Shattering barriers toward clinically meaningful MSC therapies

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More than 1050 clinical trials are registered at FDA.gov that explore multipotent mesenchymal stromal cells (MSCs) for nearly every clinical application imaginable, including neurodegenerative and cardiac disorders, perianal fistulas, graft-versus-host disease, COVID-19, and cancer. Several companies have or are in the process of commercializing MSC-based therapies. However, most of the clinical-stage MSC therapies have been unable to meet primary efficacy end points. The innate therapeutic functions of MSCs administered to humans are not as robust as demonstrated in preclinical studies, and in general, the translation of cell-based therapy is impeded by a myriad of steps that introduce heterogeneity. In this review, we discuss the major clinical challenges with MSC therapies, the details of these challenges, and the potential bioengineering approaches that leverage the unique biology of MSCs to overcome the challenges and achieve more potent and versatile therapies.

THE LANDSCAPE OF MSC THERAPIES

Multipotent mesenchymal stromal cells (MSCs) have been extensively investigated as a cell therapy, showing promise in treating an array of diseases by restoring organ homeostasis in inflamed, injured, or diseased tissues. Bone marrow–derived MSCs (BM-MSCs) were first described by Friedenstein et al (1) in the late 1970s and continue to be the most commonly studied MSC source in preclinical and clinical studies. MSCs can also be easily isolated from multiple tissues including adipose tissue (AT), umbilical cord (UC), Wharton’s jelly, and the placenta (2). While initial therapeutic efforts were based on their multipotency, the discovery of their immunomodulatory and trophic properties motivated harnessing MSCs as a treatment for neurodegenerative and inflammatory diseases. To this end, MSCs have been investigated as a treatment for graft-versus-host disease (GvHD), multiple sclerosis (MS), Crohn’s disease (CD), amyotrophic lateral sclerosis (ALS), myocardial infarction (MI), and acute respiratory distress syndrome (ARDS), among others (Table 1) (3–5). MSCs are generally distinct from other cell therapies as their therapeutic effect not only is dictated by cell–cell contact but also may include a so-called hit-and-run mechanism. Here, paracrine effectors from their secretome, including soluble cytokines, growth factors, hormones, and miRNA, are transferred to target cells such as immune cells and cells of damaged tissues through secretion, the uptake of biologically-loaded submicrometer extracellular vesicles (EVs), and immune-mediated phagocytosis (6–9), which can lead to long-term effects. In line with this, many studies have shown that secreted biologics and MSC-derived EVs containing biologically active molecules (such as proteins, lipids, and nucleic acids) retain the biological activity of parental MSCs and demonstrate a similar therapeutic effect in selected animal models (10). Because the properties of secreted biologics and MSC-derived EVs have been thoroughly reviewed elsewhere (11–13), the current article focuses on MSC therapies.

With more than 300 completed clinical trials using MSCs as of 2020, there is a wealth of information available to better understand what dictates their success and failure when investigated in humans. The TiGenix/Takeda phase 3 clinical trial that studied the use of MSCs for complex perianal fistulas in CD is arguably the most successful late-stage MSC trial to date (NCT01541579). In this study, adult CD patients with treatment-refractory, draining, complex perianal fistulas were enrolled in a randomized, double-blind, placebo-controlled phase 3 trial and treated with either a single intrasural injection of 120 million allogeneic AT-MSCs (Alofisel) or saline (14). At the primary end point of 24 weeks, combined remission was significantly higher in patients treated with Alofisel (~50% in treated group versus ~34% in placebo group). Greater incidences of remission in the Alofisel treatment group persisted in a subsequent 52-week follow-up (~56% in treated group versus ~38% in placebo group), demonstrating the potential of MSCs to substantially improve the standard-of-care in chronic illnesses like CD. As complex perianal fistulas refractory to conventional medical treatment strategies often require surgery with suboptimal outcomes, the success of this trial validated Alofisel as a novel therapeutic for addressing an unmet clinical need. As a result, Alofisel was granted both orphan drug status and central marketing authorization approval for CD by the European Medicines Agency (EMA), becoming the first allogeneic stem cell therapy to do so in the European Union. This designation enabled Alofisel to be processed through an expedited regulatory path, and Alofisel was approved in Europe for the treatment of complex perianal fistulas refractory to CD in 2018. The cost-effectiveness of Alofisel compared to standard of care will ultimately dictate its successful integration.
as a viable treatment for eligible patients. Alofisel has thus far been approved by the EMA for use in <10,000 patients under an orphan medicinal product designation (15). Although the mechanism of action in human patients is not well elucidated, results from pre-clinical studies of Alofisel indicate that induction of indoleamine 2,3-dioxygenase (IDO) in the presence of inflammatory factors such as interferon-γ (IFN-γ) is critical for the therapeutic effect of MSCs. This is because the enzymatic activity of IDO can inhibit

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**Table 1. Representative indications of MSCs in clinical trials.** MPC, mesenchymal precursor cells; UC, umbilical cord; BM, bone marrow; AT, adipose tissue; Y, yes; N, no; TBD, to be determined; N/A, not available.

| General indication       | Clinical indication                  | Cell source       | Administration route | Clinical efficacy (Y/N) | Engineered (Y/N) | Year started | Phase | Status | Trial number |
|-------------------------|--------------------------------------|-------------------|----------------------|-------------------------|------------------|--------------|-------|--------|--------------|
| Autoimmune disease      | Rheumatoid arthritis                 | Allogeneic        | MPC                  | Y                       | N                | 2013         | 2     | Complete | NCT1851070   |
|                         | Systematic lupus erythematosus       | Allogeneic        | UC                   | Promising               | N                | 2017         | 1     | Complete | NCT03171194 |
| Cancer                  | Advanced gastrointestinal cancer      | Autologous        | BM                   | N                       | Y                | 2013         | 1/2   | Terminated | NCT02008539 |
|                         | Metastases solid tumors              | Autologous        | BM                   | Y                       | Y                | 2013         | 1/2   | Complete | NCT01844661 |
| Cardiac disorders       | Acute myocardial infarction          | Allogeneic        | BM                   | N                       | N                | 2009         | 2     | Complete | NCT00877903 |
|                         | Chronic heart failure                | Allogeneic        | MPC-BM               | Y                       | N                | 2014         | 3     | Ongoing, not recruiting | NCT02032004 |
|                         | Class 2 or 3 heart failure           | Autologous        | BM                   | N                       | N                | 2008         | 2/3   | Complete | NCT00810232 |
|                         | Ischemic stroke                      | Allogeneic        | BM                   | N                       | N                | 2011         | 2     | Complete | NCT01436487 |
|                         | Chronic GvHD                         | Allogeneic        | BM                   | Unknown                 | N                | 2012         | 2/3   | Unknown  | NCT01526850 |
|                         | Grade B to D acute GvHD              | Allogeneic        | BM                   | Y                       | N                | 2006         | 3     | Complete | NCT00366145 |
|                         | Grade B to D acute GvHD              | Allogeneic        | BM                   | Y                       | N                | 2015         | 3     | Complete | NCT02336230 |
|                         | Crohn’s disease                      | Allogeneic        | AT                   | Y                       | N                | 2012         | 3     | Complete | NCT01541579 |
|                         | Crohn’s disease                      | Allogeneic        | BM                   | TBD                     | N                | 2007         | 3     | Ongoing, not recruiting | NCT00482092 |
| IBD                     | Ulcerative colitis                   | Allogeneic        | BM                   | N                       | N                | 2010         | 2     | Complete | NCT01240915 |
| Kidney disorders        | Acute kidney injury                  | Allogeneic        | BM                   | N                       | N                | 2012         | 2     | Terminated | NCT01602328 |
|                         | Diabetic nephropathy                 | Allogeneic        | MPC-BM               | Y                       | N                | 2013         | 1/2   | Complete | NCT01843387 |
|                         | Liver/kidney failure                 | Allogeneic        | BM                   | N                       | N                | 2011         | 1/2   | Complete | NCT01429038 |
|                         | Renal transplantation                | Autologous        | BM                   | Y                       | N                | 2008         | N/A   | Complete | NCT00658073 |
| Neurodegenerative disease | Alzheimer’s disease                 | Allogeneic        | UC                   | Unknown                 | N                | 2012         | 1/2   | Ongoing, not recruiting | NCT01547689 |
|                         | ALS                                   | Autologous        | BM                   | Y                       | Y                | 2013         | 2     | Complete | NCT02017912 |
|                         | Chronic progressive MS               | Autologous        | BM                   | Local                   | TBD              | 2019         | 2     | Recruiting | NCT03799718 |
|                         | Degenerative disc disease             | Allogeneic        | MPC                  | Local                   | Y                | 2011         | 2     | Complete | NCT01290367 |
|                         | MS                                    | Autologous        | BM                   | Systemic                | TBD              | 2014         | 2     | Ongoing, not recruiting | NCT02239393 |

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T cell function and proliferation and increase the number of regulatory T cells, leading to an increase in anti-inflammatory cytokines [e.g., interleukin-10 (IL-10)] and decrease in pro-inflammatory cytokines [e.g., IFN-γ and tumor necrosis factor-α (TNF-α)] (14).

