; HMGB-1, high-mobility group box-1; HSCs, hematopoietic stem cells; IA, intra-articular; IAA, indoleacetic acid; ID, intradermal; IDO, indoleamine 2,3-dioxygenase; IFA, incomplete Freund's adjuvant; IFI, indirect immunofluorescence; IFN-$\gamma$, interferon gamma; Ig, immunoglobulin; IHC, immunohistochemical analysis; IL, interleukin; ILA, indole-3-lactic acid; iLNs, inguinal lymph nodes; IL-1RA, IL-1 receptor antagonist; IM, intramuscular; IN, intranodal; iNOS, inducible nitric oxide synthase; IP, intraperitoneal; IP10, IFN-$\gamma$-induced protein 10; ISCT, international society for cellular therapy; IV, intravenous; KC, keratinocyte chemo-attractant; LDL, low density lipoprotein; LP, lamina propria, LPS, lipopolysaccharide; M, male; mAIA, antigen-induced arthritis in mouse; mBSA, methylated bovine serum albumin; MCP-1, monocyte chemotactic protein 1; $\mu$CT, micro computed tomography imaging; MDA, malondialdehyde; MensMSCs, MSCs from menstrual fluid; MHC, major histocompatibility complex; MIP-2, macrophage inflammatory protein 2; miR, microRNA; mLNs, mesenteric lymph nodes; MMP, matrix metalloproteinase; MPO, myeloperoxidase; MPs, microparticles; MPCs, mesenchymal precursor cells; MRI, magnetic resonance imaging; MSCs, mesenchymal stem/ stromal cells; MTC, Masson's trichrome; Mtx, methotrexate; MVs, microvesicles; NA, not available; NF-$\kappa$B, nuclear factor-kappa B; NK, natural killer cells; NRP-1, neuropilin-1; NSAIDs, non-steroidal anti-inflammatory drugs; NuMa, nuclear mitotic apparatus; OCPs, osteoclast precursors; O-MSCs, MSCs from nasal tissue; OPG, osteoprotegerin; OVA, ovalbumin; OVAIA, ovalbumin-induced arthritis; P, placenta; PA, peri-articular; PB, peripheral blood; PBMCs, peripheral blood mononuclear cells; PG, proteoglycan; PGIA, proteoglycan-induced arthritis; PLGA, poly-lactic-co-glycolic acid; pLNs, popliteal lymph nodes; P-MSCs, MSCs from placenta tissue, POM-1, sodium polyoxotungstate (CD39 inhibitor); PP, peyer's patch; PPAR, peroxisome proliferator-activated receptor; PTPN22, protein tyrosine-phosphatase 22; RA, rheumatoid arthritis; RAGE, receptor for advanced glycation products; rAIA, adjuvant-induced arthritis in rats; RANKL, receptor activator of nuclear factor $\kappa$B ligand; RANTES, regulated upon activation normal T cells expressed and presumably secreted; rCIA, collagen-induced arthritis in rats; RF, rheumatoid factor, RNA, ribonucleic acid; ROR$\gamma$T, retinoic acid-related orphan receptor; S, syngeneic; SAA, serum amyloid-A; SC, subcutaneous; sCIA, collagen-induced arthritis in sheep; SF, synovial fluid; SF-MSCs, MSCs from synovial fluid; sIL6R, soluble IL-6 receptor; SIRT1, sirtuin 1; SM, synovial membrane; SM-MSCs, MSCs from synovial membrane; SNP, sodium nitroprusside; SO, safranin; SP, spleen; SOD, superoxide dismutase; SSEA, severe spontaneous erosive arthritis; STAT3, signal transducer and activator of transcription 3; SVF, stromal vascular fraction; T-AOC, total antioxidant capacity; TBO, toluidine blue O; TCR, T cell receptor; TF, tissue factor; Tfh, follicular T helper cells; TGF-$\beta$, transforming growth factor $\beta$; Th, T helper cells, TLR, toll-like receptors; T-MSCs, MSCs from tonsil tissue; TNF-$\alpha$, tumour necrosis factor $\alpha$; TNFR, tumour necrosis factor receptor; Tol-DCs, tolerogenic dendritic cells; Tr1, IL-10-expressing T cells; TRACP, tartrate-resistant acid phosphatase; TRAF, tumour necrosis factor receptor-associated factor; Tregs, regulatory T cells; UC, umbilical cord; UC-MSCs, MSCs from umbilical cord; VEGF, vascular endothelial growth factor; WT, wild type; X, xenogeneic; >, better than; <, worse than; =, similar to or equal

* Corresponding authors
*E-mail addresses:* mercedes.lopezsantalla@externos.ciemat.es (M. Lopez-Santalla), marina.garin@ciemat.es (M.I. Garin).

