

# Mesenchymal Stromal Cells Anno 2019: Dawn of the Therapeutic Era? Concise Review

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## SUMMARY

2018 was the year of the first marketing authorization of an allogeneic stem cell therapy by the European Medicines Agency. The authorization concerns the use of allogeneic adipose tissue-derived mesenchymal stromal cells (MSCs) for treatment of complex perianal fistulas in Crohn's disease. This is a breakthrough in the field of MSC therapy. The last few years have, furthermore, seen some breakthroughs in the investigations into the mechanisms of action of MSC therapy. Although the therapeutic effects of MSCs have largely been attributed to their secretion of immunomodulatory and regenerative factors, it has now become clear that some of the effects are mediated through host phagocytic cells that clear administered MSCs and in the process adapt an immunoregulatory and regeneration supporting function. The increased interest in therapeutic use of MSCs and the ongoing elucidation of the mechanisms of action of MSCs are promising indicators that 2019 may be the dawn of the therapeutic era of MSCs and that there will be revived interest in research to more efficient, practical, and sustainable MSC-based therapies. *STEM CELLS TRANSLATIONAL MEDICINE* 2019;8:1126–1134

## SIGNIFICANCE STATEMENT

This article provides an overview of the considered mechanism of action of mesenchymal stromal cells (MSCs) and the status of the development of MSC therapy as of 2019.

## INTRODUCTION

Mesenchymal stromal cells (MSCs) reside in all tissues, where part of them has a perivascular localization [1]. These cells have been described to be present within the walls of the microvasculature where they function to stabilize endothelial networks [2]. Tissues contain, in addition, nonpericyte-derived MSC populations, which are more abundant in tissues with low vascularity [3, 4]. In general, MSCs lack hematopoietic and endothelial markers and share expression of a range of markers with fibroblastic cells, although rare MSC populations with different phenotype exist [5]. MSCs are precursor cells for osteoblasts, adipocytes, and chondrocytes and will also give rise to tissue fibroblasts [6]. Via their differentiation into tissue fibroblasts, MSCs contribute to tissue maintenance and repair by depositing tissue matrix. A delicate balance exists between the tissue repair and fibrotic potential of MSCs [7]. The role of MSCs in injury-induced tissue fibrosis has been elegantly demonstrated by genetic ablation of Gli1<sup>+</sup> MSC, which resulted in

the abolishment of fibrosis [8]. Targeting pro-fibrotic signaling in perivascular stromal cells through inhibition of the C-type lectin transmembrane receptor Endosialin has recently been shown to inhibit their proliferation and differentiation toward myofibroblasts, which may offer a potential therapeutic target for inhibition of MSC mediated fibrosis [9].

MSCs also play a role in the control of tissue inflammation. In response to inflammatory factors such as Interferon (IFN $\gamma$ ) and Tumour Necrosis Factor (TNF $\alpha$ ) secreted by activated immune cells and tissue cells, MSCs adopt an immunoregulatory phenotype [10]. They elevate the expression of anti-inflammatory factors including programmed death ligand 1 and prostaglandin E2, and inhibit immune cell activity and proliferation through metabolic regulation, such as via indolamine 2,3-dioxygenase-dependent catabolism of tryptophan [11–13]. MSCs furthermore express ATPases and possess ecto-nucleotidase activity through CD73 expression, through which they have the capacity to deplete ATP from

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their environment and convert it into adenosine, which modulates the function of innate immune cells [14–16]. Via these diverse pathways, MSCs act in a feedback loop to downregulate ongoing inflammation and restore tissue homeostasis.

The combination of regenerative and immunomodulatory properties has triggered exploration of the therapeutic use of MSCs. MSCs are relatively easily isolated from tissues such as the bone marrow and adipose tissue [6, 17, 18] and more recently umbilical cord tissue has been indicated as a useful source from which juvenile MSCs can be isolated noninvasively for therapeutic use [19]. MSCs can be expanded under adherent cell culture conditions to great numbers, and they exhibit a robustness that enables them to survive a freeze-thawing cycle after cryopreservation, which is crucial for storage and transportation of the cells. These properties make MSCs attractive candidates for cellular therapy of degenerative and immune diseases. Although MSCs from various tissue sources show some differences with respect to cell surface marker expression, proliferation rate, and differentiation capacity, it is not known whether these differences lead to different therapeutic efficacy as no head-to-head comparisons have been made in clinical settings.