In addition to Alofisel, there are 10 globally approved MSC therapies including Prochymal (Osiris, approved in Canada and New Zealand), Temcell HS (ICR Pharmaceuticals, approved in Japan), Cartistem (Medipost, approved in South Korea), and Cellgram-AMI (FCB-Pharmicell, approved in South Korea) (16). The major approved indications are GVHD, CD, ALS, and MI (Table 2).

Several companies including Mesoblast, Athersys, Pluristem, Stempeutics, Cynata, and others are repurposing their MSC products for new indications. For example, Mesoblast has recently investigated the use of remestemcel-L to treat coronavirus disease 2019 (COVID-19) patients with moderate to severe ARDS, which is the major cause of death. The survival rate was 83% in ventilator-dependent COVID-19 patients when treated with two intravenous infusions of remestemcel-L. By comparison, the survival rate was only 12% in ventilator-dependent COVID-19 patients receiving standard of care during the same period (17). The capability of remestemcel-L to down-regulate pro-inflammatory cytokines and increase anti-inflammatory cytokines is believed to be the key mediator of the promising therapeutic efficacy for COVID-19 patients (18).

Despite the success of these therapies, most of the MSC therapies either have had no success in late-stage clinical trials or did not progress beyond preclinical studies. While MSCs demonstrate an exceptional safety profile, they have generally been therapeutically ineffective in humans. There are several factors that likely contribute to their suboptimal clinical outcomes, including heterogeneity in the potency of the MSC product, variable biodistribution and pharmacokinetics with different administration routes, and a limited understanding of the impact the host response has on therapeutic efficacy after administration (19, 20).

In this review, we summarize major clinical challenges for MSC therapies. These challenges are divided into three different categories, including challenges resulting from the manufacturing of MSCs (Fig. 1A), from administration of MSCs (Fig. 1B), and from the recipients (Fig. 1C). Under each challenge, we discuss major factors leading to the heterogeneity of the clinical outcome. We then highlight several examples that leverage bioengineering solutions to address the clinical challenges arising from MSC product quality, administration, and host factors and conclude that bioengineering strategies can and should be used to develop more potent and predictable MSC therapies (21–23).

### OVERCOMING CLINICAL CHALLENGES RESULTING FROM THE MANUFACTURING OF MSCS

While the current clinical successes of MSC therapies are encouraging, albeit limited, the predominating failures emphasize the difficulty in predicting immunomodulatory and regenerative effects within human trials. This unpredictability is partially rooted in defining meaningful critical quality attributes (CQAs) of the MSC product. The International Society of Cell and Gene Therapy (ISCT) initially defined human MSCs (hMSCs) according to three minimal phenotypic criteria based on their morphology, surface markers, and trilineage differentiation (24). These criteria were reflective of the MSC “stemness,” not their immunomodulatory and regenerative effects that dictate their therapeutic properties. In 2019, ISCT updated their criteria for defining MSCs to include (i) tissue origin and (ii) associated functional assays to define their relevant therapeutic mode of action (25). ISCT also called for a moratorium on referring to MSCs as “mesenchymal stem cells” in literature, unless rigorous evidence for stemness both in vitro and in vivo is presented. Furthermore, as MSC therapies have reached a critical mass in clinical trials, regulatory authorities have mandated new minimal CQAs for MSCs related to in vitro potency (e.g., suppression of T cell proliferation or IDO expression), in addition to the evaluation of identify, viability, purity, potency, proliferative capacity, genomic stability, and microbiological testing (26, 27). Measuring these CQAs is a unique challenge in the field of cell therapy, as MSCs, for example, unlike traditional chemical drugs whose structure and potency can be narrowly defined, are dynamic “living therapies.”

#### Impact of sourcing and manufacturing/storage on the functions of MSCs

**Heterogeneity in the MSC product**

Living MSC therapies are an inherently heterogeneous population of cells whose therapeutic gene and protein expression profiles vary with the characteristics of the donor, MSC tissue of origin (2, 20), isolation method (28), and in vitro preparation methods (e.g., cell culture protocol and scale-up) (26, 29). The extraordinary heterogeneity
of MSC product introduced during the manufacturing process emphasizes the need for both meaningfully characterizing and ultimately controlling the therapeutic potency of the MSC product (Fig. 1A).

Standardizing therapeutic potency of the MSC product is crucial before beginning clinical trials (30). This need has prompted research efforts toward developing improved in vitro potency assays that can accurately correlate the CQAs of the MSC product with their therapeutic function (31–35). Currently, the most widely used potency assay for the MSC product is based on in vitro inhibition of T cell proliferation using activated CD4+ T cells (26, 36, 37). This measurement is believed to be more representative of potency compared to surrogate markers for immunomodulatory function (i.e., IDO expression or TNF-α receptor expression) as it provides a direct read-out of bioactivity (26).

While each MSC product is completely different, other than useful observations, currently accessible data are insufficient to conclusively determine how tissue source, isolation method, and culture/scale-up during MSC product development can affect the therapeutic efficacy of MSCs (Fig. 1A). To highlight this point, we consider the phase 3 trial using remestemcel-L conducted for both adult and pediatric GvHD populations (NCT00366145) that was unable to meet primary clinical end points in a mixed-age patient population, despite demonstrating a positive impact on the liver and gut in earlier stages of the study (38). All remestemcel-L MSCs used in the phase 3 trial were derived from a single donor, requiring cells to be expanded to passages 3 and 4 during manufacturing to yield enough MSCs to treat all 240 participants. Conversely, a separate phase 2 trial on therapeutic MSCs conducted by the University Hospital Frankfurt avoided this failure for the same clinical indication in a similar patient population. In this study, pooled MSCs were used to treat 26 patients with GvHD using passage 1 and 2 MSCs from eight donors to yield sufficient quantities of MSCs (39). Results from this study demonstrated improved clinical efficacy in GvHD patients at the primary end point (day 28), with an overall response rate of 77%. While this comparison appears to illustrate the importance of MSC sourcing and manufacturing at the commercial scale (29, 40), differences highlighted here as well as subtle differences in the preparation of the MSC product make it challenging to draw conclusions.

