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Concise Review: Adult Mesenchymal Stromal Cell Therapy for Inflammatory Diseases: How Well Are We Joining the Dots?

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ABSTRACT

Mesenchymal stromal (stem) cells (MSCs) continue to be a strong area of focus for academic- and industry-based researchers who share the goal of expanding their therapeutic use for diverse inflammatory and immune-mediated diseases. Recently, there has been an accelerated rate of scientific publication, clinical trial activity, and commercialisation in the field. This has included the reporting of exciting new developments in four areas that will be of key importance to future successful use of MSC-based therapies in large numbers of patients: (a) fundamental biology of the primary cells in bone marrow and other tissues that give rise to MSCs in culture. (b) Mechanisms by which MSCs modulate immune and inflammatory responses in vivo. (c) Insights into MSC kinetics, safety, and efficacy in relevant animal disease models. (d) Isolation, definition, and clinical trial-based testing of human MSCs by biomedical companies and academic medical centers. Despite this progress, it remains unclear whether MSCs will enter mainstream therapeutic practice as a frequently used alternative to pharmacotherapy or surgical/radiological procedures in the foreseeable future. In this review, we summarize some of the most significant new developments for each of the four areas that contribute to the process of translating MSC research to the clinical arena. In the context of this recent progress, we discuss key challenges and specific knowledge gaps which, if not addressed in a coordinated fashion, may hinder the creation of robust "translational pipelines" for consolidating the status of MSC-based therapies. STEM CELLS *2013;31:2033–2041*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

The past two decades have seen an explosion of scientific and clinical interest in mesenchymal stromal (stem) cells (MSCs) driven by the over-riding premise that culture-expanded MSCs will soon become a widely prescribed therapeutic agent for diverse acute and chronic diseases. While therapeutic interest in MSCs initially centered upon harnessing their capacity for multilineage differentiation to directly regenerate tissues and organs [1,2], they are now also viewed as potent "trophic" modulators of disease-associated tissue microenvironments [3]. Thus, the current translational landscape for MSCs includes therapeutic models involving direct tissue regeneration as well as indirect, modulatory effects on damaged and diseased tissues [4]. This latter concept has placed the anti-inflammatory and immunomodulatory properties of MSCs front and center in much of the recently published literature (Fig. 1A) [5–7]. The capacity of MSCs to broadly modify the activity of most major components of the innate and adaptive immune system is now seen, along with their proangiogenic, cytoprotective, and antifibrotic effects, as an essential component of their therapeutic potential for many disease targets [3,6]. The observations that MSCs migrate toward sites of inflammation and are triggered (or "licensed") to become more potent by inflammatory cytokines and pattern recognition receptor (PRR) ligands [6,7] have further strengthened the belief that a single localized or systemic administration of unmodified MSCs can be expected to actively "reprogram" an inflammatory milieu towards repair and regeneration.

However, while quite a large number of clinical trials based on this concept have been completed or are under way [6,8–13], it remains unclear whether MSCs will soon become a successful, widely prescribed therapy for inflammatory diseases [14]. In comparison with new pharmacological agents, it appears worrisome that MSC therapies are being trialed simultaneously for such a wide range of diseases in the context of incomplete understanding of their mechanisms of action and in an evolving regulatory environment [4,15,16]. In this Concise Review, we critically examine the progress that is being made toward translating knowledge of MSC biology and immunomodulatory effects to widespread clinical use for diseases which share the common feature of damaging