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## 1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease caused by loss of immunologic self-tolerance that generates chronic inflammation in the joints followed by cartilage and bone destruction with associated vascular, metabolic, bone and psychological comorbidities. Several loci related to immune system effector and regulatory genes such as human leukocyte antigen II (HLA-II), protein tyrosine-phosphatase 22 (PTPN22), signal transducer and activator of transcription 3 (STAT3), among others are involved in the aetiology of the disease. In addition, environmental stimuli and epigenetic changes can also contribute to the pathogenic profile of RA. During RA development, the synovium exhibits characteristics of chronic inflammation, including leukocyte infiltration of innate cells and adaptive immune responses mainly mediated by T and B cells [1,2]. The prevalence varies between 0.3 and 1% in western countries, increases with age and is more common in women than in men [3].

Current treatments for RA include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying antirheumatic drugs (DMARDs) and, more recently, biologic DMARDs. However, there is still no cure for this disease, less than half of patients with RA are in remission, and 10%–15% are refractory or even develop severe adverse effects to the treatments [4–6]. In the last 30 years, mesenchymal stem/stromal cells (MSCs) have emerged in the field of regenerative medicine due to their capacity to differentiate into specific cell types [7]. Additionally, MSCs can secrete soluble factors and cytokines, exhibit haematopoiesis-supporting properties [8,9] and have immunomodulatory effects, thus representing a promising tool to treat immune-mediated disorders such as RA [9,10].

This review analyses the scientific literature regarding the characteristics of MSC therapy in preclinical studies of RA, focusing on experimental protocols that have paved the way for clinical translation of MSC-based therapy in RA.

## 2. Animal models of arthritis used in cell therapy protocols with MSCs

Among RA-like disease models, the collagen induced-arthritis (CIA) model is the most frequently-used experimental model of RA used to study MSC-based therapy (76% of the studies, Fig. 1 and Supplementary Tables). Regarding the species used, most of the studies using the CIA model with MSC-based therapy were conducted in mice (77%) followed by rats (20%, Supplementary Table 1) [11,12]. From 2005 to 2011, the number of studies published increased linearly each year and nearly quadrupled from 2010 to 2016. Since 2017, this tendency has dropped as a consequence of the clinical translation of MSC-based therapy for RA [13] (Fig. 1). In the CIA model, arthritis is induced by one/multiple subcutaneous (SC), intradermal (ID), intraperitoneal (IP) or intra-articular (IA) injections of type II collagen (CII; the major constituent collagen form of articular cartilage), either of chicken or bovine origin emulsified in complete Freund's adjuvant (CFA) containing *Mycobacterium tuberculosis* one, two or three weeks apart [14] (Table 1). Immunization with heterologous CII activates both CII-reactive T (mostly Th17) and B cells resulting in breach of immunological tolerance with systemic autoantibody-driven arthritis. The CIA model is widely acepted as the animal model that best resembles the systemic immune responses of human RA [15,16].

Although the CIA model is the archetypical model of RA and most of the studies with MSCs were done in this model, the high variability in terms of time required for the induction phase, variable incidence and the restriction of mouse strains used in this model have resulted in the development of alternative experimental models of RA in which MSC-based therapy has also been assessed (Fig. 1).

The adjuvant-induced arthritis model in rats (rAIA) and the antigen-induced arthritis model in mice (mAIA) are the second experimental models of arthritis most frequently-used to study MSC therapy (12% of the studies with rAIA and 3% of the studies with mAIA, Fig. 1 and Supplementary Tables). In these models, arthritis is induced by one or two subcutaneous injections of CFA or *mycobacterium butyricum* in incomplete Freund's adjuvant (IFA) with or without methylated bovine serum albumin (mBSA) (Table 1) [17]. In the AIA models, arthritis is induced only locally with articular T cell-mediated damage [18].

The collagen antibody-induced arthritic model (CAIA), has been also used to study MSC-based therapy in 3% of the studies. In the CAIA model, arthritis is induced by injection of serum antibodies against endogenous CII (Table 1) resulting in immune complex formation and complement activation, but the induction phase of the disease is B cell and T cell independent. Therefore, this model does not completely recapitulate the complexity of immune and tissue remodelling responses during human RA [16].

In most of the RA preclinical studies, the efficacy of MSC-based therapy was evaluated by measuring paw swelling, histologic analysis, X-ray, and micro computed tomography ($\mu$CT) of joints. Moreover, quantification of the levels of auto-antibodies against CII, rheumatoid factor, erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) in the sera in arthritic animals (Supplementary Table 1) were also evaluated similarly to the EULAR and ACR