While potency assays may improve product quality by excluding MSCs with low potential therapeutic efficacy, strategies are needed to instead generate high-quality MSCs in sufficient quantities for large clinical trials. Several biomaterial strategies have been explored to maintain more homogeneous MSCs during the expansion phase of MSC manufacturing. For example, Rao et al. (41) showed that expanding hMSCs on soft poly(ethylene glycol) hydrogel matrices was able to avoid reductions in cell surface marker expression and cytokine expression that was observed when hMSCs were serially passaged on polystyrene. Growing MSCs in three-dimensional (3D) culturing systems has also been beneficial in maintaining early-passage MSC phenotype during expansion (42, 43). At the clinical stage, efforts to overcome MSC product heterogeneity have been best exemplified by Cynata Therapeutics, who developed a strategy for obtaining highly homogeneous MSCs using induced pluripotent stem cells (iPSCs). Because iPSCs have an exponentially larger proliferation capacity without undergoing differentiation compared to MSCs, they can be easily expanded to generate large quantities of iPSCs and then differentiated into MSCs after expansion to yield commercial quantities of MSCs (iPSC-MSCs) with a low passage number (44). For example, upward of 1 × 10^22 passage 1 MSCs can be produced from a single iPSC population, with similar potency to low-passage BM-MSCs harvested from donors as assessed by T cell suppression (44, 45). The iPSC-MSC approach serves as an excellent solution for scaling MSC manufacturing without sacrificing therapeutic potency through the passage and expansion of cells (46). One caveat is that the intrinsic self-renewal and pluripotency of iPSCs may also be responsible for tumorigenic potential (47). However, this appears to be de-risked by clinical studies, showing that iPSC-MSCs are safe and effective. For example, Cynata’s lead product CYP-001 was shown to be effective for the treatment of steroid-resistant GvHD in a phase 1 clinical trial (NCT02923375) without showing any sign of tumorigenesis. Another phase 2 clinical trial including 448 osteoarthritis patients is expected to commence in early 2020 (48). Other approaches have used CRISPR-Cas9 technology to create a reversibly immortalized BM-MSC line, which avoids the
phenotypic changes that occur with continued passaging and expansion (49).

**Cryopreservation and culture rescue**

The loss of MSC potency following cryopreservation is another important challenge in the development of high-quality MSC products. This clinical obstacle may be best addressed by optimizing the handling of MSCs rather than engineering their physical and functional properties. The preparation of most MSC therapeutics involves expanding cells ex vivo, cryogenically banking them until needed, thawing the banked MSCs at the bedside, and administering them to the patient (29). MSC processing between thawing and administration varies widely between clinical trials, which can have significant implications on the therapeutic effect of MSCs once administered. For example, a phase 2 study assessing MSCs as a treatment against the chronic inflammatory disorder ARDS was unable to achieve a significant clinical improvement compared to control groups, despite promising results in phase 1 and a satisfactory safety profile (50). Retrospective analysis of the MSC doses found that the viability of the freshly thawed and washed MSCs ranged widely from 36 to 85%, despite no significant changes in viability between doses before cryopreservation. Furthermore, only MSCs with the highest viability (70 to 85%) were able to improve oxygenation in patients compared to the placebo (51). Fundamental studies on MSC cryopreservation have demonstrated that freshly thawed MSCs have stunted immunosuppressive capabilities, with a reduced capacity to suppress T cell proliferation (52). Cell damage following cryopreservation can also alter their post-infusion biodistribution, engraftment, and clearance kinetics (22, 53). Furthermore, thawed MSCs exhibit diminished structural integrity upon rewarming, as the freezing process disrupts the actin cytoskeleton (53). This abnormal membrane structure marks thawed MSCs as a target for activated T cells, expediting the onset of immune clearance and significantly diminishing the lifetime of intact MSCs in patients following infusion (54).

Some investigators have avoided the detrimental effects caused by cryopreservation by changing the way in which MSCs are handled before administration. For example, the successful phase 3 clinical trial on Alofisel for perianal fistulas “culture-rescued” MSCs after thaw, a process that involves the “recovery” of freshly thawed MSCs under cell culture for a period of at least 24 hours between thawing and infusion (14). The clinical success of MSCs handled in this manner suggests that the associated detrimental effects of thawing not only are reversible but also introduce a feasible strategy for improving MSC product quality (52, 54).

**Bioengineering solutions to boost the functions of MSCs**

**Engineering MSCs to boost the innate functions**

Clinical trials to date demonstrate that MSCs can be safely infused in high doses (55) and display promising responses in some clinical indications. Quality control protocols to standardize MSC product potency may help reduce the risk of clinical failure, but they are unlikely to resolve the problem completely, as the innate function of MSCs is not always therapeutically sufficient for disease treatment (51, 56). To maximize clinical potency while preserving ease of use, simple alternative bioengineering strategies should be explored that can boost the innate function of MSCs, independent of cryopreservation, passage number, and donor and tissue source. Furthermore, bioengineering can serve as a powerful platform for translating new
insights gained from the fundamental understanding of MSC behavior after infusion into more effective therapies (Fig. 2).

“Priming” MSCs with small molecules represents a simple strategy to exogenously boost their therapeutic function. Several “primed” neuroregenerative MSC products already have reached clinical investigation, with the most notable being Brainstorm Cell Therapeutics MSC product, NurOwn. NurOwn is a primed MSC product in which the innate regenerative capacity of MSCs is boosted using proprietary culture medium to express multiple neurotrophic factors (NTFs) including glial-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) (57, 58). When administered to patients with neurodegenerative diseases, NurOwn has been demonstrated to simultaneously deliver multiple NTFs, in addition to the immunomodulatory components innately secreted by MSCs (59). This combination has demonstrated impressive therapeutic efficacy in clinical trials, evidenced by a phase 2 clinical trial (NCT02017912) where ALS patients who received NurOwn demonstrated reduced ALS progression 24 months after infusion compared to controls. Brainstorm Cell Therapeutics is currently in the process of expanding the therapeutic scope of NurOwn, recruiting patients for a phase 2 clinical trial for MS (NCT03799718).

While small-molecule priming has utility, the effects only last several hours to a few days (60). With the emergence of techniques to improve the survival of transplanted cells, approaches to extend the exposure of small molecules to transplanted cells have also been developed. For example, loading MSCs with small-molecule encapsulating microparticles (MPs) has been used to boost the duration of product potency (61, 62). MPs comprise biocompatible materials that can be therapeutically tuned according to their composition, polymer molecular weight, extent of drug loading, and drug release. MSCs loaded with degradable MPs containing the steroid budesonide exhibited fourfold enhanced IDO activity in vitro compared to free budesonide-preconditioned MSCs and native MSCs. This led to a twofold improvement in the suppression of stimulated peripheral blood mononuclear cells (PBMCs) following IFN-γ stimulation (63).

MSCs can also be engineered to serve as a producer and carrier of biologics (Table 3). To produce the desired biologic within the MSC, viral DNA transduction and mRNA/DNA transfection are the most common approaches. For example, a study by Suresh et al. (64) reported that MSCs genetically engineered to express thioredoxin-1 (Trx1)—a powerful antioxidant, transcription factor, and growth factor regulator—improved cardiac function following MI in a rat model compared to unmodified MSCs. Although preclinical

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Fig. 2. Bioengineering solutions to boost the functions of MSCs. (A) Priming MSCs with small molecules is a simple and promising approach to induce the secretion of immunomodulatory and regenerative molecules, but the effect of small molecules only lasts a few hours to a few days. (B) MSCs can also be engineered with drug-loaded particles. These particles are intracellularly loaded into MSCs to sustain their immunosuppressive profile for an extended period of time, regardless of the source of MSCs, but particle preparation can increase the cost and complexity when compared to the use of free small molecules. (C) MSCs can be genetically engineered to overexpress a variety of different therapeutic molecules, including anti-inflammatory cytokines and growth factors, either to boost their innate functions or to overexpress other therapeutics and broaden their application to other diseases such as cancer. Viral vector–based genetic engineering typically has more efficient and durable gene expression but has some safety concerns because genes are integrated into the target cell genome. Nonviral vectors are safer, but the transfection efficiency is typically lower and gene expression is less durable. (D) OVs have also been used to engineer MSCs. MSCs function by shielding viruses to avoid immunogenicity and by releasing the virus in tumor tissue to kill tumor cells. One limitation is that regular OVs have only moderate infectivity, although this can be overcome by using certain viral variants with higher infectious capacity.
Engineering MSCs to go beyond the innate functions

Bioengineering is a powerful approach for expanding the therapeutic scope of MSCs beyond their innate functions. This can be achieved by engineering MSCs to secrete either poorly expressed or non-native therapeutic proteins (Fig. 2). A key example of this approach is in the use of MSCs to generate anticancer therapeutics. Systemic drug toxicity is a pressing concern in chemotherapy and related cancer treatment (65). Unlike synthetic biomaterials such as nanoparticles, MSCs have intrinsic capability to temporarily evade the immune response and home to tumors (66). With these unique carrier features, engineered MSCs have been reimagined as anticancer “Trojan horses” that are able to safely deliver large doses of cancer-targeting biologics with a single MSC dose (Table 4). This approach was validated by Sasportas et al. (67), in which engineered MSC Trojan horses delivered TNF-related apoptosis-inducing ligand (TRAIL) to cancer cells, suppressing tumor growth in a highly malignant glioblastoma mouse model. MSCs have also been used to express other proteins, including herpes simplex virus–thymidine kinase (HSV-TK), enzymes for converting chemotherapy prodrugs into their active toxic compound (i.e., ganciclovir) (68, 69), soluble VEGF receptor-1, and thrombospondin-1, all of which have an antitumor effect (Table 4).