In recent years, significant advances have been made in the elucidation of the mechanisms of action of MSCs. Furthermore, in 2018 the first allogeneic MSC product received marketing approval in the European Union. These events represent major breakthroughs in the field and therefore in the present article we pose the question whether 2019 will be the start of the therapeutic era of MSCs. To address this question, we will evaluate the state of the art of the mechanism of action of MSCs and discuss aspects that still pose challenges for the implementation of MSC therapy.

## MECHANISM OF ACTION; CURRENT STATE OF THE ART

### MSC Administration

MSCs are under consideration as a treatment for a wide variety of conditions. The type of condition determines the route of administration of the cells. For most immunological disorders, intravenous administration has been the route of choice whereas for bone repair purposes, MSCs are seeded on transplantable scaffolds [20] or administered as *in vitro* generated cartilaginous templates that undergo osteogenic differentiation after implantation [21]. For treatment of other types of tissue injuries, MSCs have been applied into the wound area via local injections [22].

The paradigm of how exogenously administered MSCs are thought to act has changed considerably over the years. Up to 10 years ago, MSCs were believed to migrate to sites of injury, engraft long-term, and differentiate into functional tissue cells. However, cell tracking technology and long-term follow-up studies have demonstrated little evidence that this is indeed the case. Intravenously administered MSCs accumulate in the lungs from where the large majority of cells do not migrate to other sites and do not survive for more than 24 hours [23–25]. There is also evidence that MSCs that are administered as endochondral bone constructs are replaced by host cells [26]. On the basis of these findings, the prevailing theory on the mechanism of action of MSCs evolved to the idea that MSCs act as trophic mediators and in this role modulate the function of immune cells and tissue resident progenitor cells [27, 28].

### Interaction with Host Cells

MSCs are capable of secreting a range of growth factors, angiogenic factors, and immune regulating factors. The secretome of MSCs also includes extracellular vesicles, which contain proteins and  $\mu$ RNAs that control target cell function. There are multiple examples of therapeutic effects of MSCs in preclinical models that are attributed to the MSC secretome, such as for instance the inhibition of colitis via the release of tumor necrosis factor-induced protein 6 by intraperitoneal administered MSCs [29] or via the release of the osteoclastogenic factor Receptor Activator of Nuclear factor Kappa-B Ligand by MSCs seeded on biomimetic scaffolds for the treatment of osteopetrosis [30]. Other studies have demonstrated that the effects of MSCs can be mimicked by infusion of MSC conditioned culture medium [31, 32]. The MSC secretome may therefore be effective as a cell-free therapy in regenerative medicine [33].

Many clinical trials have used the intravenous route of injection to administer MSCs [34–38]. In the setting of a clinical trial, it is difficult to provide scientific evidence that the effects of intravenously infused MSCs are indeed mediated via secreted factors. Dosing of MSCs in clinical trials is typically several fold lower than in animal experiments, and, furthermore, MSCs have an estimated half-life of approximately 12 hours after infusion [39, 40] and therefore there may not be enough cells around to buildup relevant concentrations of secreted factors in the blood compartment. Nevertheless, after MSC infusion, transient elevations in serum cytokine and chemokine levels can be observed [41], but these are derived from host cells rather than from MSCs themselves, as secretome deficient MSCs evoke the same responses [39]. These observations suggest that some of the effects attributed to the MSC secretome may have in fact another origin.

### Immunomodulatory Effects of MSCs

Evidence is accumulating that many of the immunomodulatory effects of MSCs are mediated by host cells. It has been demonstrated that the induction of apoptosis of intravenously infused MSCs and the subsequent engulfment of MSCs by phagocytic cells is crucial for the therapeutic effect of MSCs in graft versus host disease [42]. Engulfment of MSCs induces expression of the regulatory markers CD163 and CD206 on monocytes and increases IL10 and TGF $\beta$  expression and reduces TNF $\alpha$ , which strongly suggests the adaptation of a regulatory function of monocytes upon uptake of MSCs [40] (Supporting Information Fig. S1). Parallels can be drawn with the response of macrophages to tissue injury. Although damage associated molecular patterns, which are typically released after necrotic cell death [43], induce inflammatory macrophages, the more controlled signals stemming from phagocytosis of apoptotic cells or immune complexes lead to damage resolving and repair macrophages [44]. Intravenous administration of MSCs may thus mimic a controlled tissue injury event to which the immune system responds by adapting an immune regulatory and regeneration-supporting status. Monocytes and neutrophils are the dominant cells in clearing infused MSCs and whereas neutrophils appear to deposit in the lungs after engulfment of MSCs, monocytes enter the circulation and can be detected in the liver [40]. It is tempting to propose that monocytes, which adopt an immunoregulatory phenotype through engulfment of MSCs migrate to distant sites of injury where they exert their acquired immunoregulatory