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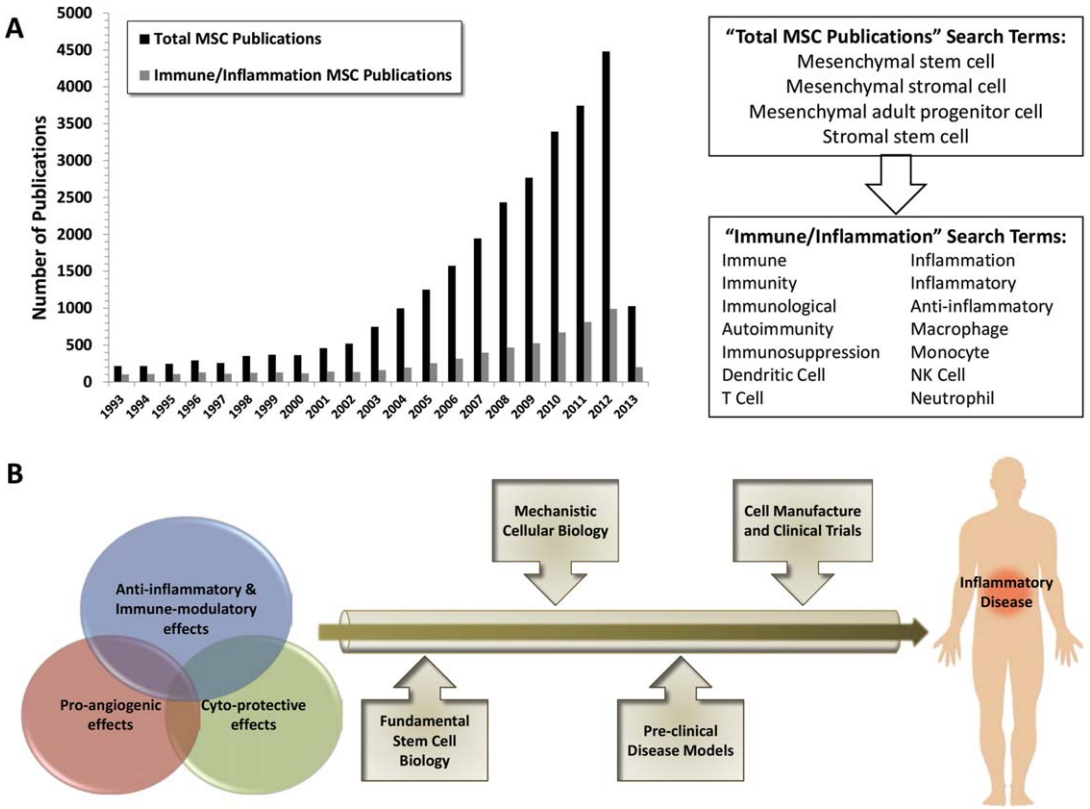

Figure 1. Progression of interest in the immunological properties of MSCs: **(A):** Left: A graphical representation of total mesenchymal stem cell (MSC)-related publications between January 1993 and March 2013 and the subset of these that address immune response and inflammation. Right: A summary of the PubMed search strategy used to generate data on MSC-related publications. **(B):** An illustration of the "Pipeline" of key research areas contributing to the process of translating knowledge of MSC paracrine properties toward the treatment of human inflammatory diseases. Abbreviation: MSC, mesenchymal stem cell.

acute or chronic immunological activity. Initially, we highlight some of the most exciting recent developments that have occurred in four key areas of relevance to successful clinical translation - fundamental stem cell biology, mechanistic cellular studies, preclinical diseases models, and cell manufacture/clinical trials (Fig. 1B). We then discuss how these new developments are influencing therapeutic protocols, the degree to which a robust translational pipeline is emerging for a range of inflammatory diseases and the future strategies that could positively impact eventual benefits to patients and communities.

Recent Progress in Understanding Primary MSCs and Their In Vivo Locations and Functions

Until quite recently, the identity, diversity and physiological functions of the cells in bone marrow and other tissues that expand in plastic-adherent culture to generate MSCs were not clearly known and there were important misconceptions about their status as stem cells [4]. In the past 5 years, however, significant progress has been made by groups with expertise in fundamental stem cell biology in elucidating the in vivo biology of the tissue-resident cells from which culture-expanded MSCs are derived [4].

In the mouse, Morikawa et al. demonstrated that a subset of MSCs could be prospectively identified and purified from bone marrow using the surface proteins platelet-derived growth factor receptor (PDGFR)α and stem cell antigen (Sca)-1 [17]. This identification subsequently allowed for the localization of the primary MSC subset to the peri-vascular region of arteries in cortical bone. In this study, the PDGFRα^+/Sca-1^+ cells were capable of repopulating a similar bone marrow niche when transplanted into new recipients directly after purification [17]. Using an in vivo ablation approach, Omatsu et al. showed that mouse bone marrow cells that abundantly express CXCL12 and are primarily located close to peri-sinusoidal endothelium (which they termed CXCL12-abundant reticular cells (CAR) cells) play an important role in maintaining hematopoietic stem cell (HSC) proliferation in addition to serving as progenitors for osteoblasts and adipocytes [18]. Similarly, Méndez-Ferrer et al. reported that peri-vascular bone marrow stromal cells expressing nestin are closely co-associated with HSCs and account for all of the MSC content of mouse bone marrow cultures. Furthermore, cultured nestin$^+$ MSCs were capable of self-renewal, contributed to physiological bone and cartilage turnover following transplantation and were required for HSC maintenance in vivo [19]. Subsequently, Ding et al. demonstrated that stem cell factor (SCF)-producing peri-vascular stromal cells expressing multiple MSC-associated markers were required for maintenance of the mouse bone marrow HSC niche. In this study, however, the identified cells were found to express the leptin receptor but not nestin [20]. Greenbaum et al. further revealed the complexity of bone marrow stromal subsets by selectively deleting CXCL12 expression from osteoblasts, endothelial cells and individual stromal subpopulations in the mouse. This study indicated the presence of separate MSC subpopulations that express the

