Mesenchymal stem cells for systemic therapy: Shotgun approach or magic bullets?

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Given their heterogeneity and lack of defining markers, it is surprising that multipotent mesenchymal stem/stromal cells (MSCs) have attracted so much translational attention, especially as increasing evidence points to their predominant effect being not by donor differentiation but via paracrine mediators and exosomes. Achieving long-term MSC donor chimerism for treatment of chronic disease remains a challenge, requiring enhanced MSC homing/engraftment properties and manipulation of niches to direct MSC behaviour. Meanwhile advances in nanoparticle technology are furthering the development of MSCs as vehicles for targeted drug delivery. For treatment of acute injuries, systemic cell-free exosome delivery may ultimately displace current emphasis on empiric donor-cell transplantation for anti-inflammatory, immunomodulatory and repair-promoting effects. Exploration of potential clinical sources of MSCs has led to increased utilisation of perinatal MSCs in allogeneic clinical trials, reflecting their ease of collection and developmentally advantageous properties.

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- mesenchymal stem cell; regenerative medicine; stem cell therapy

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Abbreviations:
BM, bone marrow; **GvHD**, graft-versus-host disease; **ISCT**, International Society for Cell Therapy; **MSC**, mesenchymal stem cell; **OI**, osteogenesis imperfecta.

Introduction

There is still no precise definition for mesenchymal stem cells (MSCs) despite almost half a century having passed since the first isolation of plastic-adherent non-haematopoietic bone marrow (BM) cells capable of clonal expansion [1, 2]. There is also no consensus on nomenclature, with terms including *mesenchymal stem cell*, *mesenchymal stromal cell* and *multipotent mesenchymal stromal cell* – preferred by the International Society for Cell Therapy (ISCT) – all being known by the abbreviation MSC [3]. These names reflect differing emphasis on two key properties of MSCs: (i) the ability to offer stromal support for expansion of other cell types [4] and (ii) their capacity for multi-lineage differentiation [5, 6]. While broadly similar MSC populations have been isolated from a wide anatomical and ontological array of tissues, the lack of a unique marker means that cells fulfilling the minimal in vitro criteria for defining MSCs, set out by the ISCT [7], necessarily comprise heterogeneous populations. Notwithstanding this, the well-described in vitro differentiation capacity of MSCs along with a decade's experience with in vivo models attests to their potential therapeutic use to replace damaged tissues. This has been validated in a range of organs, particularly heart, kidney, bone, cartilage, gut and brain. MSCs have also been used clinically as tissue-engineering substrates to bridge large tissue defects, such as the trachea [8, 9]. This review addresses our changing understanding of how MSCs work, how they can be exploited for systemic therapy, and optimal sourcing of cells for clinical trials.

Do donor MSCs directly contribute to primary tissue repair?

Achieving high levels of donor engraftment and differentiation is challenging

If MSCs facilitate healing by direct incorporation into regenerating tissue, then they must home to, and engraft at, sites of

tissue injury. While MSCs have an inherent ability to home to inflamed tissues in wounds, tumours [10, 11], and a range of neuropathologies [12], the rate of MSC engraftment in pre-clinical disease models has been low. In a porcine model of myocardial infarction, only 6% of the total number of injected MSCs showed cardiac engraftment at ten days post infusion [13], suggesting that homing efficiency or a lack of engraftment sites within damaged tissue may be limiting.

Inefficiencies in MSC homing have been demonstrated, with substantial transient localisation to organs such as lung, spleen, liver and kidney following intravenous infusion, prior to systemic clearance [10, 14]. Cytokine/receptor pairs (SDF-1/CXCR4, SCF/c-Kit, HGF/c-Met, MCP2/CCR, HMGB1/RAGE) and adhesion molecules (β1 and β2 integrins, L-selectin) [15–18] involved in MSC homing are targets for enhancing homing efficiency. For instance, genetically modifying MSCs to overexpress CXCR4 enhances migration of MSCs to infarcted myocardium twofold [19]. Novel alternatives to genetic engineering, such as modification of cell surface properties, also have significant clinical translation potential. Conjugation of selectin-binding peptides to the MSC surface enhances their ability to adhere to, and roll on, selectin substrates in vitro [20, 21]. In vivo, these MSCs showed a fourfold increase in transit time through inflamed blood vessels and 40–50% increased homing to inflammatory sites [21]. Enhancing homing efficiency alone may not be sufficient to enhance long-term engraftment if it is the number of engraftment sites that is limiting. A recently described strategy circumvents this issue by tethering MSCs to a target tissue; a synthetic ligand for α4β1 integrin covalently coupled to alendronate, which has high affinity for bone, enhanced homing of human MSCs to murine skeleton [22].