effect. This novel hypothesis on the mechanism of action of intravenously infused MSCs suggests that maximal therapeutic effects of MSCs can be obtained not by optimizing the migratory capacity and secretome profile of MSCs, but by generating MSCs that are optimally capable of inducing an immune regulatory and regenerative phenotype and function in phagocytic cells.

### Clinical Trials up to 2019

In parallel to these changes of paradigm regarding the mechanism of action of MSC treatment over the last decade, numerous pre-clinical studies testing MSCs in a great variety of experimental animal models of immune-mediated diseases have been carried out, showing in most cases good safety and efficacy results [45–49]. These encouraging results prompted researchers to test the feasibility, safety, and efficacy of MSCs treatment in human clinical trials in a variety of indications (920 at the start of 2019 according to). These trials, mostly phase I and phase II, confirmed a positive safety profile, but provided rather underwhelming efficacy outcomes. This hampered the progression of MSCs as a marketed therapy. Marketing approval for the use of MSCs for pediatric graft versus host disease patients in Canada and New Zealand in 2012 did not lead to the use of MSCs outside the context of clinical trials [49]. An innovation-stimulating framework for regenerative medicine that was enacted in Japan in 2014 allowed the approval of MSCs for treatment of graft versus host disease in 2015, but no other countries followed Japan [49].

It has not been until recently that the first statistically significant therapeutic effects of MSC treatment in phase III trials have been reported. The TiGenix-sponsored randomized, double-blind, parallel-group, placebo-controlled phase III clinical trial, NCT01541579, reported statistically significant improvement of intralesional administration of 120 million allogeneic expanded adipose mesenchymal stem cells (darvadstrocel, formerly Cx601) over control in the treatment of complex perianal fistulas in Crohn's disease patients [50]. Thus, a significant difference was observed in combined remission in patients treated with darvadstrocel (50%) versus control patients (34%) after 24 weeks. In the darvadstrocel group, less treatment-related adverse events were observed. Importantly, the therapeutic benefit and the good safety profile of darvadstrocel were maintained after 1 year of treatment [50]. These results allowed TiGenix (recently acquired by Takeda) to receive central marketing authorization approval for darvadstrocel by the European Medicines Agency (EMA) in March 2018 for its commercialization of the treatment of complex perianal fistulas in adult patients with nonactive/mildly active luminal Crohn's disease, becoming the first approved allogeneic stem cell therapy in Europe. In addition, in September 2018 Mesoblast announced the positive results of its open-label phase III trial in 55 children with steroid-refractory acute GvHD, NCT02336230. Treatment with allogeneic bone marrow mesenchymal stem cells (remestemcel-L) not only significantly improved the overall response rate at day 28 (69%) compared with the protocol-defined historical control rate of 45% ( $p = .0003$ ), but also provided a sustained therapeutic effect at 6 months after the treatment with an overall survival rate for the MSC-treated group of 69%, compared with the historical survival rates of 10%–30% in patients with grade C/D disease and failure to respond to steroids (press release, data not published). With these results, Mesoblast announced that the preparation of a biologics license application to the Food and Drug Administration (FDA) in the United States is underway.

**Table 1.** Distribution of preclinical mesenchymal stem cells (MSC) studies in inflammatory diseases with a focus on sepsis, acute lung injury, acute respiratory distress syndrome, arthritis, and colitis, based on MSC origin (autologous, allogeneic or xenogeneic) with corresponding therapeutic outcome

	# of papers	%	Efficacy # (%)
Autologous	40	43.5	35 (87.5)
Allogeneic	7	7.6	7 (100)
Xenogeneic	39	42.4	39 (100)
Autologous/allogeneic	2	2.2	2 (100)
Xenogeneic/autologous	1	1.1	1 (100)
Xenogeneic/allogeneic	1	1.1	1 (100)
Xenogeneic/autologous/allogeneic	2	2.2	2 (100)
Total # (%)	92		87 (94.6)