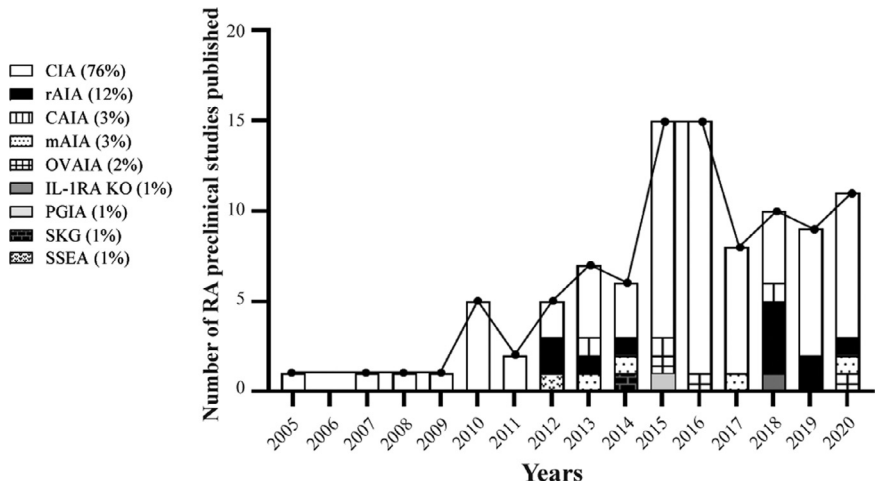


**Fig. 1.** Published preclinical studies of RA with MSC-based therapy classified according to the arthritis models and publication date. Data are expressed as percentages (%).

**Table 1**
Animal models of RA used in MSC-based therapy studies.

| RA model | Specie | Strains | Immunogen and protocol | Onset of arthritis from induction |
|---|---|---|---|---|
| Antigen-induced arthritis (mAIA) [96,97] | Mouse | C57BL/6 | SC/IP-injected mBSA in/or CFA with *Bordetella pertussis* toxin and IA-injected mBSA three weeks later | 21 days |
| Adjuvant-induced arthritis (rAIA) [17] | Rat | -Albino, Fisher-Lewis, Sprague dawley, Wistar | -SC-injected *Mycobacterium butyricum* in IFA -SC-injected mBSA in CFA & IA-injected mBSA -One or two IA injections of CFA 5 or 14 days apart | 6-15 days |
| Collagen antibody-induced arthritic mouse (CAIA) [98] | Mouse | DBA/1 | IV-injected antibodies against CII and IP-injected LPS 24, 48 or 72 hours later | 4 days |
| Collagen-induced arthritis (CIA) [14] | Mouse | -Albino, C57BL/6 DBA/1 | -One or two SC/ID/IV injections of mouse, chicken or bovine CII with CFA or IFA two or three weeks apart and, in some cases, IP LPS 1 week later | 21 days |
| | Rat | -Lewis, Sprague-dawley Wistar | -One or two SC/ID/IA injections of chicken or bovine CII with CFA or IFA and other IP/IA injections one, two or three weeks apart | 7-12 days |
| | Sheep | Merino | -Two SC injections of bovine CII with CFA or IFA two weeks apart and other IA injection one week later | 29 days |
| IL-1RA knockout mice [99] | Mouse | BALB/C | IL-1RA gene deleted | 5 weeks after birth |
| Ovalbumin (OVA)-induced arthritis (OVAIA) [100] | Rabbit | Japanese white | Three doses of SC-injected OVA in CFA weekly and IA-injected OVA two weeks later | 5 weeks |
| Proteoglycan-induced arthritis (PGIA) [101] | Mouse | BALB/C | Two IP injection of PG 3 weeks apart | 26 days |
| Severe arthritis spontaneously (SSEA) [102] | Mouse | K/BxN | Crossing KRN mice with NOD.Ea16 transgenic mice | 5 weeks after birth |
| SKG strain mice [103] | Mouse | BALB/C | IP injection of Curdlan | 14 days |

recommendations for the management of rheumatoid arthritis patient treatments [4,5].

Overall, MSC-based therapy can reduce arthritis inflammation by 30% in the majority of the published RA animal models (Supplementary table 1). The different RA-like disease models offer advantages for modelling different aspects of human disease although the CIA model is the most frequently used due to the fact that it shares local and systemic immunological aspects of human RA disease.

## 3. Mechanism of action of MSC-based therapy in preclinical studies of RA

Although different immune responses and mechanisms of action have been implicated in the immunomodulatory capacity of MSCs, nowadays the exact mechanism of action is still not fully defined. An increase in regulatory adaptive T cells (regulatory T cells (Treg) and IL10-expressed $CD4^+$ T cells (Tr1)) and a decrease of inflammatory cells such as T helper 1 (Th1) and Th17 in peripheral blood (PB), spleen (SP), draining lymph nodes (dLNs) and in the joints have been amply described in preclinical studies of RA following MSC-based therapy (Supplementary table 1). Furthermore, a decrease of pro-inflammatory cytokines such as interferon (IFN) $\gamma$, tumour necrosis factor (TNF) $\alpha$, interleukin (IL) 4 and IL17 levels together with an increase of anti-inflammatory cytokines such as transforming growth factor beta (TGF-$\beta$1) and interleukin 10 (IL10) in PB and joints are the most common mechanisms of action described in MSC-based studies (Supplementary Table 1). These mechanisms have also been analysed and most of them confirmed in clinical trials [13] emphasizing the importance of preclinical studies for the development of MSC-based therapies for RA.

