STEM CELLS TRANSLATIONAL MEDICINE®

PERSPECTIVES

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

MARTIN J. HOOGDUIJN [a], ELEUTERIO LOMBARDO [b]

[a]Nephrology and Transplantation, Department of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands; [b]Takeda Madrid, Cell Therapy Technology Center, Madrid, Spain

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 to 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;00:1–9*

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 $Gli1^+$ 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 (IFNγ) and Tumour Necrosis Factor (TNFα) 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

Correspondence: Martin J. Hoogduijn, Ph.D., Department of Internal Medicine, Room Na516, Erasmus Medical Center, Wytemaweg 80, 3015 CN Rotterdam, The Netherlands. Telephone: 31 107035418; e-mail: m.hoogduijn@erasmusmc.nl; or Eleuterio Lombardo, Ph.D., Takeda Madrid, Cell Therapy Technology Center, Calle Marconi 1, 28760 Madrid, Spain. Telephone: 34 918049264; e-mail: eleuterio.lombardo@takeda.com Received March 11, 2019; accepted for publication June 17, 2019. http://dx.doi.org/10.1002/sctm.19-0073

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 μRNAs 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 TGFβ expression and reduces TNFα, 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 preclinical 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 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)

Abbreviation: MSC, mesenchymal stem cell.

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 preclinical 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 seven 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 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			

Abbreviation: MSC, mesenchymal stem cell.

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-sensitisation, 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]. Nonbone 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, in particular 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?

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 IFNγ, 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

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. 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.

References

1 Crisan M, Yap S, Casteilla L et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008;3: 301–313.

2 Traktuev DO, Merfeld-Clauss S, Li J et al. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. Circ Res 2008;102:77–85.

3 Feng J, Mantesso A, De Bari C et al. Dual origin of mesenchymal stem cells contributing to organ growth and repair. Proc Natl Acad Sci USA 2011;108:6503–6508.

4 Rasini V, Dominici M, Kluba T et al. Mesenchymal stromal/stem cells markers in the human bone marrow. Cytotherapy 2013; 15:292–306.

5 Churchman SM, Ponchel F, Boxall SA et al. Transcriptional profile of native CD271+ multipotential stromal cells: Evidence for multiple fates, with prominent osteogenic and Wnt pathway signaling activity. Arthritis Rheum 2012; 64:2632–2643.

6 Pittenger MF, Mackay AM, Beck SC et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–147.

7 Packer M. The Alchemist's nightmare: Might mesenchymal stem cells that are recruited to repair the injured heart be transformed into fibroblasts rather than cardiomyocytes? Circulation 2018;137:2068–2073.

8 Schneider RK, Mullally A, Dugourd A et al. Gli1(+) mesenchymal stromal cells are a key driver of bone marrow fibrosis and an important cellular therapeutic target. Cell Stem Cell 2017;20:e8.

9 Di Benedetto P, Liakouli V, Ruscitti P et al. Blocking CD248 molecules in perivascular stromal cells of patients with systemic sclerosis strongly inhibits their differentiation toward myofibroblasts and proliferation: A new potential target for antifibrotic therapy. Arthritis Res Ther 2018;20:223.

10 Krampera M, Cosmi L, Angeli R et al. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells 2006;24: 386–398.

11 Meisel R, Zibert A, Laryea M et al. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood 2004;103:4619–4621.

12 Franquesa M, Mensah FK, Huizinga R et al. Human adipose tissue-derived mesenchymal stem cells abrogate plasmablast formation and induce regulatory B cells independently of T helper cells. Stem Cells 2015;33:880–891.

13 Mancheno-Corvo P, Menta R, del Rio B et al. T lymphocyte prestimulation impairs in a time-dependent manner the capacity of adipose mesenchymal stem cells to inhibit proliferation: Role of interferon gamma, poly I:C, and tryptophan metabolism in restoring adipose mesenchymal stem cell inhibitory effect. Stem Cells Dev 2015;24:2158–2170.

14 Regateiro FS, Cobbold SP, Waldmann H. CD73 and adenosine generation in the creation of regulatory microenvironments. Clin Exp Immunol 2013;171:1–7.

15 Kumar V, Sharma A. Adenosine: An endogenous modulator of innate immune system with therapeutic potential. Eur J Pharmacol 2009;616:7–15.

16 Crop MJ, Baan CC, Korevaar SS et al. Inflammatory conditions affect gene expression and function of human adipose tissue-derived mesenchymal stem cells. Clin Exp Immunol 2010;162:474–486.

17 Hoogduijn MJ, Crop MJ, Peeters AM et al. Human heart, spleen, and perirenal fat-derived mesenchymal stem cells have immunomodulatory capacities. Stem Cells Dev 2007;16: 597–604.