### Discrepancy in Outcome Between Clinical Trials and Preclinical Models

In recent years, it has been suggested that the discrepancy between the consistently positive MSCs efficacy outcomes from nonclinical experimental animal models (mostly in mice) and the failure to demonstrate efficacy in human phase III clinical trials is due, at least in part, to MSCs preparation [49]. In this publication, the authors suggested that nearly all pre-clinical studies have been performed with syngeneic (autologous), exponentially expanding, cultured (trypsinized prior to administration) MSCs, whereas in clinical trials, human MSCs are usually expanded to their replicative limit, cryopreserved and thawed immediately before administration and mostly of allogeneic origin [49], which became the concept of "MSC, fresh is best," a repeated mantra in the MSC therapy field. In our view, those statements are not strictly supported by the literature. To clarify this, we performed a comprehensive survey for publications using MSCs in experimental animal models of inflammatory diseases (focusing mainly on sepsis, acute lung injury, acute respiratory distress syndrome, arthritis, and colitis). We identified the methodological details provided in each publication regarding origin and immunological matching of the MSCs used, the status of the cells prior to administration (trypsinized from culture or thawed after cryopreservation), and therapeutic outcome (Supporting Information Table S1; refs: 51–141). Of the 92 publications reviewed, 40 used autologous/syngeneic (43%), 39 xenogeneic (42%), and 7 allogeneic (8%) MSCs (Table 1). Notably, 87.5% of the publications using autologous MSCs (35 out of 40) reported beneficial outcomes, whereas 100% of publications using xenogeneic or allogeneic MSCs did (Table 1). Moreover, the majority of publications evaluated did not clearly state whether MSCs were trypsinized from culture or thawed after cryopreservation prior to administration (72%; Table 2). Thus, only 28% of the publications reported whether MSCs were cultured (21%) or thawed (5%). All publications reported therapeutic effects, despite the alterations that have been described in thawed MSCs compared with cultured cells [142–146]. Only two publications (2%) were found that compared cultured and thawed MSCs side-by-side, reporting similar therapeutic effects [67, 80]. These results indicate that xenogeneic MSCs seem to be equally efficacious as autologous and allogeneic MSCs in the

**Table 2.** Distribution of preclinical mesenchymal stem cells (MSC) studies in inflammatory diseases with a focus on sepsis, acute lung injury, acute respiratory distress syndrome, arthritis, and colitis, based on MSC preparation prior to administration (used straight from culture or after thawing) and corresponding efficacy

	# of papers	%	Efficacy	No efficacy
Not stated	66	71.7	61	5
Cultured	19	20.7	19	0
Thawed	5	5.4	5	0
Cultured vs. thawed	2	2.2	2	0
Total	92			

animal models included in the survey. That said, in other models this may not be the case, as has been shown in a rat corneal transplantation model where human MSCs in contrast to rat MSCs failed to prolong allograft survival [147]. In this model, freshly cultured allogeneic rat MSCs showed equal efficacy as the same cells after cryopreservation [148].

Furthermore, it appears that MSCs administered immediately after thawing retain therapeutic potency that, at least in the animal models studied, is equivalent to cultured MSCs. With regard to the *in vitro* expansion of the cells, 55 publications reported the use of MSCs in an ample range of passages (from p2 to p25), 31 did not indicate the passage used, and six stated the population doublings of the cultures, making it difficult to draw conclusions on the effect of expansion rate on the efficacy of the cells. Despite the limitations of our survey (relatively small number of publications, variety of animal models and the unavoidable publication bias causing underreporting of studies with a negative outcome), given these observations, in our opinion the argument that the disparity between preclinical and clinical therapeutic effects is associated with the use of autologous MSCs straight from culture in animals, whereas in humans allogeneic cryopreserved MSCs are used, should be reconsidered as the evidence is not conclusive. Further research comparing side-by-side cultured and thawed MSCs in experimental animal models is needed. Many studies were methodologically poorly described, and we urge researchers to provide more detailed methodological information.