## 4. Tissue sources for MSC isolation and expansion

In 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) proposed a set of standards and minimal criteria to define human MSCs for cell-based therapy protocols. According to these criteria, MSCs must be plastic-adherent when maintained in standard culture conditions, must express CD105, CD73 and CD90 surface markers, and lack expression of hematopoietic and endothelial surface molecules such as CD45, CD34, CD14, CD11b, CD79$\alpha$, CD19, HLA-DR and CD31. *In vitro*, under specific conditions, the expanded MSCs must differentiate to osteoblasts, adipocytes and chondroblasts to confirm their multipotency [19]. These criteria have been demonstrated suitable for laboratory-based and preclinical studies as well as clinical translation of MSC-based protocols, thus facilitating the comparison among laboratories and hospitals. Nevertheless, there is a great variety in the culture media for the *in vitro* expansion of the MSCs, the number of MSC passages and infusion vehicle of MSCs used in preclinical studies which hampers this comparison.

Bone marrow (BM) was the first tissue source used to isolate and expand MSCs (BM-MSCs) for cell-based therapy; consequently, it has been the most frequently used in animal models of RA [11] (53% of the studies, Fig. 2 and Supplementary Tables).

Since the collection of BM tissue is a highly invasive procedure for the donor and the frequency of MSCs is extremely low, alternative tissue sources have been subjected to intensive investigation. The most frequent alternative sources of MSCs that have been used in RA preclinical studies are umbilical cord (UC, 17%) and adipose (AD, 17%) tissues (Fig. 2). The frequency of MSCs in adipose tissue is higher than in BM [20]. UC-MSCs can be obtained from Wharton's jelly, artery, vein, or cord lining but this requires time-consuming tissue dissection. Consequently, in general, MSCs from umbilical cord (UC-MSCs) are obtained easily by digesting the complete segments of the umbilical cord containing heterogeneous populations of MSCs. MSCs derived from adipose tissue (AD-MSCs) and UC-MSCs display similar functionality to BM-MSCs [21–23]. The use of adipose and umbilical cord tissues as sources of MSCs in preclinical studies of RA have been lesser-used sources than BM [11] (Fig. 2 and Supplementary Tables) since they have been identified more recently.

Several groups have isolated MSCs from alternative tissue sources such as gingiva (G-MSCs) [24], palatine tonsil (T-MSC) [25] and

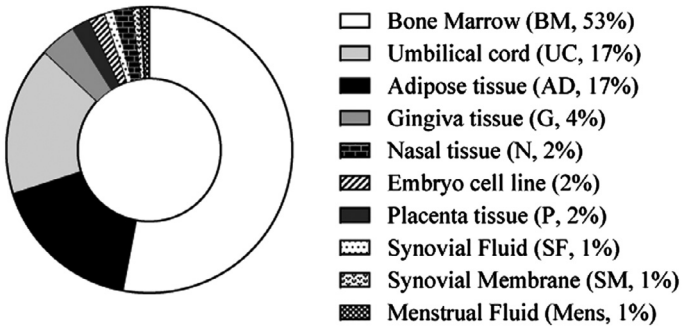


**Fig. 2.** Published preclinical studies of RA with MSC-based therapy classified according to the tissue sources. Data are expressed as percentages (%).

placenta tissues (P-MSCs) [26], as well as synovial fluid (SF-MSCs) and synovial membrane (SM-MSCs) [27], nasal (https://patentscope.wipo.int; patent number: WO2019022386 [28]) and embryo tissues [29]. The MSCs derived from these alternative sources have similar characteristics to BM-MSCs, AD-MSCs and UC-MSCs which make them potentially applicable for cell-based therapies in RA. In preclinical studies, these alternative tissue sources for MSCs have also shown amelioration of arthritis severity in RA animal models (Supplementary Tables), which warrants future translation into clinical trials for RA [13].

Interestingly, the stromal vascular fraction (SVF) from liposuction surgery contains MSCs apart from hematopoietic stem cells (HSCs) and elements directly capable of promoting tolerogenesis such as Tregs and inhibitory macrophages [30]. Although no RA preclinical studies with SVF therapy have been reported, SVF therapy has been used in five clinical trials for treatment of RA, one of which is currently ongoing [31].

Today, all tissue sources of MSCs have shown analogous efficacy in RA experimental models; consequently, any of them could be used for future treatments of patients with RA. Umbilical cord is the main source of MSCs used in RA clinical trials although BM-MSCs are the most frequently used in RA preclinical studies. This might be due to the fact that UC is more accessible and less aggressive for the donor compared to BM-derived MSCs.