Following engraftment, the cues MSCs receive from the local microenvironment heavily influence the extent to which those cells divide, differentiate and functionally contribute to tissue repair. For instance, stem cells have shown evidence of neural differentiation in murine models of neural ischemia and demyelinating lesions, while stem cells localised to brain tumours in mice remained in a quiescent and undifferentiated state [12]. In a porcine myocardial infarction model, the vast majority (76%) of MSC-derived engrafted cells were observed as undifferentiated cell clusters in interstitial spaces three months post injection [23]. Nonetheless, pigs that had received MSC treatment in the above example showed improved infarct size and myocardial blood flow that correlated with engraftment. Either the small number of differentiated cells was sufficient to generate a therapeutic benefit or the MSCs contribute to healing by other means.

Are high levels of MSC donor engraftment required?

The minimal level of donor chimerism required for MSC transplantation to deliver a therapeutic outcome differs depending on the clinical application. For treatment of acute injuries, establishment of donor chimerism may not be required at all [24–26]. Although establishment and maintenance of donor chimerism is critical for replacing defective cells in treatment of genetic disorders and chronic conditions, low levels of chimerism may suffice. Intrauterine delivery of MSCs in a mouse model of osteogenesis imperfecta (OI) resulted in a reduction in fracture rates, albeit at ~1–5% donor chimerism in adult mice [27, 28]. The disproportionate benefit from low levels of MSC, or indeed BM [29], engraftment in OI models likely stems from engrafted cells producing greater quantities of type I collagen than defective endogenous cells [29]. Similarly, an early clinical study, reported increased growth velocity in paediatric OI patients in the six months following MSC transplantation, despite less than 1% donor chimerism 4–6 weeks after transplantation [30]. More recently it has been suggested that the transient stimulation of growth velocity by post-natal MSC therapy results not from engraftment and differentiation, but from indirect stimulation of chondrocyte proliferation [31]. These studies highlight difficulties in assessing the significance of low levels of donor chimerism with regard to clinical benefit.

Is donor chimerism maintained?

Steep declines in donor chimerism with time have been observed in animal models [32, 33], and are also suggested by limited patient data [34]. Long-term maintenance of donor cells following transplantation requires engrafted cells to avoid immune rejection and persist within a stem cell niche. MSCs have a well-documented immunomodulatory capacity [35], fail to illicit responses in mixed lymphocyte reactions in vitro and have been successfully transplanted across MHC barriers [19, 23, 26, 27, 32]. However, the extent to which allogeneic MSCs, and their progeny, evade immune rejection remains controversial.

Allogeneic MSCs persist for longer than transplants of other allogeneic cells [33, 36], demonstrating at least a limited capacity for avoidance of immune surveillance. Benefits of allogeneic MSC transplants have been comparable to those of syngeneic transplants in pre-clinical studies on cutaneous wound healing, sepsis and experimental autoimmune encephalomyelitis [32, 37, 38], suggesting no significant immune barrier for allogeneic therapy. Conversely, other studies on acute myocardial and kidney injury found allogeneic MSCs to be less beneficial than syngeneic MSCs [26, 33]. Furthermore, in vivo differentiation of allogeneic MSCs in a myocardial infarction model was associated with allo-antibody production and immune rejection [33]. Most studies assessing in vivo immunogenicity of allogeneic MSC transplants noted some responses [39]. Hence, while MSC immunogenicity is clearly attenuated, rejection may nevertheless limit the utility of allogeneic MSCs for regenerative applications.

Adaptive immune rejection is not the only limiting factor in maintenance of donor chimerism, as syngeneic MSCs also decline with time. After injection of three million syngeneic MSCs into a rat myocardial infarct, typically 1.8 million donor cells remained in the wound one day later, while just 150,000 donor cells persisted at seven days [33]. This likely reflects clearance of cells that have failed to engraft in a suitable niche by innate immune cells, rather than an adaptive response. In fact, both syngeneic and allogeneic MSCs are susceptible to lysis by activated natural killer cells because of their low level MHC class I expression [40]. Limited long-term maintenance of donor chimerism suggests that few transplanted MSCs maintain a stem cell phenotype and contribute to long-term cell repopulation. Pragmatic approaches to overcome this are

limited at present to increased and/or serial dosing. So, even if MSC engraftment can be enhanced, their maintenance in a stem cell niche remains a substantial challenge in the treatment of chronic disease.