### Challenges of MSC Therapy

Since the discovery of MSCs, great enthusiasm and expectations were generated regarding their clinical application, which have not been fulfilled as anticipated. At a therapeutic level, the challenge will be to find the way to obtain significant, durable, disease-modifying therapeutic effects with a cell therapy product that has a short persistence, specific distribution and is normally administered once or very few times. We believe the only way to do so is by a deeper understanding and characterization of the cells through rigorous science at preclinical and clinical levels. Despite recent progress in understanding the mechanism of action of MSCs, dose determination and the choice for autologous or allogeneic MSCs still amount at some degree to deductive reasoning. Translation of dosing used in, mostly rodent, preclinical models to the clinical situation is unrealistic as often extremely high cell numbers are used in preclinical studies that can practically not be

reached in man. Although for a few indications, there is a preference for the use of autologous MSCs for fear for allo-sensitization, for other indications the choice for autologous or allogeneic MSCs is led by availability. Studies describing a head-to-head comparison between autologous and allogeneic MSCs are scarce.

Another challenge for the development of MSC therapy remains the safety profile of MSCs. Although concerns about the risk for MSC transformation and tumor formation have appeared ungrounded by the excellent safety profile of MSCs when it comes to MSC-related tumor formation, there are concerns about potential adverse inflammatory effects and thrombosis associated with intravenous infusion of MSCs. MSCs have been shown to elicit a so-called instant blood-mediated inflammatory reaction (IBMIR) after exposure to blood. This reaction is dependent on cell dose, cell passage number, and MSC donor [149]. In contrast to endothelial cells and hematopoietic stem cells, culture expanded MSCs lack expression of hemocompatibility molecules [150]. Non-bone-marrow-derived MSCs generally express higher levels of the pro-thrombotic tissue factor, which imposes a further risk for IBMIR [150]. Although the majority of clinical trials using bone-marrow MSCs have not reported infusion-related toxicity [151], the use of MSCs from other sources may increase the risk for thrombosis [152]. Many researchers have experienced thrombosis-related events in preclinical models in which MSCs are usually given in high numbers without anti-coagulation, which should be carefully taken into account in dose-finding clinical studies. Because of these challenges, further studies to key aspects of MSC biology and properties that make them therapeutically of interest are required.

As indicated above, the immunological status of the MSC recipient and the inflammatory environment MSCs encounter upon administration may be key in obtaining the desired therapeutic benefits, as suggested recently by Galleu et al. in GvHD patients [42]. Understanding the “inflammatory profile” at the time of treatment both in preclinical and clinical settings that would realize the optimal therapeutic potential of MSCs is essential. The identification of predictive biomarkers for patient stratification (i.e., activity or ratio of certain cell subsets, microRNA or cytokines at local or systemic level), which may vary from disease to disease, is undoubtedly needed [153–155]. In line with this, understanding the right posology (dosing and repeats) is of most importance. Typically, MSCs are administered systemically in rodents at a dose of 50 million cells per kilogram, whereas in clinical trials the dose ranges between 1 and 10 million cells per kilogram. Up to now, no clear translation to humans of the effective dose in rodents has been made, and information is limited. With darvadstrocel, a single administration of, 120 million ASC were applied, resulting in significant healing and closure of a local fistula [50]. One could extrapolate that treating systemic indications with doses of approximately 2 million cells per kilogram (140 million cells in a patient of 70 kg), which is nearly equivalent to the dose for a local fistula, might not be sufficient. Much higher doses may be needed for systemic indications. In addition, repeat treatments might be needed to reach and/or sustain the therapeutic benefit of MSC therapy, in particular in the context of chronic indications such as Crohn’s disease or rheumatoid arthritis. However, comprehensive studies comparing the effect of single versus repeat doses at different time points, even in experimental animal models, are missing. MSCs have been considered to be immunoprivileged or poorly

immunogenic due to the low expression of HLA-I and the lack of expression of HLA-II and costimulatory molecules [156, 157]. This feature supported the idea of using allogeneic MSCs generated from healthy donors as an off-the-shelf cell-based therapy. However, evidence is increasing that although well tolerated, allogeneic MSCs may trigger allo-immune responses, such as the generation of donor specific antibodies against donor MSCs [50, 158–161]. It is unclear whether these allo-responses impair the long-term therapeutic effect of the cells, particularly if repeat dosing is needed, or have detrimental consequences in patients in the event of a future organ or tissue transplant if an antidonor memory response is generated. Thus, the immunogenicity of allogeneic MSCs must be closely monitored in clinical trials and its relation with efficacy and safety should be established.