transcription factors osterix and PRX1 and which support different aspects of hematopoiesis [21]. In this study, the $PRX1^+$, CXCL12-expressing MSC subset was negative for both nestin and leptin receptor suggesting that it may represent a precursor to cell populations identified in the other reports [21]. Mourcin et al. identified two subsets of mouse marrow stromal cells which express galectin-1 and interleukin (IL)−7 respectively. These are differentially localized and play distinct roles in B cell development [22]. Finally, Park et al. specifically sought out marrow stromal cells responsible for bone regeneration using promoter-specific lineage tracing in mice. They demonstrated that an "MSC-like" cell population expressing the transcription factor myxovirus resistance-1 (Mx1), serves as a progenitor for osteoblasts following bone fracture and demonstrates characteristics of bona fide stem cells in single-cell and serial transplantation experiments [23].

Although it is clear that some conflicting observations remain to be resolved, these recent studies in mouse have provided essential insights into the physiological nature of MSCs—in particular, their functional roles in maintaining the HSC niche and in bone regeneration. Taken together, the emerging evidence supports the conclusions that cells which serve as precursors to culture-expanded MSCs represent bona fide progenitor cells in vivo and can be prospectively identified by their expression of several functionally important proteins. In bone marrow, they now seem likely to consist of multiple individual cell types residing in peri-vascular spaces within the central sinusoidal region and at the endosteal surfaces.

Some equally important work has been carried out in human cell and tissue samples. For example, Battula et al. [24], Maijenberg et al. [25], and Tormin et al. [26] have recently reported results of fluorescence activated cell sorting (FACS)-based purification of stromal cell populations from human bone marrow using multiple cell surface markers including CD271, CD56, and MSCA-1 (now known to be tissue non-specific alkaline phosphatase) and CD146 [24–26]. These studies appear to confirm the presence of multiple subsets of human primary MSCs, which may differ in their differentiation capacities as well as in their relative abundance during fetal development and aging [24–26]. The study of Tormin et al. also localized two distinct MSC subsets within human bone marrow—$CD271^+/CD146^+$ cells in peri-vascular regions and $CD271^+/CD146^-$ cells at endosteal surfaces [26]. Furthermore, Crisan et al. demonstrated that cells which are confined to peri-vascular regions and express the pericyte markers CD146 and NG2 can be purified by FACS from multiple human tissues including skeletal muscle, pancreas, fat, placenta and bone marrow. These cells natively co-express typical MSC surface proteins and give rise to multipotent MSCs in long-term cultures [27]. Although conceptually in keeping with findings that have emerged from the mouse literature, these studies also highlight the lack of MSC- and MSC-subset-specific markers that apply equally to experimental species and to humans.

From a translational perspective, these recent basic insights highlight the fact that a better appreciation of the heterogeneity of MSC subpopulations within the bone marrow and of MSC-like pericytes derived from other tissues will serve to improve systems for defining therapeutic MSCs at the point of isolation and for optimizing their potency for specific disease targets. Similarly, the growing understanding of the embryological origins of perivascular cells within individual organ and tissue systems, the signaling pathways involved in their recruitment and function and the roles they play in vascular development and postnatal vascular repair (reviewed in detail by Armulik et al. [28]) has potential to critically inform future clinical strategies for MSC-based tissue regeneration and immunomodulation.

Recent Progress in Understanding the Mechanisms of Interaction Between MSCs and Immune/Inflammatory Cells

Substantial progress has been made recently in our understanding of the interactions between MSCs and immune cells. As a result of such studies, MSCs are now considered to intuitively respond to their immediate environment and to adapt their response accordingly through the release of soluble factors such as prostaglandin E2 (PGE2), kyneurenine, interleukin (IL)-10, tumor necrosis factor (TNF)-stimulated gene 6 protein (TSG-6), nitric oxide (NO), and transforming growth factor (TGF-β)-1 [29–34] and/or through cell contact signaling such as Notch, and CD95/Fas [35–37]. This multifaceted responsiveness is in keeping with an emerging understanding of the role of primary MSCs in regulating, along with macrophages and other cell types, the bone marrow stem hematopoietic cell niche [38].