18 Zuk PA, Zhu M, Ashjian P et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13:4279–4295.

19 de Witte SFH, Lambert EE, Merino A et al. Ageing of bone marrow and umbilical cord derived MSC during expansion. Cytotherapy 2017;19:798–807.

20 Meinel L, Karageorgiou V, Fajardo R et al. Bone tissue engineering using human mesenchymal stem cells: Effects of scaffold material and medium flow. Ann Biomed Eng 2004;32:112–122.

21 Scotti C, Tonnarelli B, Papadimitropoulos A et al. Recapitulation of endochondral bone formation using human adult mesenchymal stem cells as a paradigm for developmental engineering. Proc Natl Acad Sci USA 2010;107:7251–7256.

22 Mazzanti B, Lorenzi B, Borghini A et al. Local injection of bone marrow progenitor cells for the treatment of anal sphincter injury: In-vitro expanded versus minimally-manipulated cells. Stem Cell Res Ther 2016;7:85.

23 Schrepfer S, Deuse T, Reichenspurner H et al. Stem cell transplantation: The lung barrier. Transplant Proc 2007;39: 573–576.

24 Eggenhofer E, Benseler V, Kroemer A et al. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol 2012; 3:297.

25 Barbash IM, Chouraqui P, Baron J et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: Feasibility, cell migration, and body distribution. Circulation 2003;108:863–868.

26 Farrell E, Both SK, Odorfer KI et al. In-vivo generation of bone via endochondral ossification by in-vitro chondrogenic priming of adult human and rat mesenchymal stem cells. BMC Musculoskelet Disord 2011;12:31.

27 Caplan AI, Correa D. The MSC: An injury drugstore. Cell Stem Cell 2011;9:11–15.

28 Keating A. Mesenchymal stromal cells: New directions. Cell Stem Cell 2012;10:709–716.

29 Sala E, Genua M, Petti L et al. Mesenchymal stem cells reduce colitis in mice via release of TSG6 independently of their localization to the intestine. Gastroenterology 2015; 149:e20.

30 Menale C, Campodoni E, Palagano E et al. MSC-seeded biomimetic scaffolds as a factory of soluble RANKL in Rankl-deficient osteopetrosis. Stem Cells Translational Medicine 2019;8:22–34.

31 Fouraschen SM, Pan Q, de Ruiter PE et al. Secreted factors of human liver-derived mesenchymal stem cells promote liver regeneration early after partial hepatectomy. Stem Cells Dev 2012;21:2410–2419.

32 Gnecchi M, He H, Liang OD et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 2005;11:367–368.

33 Vizoso FJ, Eiro N, Cid S et al. Mesenchymal stem cell secretome: Toward cell-free therapeutic strategies in regenerative medicine. Int J Mol Sci 2017;18:1–24.

34 Tan J, Wu W, Xu X et al. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: A randomized controlled trial. JAMA 2012;307:1169–1177.

35 Reinders ME, de Fijter JW, Roelofs H et al. Autologous bone marrow-derived mesenchymal stromal cells for the treatment of allograft rejection after renal transplantation: Results of a phase I study. Stem Cells Translational Medicine 2013;2:107–211.

36 Le Blanc K, Frassoni F, Ball L et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: A phase II study. Lancet 2008;371:1579–1586.

37 Weiss DJ, Casaburi R, Flannery R et al. A placebo-controlled, randomized trial of mesenchymal stem cells in COPD. Chest 2013;143: 1590–1598.

38 Wilson JG, Liu KD, Zhuo H et al. Mesenchymal stem (stromal) cells for treatment of ARDS: A phase 1 clinical trial. Lancet Respir Med 2015;3:24–32.

39 Luk F, de Witte SF, Korevaar SS et al. Inactivated mesenchymal stem cells maintain immunomodulatory capacity. Stem Cells Dev 2016;25:1342–1354.

40 de Witte SFH, Luk F, Sierra Parraga JM et al. Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells. Stem Cells 2018;36:602–615.

41 Hoogduijn MJ, Roemeling-van Rhijn M, Engela AU et al. Mesenchymal stem cells induce an inflammatory response after intravenous infusion. Stem Cells Dev 2013;22:2825–2835.

42 Galleu A, Riffo-Vasquez Y, Trento C et al. Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. Sci Transl Med 2017;9:1–11.

43 Kaczmarek A, Vandenabeele P, Krysko DV. Necroptosis: The release of damage-associated molecular patterns and its physiological relevance. Immunity 2013;38:209–223.

44 Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. Immunity 2016;44:450–462.