## 5. Immunogenicity of MSCs

The large majority of RA preclinical studies for MSC-based therapy have been performed under xenogeneic conditions (46% of the studies) (Fig. 3 and Supplementary Tables), followed by the syngeneic major histocompatibility complex (MHC) context (34%) that mimics autologous settings in the clinic.

Several studies comparing different MHC contexts have been performed. All these studies claimed that xenogeneic, allogeneic, and syngeneic MSCs have similar beneficial effects [32–35]. Despite these results and the number of studies claiming low immunogenicity of MSCs [8,36,37], Liu L et al.'s meta-analysis of RA preclinical studies pointed out that although MSCs from the different MHC contexts showed modulation in different RA models, more substantial benefit was observed under xenogeneic conditions rather than syngeneic or allogeneic contexts suggesting that an appropriate level of immune disparity between the donor and the host may benefit the efficacy of the cell therapy [11]. This is in line with the hypothesis that soon after infusion the MSCs undergo extensive caspase activation and apoptosis mainly induced by endogenous cytotoxic cells ($CD8^+$ T and NK cells), in a process that is MHC independent. Apoptotic MSCs are then engulfed by phagocytic cells which in turn produce indoleamine 2,3-dioxygenase (IDO), thus triggering immunosuppression in a non-MHC-specific manner [38,39].

The allogeneic use of MSC-based therapy resolves the clinical need to obtain a sufficient number of cells to treat RA patients with MSC therapy and offers the possibility of establishing cell banks for immediate use when needed. Furthermore, some studies have pointed out that MSCs from RA patients may be functionally defective and could potentially contribute to the progression of RA, suggesting that their use in autologous context should be avoided [40,41]. Accordingly, most of the clinical trials in RA with MSC therapy, either completed or currently ongoing, are conducted with allogeneic MSCs [13].

## 6. Routes of administration and biodistribution of infused MSCs

Intravenous (IV, 47% of the studies) and IP (30%) are the most frequent routes of administration in MSC-based therapy in preclinical models of RA. IA infusion of MSCs is the chosen route of administration in larger animals such as rats, rabbits and sheep (17%, Fig. 4). In general, MSC-based therapy has shown similar beneficial effects irrespective of the route of administration used (Supplementary Table 1). These findings are in line with the studies that have confirmed the systemic effects of MSCs [42–44]. The biodistribution of infused MSCs strongly depends on the route of administration used. The large majority of preclinical studies have shown that a very small number of infused MSCs migrate to the inflamed joints and in general no robust correlation between their biodistribution and their beneficial effect can be established. Numerous studies in preclinical and clinical trials have suggested that MSC-based therapy may generate a sustained beneficial effect in the long term [45–49], even though the infused MSCs are undetectable a few days following their systemic administration as a consequence of the MSCs being phagocytosed by host cells [38,39,50].

In preclinical studies, it is well documented that IV-infused MSCs can get entrapped in the lung, potentially inducing pulmonary embolism that may compromise the survival of MSC-treated arthritic mice. This is in part due to the larger cell size of murine MSCs with respect to human MSCs which limits their IV use under syngeneic or allogeneic conditions [51]. Based on studies demonstrating that MSCs, administered systemically, migrated to dLNs where they induced immune cells with a regulatory phenotype, we demonstrated that adipose derived-MSCs injected by intranodal (IN) route in a CIA mouse model was feasible [52].

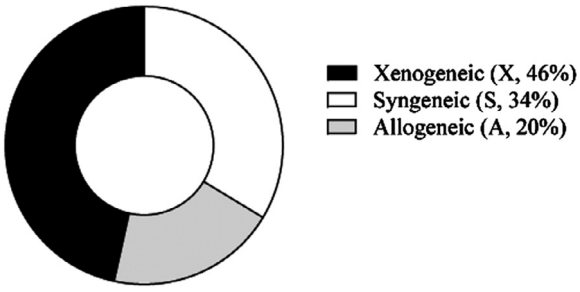


**Fig. 3.** Published preclinical studies of RA with MSC-based therapy classified according to the MHC context. Data are expressed as percentages (%).

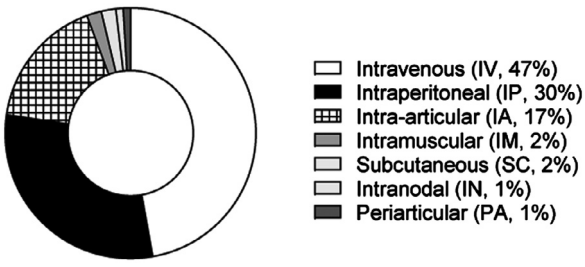


**Fig. 4.** Published preclinical studies of RA with MSC-based therapy classified according to the route of administration. Data are expressed as percentages (%).