To avoid the off-target toxicity of the antitumor biologics produced by MSCs, MSCs have been engineered to only release their biologics in response to stimuli such as mechanical cues that are unique to the tumor’s physical characteristics, rather than release the biologics nonspecifically throughout the body (70). This was accomplished using a YAP/TAZ promoter, which is activated when the cells sense the stiff, collagen-rich matrix environment of solid tumors. YAP/TAZ-engineered MSCs targeted sites of high collagen deposition in the lungs of mice inoculated with metastatic Luc-RFP-231 breast cancer cells (70). By placing expression of the prodruk-activating enzyme cytosine deaminase under the control of the YAP/TAZ promoter, the cells sense the stiff, collagen-rich matrix environment of solid tumors. YAP/TAZ-engineered MSCs targeted sites of high collagen deposition in the lungs of mice inoculated with metastatic Luc-RFP-231 breast cancer cells (70). By placing expression of the prodruk-activating enzyme cytosine deaminase under the control of the YAP/TAZ promoter, MSCs were able to locally activate the prodru 5-fluorocytosine and reduce tumor burdens in collagen-rich areas of mice, without inducing drug-related systemic toxicity (70).

Oncolytic viruses (OVs) are a relatively recent development in cancer therapy, which uses viruses that are engineered to either directly lyse tumor cells or trigger antitumor immunity against cancerous cells (71). However, humoral immunity responses can quickly neutralize the efficacy of systemically injected OVs through various processes, including inactivation by complement proteins and immune-mediated phagocytosis (72, 73). To protect OVs from the recipient humoral immune system, cell-based carriers have been demonstrated as a useful approach for both producing OVs in vivo and delivering them to the tumor site (74). One limitation of using MSCs as OV carriers is that MSCs demonstrate only moderate infectivity when

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Table 3. Examples of bioengineered MSCs as living drug factories.

| Disease model                      | Cell source     | Engineering approach | Expressed therapeutic                               | Reference         |
|------------------------------------|-----------------|----------------------|-----------------------------------------------------|-------------------|
| Neurodegenerative diseases         | Human BM-MSC    | Priming              | Neurotrophic factors (NTFs; include GDNF, BDNF, HGF, VEGF) | Gothelf et al. (59) |
| Diabetes mellitus                  | Human AD-MSC    | Priming              | Insulin                                             | Thakkar et al. (80) |
| Myocardial infarction              | Mouse BM-MSC    | Priming              | Hypoxia-inducible factor 1, angiopoietin-1, VEGF, erythropoietin. | Hu et al. (111) |
| Inflammatory diseases              | Human BM-MSC    | Microparticle (MP) delivery and priming | Indoleamine-2,3-dioxygenase (IDO)                  | Ankrum et al. (63) |
| Damaged tissue repair              | Human AD-MSC    | Biomaterial encapsulation | VEGF, HGF                                           | Kim et al. (114) |
| Colitis                            | Human BM-MSC    | Engineered hydrogel  | IDO, programmed death ligand 1 (PD-L1), CCL8, CXCL9, CXCL10 | Garcia et al. (119) |
| Parkinson's disease                | Human BM-MSC    | Genetic              | Glial cell line–derived neurotrophic factor (GDNF)  | Hoban et al. (163) |
| Juvenile Huntington's disease      | Human BM-MSC    | Genetic              | Brain-derived neurotrophic factor (BDNF)            | Deng et al. (164), Pollock et al. (165) |
| Parkinson's disease                | Human UC-MSC    | Genetic              | Hepatocyte growth factor (HGF)                      | Liu et al. (166)  |
| Multiple sclerosis                 | Human AT-MSC    | Genetic              | Interferon-β (IFN-β)                                | Marin-Banasco et al. (167) |
| Myocardial infarction              | Rat BM-MSC      | Genetic              | Thioredoxin-1 (Trx-1)                               | Suresh et al. (64) |
| Osteoporosis                       | Porcine BM-MSC  | Genetic              | Bone morphogenic protein-6 gene                     | Pelled et al. (168) |
| Osteoporosis                       | Mouse BM-MSC    | Genetic              | B cell–specific Moloney murine leukemia virus integration site 1 (Bmi1) | Chen et al. (169) |
| Hepatic fibrosis/cirrhosis         | Human BM-MSC    | Genetic              | Decorin                                             | Jang et al. (170) |

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transduced (75). To overcome unsatisfactory infectivity, certain viral variants (i.e., Ad5/3 oncolytic adenovirus) can be used that are able to infect MSCs with higher viral loads (76).

Several MSC-based anticancer therapeutics have reached the clinical stage. For example, an ongoing phase 1/2 study of intraperitoneally administered AT-MSCs expressing both the oncolytic measles meses virus and a membrane-bound sodium iodine sympporter is being investigated to enhance the measles virus' therapeutic potency in patients with recurrent ovarian cancer (NCT02068794) (77). Another phase 1/2 trial (TREAT-ME1, NCT02008539), involving intravenously administered autologous MSCs engineered to express the tumor-specific HSV-TK gene, was also investigated for the treatment of gastrointestinal tumors (78). While the study was terminated in 2016 due to an insufficient number of patients meeting the eligibility criteria, investigators reported favorable safety and tolerability in patients who received the treatment (78). Last, TRAIL-releasing MSCs (MSC-TRAIL) are also being clinically investigated as a therapeutic against inoperable lung adenocarcinomas, and recruitment is currently underway for a phase 1/2 clinical trial (NCT03298763).

These examples demonstrate that engineered MSCs may provide a novel axis for developing a reproducible product, where quality control and potency hinge on the engineered behavior of the cells, rather than their comparatively unpredictable innate immunomodulatory properties. Although we focused on examples in anticancer therapeutics, there are many other instances of MSCs being engineered beyond their innate immunomodulatory and regenerative functions to treat various diseases (79). For example, MSCs have been preconditioned toward an insulin-secreting phenotype for the treatment of diabetes mellitus. Administration of these insulin-secreting MSCs in clinical trials has been shown to be safe and provide long-term control of hyperglycemia through a decreased exogenous insulin requirement and elevated levels of C-peptide, a molecule co-released with insulin from the pancreas (80). Regardless of the application, while engineered MSCs have the potential to better control therapeutic function compared to unaltered MSCs, ideally, the approach should be simple, robust, and amenable to large-scale cost-effective manufacturing.

OVERCOMING CLINICAL CHALLENGES FROM ADMINISTRATION

Just as living cell-based therapies pose unique challenges for meaningfully assessing in vitro potency, the behavior of MSCs, including the pharmacokinetic and biological properties of the infused MSCs, can be affected by the mode of administration (81–84). Specifically, factors such as the injection site, injection device properties, and properties of the carrier materials/buffer can affect the administration of cells (Fig. 1B). For example, different injection sites can cause variations in backpressure/reflux, and injection device properties (needle size/geometry) can cause variations in shear rate and shear stress during injection that are known to affect the viability of injected cells. The impact of these factors and potential solutions has been thoroughly reviewed elsewhere (84). In this section, we will summarize the various clinical challenges encountered with locally and systemically administered MSCs, and how these clinical findings can be leveraged to engineer more therapeutically consistent and effective MSC therapies.