45 Lalu MM, Sullivan KJ, Mei SH et al. Evaluating mesenchymal stem cell therapy for sepsis with preclinical meta-analyses prior to initiating a first-in-human trial. Elife 2016;5:e17850.

46 McIntyre LA, Moher D, Fergusson DA et al. Efficacy of mesenchymal stromal cell therapy for acute lung injury in preclinical animal models: A systematic review. PLoS One 2016;11:e0147170.

47 He F, Zhou A, Feng S et al. Mesenchymal stem cell therapy for paraquat poisoning: A systematic review and meta-analysis of preclinical studies. PLoS One 2018;13:e0194748.

48 Riecke J, Johns KM, Cai C et al. A meta-analysis of mesenchymal stem cells in animal models of Parkinson's disease. Stem Cells Dev 2015;24:2082–2090.

49 Galipeau J, Sensebe L. Mesenchymal stromal cells: Clinical challenges and therapeutic opportunities. Cell Stem Cell 2018;22: 824–833.

50 Panes J, Garcia-Olmo D, Van Assche G et al. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: A phase 3 randomised, double-blind controlled trial. Lancet 2016;388:1281–1290.

51 Alcayaga-Miranda F, Cuenca J, Martin A et al. Combination therapy of menstrual derived mesenchymal stem cells and antibiotics ameliorates survival in sepsis. Stem Cell Res Ther 2015;6:199.

52 Asami T, Ishii M, Namkoong H et al. Anti-inflammatory roles of mesenchymal stromal cells during acute *Streptococcus pneumoniae* pulmonary infection in mice. Cytotherapy 2018;20:302–313.

53 Asmussen S, Ito H, Traber DL et al. Human mesenchymal stem cells reduce the severity of acute lung injury in a sheep model of bacterial pneumonia. Thorax 2014;69: 819–825.

54 Augello A, Tasso R, Negrini SM et al. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. Arthritis Rheum 2007; 56:1175–1186.

55 Banerjee A, Bizzaro D, Burra P et al. Umbilical cord mesenchymal stem cells modulate dextran sulfate sodium induced acute colitis in immunodeficient mice. Stem Cell Res Ther 2015;6:79.

56 Bouffi C, Bony C, Courties G et al. IL-6-dependent PGE2 secretion by mesenchymal stem cells inhibits local inflammation in experimental arthritis. PLoS One 2010;5:e14247.

57 Bustos ML, Huleihel L, Meyer EM et al. Activation of human mesenchymal stem cells impacts their therapeutic abilities in lung injury by increasing interleukin (IL)-10 and IL-1RN levels. Stem Cells Translational Medicine 2013;2: 884–895.

58 Castelo-Branco MT, Soares ID, Lopes DV et al. Intraperitoneal but not intravenous cryopreserved mesenchymal stromal cells home to the inflamed colon and ameliorate experimental colitis. PLoS One 2012;7:e33360.

59 Chang CL, Leu S, Sung HC et al. Impact of apoptotic adipose-derived mesenchymal stem cells on attenuating organ damage and reducing mortality in rat sepsis syndrome induced by cecal puncture and ligation. J Transl Med 2012; 10:244.

60 Chao K, Zhang S, Qiu Y et al. Human umbilical cord-derived mesenchymal stem cells protect against experimental colitis via CD5(+) B regulatory cells. Stem Cell Res Ther 2016;7:109.

61 Chao YH, Wu HP, Wu KH et al. An increase in CD3+CD4+CD25+ regulatory T cells after administration of umbilical cord-derived mesenchymal stem cells during sepsis. PLoS One 2014;9:e110338.

62 Chen B, Hu J, Liao L et al. Flk-1+ mesenchymal stem cells aggravate collagen-induced arthritis by up-regulating interleukin-6. Clin Exp Immunol 2010;159:292–302.

63 Chen QQ, Yan L, Wang CZ et al. Mesenchymal stem cells alleviate TNBS-induced colitis by modulating inflammatory and autoimmune responses. World J Gastroenterol 2013;19:4702–4717.

64 Cheng W, Su J, Hu Y et al. Interleukin-25 primed mesenchymal stem cells achieve better therapeutic effects on dextran sulfate sodium-induced colitis via inhibiting Th17 immune response and inducing T regulatory cell phenotype. Am J Transl Res 2017;9:4149–4160.

65 Choi JJ, Yoo SA, Park SJ et al. Mesenchymal stem cells overexpressing interleukin-10 attenuate collagen-induced arthritis in mice. Clin Exp Immunol 2008;153:269–276.

66 Condor JM, Rodrigues CE, Sousa Moreira R et al. Treatment with human wharton's jelly-derived mesenchymal stem cells attenuates sepsis-induced kidney injury, liver injury, and endothelial dysfunction. Stem Cells Translational Medicine 2016;5:1048–1057.