Alternative routes of MSC administration for the purposes of prolonging their survival have been assessed *in vivo*. In this sense, local administration such as IA [35,43,61–68,53–60], periarticular (PA) [64]; intramuscular (IM) [69] and SC [64,70] have also been used in MSC-based therapy in RA preclinical studies (Supplementary Tables 1 and 2).

IV route of administration is the chosen one in the clinical trials for RA conducted to date [13].

## 7. MSC dosage and time of infusion

Numerous studies have observed improved efficacy when the MSC infusion was performed during the early phases of the disease (47% and 25% in the onset and in the established phase of the disease, respectively) rather than at pre-onset (19%) or during chronic phases (9%) of the disease, although promising results in terms of efficacy were observed in all instances (Fig. 5a, Supplementary Tables 1 and 2) [33–35,71–74]. In contrast, the completed RA clinical trials with MSC-based therapy have been conducted in very refractory RA patients with a long history of the disease which may have hindered the beneficial effects of MSC-based therapy in the clinic. Interestingly, RA patients during the early phases of the disease are now being recruited in currently ongoing clinical trials. This change in the inclusion criteria may increase the efficacy of MSC-based therapy [13].

A great range of cell doses have been tested, from $0.05 \times 10^6$ up to $1{,}700 \times 10^6$ MSCs per animal upon a single or multiple infusions (up to 21), although the most commonly used MSC dose has been in the range of $1 \times 10^6$ to $10 \times 10^6$ MSCs per mouse (Fig. 5b and c and Supplementary Table 1). No adverse effects have been reported in any of the MSC doses tested. Several studies observed better efficacy when multiple doses (2, 3 and even 5) of MSCs were infused, with respect to a single infusion of MSCs during the early phases of the disease [34,35,74,75]. MSC dosage escalating to human use should be between $50 \times 10^6$-$500 \times 10^6$ MSCs/Kg based on mouse (≈20g) and human (≈70Kg) body weights. This is far above the actual MSCs dosage with beneficial effects used in RA clinical trials in MSC-based therapy [13]. This is a key issue in MSC-based therapy that needs careful consideration in translational studies.

## 8. Approaches for the improvement of MSC-based therapy in RA experimental models

At present, several studies aiming at improving the efficacy and longest-lasting beneficial effects of MSC-based therapy have emerged in RA experimental models (Supplementary Table 2). Different approaches such as pre-treatment of the MSCs with different compounds and inflammatory cytokines during the expansion of the MSCs or just before their infusion *in vivo*, combination of MSC-based therapy with other treatments for RA, the use of the MSC-conditioned medium (CM) or MSC-released microparticles as well as overexpression of molecules to increase their immunomodulatory properties by means of genetic modifications of native MSCs have been conducted to enhance their immunomodulatory properties. In addition to this, encapsulated MSCs in scaffolding structures have also been described to increase their persistence *in vivo*.