At a manufacturing level, a major challenge for clinical applicability of MSC-based products will be to guarantee a robust, comparable (from donor to donor and batch to batch) and sustainable manufacturing process not only during clinical trial investigation, but, most importantly, after eventual commercialization. Variability and heterogeneity in manufacturing and product characterization of Investigational New Drug submissions to the FDA has been reported [162]. To strengthen the manufacturing process, efforts to deeply understand the behavior of MSCs during *in vitro* expansion, and further characterization of batch-to-batch and donor to donor variability and heterogeneity within MSC preparations are extremely important. In fact, in the context of allogeneic therapies, developing tests or identifying biomarkers to select the best donors (i.e., highest immunomodulatory properties, lowest immunogenicity, best *in vitro* culture expansion, etc.) will be needed. However, defining meaningful *in vitro* test and quality specifications correlating with relevant product attributes, functionalities or characteristics *in vivo* will not be easy. The criteria for defining MSCs as proposed by the International Society for Cellular Therapy [163], are not necessarily predictors for therapeutic efficacy and therefore the use of additional markers that exhibit expression variability between donors has been proposed [164]. It has been shown that MSCs from donors with a high proliferation rate are smaller in size, have longer telomeres and show enhanced ectopic bone forming capacity compared with MSCs from donors with a lower proliferation rate [165]. For other therapeutic applications, different sets of potency tests may be required, such as proposed for the selection of MSC donors with above average immunomodulatory capacity [166, 167]. At the moment, relevant *in vitro* potency tests that enable selection of MSC donors and batches with enhanced therapeutic efficacy are very limited. In fact, at the moment, the question whether MSC characteristics are at all relevant for therapeutic efficacy or whether recipient characteristics are the more important determinant for therapeutic efficacy has not been answered sufficiently.

### Beyond 2019: Cell-Free MSC Therapy?

The year 2019 may be the start of the therapeutic era of MSCs. The future will tell whether this era will last or will be replaced by an era of novel cellular technology. Academic researchers, clinicians, and industry recognize that MSC therapy is not a straightforward treatment as it involves donor selection and cell harvesting, expansion and storage, which requires specialized labs. At patient level, identification of predictive efficacy stratification biomarkers is important and the most

appropriate posology and route of administration for the intended indication needs to be determined. Although the safety record of MSC therapy is excellent, living cells may have a small risk for cellular transformation and this could potentially lead to the administration of transformed cells with unpredictable behavior. Furthermore, in the search for efficacy there is a drive for increasing cell doses, which may induce the risk for blood incompatibility reactions. The most recent findings on the mechanisms of action of MSCs provide new leads for designing MSC therapy with optimal immunomodulatory and regenerative effects customized to specific diseases. This will include indication of specific routes of administration and the use of active components of MSCs. For particular indications, the secretome of MSCs may be sufficient to initiate immunomodulatory or regenerative responses whereas for other indications MSC therapy may be replaced by phagocytosis-inducing components of MSCs that shift the status and function of immune cells. Recent work demonstrated that isolated fragments of MSC membranes form 100–200 nm sized lipid bilayer vesicles, which are phagocytosed by monocytes and subsequently modulate their function [168]. It was also shown that pretreatment of MSCs with IFN $\gamma$ , which is well recognized to lead to modification of MSC membrane protein composition, leads to the generation of membrane vesicles with distinct function. Results from ongoing clinical trials with MSCs and preclinical and *in vitro* models will step-by-step allow researchers to attribute the therapeutic effects of MSCs to specific components of the cells. This is expected to lead to more specific, easier to handle cell-free MSC therapy in the future.

### CONCLUSION

The year 2018 was a milestone in the field of MSC therapy with the first EMA marketing approval of an MSC product. In the coming years it will become clear whether MSC therapy will take flight and become available for multiple indications. Although clinical trials proceed, we learn more about the mechanism of action of MSCs. It appears that the host immune system plays a crucial role in the efficacy of MSC therapy. Donor selection and preparation may be equally important for the success of MSC therapy as MSC phenotype. From the perspective of 2019, we expect to see continuing efforts to find novel therapeutic uses for MSCs. The mechanism of action of MSCs will be further elucidated in preclinical and clinical studies and this will lead to rational predictions of both patient characteristics and MSC properties that are supportive for MSC therapy. The year 2019 is the dawn; the future will tell whether the era will be long and prosperous.

### AUTHOR CONTRIBUTIONS

M.J.H., E.L.: conception and design, manuscript writing.

### DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

E.L. declared employment, patent holder and stock ownership with TiGenix/Takeda. The other author indicated no potential conflicts of interest.

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