MSC paracrine factors and exosomes facilitate tissue repair

Promotion of repair by MSCs in the absence of large-scale donor chimerism has been attributed to production of paracrine factors. These may act by promoting angiogenesis, dampening inflammatory responses, enhancing survival of parenchymal cells and supporting endogenous stem cells/progenitors. Soluble factors released into the circulation can function at sites of injury distant to the MSCs. For instance, production of the anti-inflammatory protein TSG-6 (tumour necrosis factor stimulated gene 6) by MSCs sequestered in the lung proved a critical mediator of improved cardiac function in a mouse model of cardiac infarction [24]. TSG-6 has also been reported as a primary mediator of MSC-induced benefits in rodent models of corneal injury [25] and peritonitis [41]. MSCs are rich sources of several other anti-inflammatory mediators and display regulatory activity against cells of both innate and adaptive immune systems [35, 42, 43]. Immunomodulatory properties typically manifest in suppressing inflammatory responses [35], although a pro-inflammatory MSC phenotype has also been described [44]. These contribute to the ability of MSCs to suppress inflammation and induce tolerance in autoimmune diseases, organ transplantation and graft-versus-host disease (GvHD) [45].

Characterising the paracrine actions of MSCs may give rise to non-cellular therapies. Conditioned medium from human MSCs reproduce at least some of the benefits reported with MSC therapy for myocardial infarction in swine, including reduced infarct size, reduced myocardial apoptosis, increased myocardial capillary density and preserved cardiac performance [46, 47]. This suggests that stable paracrine factors within conditioned medium mediate therapeutic benefit. Microvesicle or exosome shedding by MSCs allows for horizontal transfer of proteins, bioactive lipids, messenger RNA and microRNA between MSCs and cells in damaged tissue [48]. Recently, Gatti et al. [49] reported that microvesicles, purified by ultracentrifuging MSC-conditioned medium, were sufficient to protect against acute kidney injury in rats. This effect was associated with increased proliferation and decreased apoptosis of renal cells, and at least in part mediated by the RNA cargo of the microvesicles/exosomes. Another study implicated the pro-angiogenic microRNAs, miR-126 and miR-296, in microvesicle-mediated protection following acute kidney injury [50]. Others have implicated microvesicle-mediated transfer of mitochondria in damaged lung tissue [51].

Paracrine signalling stimulates angiogenesis and promotes survival of parenchymal cells, enhancing repair independent of MSC differentiation. Such signalling appears at least in part to be mediated by exosomes shed from their surface [48, 51]. Soluble factors and MSC-derived exosomes warrant further investigation as translational tools, and are particularly relevant to acute conditions where support of endogenous repair mechanisms may be more critical than long-term, large-scale donor chimerism.

MSCs deliver therapeutic agents

Gene therapy to enhance tissue repair

Clinical translation of the clear benefits of genetically modified MSCs in pre-clinical models has been curtailed by questions over the safety of ex vivo gene therapy. Genetic manipulation of MSCs has been investigated both as an approach to long-term gene replacement for treatment of genetic disorders and to enhance the regenerative capacity of MSCs. Strategies have been reported for inactivating disease-causing mutations in MSCs from OI patients [52], raising the possibility of gene-corrected autologous MSC therapy. Significant challenges to therapeutic application of autologous MSCs for genetic disorders remain, including homing, engraftment and long-term maintenance of chimerism. Modification of MSCs to express chemokine receptors, such as CXCR4 [19] or CCR1 [53], enhances their engraftment and therapeutic benefit in rodent models of myocardial infarction. Enhanced regenerative capacity has been demonstrated in other studies modifying MSCs for a range of aims including promoting angiogenesis [54, 55], suppressing inflammation [56, 57] and inducing lineage-specific differentiation [58–60].

Safety concerns have centred on the potential for unintended insertional mutagenesis from gene modification using methods that offer no control over the insertion site. This can result in malignancy, as highlighted by the incidence of leukaemia in patients participating in early clinical trials of retrovirally modified autologous HSC (haematopoietic stem cells) in paediatric immunodeficiencies [61]. Nevertheless, large-scale transplantation studies of gene-modified MSCs in immunodeficient mice observed no MSC-derived tumours over a lengthy observation window [62]. Recent advances in rapid high-throughput genomic and transcriptome screening may better ensure the genetic stability of gene-modified MSCs. Alternative methodologies for gene modification also exist. Insertional mutagenesis may be avoided by targeting gene insertion into 'safe-harbour' loci, as recently demonstrated for human MSCs from a variety of sources [63].

Finally, where prolonged expression of a therapeutic gene is not required, it may be preferable to modify MSCs by transient transfection, with advances in nanoparticle technology attractive for this purpose [55]. For longer term transduction, advances in gene-modification techniques and in genome-wide screening may allow realisation of the clinical potential of combining MSC and gene therapy.