67 Cruz FF, Borg ZD, Goodwin M et al. Freshly thawed and continuously cultured human bone marrow-derived mesenchymal stromal cells comparably ameliorate allergic airways inflammation in immunocompetent mice. Stem Cells Translational Medicine 2015;4:615–624.

68 Curley GF, Ansari B, Hayes M et al. Effects of intratracheal mesenchymal stromal cell therapy during recovery and resolution after ventilator-induced lung injury. Anesthesiology 2013;118:924–932.

69 Curley GF, Jerkic M, Dixon S et al. Cryopreserved, xeno-free human umbilical cord mesenchymal stromal cells reduce lung injury severity and bacterial burden in rodent *Escherichia coli*-induced acute respiratory distress syndrome. Crit Care Med 2017; 45:e202–e212.

70 Devaney J, Horie S, Masterson C et al. Human mesenchymal stromal cells decrease the severity of acute lung injury induced by *E. coli* in the rat. Thorax 2015;70:625–635.

71 Djouad F, Fritz V, Apparailly F et al. Reversal of the immunosuppressive properties of mesenchymal stem cells by tumor necrosis factor alpha in collagen-induced arthritis. Arthritis Rheum 2005;52:1595–1603.

72 dos Santos CC, Murthy S, Hu P et al. Network analysis of transcriptional responses induced by mesenchymal stem cell treatment of experimental sepsis. Am J Pathol 2012;181:1681–1692.

73 Duijvestein M, Wildenberg ME, Welling MM et al. Pretreatment with interferon-gamma enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis. Stem Cells 2011;29:1549–1558.

74 Fan H, Zhao G, Liu L et al. Pre-treatment with IL-1beta enhances the efficacy of MSC transplantation in DSS-induced colitis. Cell Mol Immunol 2012;9:473–481.

75 Forte D, Ciciarello M, Valerii MC et al. Human cord blood-derived platelet lysate enhances the therapeutic activity of adipose-derived mesenchymal stromal cells isolated from Crohn's disease patients in a mouse model of colitis. Stem Cell Res Ther 2015;6:170.

76 Goncalves Fda C, Schneider N, Pinto FO et al. Intravenous vs intraperitoneal mesenchymal stem cells administration: What is the best route for treating experimental colitis? World J Gastroenterol 2014;20:18228–18239.

77 Gonzalez MA, Gonzalez-Rey E, Rico L et al. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis Rheum 2009;60:1006–1019.

78 Gonzalez MA, Gonzalez-Rey E, Rico L et al. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. Gastroenterology 2009;136:978–989.

79 Gonzalez-Rey E, Anderson P, Gonzalez MA et al. Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut 2009;58:929–939.

80 Gramlich OW, Burand AJ, Brown AJ et al. Cryopreserved mesenchymal stromal cells maintain potency in a retinal ischemia/-reperfusion injury model: Toward an off-the-shelf therapy. Sci Rep 2016;6:26463.

81 Gupta N, Krasnodembskaya A, Kapetanaki M et al. Mesenchymal stem cells enhance survival and bacterial clearance in murine *Escherichia coli* pneumonia. Thorax 2012;67:533–539.

82 Gupta N, Sinha R, Krasnodembskaya A et al. The TLR4-PAR1 axis regulates bone marrow mesenchymal stromal cell survival and therapeutic capacity in experimental bacterial pneumonia. Stem Cells 2018;36:796–806.

83 Gupta N, Su X, Popov B et al. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. J Immunol 2007;179:1855–1863.

84 Hall SR, Tsoyi K, Ith B et al. Mesenchymal stromal cells improve survival during sepsis in the absence of heme oxygenase-1: The importance of neutrophils. Stem Cells 2013;31: 397–407.

85 Hayes M, Curley GF, Masterson C et al. Mesenchymal stromal cells are more effective than the MSC secretome in diminishing injury and enhancing recovery following ventilator-induced lung injury. Intensive Care Med Exp 2015;3:29.

86 Ionescu L, Byrne RN, van Haaften T et al. Stem cell conditioned medium improves acute lung injury in mice: in vivo evidence for stem cell paracrine action. Am J Physiol Lung Cell Mol Physiol 2012;303:L967–L977.

87 Jackson MV, Morrison TJ, Doherty DF et al. Mitochondrial transfer via tunneling nanotubes is an important mechanism by which mesenchymal stem cells enhance macrophage phagocytosis in the in vitro and in vivo models of ARDS. Stem Cells 2016;34:2210–2223.