A number of recent studies have elegantly demonstrated the response of culture-expanded MSCs to different environmental cues. Utilizing a mouse sepsis model, Nemeth et al. conclusively revealed an in vivo anti-inflammatory effect mediated by MSCs through promotion of IL-10-producing alternatively activated macrophages in the lung. In this model, lipopolysaccharide- and TNF-α activated NF-κB signaling through toll like receptor-4 and TNF receptor 1, led to expression of cyclooxygenase (COX)−2, and subsequent PGE-2 production by MSCs. This facilitated MSC interaction with macrophages through EP2 and EP4 receptors and resulted in increased macrophage production of IL-10 [34]. The group of Prockop et al. have documented an alternative anti-inflammatory mechanism of human MSCs transferred into rodent recipients in the context of inflammatory injury models such as acute myocardial infarction, ischemic stroke and peritonitis [7]. This mechanism involves secretion by MSCs of TSG-6, an anti-inflammatory glycoprotein, in response to TNF-α produced by macrophages [7].

Context-dependent modification of T-helper (Th)1/Th2 balance by MSCs has also been demonstrated in a number of disease models, including a Th2-driven model of ragweed-induced allergic asthma. In this report, MSC administration was associated with reduced serum and bronchioalveolar lavage fluid levels of IL-4 and IL-13, reduced levels of IgG_1 and IgE and impeded inflammatory cell infiltration and mucus deposition in the lungs. The activation of IL-4R/STAT-6 signaling in MSCs resulted in an increase in TGF-β1 production, which was proposed to be responsible for an increase in regulatory T-cell (Treg) numbers [33]. A significant number of additional studies have also demonstrated the capacity for MSCs to promote/expand Treg in vitro and in vivo. This effect has been linked with induction of TGF-β1 [30,32,35,39]. as well as idoleamine 2,3 dioxygenase [31]. In a number of recent reports, the importance of Treg in mediating beneficial effects of MSCs in vivo has been unequivocally demonstrated using Treg depletion [31,32]. Proinflammatory T-helper 17 (Th17) cell differentiation is also modulated by MSCs, in some cases in favor of the generation of Treg [6,39,40]. In the case of Th17 suppression, MSCs have been shown to mediate their effect through a contact-dependent mechanism involving induction in MSCs of COX-2/PGE2 and modulation of responder T-cells via the PGE2 receptor EP4 [40]. In human T-cells, MSC-mediated inhibition of Th17 differentiation was associated with upregulation of the transcription factor FOXP3 and induction of an IL-10-producing Treg phenotype [39]. Further evidence of a

role for cell contact-dependent signaling was recently reported by Akiyama et al. who showed that MSC administration in a mouse model of experimental colitis led to reduction in Th17 cells and increase in Treg. In this model, CD95L (FAS Ligand) signaling by MSCs resulted in increased apoptosis of activated effector T-cells and macrophage-derived TGF-β1, produced in response to T-cell apoptosis, leading to an increase in Treg [35]. Using human cells in vitro, Quaedackers et al., demonstrated that activated $CD8^+$ and $CD4^+$ T-cells were specifically modulated through binding to adipose-derived MSCs [41]. Finally, Ren et al. have demonstrated that MSCs suppress T-cell responses through the coordinated production of chemoattractants and NO in the context of an inflammatory environment. Interestingly, however, in the absence of NO, the influence of MSCs was reversed and resulted in increased T-cell proliferation [42].

Along with macrophages and T-cells, dendritic cells (DC) play a key role in disease-associated immune and inflammatory responses and are also subject to MSC modulation. In various studies, MSCs have been demonstrated to modulate DC maturation, migration, and antigen presentation and to induce a tolerogenic DC phenotype [36,37,43]. Initial in vitro observations regarding MSC attenuation of DC migration [43] have now been substantiated in an in vivo model [44]. Regarding the mediators of MSC-associated DC modulation, IL-6 has been shown to play a partial role in the inhibition of DC maturation [43,45], while more recent studies have also identified a role for the Notch signaling pathway [36,37].

MSCs may also mediate context-dependent effects on the activity of other innate and adaptive immune cells that are involved in inflammatory disease pathogenesis [6]. For example, in vitro studies of MSC interactions with natural killer (NK)-cells indicate cross-talk between the two cell types that is mediated both by cell–cell contact and by soluble mediators [6,46]. Depending on the cytokine milieu and the activation state of the NK-cell, this cross-talk may result in inhibition of NK-cell proliferation and cytolytic capacity or in NK-cell-mediated lysis of MSCs [46]. Although relatively under-investigated, direct and indirect modulatory effects of MSCs on B-cells and antibody responses have also been demonstrated [47]. Interestingly, results of in vitro studies to date have provided somewhat contradictory results with evidence for both suppression and promotion by MSCs of key B-cell biological responses including survival, proliferation, immunoglobulin production, and plasma cell differentiation. Similarly, in vivo models of antibody-mediated disease, most notably models of systemic lupus erythematosis and organ transplantation, have yielded evidence of both beneficial and detrimental effects of MSC administration [47].