### Challenges associated with different administration routes

#### Local administration

Local administration is commonly used in clinical indications as it provides direct access to the disease site. As of 2018, 49% of registered MSC clinical trials use localized delivery (85). Most MSC therapies that have progressed to late-stage clinical trials have used local MSC administration (i.e., intrathecal, intralesional, and endocardial) within various clinical indications including lower back pain, perianal fistulas, and chronic heart failure (86). Local administration of MSCs is a more controlled delivery approach, making it easier to access the

| Disease model                   | Cell source                | Expressed therapeutic                                                                 | Reference          |
|---------------------------------|---------------------------|--------------------------------------------------------------------------------------|--------------------|
| Pancreatic carcinoma            | Murine BM-MSC             | HSV-TK activating ganciclovir                                                       | Conrad et al. (171) |
| Hepatocellular carcinoma        | Murine BM-MSC             | HSV-TK activating ganciclovir                                                       | Niess et al. (69)  |
| Pulmonary melanoma metastasis   | Rat BM-MSC                | HSV-TK activating ganciclovir                                                       | Zhang et al. (21)  |
| Glioblastoma                    | Human BM-MSC              | HSV-TK activating ganciclovir + TRAIL secretion                                     | Shah (172)         |
| Lewis lung cancer metastasis    | Murine BM-MSC             | Soluble vascular endothelial growth factor receptor-1 (sVfgf-1)                      | Hu et al. (173)    |
| Glioblastoma                    | Human BM-MSC              | Thrombospondin-1                                                                      | Choi et al. (174)  |
| Metastatic breast cancer        | Human BM-MSC              | Cytosine deaminase under the control of the YAP/TAZ promoter activating 5-fluorocytosine | Wu et al. (94)     |
| Prostate cancer                 | Human BM-MSC              | Thapsigargin-based prostate-specific antigen (PSA)–activated prodrug (G114)         | Levy et al. (61)   |
| Glioblastoma                    | Human AT-MSC              | Adenovirus expressing soluble hyaluronidase (ICOVIR17)                              | Martinez-Quintanilla et al. (23) |
| Glioblastoma                    | Human BM-MSC              | Oncolytic herpes simplex virus (oHSV)                                               | Duebgen et al. (175) |
| Ovarian cancer                  | Human menstrual blood MSC | Oncolytic adenovirus                                                                 | Alfano et al. (176) Moreno et al. (177) |
disease site that often results in better therapeutic responses (87). In ischemic stroke, for example, locally administering MSCs to the damaged site has shown to be more effective than intra-arterial and intravascular MSC injection in improving the neurological severity score (88). However, there are still clinical challenges associated with local administration that hinder therapeutic efficacy, primarily due to insufficient retention and survival of transplanted MSCs at the site of administration.

**Insufficient retention and survival**

Retention here is defined as the duration of localization of cells at the target site. The lack of retention following local administration has been attributed to multiple issues after administration, including cell death due to the hostile environment encountered at the disease site and poor engraftment into the tissue (89, 90). Although highly dependent on the clinical application, some cases have shown that less than 5% of administered cells remain at the site of injection in the hours following transplantation (91). For example, in a study using intracoronary injection of bone marrow stem cells in patients with MI, only 2.1% of radiolabeled stem cells remained at the site of injection after ~1 hour. In addition, most of the remaining signal was found primarily in the liver and spleen (92). This clinical study highlights that, despite injecting MSCs directly at the damaged tissue site, retention in these regions is still a major concern. Furthermore, the cells that are at the target site are often no longer viable due to immune-mediated damage and apoptosis (89, 91, 93). For instance, an in vivo rat study showed an undetectable level of viable MSCs 2 days following local injection at the site of MI (94). Moreover, limited diffusion of nutrients and oxygen can also affect the survival of cells following administration. Cells must be within ~200 μm of the nearest blood vessel for sufficient nutrients and oxygen, but it may take many days for vascularization to reach the cells, leading to cell death (95). Because nonviable cells have a reduced capacity to produce therapeutic biologics, this can compromise the potency of the MSCs. Together, these studies highlight that both the retention and survival of MSCs following local administration of MSCs must be enhanced to improve the therapeutic outcome.

**Systemic administration**

While local delivery of MSCs can help deliver paracrine factors directly to the diseased tissue, local administration is not a feasible option for many clinical indications, as more invasive injections can cause serious complications in many diseases (96). Alternatively, intravenous injection of MSCs is used, but the therapeutic utility has been limited due to insufficient homing to the target site. Furthermore, using systemic administration of MSCs has led to key challenges, namely, the instant blood-mediated inflammatory reaction (IBMIR) and insufficient residence time at the target site.

**Instant blood-mediated inflammatory reaction**

Elevated concentrations of procoagulants like tissue factor (TF) on the surface of MSC serve as a potent trigger for IBMIR, compromising cell engraftment, cell lifetime, and therapeutic potency (97–99). TF triggers the extrinsic pathway of coagulation, leading to thrombin generation, platelet activation, and fibrin cross-linking, which all contribute to adverse clinical outcomes. For instance, the intravenous infusion of allogeneic UC-MSCs into two patients with renal transplantation and chronic kidney disease triggered thrombosis in both patients and required emergency treatment to dissolve the resulting thromboemboli (98). Interestingly, both TF expression and the magnitude of IBMIR with MSC products used in clinical trials depend on the tissue and donor source as well as the passage number (100–102).

In addition to triggering coagulation, IBMIR following MSC contact with human serum has been demonstrated to activate complement in all three classical, alternative, and lectin pathways (103). Coagulation and complement pathways are known to strongly interact in vivo, with cross-talk between the two pathways leading to synergistic effects that enhance therapeutic MSC dysfunction and cytotoxicity (104). Consequently, the clinical safety of systemic MSC therapies in patients relies on the optimal control of IBMIR.

**Insufficient residence time and homing**

When MSCs are delivered systemically, a key factor for exerting maximal therapeutic benefit is their ability to remain in circulation for long enough to deliver therapeutic payloads to the damaged tissue. However, it is well known that intravenously administered MSCs are immediately concentrated in the lung capillaries and phagocytosed by monocytes within 24 hours (88, 105–107). This limits the MSC’s ability to deliver therapeutic payloads to the host environment via secreted paracrine factors to a short period of time following injection (106, 108) and limits cell homing to target tissues (i.e., bone marrow and nervous system). Entrapment of MSCs in the lung capillaries also increases susceptibility to immune clearance (83, 108). For example, clinical studies on intravenous administration of radiolabeled MSCs for MI showed a complete lack of MSC homing in the infarcted myocardium following intravascular injection (92, 109). In addition, a recent phase 1 clinical trial by Schweizer et al. (110) indicated that unmodified MSCs did not home to primary prostate tumors in adequate levels to warrant further development, although this may have been due to insufficient sensitivity for MSC detection. Alternatively, it is also possible that mouse tumors are not representative of human tumors, and therefore, promising preclinical results fail to be translated to human patients, highlighting the need to use large animal models or develop alternative models that can recapitulate key features of human diseases before the clinical study. Nevertheless, these clinical findings illustrate that systemically injected MSCs often fail to properly home to target tissues, making them insufficient at delivering therapeutic payloads to diseased sites.

**Bioengineering approaches for improving MSC administration**

**Strategies to improve local administration**

To improve the local administration of MSCs, multiple strategies have been investigated (Fig. 3). Among these strategies, priming MSCs in vitro is a simple approach. For example, hypoxic priming up-regulated expression of prosurvival factors such as hypoxia-inducible factor 1, which can help MSCs adapt to the disease site that is typically hypoxic. Consequently, hypoxia-primed MSCs exhibited ~40% less cell death on day 3 after intramyocardial injection compared with nonprimed MSCs in a rat model of MI, resulting in improved vascularization in the infarcted myocardium and better therapeutic efficacy (111). However, the effect of priming may not be preserved upon cryopreservation/thawing because the expression of prosurvival factors is highly dependent on the environment.