MSCs as kamikaze tools to deliver chemotherapy

A novel approach lies in harnessing the trophic abilities of MSCs for targeted delivery of anti-tumour agents, where long-term engraftment and survival of genetically modified MSCs is neither required nor desirable. Tumours secrete many of the same cytokines, chemokines and inflammatory mediators that attract MSCs to sites of tissue damage, and MSCs have a demonstrated ability to home to, and cluster at, tumour sites

[10, 64]. The action of exogenously delivered unmodified MSCs on tumour biology is unclear, with different in vivo tumour model studies suggesting MSCs to have either pro-tumourogenic or anti-tumourogenic activity, possibly as a result of MSC polarisation [65]. However, to ensure anti-tumourogenic effects, MSCs can be genetically modified to express pro-apoptotic, anti-angiogenic, immunostimulatory or pro-drug converting protein products for delivery to tumours. Such strategies have been validated in rodent models with a diverse range of cancers including breast, colon, prostate, melanoma and glioma [66]. Pre-clinical studies attest to the efficacy of anti-cancer MSC therapy against both primary tumours and disseminated tumours. For instance, MSCs engineered to secrete a pro-apoptotic factor, S-TRAIL (secretable tumour necrosis factor apoptosis inducing ligand), are capable of tracking and clearing disseminated gliomas in mice [67].

Perhaps the most intellectually appealing strategy is to modify MSCs to express a pro-drug converting enzyme. This would comprise two steps, patients first receiving a dose of MSCs expressing a pro-drug converting enzyme, followed by treatment with the pro-drug. The latter would be given at doses expected to have few systemic side effects, while conversion of the pro-drug to an active cytotoxic agent by MSCs would induce MSC apoptosis and the death of surrounding (tumour) cells due to a bystander effect. Well-characterised non-mammalian pro-drug systems include 5-fluorocytosine conversion to the cytotoxic 5-fluorouracil by cytosine deaminase and ganciclovir activation by herpes simplex virus thymidine kinase (HSVtk), which leads to inhibition of DNA synthesis and apoptosis. 5-Fluorocytosine and ganciclovir have well-established safety profiles from their current clinical use as anti-mycotic and anti-viral agents, respectively.

Undesirable side effects with such a pro-drug strategy could result from non-specific entrapment of MSCs in organs such as the lung, liver, spleen and kidney. In vivo imaging has demonstrated transient localisation of MSCs in these organs, prior to persistent MSCs localizing to tissue injury/tumour sites [10, 68]. Given a thorough understanding of MSC bio-distribution following administration, pro-drug treatment could be delayed to avoid damage to the organs that are subject to MSC trapping; a clear advantage over therapy with MSCs constitutively expressing a pro-apoptotic factor. Unfortunately, the stem cell tracking modalities of bioluminescence and fluorescence used in the laboratory setting (e.g. [10] and [64]) have limited clinical applicability because of their shallow tissue penetration depth. Clinical imaging will require labelling MSCs by alternative means. Labelling MSCs with iron oxide to facilitate magnetic resonance imaging (MRI) results in little change to their biological properties [69]. Recently, a proof-of-concept study demonstrated ultrasound-guided photo-acoustic imaging of MSCs labelled with gold-nanotracers [70], an imaging modality with higher sensitivity than MRI. In the context of anti-cancer therapy, the application of MSC imaging modalities will be of value clinically in monitoring MSC bio-distribution following administration, in determining the optimal timing for commencing the pro-drug and in monitoring the therapeutic effect following pro-drug administration.

Nanoparticle modification is a promising alternative to gene therapy

Combining MSCs with nanoparticulate drug delivery systems offers an alternative to genetic modification of MSCs for delivery of therapeutic agents. After a decade of research into the application of polymeric nanoparticles [71, 72], a key challenge to their biomedical application remains the ability to target these particles efficiently. One solution is to harness the trophic abilities of MSCs for nanoparticle delivery. An added advantage is that nanoparticles tethered to an MSC carrier will not be sequestered by the mononuclear phagocyte system. This strategy was recently validated in a pre-clinical glioma xenograft model [73]. Nanoparticles loaded with the chemotherapeutic agent, doxyrubicin, were anchored to MSCs using an antibody against the MSC cell surface marker CD90. Intratumoural injection of nanoparticle-carrying MSCs induced apoptosis within the tumour and greater retention of doxyrubicin within the tumour compared to injection of the doxyrubicin-loaded nanoparticle without a cellular carrier.

Similar strategies can be devised to deliver agents to enhance tissue repair or stimulate cell differentiation. For instance, Sarkar et al. [74] loaded MSCs with intracellular depots of microparticles containing dexamethasone. In culture, these cells showed sustained release of dexamethasone for at least 14 days with osteogenic differentiation as stimulated by both particle-carrying MSCs and MSCs co-cultured with the particle-carrying cells. Nanoparticles present a promising alternative to genetic modification of MSCs for a range of clinical purposes such as enhancing MSC regenerative capacity [74], targeted delivery of chemotherapeutics [73] and labelling MSCs to enable real-time cell tracking [69, 70].