Taken together, recent mechanistic studies using both in vitro and in vivo systems document complex interactions between MSCs and their immediate environment, which result in key modulatory effects on a range of immune effector cell types. Importantly, earlier studies focused on identifying individual factors responsible for immunosuppressive effects of MSCs have progressed to mechanistic insights that reveal coordinated "cross-talk" between MSCs and immune/inflammatory cells involving signals from multiple cell-surface and secreted factors [34]. On-going elucidation of the relative roles of contact-dependent signals and soluble mediators in directing these effects remains very cogent to their successful clinical translation. Specifically, new insights into mechanism of action will continue to influence key aspects of clinical trial design such as route and site of MSC administration, optimization of MSC anti-inflammatory phenotype and, eventually, the potential for replacing the cells themselves with individual mediators, mediator "cocktails" or subcellular vesicles.

Recent Progress in the Use of Preclinical Models of Inflammatory Disease to Guide Clinical Translation

In addition to serving as a testing ground for therapeutic efficacy and mechanism of action, preclinical animal models also provide important indications of MSC safety, toxicity, pharmacodynamics, and pharmacokinetic profiles that may be of relevance to future widespread human clinical use. One important consideration in this regard relates to the influence of MSC administration on new or preexisting cancers [48–50]. Recent reports of the context-dependent role of MSCs in regulating tumor growth speak to the complexity of this issue [48,51,52]. While Lee et al. reported that weekly infusions of preactivated human MSCs enhanced tumor-suppressive activity in a mouse xenograft tumor model [51], Ren et al. demonstrated that tumor-resident MSCs recruit monocyte/macrophages via the chemokine CCR2 with resulting enhancement of tumor growth [52]. The latter finding is in keeping with the reprogramming effect of MSCs on macrophages in an inflammatory environment but emphasizes the fact that such effects may be detrimental in settings such as neoplasia.

Preclinical models have also provided important opportunities for evaluating the immune responses induced by allogeneic MSCs (allo-MSCs) under varying conditions [5]. Allo-MSCs are considered to be poorly immunogenic in comparison with commonly transplanted cells and tissues and the concept of immune privilege, whereby allo-MSCs fail to elicit any active anti-donor immune response in vivo, has been an important tenet of the "off-the-shelf" model of human MSC therapies [3,6]. However, a number of recent animal model studies provide evidence that allo-MSCs do, in fact, promote detectable antidonor immunity in nonimmunosuppressed hosts [5]. For example, Schu et al. demonstrated that intravenous (i.v.) injection of allo-MSCs in rat results in the formation of alloantibodies, which may contribute to the rapid clearance of a second cell inoculum [53]. Moreover, in a rat model of post-myocardial infarction (MI) cardiac repair, Huang et al. showed that in vivo differentiation of locally implanted allo-MSCs is associated with the development of antidonor T-cell and antibody responses which resulted in loss of long-term benefits for cardiac function [54].

A related area for which animal models represent an essential testing ground is the in vivo distribution, fate and longevity of MSCs administered by various routes. Within the past year, some notable studies have revisited the phenomenon of MSC entrapment in the liver and lungs of rodents following i.v. injection to better determine its importance for clinical applications [53,55–58]. In most settings, almost all i.v. injected MSCs have been shown to remain in the lungs and liver, casting doubt on whether direct MSC/immune cell contact is likely to occur at other sites [55,57]. This phenomenon of entrapment is not inconsistent with immunosuppressive/anti-inflammatory effects of administered MSCs at distant sites but would imply that the entrapped cells either inherently produce soluble immunosuppressive mediators that act systemically or, more likely, interact with monocyte/macrophages in the lungs and/or liver to induce the production of anti-inflammatory factors such as IL-10 [34]. However, as

recently reported by Nystedt et al., the extent of lung adhesion may vary depending on cell size, passage number, MSC source and expression of adhesion molecules and matrix components such as CD49 and fibronectin [56]. Furthermore, in both rat and mouse, distribution and persistence of injected MSCs to multiple lymphoid and nonlymphoid, including perivascular engraftment, have been demonstrated [53,58]. The degree to which these diverse observations from animal experimentation are replicated in human MSC recipients is poorly understood at present.

Taken together, the recent literature from a wide variety of preclinical animal models provides a wealth of potentially valuable information regarding mechanisms of action, safety, immunogenicity, and in vivo kinetics of therapeutically administered MSCs. Preclinical studies also continue to reveal promising new disease targets with some notable recent examples being acute lung injury [59,60] and corneal injury/transplantation [61,62]. As we discuss in the concluding section of this review, however, the diffuse and variable nature of animal experimentation has also created a complex landscape for progress from preclinical to clinical studies. In addition, results pertaining to MSC influences on disease pathophysiology or potential for adverse effects such as entrapment and tumorigenesis in animal models should be interpreted with a measure of caution as regards to their direct applicability to human subjects.