88 Kehoe O, Cartwright A, Askari A et al. Intra-articular injection of mesenchymal stem cells leads to reduced inflammation and cartilage damage in murine antigen-induced arthritis. J Transl Med 2014;12:157.

89 Kim ES, Chang YS, Choi SJ et al. Intratracheal transplantation of human umbilical cord blood-derived mesenchymal stem cells attenuates *Escherichia coli*-induced acute lung injury in mice. Respir Res 2011;12:108.

90 Kim H, Darwish I, Monroy MF et al. Mesenchymal stromal (stem) cells suppress proinflammatory cytokine production but fail to improve survival in experimental staphylococcal toxic shock syndrome. BMC Immunol 2014;15:1.

91 Krasnodembskaya A, Samarani G, Song Y et al. Human mesenchymal stem cells reduce mortality and bacteremia in gram-negative sepsis in mice in part by enhancing the phagocytic activity of blood monocytes. Am J Physiol Lung Cell Mol Physiol 2012;302: L1003–L1013.

92 Krasnodembskaya A, Song Y, Fang X et al. Antibacterial effect of human mesenchymal stem cells is mediated in part from

secretion of the antimicrobial peptide LL-37. Stem Cells 2010;28:2229–2238.

93 Lee FY, Chen KH, Wallace CG et al. Xenogeneic human umbilical cord-derived mesenchymal stem cells reduce mortality in rats with acute respiratory distress syndrome complicated by sepsis. Oncotarget 2017;8: 45626–45642.

94 Lee HJ, Oh SH, Jang HW et al. Long-term effects of bone marrow-derived mesenchymal stem cells in dextran sulfate sodium-induced murine chronic colitis. Gut Liver 2016;10: 412–419.

95 Lee SH, Jang AS, Kim YE et al. Modulation of cytokine and nitric oxide by mesenchymal stem cell transfer in lung injury/fibrosis. Respir Res 2010;11:16.

96 Li J, Li D, Liu X et al. Human umbilical cord mesenchymal stem cells reduce systemic inflammation and attenuate LPS-induced acute lung injury in rats. J Inflamm 2012;9:33.

97 Li S, Wu H, Han D et al. A novel mechanism of mesenchymal stromal cell-mediated protection against sepsis: Restricting inflammasome activation in macrophages by increasing mitophagy and decreasing mitochondrial ROS. Oxid Med Cell Longev 2018;2018: 3537609.

98 Liu FB, Lin Q, Liu ZW. A study on the role of apoptotic human umbilical cord mesenchymal stem cells in bleomycin-induced acute lung injury in rat models. Eur Rev Med Pharmacol Sci 2016;20:969–982.

99 Liu W, Zhang S, Gu S et al. Mesenchymal stem cells recruit macrophages to alleviate experimental colitis through TGFbeta1. Cell Physiol Biochem 2015;35:858–865.

100 Lopez-Santalla M, Mancheno-Corvo P, Escolano A et al. Biodistribution and efficacy of human adipose-derived mesenchymal stem cells following intranodal administration in experimental colitis. Front Immunol 2017;8:638.

101 Lopez-Santalla M, Mancheno-Corvo P, Menta R et al. Human adipose-derived mesenchymal stem cells modulate experimental autoimmune arthritis by modifying early adaptive T cell responses. Stem Cells 2015;33:3493–3503.

102 Lopez-Santalla M, Menta R, Mancheno-Corvo P et al. Adipose-derived mesenchymal stromal cells modulate experimental autoimmune arthritis by inducing an early regulatory innate cell signature. Immun Inflamm Dis 2016;4:213–224.

103 Luo CJ, Zhang FJ, Zhang L et al. Mesenchymal stem cells ameliorate sepsis-associated acute kidney injury in mice. Shock 2014;41: 123–129.

104 Mancheno-Corvo P, Lopez-Santalla M, Menta R et al. Intralymphatic administration of adipose mesenchymal stem cells reduces the severity of collagen-induced experimental arthritis. Front Immunol 2017;8:462.

105 Manukyan MC, Weil BR, Wang Y et al. Female stem cells are superior to males in preserving myocardial function following endotoxemia. Am J Physiol Regul Integr Comp Physiol 2011;300:R1506–R1514.

106 Mao F, Wu Y, Tang X et al. Exosomes derived from human umbilical cord mesenchymal stem cells relieve inflammatory bowel disease in mice. Biomed Res Int 2017;2017:5356760.

107 Maron-Gutierrez T, Silva JD, Asensi KD et al. Effects of mesenchymal stem cell therapy on the time course of pulmonary remodeling depend on the etiology of lung injury in mice. Crit Care Med 2013;41:e319–e333.