Are there clinically relevant differences in MSCs from different sources?

MSCs can be isolated from numerous somatic tissues

There are many potential sources of MSCs, some already under assessment in clinical trials. Those trials registered on clinicaltrials.gov typically use adult BM-derived MSCs (BM-MSCs), either allogeneic or autologous, or more recently allogeneic perinatal sources. Perinatal MSCs can be derived from the umbilical cord (whole umbilical cord, umbilical cord blood or Wharton's Jelly), the placenta (fetal chorion or maternal decidua) or the membranes (amniotic or chorionic). Additional adult sources under investigation in clinical trials include adipose tissue-derived MSCs and endometrium-derived cells isolated from menstrual blood. The bias towards use of BM-MSCs in current clinical trials is largely a reflection of the fact that BM was the first source from which MSCs were derived. Comparative characterisation of MSCs from this myriad of sources reveals both similarities and differences. At a minimum, MSCs from different sources share adherence to plastic, cell surface marker expression and in vitro multipotent differentiation potential, as defined by the ISCT. Global transciptome analysis demonstrates around 85% of genes with detectable levels of expression show unchanged expression

levels between BM-MSCs, adipose tissue MSCs and umbilical cord blood MSCs [75]. Both global transcriptome and global proteomic analysis of MSCs from different tissue sources and different donors clearly show more variation due to source rather than donor [75–77]. It is likely that the differences between MSCs from various sources will influence their clinical utility.

In addition to the various sources, current clinical trials process MSCs by vastly different procedures, with precise strategies rarely detailed on trial registries due to their commercially sensitive nature. Indeed, culture expansion of MSCs from the same source in different media can cause as much variation in the transcriptome as isolation of MSCs from different tissue sources [77]. Even in the absence of a unique cell-surface marker for MSCs, selection can be used to enrich the frequency of MSCs in a cell population. For instance, positive selection of BM for Stro-1 and CD106 cell surface expression prior to expansion increases the frequency of colony-forming units 1,000-fold [78]. Hence, MSC preparations from different trials will vary significantly due not only to differences in source, but also in density and purity.

Limitations of autologous MSC therapy

The use of autologous cells obviates the risks of immune rejection and communicable disease transmission from donor to recipient, but has limitations. Because of their low frequency in adult tissue, ex vivo expansion is required to obtain sufficient numbers of MSCs for therapy, necessitating a delay in treatment that may be problematic in acute injury situations. In other settings, the use of autologous MSCs may not be feasible, such as in patients with genetic disorders or where the patient's MSCs have been adversely affected by aging and/or disease. These limitations and the purported immune-privileged status of allogeneic MSCs have led to increasing interest in allogeneic sources.

Perinatal MSCs have advantageous properties for an off-the-shelf stem cell product

Allogeneic MSCs offer the promise of 'off-the-shelf' products suitable for a range of disease scenarios, including genetic disorders. As a clinical source, perinatal MSCs demonstrate advantageous properties such as ease of isolation at high frequency from readily available tissues and extensive proliferative capacity. However, differences in immunomodulatory capacity or lineage plasticity between MSC sources may be relevant for specific clinical applications.

Tissue availability is one determinant of the ease of isolation of MSCs. While tissue can be readily obtained from fetal, perinatal and adult sources, potential ethical objections in some quarters may arise in relation to the clinical use of MSCs derived from abortal tissue. Such concerns typically engender strict ethical guidelines on fetal MSC collection. Reports of isolation of fetal MSCs from excess diagnostic tissue sampled from early pregnancy [79, 80] suggest that it may be possible to obtain fetal MSCs for clinical use from sources free of such reservations. Extra-embryonic perinatal tissues (umbilical cord, placenta) are easily obtained at term without ethical objections, as these would otherwise be discarded. Adult MSCs are isolated from tissues harvested by simple but invasive procedures such as BM aspirates or liposuction. Hence, extra-embryonic perinatal tissues are the most readily available tissue source for clinical exploitation.

Umbilical cord MSCs and adult adipose tissue MSCs have the highest reported isolation yields, as outlined in Table 1.