Recent Progress in the Development of Commercially Viable Cell Products and the Execution of Clinical Trials

Human therapy with culture-expanded MSCs has moved well beyond the proof-of-concept phase. Since the first reported clinical trial of MSCs in 1995 [63], more than 100 clinical trials involving over 3,000 human subjects have met safety endpoints [10] and a large number of phase two third efficacy trials are close to conclusion. Strikingly, no severe adverse events caused by MSC administration have been reported from these trials to date, providing an impetus for large-scale development of MSC-based products [10]. In May 2012, the biotechnology company Osiris Therapeutics Inc. (Columbia, MD, http://www.osiris.com/) received market approval in Canada and New Zealand for a proprietary MSC formulation (Prochymal) to treat specific pediatric cases of graft versus host disease (GvHD)—representing the first market-approved allogeneic ("off-the-shelf") stem cell medicine. Prochymal is now undergoing phase II/III trials for inflammatory bowel disease (IBD), MI, chronic obstructive pulmonary disease, and type-1 diabetes mellitus. Several other major commercial developers of MSC-based therapeutics with competing sources and methods of formulation are also trialling their products across a range of clinical indications. These include Athersys Inc. (Celveland, OH, http://www.athersys.com/, product Multistem, in phase II trials for IBD and ischemic stroke); Stempeutics (Bangalore, India, http://www.stempeutics.com/, product Stempeucel, in phase II trials for osteoarthritis, ischemic stroke, liver cirrhosis, and critical limb ischemia); Mesoblast Inc. (Melbourne, Australia, http://www.mesoblast.com/, products Revascor and NeoFuse, in phase II trials for heart failure, MI, type 2 diabetes mellitus and degenerative disc and joint disease); TiGenix NV (Leuven, Belgium, http://www.tigenix.com/, product Cx601, in phase II and III trials for rheumatoid arthritis and fistulizing IBD); Celgene Cellular Therapeutics (Warren, NJ, http://www.celgene.com/, product PDA-001, in phase I and II trials for MS, sarcoidosis, ischemic stroke, rheumatoid arthritis and IBD) and Pluristem Therapeutics (Haifa, Israel, http://www.pluristem.com/, product PLX-PAD cells, in phase II trial for peripheral artery disease).

In parallel with these industry-driven initiatives, there have been numerous recent clinical trials of culture-expanded MSCs completed entirely within university- and academic medical center-based systems. Although it is not possible to comprehensively review all of these studies here, it is notable that human trials of MSC therapy have been reported from academic centers across Europe, North America, and Asia and have involved both autologous and allogeneic MSC sources. Some important recent examples that have specifically provided new evidence of MSC safety and/or efficacy include a large trial of autologous MSC "induction" therapy in living donor kidney transplant recipients from China [11], a phase I/II trial comparing trans-endocardial injection of autologous and allogeneic MSCs for ischemic cardiomyopathy from the USA [13], a multicenter phase II study of MSC infusions for steroid-resistant GvHD in recipients of allogeneic bone marrow transplant from Europe [9], and phase I/II studies of i.v. and localized autologous MSCs for Crohn's disease from Italy and the Netherlands [8,12].

How can Cohesive Translational Pipelines be Developed for MSC Therapeutics?

As we have reviewed in the preceding sections, research and technology focused on MSCs has yielded a wealth of exciting new insights, concepts and clinical observations which appear to lend further support to their therapeutic value in inflammatory diseases. Despite this progress, however, the future of MSC administration as a widely applied therapeutic intervention currently seems far from assured. To the unbiased eye, the field appears vulnerable on several fronts including: (a) the unequivocal documentation of safety and efficacy in Phase III and long-term (Phase IV) post-marketing studies, (b) the ability of cell manufacturers and clinical trialists to keep pace with emerging regulatory frameworks for advanced therapy medicinal products, and (c) the degree to which new scientific insights can be practically incorporated into therapeutic products. Furthermore, and in contrast to translational pathways that have proved successful for other therapies such as new drug classes, disease-modifying biological agents and medical devices, the anti-inflammatory and immunomodulatory properties of MSCs are being investigated and applied with varying degrees of cohesion to a very broad range of disease processes with diverse pathophysiological features. Thus, it is reasonable to ask whether strong translational "pipelines" will emerge from current worldwide research trends. For each of the four areas we have addressed in this article, there have been developments that could clearly facilitate future success in the clinical arena. However, there are also persistent knowledge gaps which, if left unaddressed, may significantly hamper progress (Fig. 2). Thus, we believe that there are some important challenges to be considered by both the academic- and industry-based communities to ensure a more cohesive overall effort toward widespread clinical application of MSC-based therapies in the coming years.