Using biomaterials to encapsulate MSCs is another promising strategy to overcome challenges associated with local administration. For example, when a rat model of MI was treated with alginate-encapsulated human BM-MSCs or non-encapsulated MSCs, alginate encapsulation significantly increased the retention of MSCs at the infarction site and improved the therapeutic outcome with respect to increased microvascularization and decreased scar formation (112).
While it was not clear in this study whether cell survival was also improved, biomaterial encapsulation has been demonstrated to prolong MSC survival by providing a mechanical barrier that helps both position cells at the target site and shield them from immune attack (91, 93). For example, in a separate rat MI model, immunohistology studies in vivo showed that MSC survival was sustained for up to 16 days following delivery of HGF-overexpressing MSCs in a synthetic peptide-based hydrogel compared to native MSCs that did not survive past day 2. This engineered MSC therapy demonstrated superior reduction in scar formation, accelerated angiogenesis, and increased ventricular wall thickness compared with native MSC (94). In another study, when alginate encapsulated MSCs were subcutaneously inoculated in mice with GvHD, a high percentage of encapsulated MSCs were alive 30 days after subcutaneous inoculation. Interestingly, subcutaneous inoculation of free MSCs also had a similar therapeutic effect in the same study, indicating that alginate encapsulation of MSCs may not be required in this specific condition. However, the lack of viability data for free MSCs makes this conclusion questionable, as free MSCs can survive for less than 30 days in the injection site; thus, the lack of difference may simply be because the GvHD animal model is relatively easy to treat, and it is hard to see the benefit of alginate encapsulation (113).

Compared with the above in vivo studies, where some key quantitative information is missing due to difficulty of extracting non-encapsulated MSCs, the difference between free MSCs and encapsulated MSCs has been more precisely characterized in vitro. For example, when MSCs were encapsulated in a fibrin-based scaffold, more than 95% of MSCs were viable for 14 days; in contrast, non-encapsulated MSCs had a viability of ~80.4 ± 10% on day 6 and 76.7 ± 3.6% on day 14 in vitro. Moreover, when encapsulated MSCs or non-encapsulated MSCs were added to the upper chamber of a Transwell system, whose lower chambers were filled with PBMCs stimulated with phytohemagglutinin, only 1.8 ± 0.7% of encapsulated MSCs migrated out of the scaffolds, while 88.7 ± 8.1% of non-encapsulated MSCs migrated away from the initial site after 2 days. Consequently, encapsulated MSCs exhibited up to 10-fold higher local secretion of beneficial soluble factors, such as VEGF and HGF, in comparison to non-encapsulated cells, with the secretion highly dependent on cell number (114). Furthermore, hydrogels have been engineered more recently to boost MSCs potency in vitro. Tuning the in situ mechanical properties, such as pore size and stiffness, of 3D hydrogels that encapsulate injected MSCs has been shown to influence the relative cell proliferation, cell survival, and secretion of beneficial cytokines (115–117). For instance, Cai et al. (116) varied the amount of the thermoresponsive polymer poly(N-isopropylacrylamide) to alter the shear storage moduli of an encapsulating hydrogel. The best formulations showed more than twofold increases in cell proliferation on day 14 and up to threefold higher mRNA expression of relevant factors, such as angiopoietin and fibroblast growth factor-2, compared to formulations without the thermoresponsive polymer. Along with tuning mechanical properties, synthetic hydrogels with added linker molecules, including activated peptides and pro-inflammatory cytokines like IFN-γ, have also been explored as a technique to enhance MSC survival, persistence at the target site, and cytokine secretion (118, 119). For example, hydrogels engineered with the adhesive integrin-specific peptide GFOGER compared to the non-adhesive peptide GAOGER have been shown to significantly enhance the in vitro secretion of relevant cytokines, such as IL-8 and VEGF, and subsequently enhance MSC survival, engraftment, and bone repair in an in vivo mouse bone defect model (118).

While these studies demonstrated the impact of biomaterial encapsulation on the retention and survival of MSCs in vitro, additional work is still needed to further validate this in vivo. Nevertheless, promising results from the above preclinical studies using biomaterials to encapsulate MSCs have motivated their advancement to clinical trials. For example, Anterogen Ltd. is investigating the use of a hydrogel sheet containing allogeneic AT-MSCs in a multitude of diseases, including a phase 3 study on diabetic foot ulcers (NCT03370874), a phase 2 study on burn injury (NCT03183648), a...
Bioengineering strategies are being studied to address challenges from systemic administration related to both IBMIR and the insufficient residence time and homing of MSCs (Fig. 3). To attenuate IBMIR, Moll et al. (85) have recently advocated for the regular use of low-dose anticoagulants like heparin in the clinical setting. In addition, ABO antigens, complement, and coagulation factors that may be found in the AB plasma (ABP) used in MSC culture medium can amplify IBMIR in vitro (120). Replacing the ABP with a more defined, nonimmunogenic human serum albumin (HSA)–based supplement for MSC culture medium has been suggested as an approach to reduce the risk of IBMIR in MSC products. Recent clinical studies on GvHD showed an improved therapeutic effect when replacing ABP with HSA-supplemented medium and adding a low dose of heparin to MSC therapies (121, 122). This study highlights a simple, scalable, and clinically translatable technique for potentially improving the outcomes of many MSC therapies. In addition to the solutions discussed above, other bioengineering approaches have served as a useful platform in addressing MSC-mediated IBMIR. For instance, genetic engineering approaches (i.e., CRISPR-Cas9 or antisense RNA) to reduce expression of TF by MSCs, as well as engineering the MSCs with heparin cell surface coatings to prevent coagulation and complement properties, are bioengineering alternatives to systemic anticoagulation that have shown promise in preventing MSC-mediated IBMIR (97–99, 123). Furthermore, engineering MSCs to express blood regulatory molecules, such as CD46 (124), or pathway inhibitor (85, 124), may be a beneficial strategy for suppressing IBMIR following systemic MSC administration. There are many promising bioengineering solutions to mitigate the effects of IBMIR. Translating these approaches into a clinical setting may provide a new tool for avoiding adverse IBMIR-related events following systemic MSC administration.

Independent of IBMIR, additional protective bioengineering strategies to increase MSC residence time and sufficiently deliver MSCs to target tissues have been developed in recent years. To enhance residence time, Mao et al. recently demonstrated a microencapsulation technique, in which individual MSCs were encapsulated in alginate-poly-γ-lysine (PDL)-alginate (APA) microgels (particulate hydrogels with dimensions in the range of 30 to 50 μm). Using a single-cell microgel encapsulation approach has several distinct advantages compared to typical larger multicellular hydrogels for systemic administrations: The advantages include a reduced fibrotic capsule formation, a reduction in diffusion limitations that lead to hypoxic effects, and a higher surface area to volume ratio, which facilitates the release of biologics from encapsulated cells (125). Specifically, in the study by Mao et al., unlike a regular hydrogel that has a large volume and is not suitable for intravenous injection, the encapsulating microgel layer is on the order of 10 μm and can be easily injected intravenously. Furthermore, the encapsulating material did not interfere with the ability of MSCs to secrete therapeutic anti-inflammatory cytokines. Encapsulating MSCs into microgels significantly increased their residence time in vivo. Interestingly, encapsulating multiple cells per microgel had a half-life of >50 hours, whereas encapsulating a single cell per microgel had a half-life around 20 hours, although both were longer than unmodified MSCs that had a half-life of <2 hours in a mouse model (88, 108). Further analysis indicated that, compared with single-cell microgel encapsulates, multicellular microgel encapsulates had higher levels of collagen I and lower oxygen tension, both of which positively contributed to the prolonged residence time of encapsulated MSCs. These results indicate that controlling the cell number inside microgels is an important consideration to achieve the desired residence time of MSCs. Moreover, this improved in vivo residence time of APA-treated MSCs occurred despite the presence of innate and adaptive immune clearance mechanisms, leading to a significant improvement of therapeutic outcome in a bone marrow transplant model (108).