Table 1. Frequency and proliferative characteristics of human MSC isolated from different sources

MSC source	Frequency/isolation yield[a]	Expansion potential (cumulative PD)[b]	Proliferative rate (doubling time)	CFU-F/passaged MSC
Adult-BM	0.001% [81] 0.003% [83] 0.008% [82]	27 ± 4 [81] 12 [84]	75–85 hours [85] ~58 hours [86] ~40 hours [83] 117 ± 22 hours [87]	40 ± 5% [87]
Adult-adipose tissue	0.06% [82]	Similar to adult-BM [82]	Similar to adult-BM [82] 96–120 hours [114] 70 ± 4 hours [87]	38 ± 6% [87]
Placenta (maternal)		Up to 64 [84]	~50 hours [84]	
Placenta (fetal)	~25,000 cells isolated per 40 mg term chorionic villi [115]	>35, first trimester tissue [79]	≥40 hours, first trimester tissue [79]	
Umbilical cord/Wharton's jelly	0.06% [83]	~ 60 (not reported clearly) [83]	~34 hours [86] ~24 hours [83] 55 ± 4 hours [87]	48 ± 8% [87]
Umbilical cord blood	$<10^{-6}$ % [82]		~2-fold faster than adult BM [82]	
Fetal-first trimester	~0.001% Similar for blood, liver and BM [116]	~ 50 [116]	21–25 hours [85] 32 ± 2.5 hours [87] 3–5-fold faster than adult BM-MSC [101]	75 ± 5% [87]

[a] Expressed as percentage of plated mononuclear cells that form CFU-F (colony-forming unit – fibroblast) unless otherwise stated.
[b] Cumulative PD (population doublings) counted from first passage to senescence.

The frequency at which mononuclear cells generate colony-forming MSC units is generally reported to be 0.001–0.008% for adult BM [81–83], although this declines with age [5]. Similar frequencies are reported for first trimester fetal tissues, whereas MSC frequency in umbilical cord [83] or adult adipose tissue [82] is around tenfold higher.

MSCs of earlier ontological origins have favourable proliferative properties for ex vivo expansion (Table 1), desirable for expanding MSCs for transplantation. Ex vivo expansion potential, denoted as population doublings between first passage and senescence, is much greater for MSCs from fetal and perinatal sources than from adult sources. This is also the case for maternally derived placental MSCs [84]. Consistent with this, fetal MSCs have long telomeres and high telomerase [85]. Proliferation rates (doubling times) can vary for a given source between studies, likely due to variation in culture methodology. In comparative studies, cells from fetal and perinatal sources always demonstrate faster doubling times than adult MSCs [83, 85–87]. Direct comparison of fetal (first trimester BM) and perinatal (umbilical cord) MSCs revealed that fetal cells proliferate faster and maintain a higher colony-forming unit ability.

The greater proliferative capacity of primitive stem cells, such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, has fuelled interest in using them to derive MSCs [52, 88–90]. This strategy carries a potential oncogenic risk if MSC populations are contaminated with pluripotent parent cells. Inherent challenges particular to the generation of iPS cells have been reviewed elsewhere [91–93]. The goal of ex vivo expansion of stem cells is to generate a therapeutically relevant quantity of a consistent cell product. However, ex vivo expansion can also be associated with adverse changes in cell characteristics of consequence to their safety and efficacy. Reported changes include loss of differentiation potential, loss of chemokine receptors required for homing [94], changes in epigenetic stability [95], and chromosomal aberrations [96]. Interestingly, fetal MSCs were found to have greater epigenetic stability than ES cells [95], suggesting that they may be a superior source for large quantities of ex vivo expanded cells. The challenges of ex vivo expansion of MSCs are discussed elsewhere in this special issue [97].

Overall, perinatal tissues display the most advantageous array of properties for clinical-grade MSCs. Perinatal-derived MSCs can be isolated with high frequency from readily available tissues and subsequently demonstrate great expansion potential and high proliferative rates. MSCs are present at similar frequency in adult adipose tissue, but these cells lack the ex vivo expansion potential. Fetal MSCs have similar expansion potential and faster doubling times, but these properties are counter-balanced by a low frequency at isolation.

Are MSCs from different sources optimal for different clinical applications?

While ease of isolation and ex vivo expansion capacity are desirable for clinical-grade MSCs, other biological properties influence the suitability of MSCs for particular applications. Envisioned MSC therapies are diverse in their method of cell preparation and application, and in desired clinical outcome. The MSC secretome is critical to their promotion of healing via paracrine mediators. Tissue engineering applications require functional differentiation of MSCs and low immunogenicity. Immunomodulatory therapy exploits both the ability of MSCs to secrete anti-inflammatory cytokines and their direct interactions with immune cells. To date, significant differences in lineage potential and immunomodulatory capacity have been noted for different-source MSCs.