For researchers focusing on cell mechanism and preclinical MSC studies, the means by which experimental observations can be linked to clinical applications remains

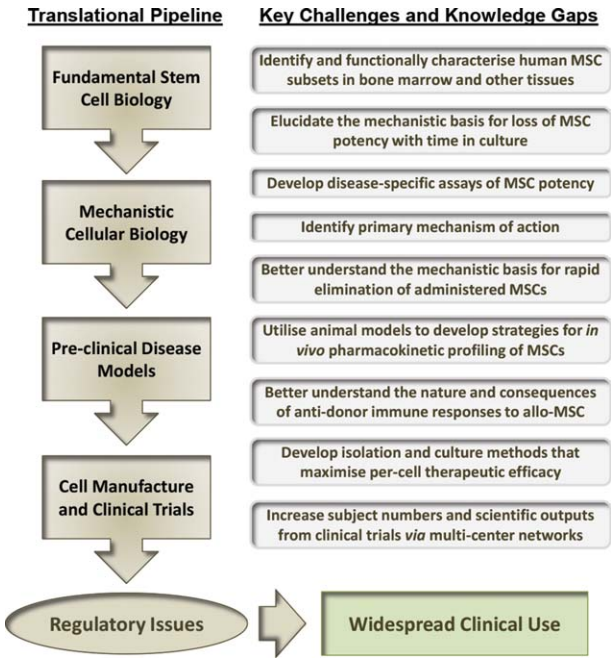

Figure 2. An illustration of key challenges and knowledge gaps related to four major research themes associated with clinical translation of mesenchymal stem cells to widespread clinical use. Abbreviations: MSC, mesenchymal stem cell; NK, natural killer.

problematic. As interest in the field has grown, studies performed in culture systems and in animal models have raised many, sometimes contradictory, possibilities regarding MSC diversity, mechanism of action, efficacy, and safety that merit testing in human subjects. While the cumulative knowledge gained is of undoubted value on several fronts, a large number of studies are performed in isolation from focused translational projects. One strategy that could be pursued to better connect preclinical and clinical investigations in the MSC field is the formation of multidisciplinary, interinstitutional networks to collaboratively target specific disease categories across a full spectrum of research and technical skills. In fact, some good examples exist of collaborative groups driving the rational translation of MSC-based therapy in the areas of organ transplantation and hematological malignancies [9,64]. The combined expertise of such networks allows for optimal selection and interpretation of animal models as well as purposeful preclinical testing of administration regimens that can be subsequently translated to human subjects. A network-based approach is also likely to exert a more cohesive and positive influence upon the development of regulatory standards and best practice guidelines as the field evolves [15]. A related area for which network-based collaboration between basic and clinical research groups would be of high value is in the development of mechanistically informed strategies for monitoring MSC potency, efficacy, and safety in human clinical trial subjects. An important issue which remains poorly addressed in the field at present is the gap that exists between our understanding of MSC anti-inflammatory and immune modulatory mechanisms in culture/animal model systems and our ability to measure the contribution and potency of such mechanisms in human subjects receiving MSC therapies. Additionally, the clinical implications of anti-donor T-cell and antibody responses induced in vivo by allogeneic MSCs remain poorly understood [5]. Further coordinated effort from laboratory-based immunologists and clinical researchers will be of value in better addressing these areas.

For clinical researchers and commercial entities developing therapeutic MSC products, several key issues threaten to impede progress. In the first place, the majority of clinical-grade MSCs generated by commercial entities or academic medical centers are isolated by plastic adherence and constitute heterogeneous cell populations. As future regulatory guidelines are likely to dictate that methods to prospectively define and purify MSCs from their primary source tissues be developed [15,16], such plastic adherent cells may fall short of emerging standards. Prospective purification of primary MSCs on the basis of specific cell surface markers such as alkaline phosphatase (Stro3) and CD271 represents one promising strategy for addressing this issue [4,24,25,65,66]. It is important to emphasize, however, that these and other individual markers are not unique to MSCs in their various in vivo niches

Table 1. Recent research and technology contributions to the rational clinical translation of immunomodulatory mesenchymal stem cell therapy for graft versus host disease