Engineering approaches that up-regulate ligands on MSCs have also improved homing by improving the interaction between MSCs and the inflamed endothelium or chemokines proximal to the disease site. Many studies have used various cocktails of soluble factors to increase the expression of CXCXCR4 and matrix metalloproteinases (MMPs), an approach that has yielded preclinical success in homing and disease recovery (126–130). For example, priming MSCs with valproic acid and lithium induced CXCXCR4 and MMP-9 up-regulation, which subsequently increased MSC homing, improved functional recovery, and reduced the infarct volume in the brain when tested in a rat model of cerebral ischemia (128). Alternatively, RNA- or DNA-based genetic engineering is a well-established approach that can be used to induce the continuous synthesis of homing ligands in vivo. For example, mRNA transfection was used to induce the expression of the homing ligands PSGL-1/SLeX as well as the anti-inflammatory cytokine IL-10 in MSCs (131). The engineered MSCs displayed increased homing to sites of inflammation/disease and showed an improved therapeutic impact in mouse models of skin inflammation (131) and experimental autoimmune encephalomyelitis (132). In addition, viral transduction protocols have been used to permanently overexpress homing factors in MSCs. Most notably, overexpression of CXCXCR4 increased the homing of engineered MSCs to the ischemic myocardium (133, 134), bone marrow (135, 136), and damaged intestinal mucosa (137) in preclinical models. Other bioengineering techniques have been investigated to chemically engineer the cell surface to improve MSC adhesion and homing, including hyaluronic acid coatings (138), enzymatic modifications (139, 140), binding of adhesion molecules (141–143), and the attachment of docking systems to bind homing antibodies such as PSGL-1 (22, 144).

Magnetically labeled MSCs have also been investigated as a means to direct cells to the target tissue with an external magnetic field (145–147). Iron oxide has no perceivable effects on MSC function in vitro and in vivo at treatment doses of ~23 pg per cell, and is deemed a safe material for MSC bioengineering (146, 148). Iron oxide–labeled MSCs demonstrated a 10-fold increase in retinal homing following intravenous infusion in a rat model, with magnetic MSCs better penetrating the inner and outer retina compared to nonmagnetic MSCs 1 week after injection (147). Furthermore, the improved homing of magnetic MSCs enhanced the overall expression of IL-10 in the retina, although this is believed to be largely due to increased endogenous expression of cytokines from retinal cells rather than from the MSCs directly (149).

Overall, there has been a vast amount of preclinical investigation into bioengineering strategies to improve the residence time and homing, but few have been translated into clinical trials. These bioengineering strategies may help to improve clinical outcomes for various diseases by addressing the challenges of insufficient MSC residence time and homing to diseased sites.
OVERCOMING CLINICAL CHALLENGES FROM THE HOST

Host cytotoxic responses against MSCs

While the potency of the MSC product and the route of administration are critical parameters for the efficacy of MSC therapies in clinical trials, host factors are also an important consideration. Variations in the host cytotoxic response, inflammation status, and tissue microenvironment such as hypoxia and extracellular matrix (ECM) (stiffness) have been demonstrated as important factors in the efficacy of MSCs after administration (Fig. 1C) (58). This was most recently demonstrated in a study characterizing the PBMCs of patients who had variable therapeutic responses to intravenously administered MSCs (7). In this study, 16 patients with severe steroid-resistant grade 3 to 4 GvHD were treated with MSCs, and PBMCs were collected from each individual 24 hours after intravenous MSC administration. Subsequent analysis of the host PBMCs revealed that clinical responders to the MSC treatment elicited ex vivo cytotoxicity against MSCs that was almost fourfold higher than clinical nonresponders (7). This study demonstrated that the magnitude of immunosuppression in vivo is correlated to the recipient cytotoxic response against the infused MSCs.

Although the mechanisms underlying the role of the host response in a clinical setting are still unclear, several preclinical observations provide useful insight into its correlation with therapeutic outcome. In 2017, Galleu et al. (7) published an important study that identified a mechanism contributing to the immunosuppressive efficacy of intravenously administered MSCs that was not dependent on the typical CQAs of the cell product in isolation (i.e., IDO and TNF-α receptor expression). In a mouse model, it was observed that intravenously injected hMSCs underwent perforin-dependent apoptosis induced by the recipient immune system. The subsequent phagocytosis of apoptotic MSCs by recipient macrophages triggered the immune cells to produce additional IDO intracellularly, increasing the overall systemic IDO expression by ~2.5-fold. Furthermore, de Witte et al. (82) demonstrated in a mouse model that the phagocytosis of infused hMSCs by monocytes in the first 24 hours following injection triggered the immune cells to adopt an immunoregulatory phenotype. A separate study found that monocyes containing phagocytosed MSC debris then migrated to multiple tissues and established further immunotolerance in the adaptive immune system by promoting an immunoregulatory phenotype in lymphocytes (82). Together, these observations demonstrate that, in some clinical indications, stronger cytotoxic responses against MSCs improve the therapeutic effect of immunosuppressive MSCs by facilitating the adoption of a systemic immunoregulatory phenotype in the host. To this end, evaluating in vitro PBMC-mediated cytotoxicity using host immune cells may potentially be used as an indicator for success when recruiting patients for MSC treatment.

The importance of the host immune response on the efficacy of MSC therapy is also demonstrated in other diseases, such as cardiac ischemia (150). For example, a recent study has shown that the therapeutic benefit of cardiac stem cell therapy is not due to the production of new cardiomyocytes but through an acute sterile immune response mediated by host derived CCR2+ and CX3CR1+ macrophages. These macrophages resulted in the alteration of cardiac fibroblast activities, reduction in the ECM content, and functional improvement. Intracardiac injection of a chemical inducer, which induced a similar level of CCR2+ and CX3CR1+ macrophages locally, also provided functional improvement in the cardiac ischemic injury model.

Together, while the exact mechanism may vary depending on the disease and cell therapies used, the host immune responses play important roles in mediating the therapeutic benefit provided by cell-based therapies. Therefore, variations in the host immune response can also be responsible for the variability of cell therapies.

Host disease stage/severity

In addition to the cytotoxic response, host factors related to the stage of disease progression and disease microenvironment may also have implications in better predicting the clinical efficacy of MSC therapies. For example, many studies have suggested that early treatment is better than late treatment to achieve maximal therapeutic efficacy in certain indications. This has been best illustrated by a recent phase 2 study conducted by Athersys Inc. (NCT01436487), which investigated their allogeneic MSC product, Multistem, for treating ischemic stroke. The study was a clinical failure, showing no improvement in neurological outcome compared to placebo controls (151). However, retrospective analysis demonstrated that patients treated <36 hours following the onset of stroke had improved secondary outcomes compared to those treated between 36 and 48 hours. Hoping to leverage the temporal dependency of Multistem’s regenerative potency, the therapy will now be administered exclusively within the first 36 hours following stroke for their phase 3 study (NCT03545607).

At the clinical level, there has been limited progress in understanding the connection between therapeutic outcome and disease stage/severity, in part, due to difficulties in routinely sampling critically ill patients (151). However, evidence from preclinical studies indicate that the microenvironment of the tissue surrounding the MSCs may be a contributing factor to the therapeutic efficacy in different disease stages. Inflammation, hypoxia, and the ECM in the disease site microenvironment are dynamic, and each parameter can influence MSC function in vivo (20). First, MSCs appear to be more potent in suppressing GvHD when inflammation is high and less potent when inflammation is low (20). Second, previous studies on environmental hypoxia have indicated that exposing MSCs to hypoxic conditions can induce various soluble bioactive molecules and enhance their angiogenic and regenerative potential (152). Third, in vitro studies on the impact of the ECM demonstrate that MSCs seeded on stiff surfaces have reduced suppression of allogeneic lymphocyte activation compared to MSCs seeded in softer, collagen-based 3D scaffolds (153). The stiffness of the ECM is positively correlated with the severity of fibrotic disease, which is highly variable between patients, thus likely contributing to mixed therapeutic outcomes (154). Moreover, along with the variations in inflammation, hypoxia, and the ECM, the increase in the number of damage-inflicting cells (T cells) with increasing disease severity may also influence the therapeutic effect of MSCs in vivo. For example, effector T cells in GvHD increase from nondetectable levels in the early disease stages to 90% of the total disease population in the late stage (155). Hence, the MSC doses (i.e., the number of infused MSCs) administered in late-stage GvHD become therapeutically insufficient, as they are substantially outnumbered by effector T cells. Together, these observations demonstrate that while cell engineering is able to boost the potency of MSCs, evaluating the status of recipients—especially the disease stage—will help optimize the dosing regimen and improve the clinical predictions of therapeutic response to MSC therapies.