108 Martin Arranz E, Martin Arranz MD, Robredo T et al. Endoscopic submucosal injection of adipose-derived mesenchymal stem cells ameliorates TNBS-induced colitis in rats and prevents stenosis. Stem Cell Res Ther 2018;9:95.

109 Martinez-Gonzalez I, Roca O, Masclans JR et al. Human mesenchymal stem cells overexpressing the IL-33 antagonist soluble IL-1 receptor-like-1 attenuate endotoxin-induced acute lung injury. Am J Respir Cell Mol Biol 2013;49:552–562.

110 Mei SH, Haitsma JJ, Dos Santos CC et al. Mesenchymal stem cells reduce inflammation while enhancing bacterial clearance and improving survival in sepsis. Am J Respir Crit Care Med 2010;182:1047–1057.

111 Mei SH, McCarter SD, Deng Y et al. Prevention of LPS-induced acute lung injury in mice by mesenchymal stem cells overexpressing angiopoietin 1. PLoS Med 2007;4:e269.

112 Moodley Y, Sturm M, Shaw K et al. Human mesenchymal stem cells attenuate early damage in a ventilated pig model of acute lung injury. Stem Cell Res 2016;17:25–31.

113 Nam YS, Kim N, Im KI et al. Negative impact of bone-marrow-derived mesenchymal stem cells on dextran sulfate sodium-induced colitis. World J Gastroenterol 2015;21:2030–2039.

114 Nemeth K, Leelahavanichkul A, Yuen PS et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med 2009;15: 42–49.

115 Park JS, Yi TG, Park JM et al. Therapeutic effects of mouse bone marrow-derived clonal mesenchymal stem cells in a mouse model of inflammatory bowel disease. J Clin Biochem Nutr 2015;57:192–203.

116 Park MJ, Park HS, Cho ML et al. Transforming growth factor beta-transduced mesenchymal stem cells ameliorate experimental autoimmune arthritis through reciprocal regulation of Treg/Th17 cells and osteoclastogenesis. Arthritis Rheum 2011;63:1668–1680.

117 Pedrazza L, Lunardelli A, Luft C et al. Mesenchymal stem cells decrease splenocytes apoptosis in a sepsis experimental model. Inflamm Res 2014;63:719–728.

118 Rojas M, Parker RE, Thorn N et al. Infusion of freshly isolated autologous bone marrow derived mononuclear cells prevents endotoxin-induced lung injury in an ex-vivo perfused swine model. Stem Cell Res Ther 2013;4:26.

119 Ryu DB, Lim JY, Lee SE et al. Induction of Indoleamine 2,3-dioxygenase by pretreatment with poly(I:C) may enhance the efficacy of msc treatment in dss-induced colitis. Immune Netw 2016;16:358–365.

120 Schurgers E, Kelchtermans H, Mitera T et al. Discrepancy between the in vitro and in vivo effects of murine mesenchymal stem cells on T-cell proliferation and collagen-induced arthritis. Arthritis Res Ther 2010;12:R31.

121 Sepulveda JC, Tome M, Fernandez ME et al. Cell senescence abrogates the therapeutic potential of human mesenchymal stem cells in the lethal endotoxemia model. Stem Cells 2014;32:1865–1877.

122 Shin S, Kim Y, Jeong S et al. The therapeutic effect of human adult stem cells derived from adipose tissue in endotoxemic rat model. Int J Med Sci 2013;10:8–18.

123 Silva JD, Lopes-Pacheco M, Paz AHR et al. Mesenchymal stem cells from bone marrow, adipose tissue, and lung tissue differentially mitigate lung and distal organ damage in experimental acute respiratory distress syndrome. Crit Care Med 2018;46:e132–e140.

124 Simovic Markovic B, Nikolic A, Gazdic M et al. Pharmacological inhibition of Gal-3 in mesenchymal stem cells enhances their capacity to promote alternative activation of macrophages in dextran sulphate sodium-induced colitis. Stem Cells Int 2016;2016: 2640746.

125 Song WJ, Li Q, Ryu MO et al. TSG-6 secreted by human adipose tissue-derived mesenchymal stem cells ameliorates DSS-induced colitis by Inducing M2 macrophage polarization in mice. Sci Rep 2017;7:5187.

126 Sullivan C, Barry F, Ritter T et al. Allogeneic murine mesenchymal stem cells: Migration to inflamed joints in vivo and amelioration of collagen induced arthritis when transduced to express CTLA4Ig. Stem Cells Dev 2013;22:3203–3213.

127 Sullivan C, Murphy JM, Griffin MD et al. Genetic mismatch affects the immunosuppressive properties of mesenchymal stem cells in vitro and their ability to influence the course of collagen-induced arthritis. Arthritis Res Ther 2012;14:R167.