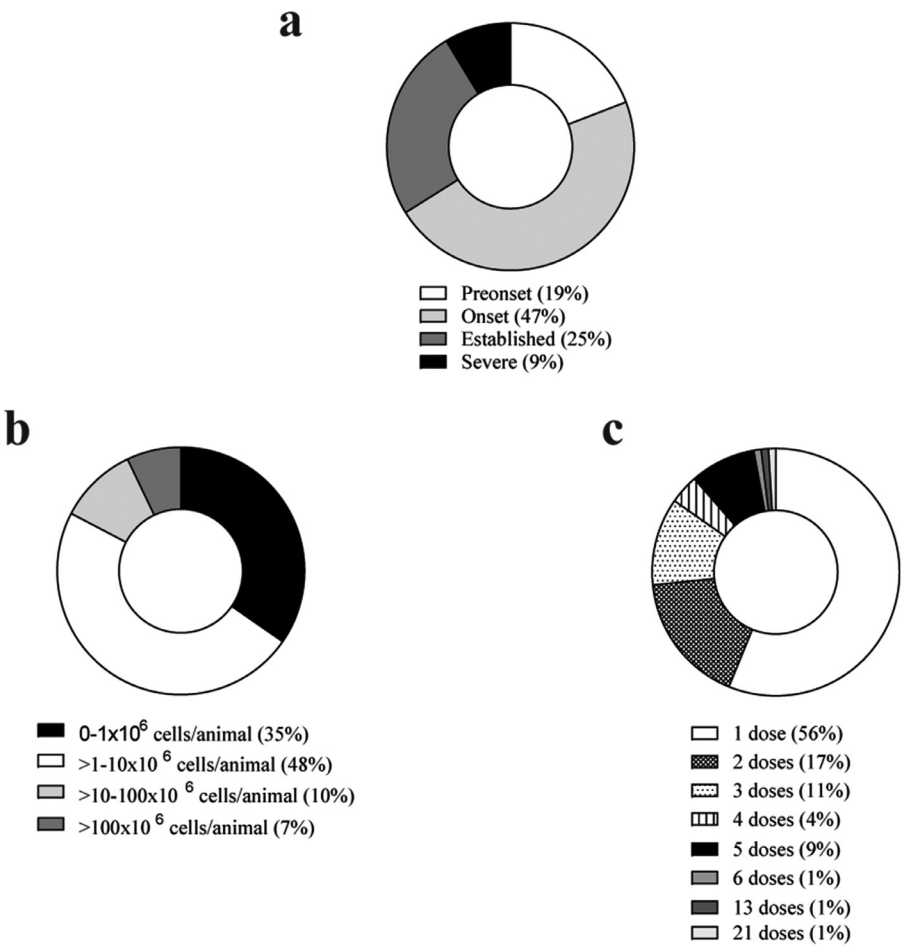


**Fig. 5.** Distribution of preclinical studies of RA with MSC therapy according to the time of treatment with MSCs related to the disease onset (a), MSC dose per mouse (b) and MSC dosage tested (c). Data are expressed in percentage (%).

### 8.1. Pre-treatment of MSCs

It has been amply described that pro-inflammatory cytokines, toll-like receptor (TLRs) ligands and some molecules can increase the proliferation, survival, differentiation and immunomodulatory functions of MSCs, thus enhancing their therapeutic efficacy *in vivo*. Cosenza et al. observed that small vesicles isolated from IFN-$\gamma$-pretreated BM-MSCs decreased inflammation in CIA arthritic mice [76] suggesting that the therapeutic efficacy of MSCs is highly dependent on the IFN-$\gamma$ levels. This was later confirmed by He X et al. where the authors observed enhanced synovial inflammation in mice treated with INF-$\gamma R^{-/-}$ MSCs with respect to wild type MSCs [77].

Alternatively, pre-treatment of MSCs with a STAT3 inhibitor (Patent numbers KR101160698B1 and KR101723265B1) or with soluble IL-6 receptor (sIL6R) [55] can enhance the therapeutic effects of MSCs in arthritis inflammation.

All these results support the notion that the immunomodulatory properties of MSCs are favoured when the infused MSCs encounter an inflammatory environment.

### 8.2. MSC therapy in combination with other molecules or alternative cell-based therapies

Several investigators have used MSC therapy in combination with treatments used in RA such as TNF-$\alpha$ inhibitors [66,78], IL-4 [79] or hesperidin [80] in preclinical models of RA aiming to increase the beneficial effects of MSC-based therapy. These groups demonstrated that the combination of MSCs with these molecules was more efficient than either of the treatments alone.

Lim JY et al. observed improved beneficial effects of MSCs co-infused with Tr1 cells [81]. Li R et al. demonstrated that MSC-based therapy in combination with tolerogenic dendritic cells (Tol-DCs) [82] in the CIA mouse model was more efficient at modulating arthritis progression than either cell-based treatment alone.

All these results support the notion that the combination of MSC therapy with other treatments currently used in the clinic for inflammatory diseases could an adequate alternative to increase the therapeutic effects of MSC-based therapy.

### 8.3. Scaffolding methods for MSC therapy

To increase the beneficial immunomodulatory effects of MSC therapy, several investigators are developing methods aiming to increase the survival of MSCs when the cells are infused locally. Zhang X et al. [64] and Yamagata K et al. [55] developed a poly-lactic-co-glycolic acid scaffold and Liu H et al. developed fibrin gel-encapsulated MSCs [63] and thermogel-encapsulated MSCs [58] that when administered locally were retained at the implanted site longer, thus reducing their migration to other tissues and enhancing the modulation of inflammation thus resulting in the remission of local inflammatory conditions in comparison to scaffold-free infused MSCs.

### 8.4. MSC-derived vesicles and MSC-condition medium

Most of the beneficial effects of MSCs are thought to be mediated by paracrine mechanisms involving the secretion of factors or the packaging of these factors in membrane-bound vesicles including microvesicles (MVs). The therapeutic potential of conditioned medium from MSCs (CM-MSCs) and MSC-derived MVs are particularly attractive as a strategy to increase the clinical benefits of MSC therapy since they reduce the risks associated with potential immune reactions against MSCs [83]. Kay AG et al. [57] and Nazemian V et al. [84] observed that CM-MSCs ameliorated the severity of inflammatory arthritis. Cosenza S et al. observed that MSC-derived exosomes (exos, diameter below 150 nm) can decrease more efficiently the clinical signs of inflammation with respect to MVs (ranging from 150 to 1000 nm in diameter) in a CIA model [76]. In addition to this, MSC-conditioned medium and MSC-derived MVs would not suffer the size exclusion in the lungs upon IV administration. Additionally, the variety of proteins, peptides, ribonucleic acids (RNA) and lipid mediators that can be concentrated, frozen, or even lyophilized without loss of activity give them a certain advantage over cellular products that require liquid nitrogen storage and an adequate infrastructure to preserve and revive frozen cells. This suggests that the molecules released from the MSCs have an important role in their beneficial effects, thus increasing the safety and clinical applicability of MSC therapy.