Other host factors

In addition to the host immune response, other host factors may also affect the recruitment of MSCs to the disease site, which, in
turn, can affect the therapeutic outcome for some clinical indications. For example, capillaries (10 to 15 μm in diameter) trap systemically infused MSCs (~20 μm in diameter) (83), compromising MSC homing to other organs. Moreover, MSC homing is also highly dependent on the secretion of chemokines, as the magnitude of chemokine secretion by the target organ in the host may be insufficient to recruit MSCs efficiently (156). Continued investigation of bioengineering strategies that can overcome these issues may represent a novel strategy to fully unleash the therapeutic potential of MSCs.

Bioengineering strategies relevant to host factors
Clinical and preclinical observations related to the role of the host in facilitating the MSC therapeutic response have inspired novel approaches to controlling these factors in a clinical setting. Patient stratification based on the host cytotoxic responses against MSCs or the disease severity/stage can be a simple strategy to help recruit patients who can likely benefit from MSC therapies (Fig. 4). This can be achieved by incubating MSCs with PBMCs from patients and testing the ability of PBMCs to induce MSC apoptosis in a cytotoxic assay (7). Priming the hosts to establish a microenvironment that can better use the therapeutic potential of MSCs represents another avenue to improve their potency and resulting response rate. For example, the water-soluble antioxidant vitamin C has the capacity to prevent oxidative stress and reduce damage to transplanted cells (157). In a spinal cord injury model, animals receiving intraperitoneal injection of vitamin C in combination with local injection of MSCs at the site of spinal cord injury had a better therapeutic outcome compared with MSCs or vitamin C alone (157). MSC recipients can also be primed with vasodilators such as sodium nitroprusside before systemic infusion of MSCs to circumvent MSC entrapment by the host capillaries (158). Preclinical studies have indicated that, following vasodilation, the accumulation of MSCs in the lungs was reduced by 15% and the recruitment of MSCs to the bone marrow was increased by 10 to 50% compared to untreated control hosts. Boosting the chemokine secretion by the target organ in the host has also been used to improve MSC homing (156). For example, preclinical studies have shown that, before systemic infusion of MSC, animals primed with irradiation had MSC homing to bone marrow increased by twofold compared to unirradiated controls. Irradiation of the animals increased the secretion of the chemotractant SDF-1 at the damaged bone marrow site, which facilitated improved homing of MSCs in the irradiated population (156). Although host priming remains at the preclinical stage, continued investigation into this approach through irradiation, vasodilation, or other methods may represent a novel strategy to fully unleash the therapeutic potential of MSCs.

Last, an improved understanding of the role of the host environment in MSC function can also be used to guide novel approaches for MSC engineering. For example, priming MSCs with inflammatory cytokines or hypoxia represents an interesting bioengineering approach to boost their potency toward therapeutic applications (152, 159). It may also be useful to engineer the MSC secretome so that it functions independently from the surrounding microenvironment. For example, MSCs overexpressing anti-inflammatory cytokines such as IL-10 or IL-35 have been shown to improve the therapeutic effect compared with native MSCs (160, 161). By exogenously secreting anti-inflammatory factors in a pro-inflammatory environment, MSCs can more efficiently reduce inflammation and better achieve their desired function.

CONCLUSIONS
Although MSCs undoubtedly have immunomodulatory and regenerative therapeutic properties, attempting to apply MSCs “as is” without a clear target has proved to be unsuccessful in most clinical studies. Without a well-defined target, developing more effective “next-generation” MSC therapies will be limited. Specifically, a clear definition of the targets, ideally from the beginning of a project, is critical to guide the design of better MSC therapies. Thinking more about the target up front will help researchers to see how the baseline levels achieved by MSCs are typically suboptimal to activate target biology. This will also help provide a solid rationale to engineer MSCs to more efficiently act on these targets by secreting relevant factors and/or by interacting with the target cells through cell-cell contact and ultimately improve the therapeutic outcome.

Fig. 4. Solutions relevant to host factors. (A) Patient stratification based on the host cytotoxic responses against MSCs or the disease severity/stage can be used to recruit patients who can benefit from MSC therapies. (B) Priming the host with vitamin C can scavenge free radicals that compromise the potency of MSCs. Furthermore, priming the host with a vasodilator or irradiation can facilitate the homing of MSCs to the target sites, resulting in better therapeutic outcomes. (C) The identified host factors that affect the function of MSCs can guide the development of better MSCs, which can complement the host priming strategy and ultimately improve the therapeutic outcome. In particular, MSCs can be engineered to improve the homing to the target sites, engineered to maximum potency, or programmed to function regardless of the host environment. However, it is not clear yet if homing can be engineered to achieve a meaningful boost in efficacy in humans.
Moreover, enormous challenges remain for MSC therapies—from the diverse origins of MSCs, the highly variable culture and cryopreservation conditions, the challenges associated with administration of MSCs, and the challenges of the host environment—that can also lead to unpredictable therapeutic outcomes. Continued exploration of engineering approaches that address these challenges should significantly improve the therapeutic efficacy for a broad range of clinical indications. In particular, boosting the potency of MSCs through engineering strategies such as small-molecule priming, MP engineering, and genetic modification (Fig. 2) provides a measurable property that can be examined throughout all stages of preclinical and clinical development, from well-defined potency assays and CQAs to therapeutic biomarkers in human clinical studies. It is also critical to ensure that these CQAs are preserved following cryopreservation and thawing at both the preclinical and clinical stages. Recent development of single-cell RNA sequencing will also enhance our understanding of MSC heterogeneity and phenotype shift during culture, which, in turn, may provide critical insights to improve the MSC manufacturing process (162). Coupled with other technologies such as iPSC-based MSCs and CRISPR-Cas9-based gene editing, there are many possibilities regarding what MSCs can functionally achieve. Furthermore, while local injection can position MSCs directly at the target site, the insufficient retention of MSCs can compromise the potency and duration of the therapeutic effect. Although strategies such as the use of biomaterials can help address these challenges, most studies have not decoupled cell survival from retention in vivo. Additional studies are needed to clarify this, which, in turn, may provide novel insights about the bottlenecking limitations or survival of MSCs and guide the design of engineering approaches to develop better MSC therapies. For systemic administration, it is critical to properly engineer MSCs to modulate the IBMIR and improve the homing of infused MSCs (Fig. 3). Learning how the host factors impact function and delivery of MSCs will help both inform engineering strategies and inspire new approaches to prime the host (Fig. 4). One caveat is that preclinical mouse models often have limitations in recapitulating the key features of human diseases. Moreover, the infusion volume and number of cells used in mouse models are also very different from those in clinical patients. For example, when MSCs are intravenously injected into rodents, the dose is typically around 50 million/kg. However, the dose for intravenous injection in human patients is typically 1 million to 2 million/kg (19). Because the paracrine factors secreted from MSCs are dependent on cell number, different dosing can significantly affect the therapeutic outcome, assuming the mechanism of action is similar for different species. Consequentially, many promising preclinical results cannot be translated into clinical success. To obtain meaningful results, future studies should explore the use of large animal models that can better mimic the host disease conditions and dosing regimen in clinical settings.

Last, it should be noted that many of the failures in characterization, cell delivery, and thawing variability are limitations in process development, which is critical for ensuring that all procedures are robust and reliable and deliver the expected and intended outcomes in a repeatable manner. Process development is particularly important when large doses of MSCs need to be manufactured for clinical use. Preclinical work should shift to using MSCs manufactured under Good Manufacturing Practice (GMP) to facilitate clinical translation. Rather than just be hopeful that MSC efficacy will be preserved from preclinical to clinical studies (and during scale-up) and that the mechanism of action will include a relevant target with a robust response, engineering strategies can and should be used to engineer the mechanism of action with specific target biology in mind. While nature provides a basic therapeutic framework for MSC-based treatments, bioengineering tools will be the key to shatter translational barriers.

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