128 Sun J, Han ZB, Liao W et al. Intrapulmonary delivery of human umbilical cord mesenchymal stem cells attenuates acute lung injury by expanding CD4+CD25+ Forkhead Boxp3 (FOXP3)+ regulatory T cells and balancing anti- and pro-inflammatory factors. Cell Physiol Biochem 2011;27:587–596.

129 Sun T, Gao GZ, Li RF et al. Bone marrow-derived mesenchymal stem cell transplantation ameliorates oxidative stress and restores intestinal mucosal permeability in chemically induced colitis in mice. Am J Transl Res 2015;7:891–901.

130 Sung PH, Chang CL, Tsai TH et al. Apoptotic adipose-derived mesenchymal stem cell therapy protects against lung and kidney injury in sepsis syndrome caused by cecal ligation puncture in rats. Stem Cell Res Ther 2013;4:155.

131 Sung PH, Chiang HJ, Chen CH et al. Combined therapy with adipose-derived mesenchymal stem cells and ciprofloxacin against acute urogenital organ damage in rat sepsis syndrome induced by intrapelvic injection of cecal bacteria. Stem Cells Translational Medicine 2016;5:782–792.

132 Toupet K, Maumus M, Luz-Crawford P et al. Survival and biodistribution of xenogenic adipose mesenchymal stem cells is not affected by the degree of inflammation in arthritis. PLoS One 2015;10:e0114962.

133 Wang WQ, Dong K, Zhou L et al. IL-37b gene transfer enhances the therapeutic efficacy of mesenchumal stromal cells in DSS-induced colitis mice. Acta Pharmacol Sin 2015;36: 1377–1387.

134 Weil BR, Herrmann JL, Abarbanell AM et al. Intravenous infusion of mesenchymal stem cells is associated with improved myocardial function during endotoxemia. Shock 2011;36:235–241.

135 Weil BR, Manukyan MC, Herrmann JL et al. Mesenchymal stem cells attenuate myocardial functional depression and reduce systemic and myocardial inflammation during endotoxemia. Surgery 2010;148:444–452.

136 Yagi H, Soto-Gutierrez A, Kitagawa Y et al. Bone marrow mesenchymal stromal cells attenuate organ injury induced by LPS and burn. Cell Transplant 2010;19:823–830.

137 Yagi H, Soto-Gutierrez A, Navarro-Alvarez N et al. Reactive bone marrow stromal cells attenuate systemic inflammation via sTNFR1. Mol Ther 2010;18:1857–1864.

138 Yang H, Wen Y, Hou-you Y et al. Combined treatment with bone marrow mesenchymal stem cells and methylprednisolone in paraquat-induced acute lung injury. BMC Emerg Med 2013;13:S5.

139 Zhao X, Liu D, Gong W et al. The toll-like receptor 3 ligand, poly(I:C), improves immunosuppressive function and therapeutic effect of mesenchymal stem cells on sepsis via inhibiting MiR-143. Stem Cells 2014;32: 521–533.

140 Zhao Y, Yang C, Wang H et al. Therapeutic effects of bone marrow-derived mesenchymal stem cells on pulmonary impact injury complicated with endotoxemia in rats. Int Immunopharmacol 2013;15:246–253.

141 Zhou B, Yuan J, Zhou Y et al. Administering human adipose-derived mesenchymal stem cells to prevent and treat experimental arthritis. Clin Immunol 2011;141:328–337.

142 Francois M, Copland IB, Yuan S et al. Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-gamma licensing. Cytotherapy 2012; 14:147–152.

143 Moll G, Geissler S, Catar R et al. Cryopreserved or fresh mesenchymal stromal cells: Only a matter of taste or key to unleash the full clinical potential of MSC therapy? Adv Exp Med Biol 2016;951:77–98.

144 Hoogduijn MJ, de Witte SF, Luk F et al. Effects of freeze-thawing and intravenous infusion on mesenchymal stromal cell gene expression. Stem Cells Dev 2016;25:586–597.

145 Chinnadurai R, Copland IB, Garcia MA et al. Cryopreserved mesenchymal stromal cells are susceptible to T-cell mediated apoptosis which is partly rescued by ifngamma licensing. Stem Cells 2016;34:2429–2442.

146 Chinnadurai R, Garcia MA, Sakurai Y et al. Actin cytoskeletal disruption following cryopreservation alters the biodistribution of human mesenchymal stromal cells in vivo. Stem Cell Rep 2014;3:60–72.

147 Lohan P, Treacy O, Morcos M et al. Interspecies incompatibilities limit the immunomodulatory effect of human mesenchymal stromal cells in the rat. Stem Cells 2018;36:1210–1215.