Pre-clinical investigations into the use of fetal MSCs for bone tissue engineering illustrate how an MSC tissue source may be ideally suited for a particular clinical application. Firstly, fetal MSCs may have lower immunogenicity than their adult counterparts as they express lower levels of human leukocyte antigen (HLA) class I and no cytosolic HLA class II [98, 99]. Furthermore, neither undifferentiated nor differentiated fetal MSCs induced proliferation of allogeneic lymphocytes [99], suggesting that fetal MSCs have favourable immunogenic properties for long-term graft survival. Fetal MSCs also demonstrate an osteogenic capacity superior to both adult and perinatal MSCs in a range of in vitro and in vivo assays [87, 100]. In vitro assays implicitly assume that the same set of cues is optimal for differentiating MSCs from all sources, making validation in in vivo assays essential. For instance, while umbilical cord MSCs lay down more calcium than adult MSCs in in vitro assays, adult MSCs were more effective in an in vivo ectopic bone formation assay [87]. The performance of fetal MSCs out-strips both umbilical cord MSCs and adult MSCs in both assays. Conversely, fetal MSCs display weaker suppression of proliferative lymphocyte responses than their adult counterparts [99, 101]. Hence, while low immunogenicity and high osteogenic capacity make fetal MSCs preferable substrates for bone tissue engineering [102], their relatively weak immunomodulatory capacity makes them less attractive candidates for treatment of GvHD or induction of transplant tolerance.

The greatest immunosuppressive capacity is found in MSCs from perinatal tissues, where maternal immune tolerance of the fetus is critical for tissue function. MSCs derived from fetal placental tissue were found to have greater immunosuppressive properties than maternally derived deciduoplacental MSCs [103]. This was associated with greater expression of the soluble cytokines, interleukin-10 (IL-10) and vascular endothelial growth factor. Nonetheless, cell–cell contact was also required for maximal inhibition of alloreactive T cell proliferation. Direct comparison of umbilical cord and adult BM-MSCs found the perinatal cells to have stronger immunosuppressive potential, and to be associated with greater IL-10 production [104]. This is in contrast to fetal MSCs derived from embryonic tissues, which fail to suppress alloreactive lymphocyte proliferation unless they have been primed with interferon-γ (IFN-γ) [99, 101]. Priming with IFN-γ enhances the immunosuppressive capacity of MSCs from other sources, and enhances their efficacy against GvHD in pre-clinical models [105]. Current comparative studies are consistent with perinatal MSCs of extra-embryonic fetal origin being superior for clinical applications requiring immunomodulation.

How significant is progress in clinical trials?

Over 200 clinical trials involving genetically unmodified MSCs are listed in the world's largest clinical trials database, clinicaltrials.gov. Systematic review of these trials (summarised in Fig. 1) shows that they are primarily Phase I and/or II trials in a broad range of conditions, including orthopaedic defects, cardiovascular conditions, neurological diseases, liver pathology and GvHD. Reports published so far attest to the overall safety of MSC therapy with both autologous and allogeneic MSCs, from adult and perinatal sources [106–110]. However, there remain a number of safety concerns. Firstly, it is feasible that the ability of MSCs to act as immunosuppressive agents while therapeutically useful might also result in inappropriate suppression of host immune responses to exogenous threats. Similarly, the ability of MSCs to support the growth of other progenitor cells may be harnessed by cancer stem cells to nurture a pre-existing undiagnosed lesion. To date, animal studies have shown conflicting results as to whether unmodified MSCs support or suppress tumour growth, so this is an important area for further study [67]. The first clinical trial to examine stem cell anti-cancer therapy has been initiated using a pro-drug converting enzyme strategy against glioma (Identifier: NCT01172964).