Contribution	Relevance to translation	
Identification of primary stromal cell subtypes along Identification of primary stromal cell subtypes along with their developmental origins, locations, marker expression, migration characteristics, and response to environmental cues	Prospective isolation of optimal MSC population Opportunities to modify biodistribution and enhance therapeutic efficacy	[17–22,24–28]
Identification of biomarkers of MSC therapeutic response in GvHD	Optimisation of patient selection Development of tailored therapeutic administration	[72]
Identification of mechanisms of MSC elimination following in vivo administration	Establishment of optimal culture conditions and dosing regimen Improvement of patient safety	[68,73]
Detailed investigation of MSC biodistribution in relevant preclinical models of GvHD and human recipients	Improved understanding of pharmacokinetic and safety profiles	[71,74]
Correlation of passage number and culture conditions with therapeutic response in GvHD	Minimisation of cell dose Optimisation of therapeutic efficacy	[69,70]
Achievement of regulatory standards for use of MSC therapeutic products in GvHD	Facilitation of phase II, III and phase IV clinical trials	[9,70,75]
Extended follow-up of patients receiving MSCs for GvHD in the context of multicenter clinical trial networks	Cumulative record of disease-specific safety and efficacy	[9,69,75]

Abbreviations: GvHD, graft versus host disease; MSC, mesenchymal stem cell.

and that their expression during culture expansion is subject to change and susceptible to variability based on culture conditions [4]. In addition, it is not clear whether the culture conditions currently used to propagate MSCs provide optimal support for their primary plating and secondary expansion. Indeed a recent article suggests that specific subpopulations of MSC fail to adhere to tissue culture plastic under standard conditions [67]. Furthermore, there is growing evidence from in vitro, preclinical, and clinical studies that the immunosuppressive and other trophic functions of culture-expanded MSCs lose potency over time in culture [68–70]. Addressing this issue will require new strategies to continuously monitor the therapeutic potency of MSCs throughout the culture period and to develop culture conditions which improve or maintain the key therapeutic effector mechanisms [70]. This process of optimization may also require tailoring for individual clinical indications. A third area which may come to bear as regulatory legislation moves beyond the current focus on manufacturing sterility and therapeutic toxicology, is the need to better document the absorption, distribution, metabolism, and excretion toxicity (ADMET) profile of clinical-grade MSC products. Currently there is little or no activity toward understanding the pharmacokinetic parameters of MSC administration such as absorption into the blood stream, biodistribution and in vivo metabolism of MSC-associated biomolecules. In addition to the regulatory issues, more attention to developing pharmacokinetic assays for cellular products is also likely to be of value for accurate calculation of appropriate MSC dosing regimens and for the testing of strategies to overcome immune-mediated clearance of systemically administered MSCs. Approaches to accurately selecting patients most likely to benefit from MSC therapies are also generally lacking and infrequently investigated across the field. Finally, the need to minimize risk of culture-expanded MSCs in treated individuals remains an important concern for the field. For instance, while i.v. infusions of MSCs appear to be well tolerated in clinical settings, the potential for acute or chronic adverse effects from entrapment/embolization of cells in the lung or liver should continue to be carefully evaluated [55,71]. Similarly, despite the paucity of evidence to date for in vivo transformation of human MSCs in animal recipients [50], it will only be possible to accurately define the risk for malignant transformation in human recipients by performing close surveillance of treated patients and collating experience from multiple clinical studies into central, shared databases.

Despite the significant gaps in knowledge and focus described above, there is also reason to be optimistic that immunomodulatory MSC therapy will establish a foothold for specific disease entities in the next several years and that this could subsequently provide the basis for targeted translation into additional clinical niches. Perhaps the best example of an immune-mediated disease for which many of the challenges depicted in Figure 2 have been recognized and are in the process of being addressed is GvHD. Table 1 summarizes some of the key developments related to the clinical translation of MSC therapy for GvHD with a view to highlighting the important connections that been made across the different components of the research pipeline. As robust and reproducible efficacy remains to be unequivocally demonstrated even for this well-studied disease [70], the continuation of this translational process for GvHD will be great significance to the broader field of MSC therapy.

Conclusion

To conclude, there can be no doubt that the last decade of worldwide MSC-related research has generated a highly valuable knowledge base that spans fundamental stem cell biology, immunology and inflammatory response, disease pathogenesis, cell manufacture, and the clinical safety of cellular therapeutic products. It has also created the foundations of an effective clinical intervention for diverse immune/inflammatory diseases. Whether this progress will be converted into widely used, cost-effective treatment protocols for large numbers of patients is likely to depend on the degree to which specific practical challenges that have recently become apparent are met in coordinated fashion by the various stakeholders.

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Disclosure of Potential Conflicts of Interest

Dr. Stephen Elliman is Head of Research and Development for Orbsen Therapeutics, Ltd., a campus-based spin-out company that is developing novel cell-based therapies, including human MSCs. The other authors of this article have no vested interest in Orbsen Therapeutics and declare no other conflicts of interest.

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