148 Lohan P, Murphy N, Treacy O et al. Third-party allogeneic mesenchymal stromal cells prevent rejection in a pre-sensitized high-risk model of corneal transplantation. Front Immunol 2018;9:2666.

149 Moll G, Rasmusson-Duprez I, von Bahr L et al. Are therapeutic human mesenchymal stromal cells compatible with human blood? Stem Cells 2012;30:1565–1574.

150 Moll G, Ankrum JA, Kamhieh-Milz J et al. Intravascular mesenchymal stromal/stem cell therapy product diversification: Time for new clinical guidelines. Trends Mol Med 2019; 25:149–163.

151 Lalu MM, McIntyre L, Pugliese C et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): A systematic review and meta-analysis of clinical trials. PLoS One 2012;7:e47559.

152 Wu Z, Zhang S, Zhou L et al. Thromboembolism induced by umbilical cord mesenchymal stem cell infusion: A report of two cases and literature review. Transplant Proc 2017;49:1656–1658.

153 Mallinson DJ, Dunbar DR, Ridha S et al. Identification of potential plasma microrna stratification biomarkers for response to allogeneic adipose-derived mesenchymal stem cells in rheumatoid arthritis. Stem Cells Translational Medicine 2017;6:1202–1206.

154 Jokerst JV, Cauwenberghs N, Kuznetsova T et al. Circulating biomarkers to identify responders in cardiac cell therapy. Sci Rep 2017;7:4419.

155 Lee RH, Yu JM, Foskett AM et al. TSG-6 as a biomarker to predict efficacy of human mesenchymal stem/progenitor cells (hMSCs) in modulating sterile inflammation in vivo. Proc Natl Acad Sci USA 2014;111:16766–16771.

156 Griffin MD, Ritter T, Mahon BP. Immunological aspects of allogeneic mesenchymal stem cell therapies. Hum Gene Ther 2010;21:1641–1655.

157 Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: Immune evasive, not immune privileged. Nat Biotechnol 2014;32:252–260.

158 Alvaro-Gracia JM, Jover JA, Garcia-Vicuna R et al. Intravenous administration of expanded allogeneic adipose-derived mesenchymal stem cells in refractory rheumatoid arthritis (Cx611): Results of a multicentre, dose escalation, randomised, single-blind, placebo-controlled phase Ib/IIa clinical trial. Ann Rheum Dis 2017;76:196–202.

159 Lohan P, Treacy O, Griffin MD et al. Anti-donor immune responses elicited by allogeneic mesenchymal stem cells and their extracellular vesicles: Are we still learning? Front Immunol 2017;8:1626.

160 Alagesan S, Sanz-Nogues C, Chen X et al. Anti-donor antibody induction following intramuscular injections of allogeneic mesenchymal stromal cells. Immunol Cell Biol 2018;96: 536–548.

161 Alvaro Avivar-Valderas CM-M, Ramírez C, Del Río B et al. Dissecting allosensitization after local administration of human allogeneic adipose mesenchymal stem cells in perianal fistulas of Crohn's disease patients. Front Immunol 2019;10:1–16.

162 Mendicino M, Bailey AM, Wonnacott K et al. MSC-based product characterization for clinical trials: An FDA perspective. Cell Stem Cell 2014;14:141–145.

163 Dominici M, Le Blanc K, Mueller I et al. Minimal criteria for defining multipotent mesenchymal stromal cells. International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–317.

164 Camilleri ET, Gustafson MP, Dudakovic A et al. Identification and validation of multiple cell surface markers of clinical-grade adipose-derived mesenchymal stromal cells as novel release criteria for good manufacturing practice-compliant production. Stem Cell Res Ther 2016;7:107.

165 Samsonraj RM, Rai B, Sathiyanathan P et al. Establishing criteria for human mesenchymal stem cell potency. Stem Cells 2015;33:1878–1891.

166 Ketterl N, Brachtl G, Schuh C et al. A robust potency assay highlights significant donor variation of human mesenchymal stem/progenitor cell immune modulatory capacity and extended radio-resistance. Stem Cell Res Ther 2015;6:236.

167 Ribeiro A, Ritter T, Griffin M et al. Development of a flow cytometry-based potency assay for measuring the in vitro immunomodulatory properties of mesenchymal stromal cells. Immunol Lett 2016;177:38–46.

168 Goncalves FDC, Luk F, Korevaar SS et al. Membrane particles generated from mesenchymal stromal cells modulate immune responses by selective targeting of pro-inflammatory monocytes. Sci Rep 2017;7:12100.

See www.StemCellsTM.com for supporting information available online.