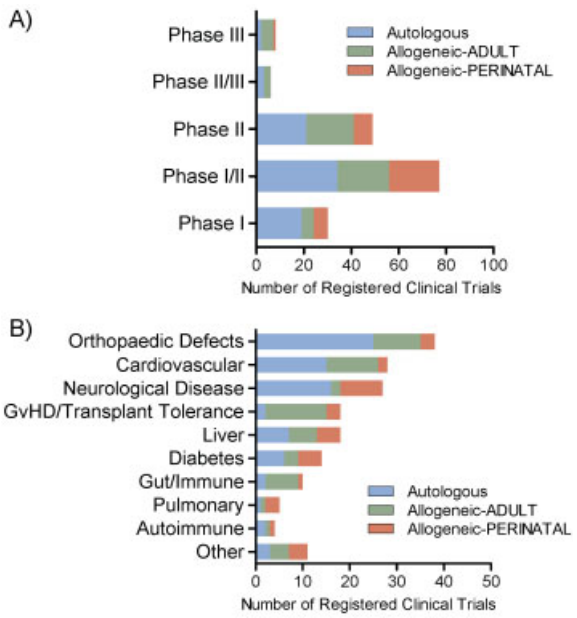

Figure 1. Registered clinical trials of MSC therapy. **A:** Trials categorised by phase. **B:** Trials categorised by targeted condition/disease. A systematic review of the registered clinical trials utilizing MSCs was conducted on 22 March 2012 using the search engine of clinicaltrials.gov (the world's largest clinical trial database). The following search terms were used: 'mesenchymal stem cells', 'mesenchymal progenitor cells' and 'mesenchymal stromal cells'. Limits were set to 'interventional studies' for the 'study type'. In total, 210 registered clinical trials were retrieved and categorised by MSC source, registered Phase and targeted disease/disorder. Indications categorised as 'Other' include tumour-associated acute renal failure, HIV infection, aplastic anaemia, sickle cell disease, acute burns, Romberg's disease, retinitis pigmentosa, limbus corneae insufficiency syndrome, epidermolysis bullosa and myelodysplastic syndromes; 34 trials were excluded from the data summary due to insufficient information.

Phase III trials have been initiated to examine therapeutic efficacy of MSCs in Crohn's disease, knee cartilage injuries, myocardial infarction, critical limb ischemia and GvHD. Preliminary results of a Phase III trial of an allogeneic adult BM-MSC product, Prochymal by Osiris Therapeutics Inc, to treat GvHD were disappointing in that they demonstrated only small statistical improvements over placebo in selected subsets of patients [111]. However, a recent meta-analysis concluded that children respond better than adults to MSC treatment for GvHD [112], and Prochymal has been granted provisional approval in Canada and New Zealand for steroid-refractory paediatric acute GvHD (Osiris 14 June 2012 press release; http://investor.osiris.com/releases.cfm?Year=2012, accessed on 24 July 2012). Despite this, the commitment of Osiris Therapeutics' commercialisation partner, Genzyme, to the development of Prochymal for GvHD is unclear (Zacks Equity Research Blog http://www.zacks.com/stock/news/75568/canada-approves-osiris-prochymal, accessed on 24 July 2012). Modest clinical benefits of autologous BM cell therapy for myocardial infarction were confirmed by a recent Cochrane review [106]. This included studies with treatment with 'any autologous human adult stem cells', with the majority of studies utilizing crude preparations of BM-mononuclear cells. Patients receiving stem cell therapy had improved cardiac function (left ventricular ejection fraction) [106]. Phase I and II clinical trials suggest that more refined allogeneic MSC preparations, including Prochymal, demonstrate similar effects to crude autologous cell preparations in treatment of acute myocardial infarction (Osiris 2 July 2012 press release; http://investor.osiris.com/releases.cfm?Year=2012, accessed on 24 July 2012, [113]). Nonetheless, given the potential of MSC therapy and the incompleteness of our understanding of their biology, it is likely that first generation MSC therapies will continue to have, at best, modest clinical benefits that fall short of over-inflated expectations.

Conclusions and outlook

Rapidly expanding numbers of clinical trials utilizing MSC transplantation attest to the broad range of clinical potential envisaged for MSC therapy. Different therapeutic paradigms seek to capitalise on three distinct mechanisms of action; MSCs acting as (i) cellular substrates for tissue repair, (ii) rich sources of paracrine factors that promote survival of and tissue repair by endogenous cells or (iii) carriers for the delivery and retention of therapeutic agents at sites of inflammation. Inefficiencies in MSC homing represent a limitation to therapies based on any of these three mechanisms. Surface modification using nanoparticles presents an exciting tool for the manipulation of MSC homing and engraftment. Design of such modifications will be informed by addressing fundamental questions about endogenous MSC behaviour and their niche: How do endogenous MSCs function? To what extent are they systemically mobilised? What limits the utility of endogenous MSCs in disease states? These may reveal mechanisms for promoting endogenous MSC participation

in healing, in addition to enhancing the therapeutic efficacy of exogenous MSCs. Furthermore, nanoparticle technology is advancing in vivo imaging to track exogenous MSCs, aiding the study of homing and engraftment and allowing monitoring of MSC-based therapies in the clinic.

The paracrine mediation of MSC effects may lead to MSC-inspired cell-free therapies or, more likely, to MSC-derived exosome therapy. MSC exosomes may be manipulated to deliver multiple factors for purposes as diverse as promoting tissue repair or inducing tumour cell death. Exosome content could be manipulated by genetic manipulation of MSCs or by surface modification with nanoparticles. Finally, perinatal source MSCs will continue to be utilised for clinical applications that cannot be superseded by cell-free therapies. Tailoring of MSC therapies over the next decade should underpin the transition of MSCs from non-specific shotgun therapy, to targeted magic bullets.

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