### 8.5. Genetic modifications of MSCs

To increase the therapeutic effect of MSC therapy, some authors have genetically modified MSCs to express molecules with immunosuppressive properties such as IL-10 [85,86], TFG-$\beta$ [87], IL-1RA [70], RAGE [88], cytotoxic T lymphocyte antigen (CTLA4Ig) [89–91] and etanercept (ETN) [92] or tumour necrosis factor receptor (TNFR) [93] which show an enhanced capacity to reduce inflammation compared to native MSCs. Additionally, the use of genetically-modified MSC-derived exosomes introduces the possibility of loading or changing the contents of the released extracellular vesicles (EVs) with factors with therapeutic interest such as miR-146a/miR-155 [94], miR-150-5p [95] and miR-192-5p [53].

All of these encouraging results in preclinical models of RA suggest that the use of modified MSCs may increase their beneficial therapeutic effects, thus overcoming potential limitations of MSC-based-therapy observed in the clinic. No clinical trials with genetically-modified MSCs in any disease have been conducted so far.

## 9. Discussion

MSC-based therapy modulates and delays arthritis inflammation independent of the tissue source, MHC context and route of administration in the majority of preclinical animal studies mimicking the pathogenesis of RA without adverse effects. This evidence provides a robust justification for the use of MSC-based therapy in the clinic. As a consequence of encouraging preclinical data, the use of MSC-based therapy in clinical trials of RA has experienced a rapid increase in the last decade. Overall, the CIA model is probably the best animal model to study MSC therapy due to similarities in the systemic immunological responses to human RA. Allogeneic context could be the best option for MSC treatment considering the possible impaired MSC function in RA patients that would also allow a cell bank establishment for immediate use. Furthermore, preclinical results suggest that the early phases of inflammation are the best moment for MSC infusion in contrast to most of the completed clinical trials with MSC-based therapy that have been conducted in RA patients with a long history of the disease which may have hidden the clinical benefit of the MSC-based therapy. Based on preclinical and clinical results, rational selection of subjects that would benefit from MSC-based therapy are indicating that recruiting RA patients during the early phases of the disease may increase of the efficacy of MSC therapy. A better understanding of the pathogenesis of the disease as well as the mechanisms of action underlying MSC therapy in RA would identify key biomarkers and would contribute to deployment of optimized protocols of MSC-based therapy for the benefit of RA patients with unmet medical needs.

## Outstanding questions

Mesenchymal stem/stromal cell (MSC)-based therapy are undergoing a rapid development as a promising therapeutic tool to treat rheumatoid arthritis (RA) patients who do not respond or are intolerant to current treatments.

Based on the comprehensive analysis of the scientific literature related to preclinical studies of MSC-based therapy in RA, key aspects of currently used preclinical protocols have demonstrated:

1. The ability of MSCs to modulate immune responses is a safe and feasible strategy to treat rheumatoid arthritis.
2. MSC-based therapy has consistently exhibited therapeutic benefits in modulating arthritis inflammation despite the significant diversity in tissue sourcing, MHC contexts, routes of administration and the animal models used.
3. The optimal timing of MSC infusion to achieve high efficacy seems to be during the early phases of inflammation. Further elucidation of this key aspect warrants future investigation.
4. Assessment of long-term efficacy of MSC-based therapies in RA also needs to be addressed in the near future.

Despite the successful results achieved in preclinical studies, a better understanding of the pathogenesis of the rheumatoid arthritis disease as well as the in-depth mechanisms underlying the beneficial effects of MSCs will contribute to develop optimized protocols for improving the clinical outcomes of MSC-based therapies in RA.

## Search strategy and selection criteria

The large majority of published data up to December 2020 that used MSC-based therapy to treat RA in animal models have been included in this review (106 articles). We have used the following terms: *'mesenchymal stromal cells'* or *'mesenchymal stem cells'* or *'mesenchymal'* and *'stromal'* and *'cells'* or *'mesenchymal'* and *'stem'* and *'cells'* and *'arthritis'* or *'arthritic'* and *'rheumatic'* or *'rheumatoid'*. We searched in the following electronic databases: PubMed, Web of Science and patent registry (https://patentscope.wipo.int). The electronic search strategy excluded non-English articles, *in vitro* studies, human RA sample studies and RA clinical trials using MSC therapy.

## Author contributions

Conceptualization, M.L-S and M.I.G.; writing-original draft preparation, M.L-S and M.I.G. writing—review and editing, M.L-S, J.A.B and M.I.G.

## Declaration of Competing Interest

None.

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All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

## Supplementary materials

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