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Mesenchymal Stem/Stromal Cells

Fate and function of exogenously administered mesenchymal stromal cells: current insights and future directions

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A B S T R A C T

The *in vivo* fate of mesenchymal stromal cells (MSCs), including their clearance, interaction with host tissues, and persistence, remains incompletely understood following systemic or local clinical administration to patients. Although immune-mediated clearance mechanisms, such as triggering of the instant blood-mediated inflammatory reaction, activation of coagulation and complement pathways, apoptosis and efferocytosis have been identified, their contributions to MSC function and efficacy are still under investigation. To address these knowledge gaps, an international panel of experts in MSC biology and clinical regenerative medicine convened to assess current evidence and define key unanswered questions. Discussions were structured around three thematic domains: (i) biodistribution and mechanisms of action following systemic delivery; (ii) biological implications of local or depot-based administration and (iii) the dynamics of MSC persistence and clearance *in vivo*. A major focus was on the role of MSC apoptosis and its immunological consequences, particularly interactions between apoptotic MSCs, phagocytes and endothelial barriers. This perspective highlights the most urgent research questions identified during the meeting and in follow-up discussions and proposes experimental strategies to move beyond traditional cell tracking toward interrogating functional persistence, immune modulation and delivery context. Addressing these gaps will deepen our understanding of MSC behavior *in vivo* and guide the development of safer, more predictable and more effective MSC-based interventions.

Key Words: apoptosis, extracellular vesicles, immune cells, instant blood-mediated inflammatory reaction, local delivery, mechanism of action, MSCs, persistence, systemic delivery.

Introduction

An invitation-only group of basic, clinical and translational scientists working on mesenchymal stromal cells (MSCs) gathered virtually in April 2025 (see author list) to discuss the cascade of events upon *in vivo* MSC delivery. The session was divided into three segments focusing on: (i) systemic delivery of MSCs; (ii) local and/or depot delivery; and (iii) *in vivo* persistence of MSCs. Each section included short presentations outlining the state of current knowledge, followed by in-depth discussions to probe critical knowledge gaps and formulate experimental questions. The result is a consolidated list of high-priority research directions to advance our mechanistic understanding of MSC behaviour *in vivo*. We anticipate that completion of these studies by session participants, as well as by other investigators, will deepen our understanding of MSC behavior and support the broader field in developing more rational, context-specific applications. The outcomes of ongoing studies aligned to this framework will be disseminated in future perspective pieces.

Across all proposed studies, comparative analyses should include MSCs from different tissue sources (e.g., bone marrow MSC (M), umbilical cord blood (CB), Wharton's jelly, adipose tissue (AT) and induced pluripotent stem cell (iPSC, iMSC), abbreviated as per ISCT's MSC committee statement [1]), and from multiple donors to identify source-specific heterogeneity, strengths and limitations. Additionally, in conjunction with other analytic methods, the use of cell-free DNA as a minimally invasive technique can be used to monitor MSC survival, clearance and biodistribution in real-time. The integration of machine learning and other artificial intelligence (AI) approaches will also be valuablefor analyzing high-dimensional data from various outcome measures [2–4].

The fate and function of MSCs following *in vivo* delivery to patients and various preclinical models have been extensively studied, including different modes of systemic and local delivery that may confer specific advantages depending on the clinical or biological context [5–9]. Importantly, the clinical modes of MSC delivery can affect the product safety and efficacy profiles, and their respective mechanisms of actions [6–9], e.g., to reduce adverse inflammation and to induce beneficial tissue repair pathways. Thereby, the mode of therapeutic cell delivery affects many clinically relevant parameters, such as MSC persistence/engraftment, cellular/tissue interactions and consequent bioactivity and mechanisms of action, which together form the foundation of and influence MSC therapeutic activity [6,7,10,11]. Hence, it is of crucial importance to systematically study the different modes of cell delivery in relation to the optimal desired therapeutic outcomes in patients [6–9].

In the following sections, we outline the current state of knowledge and open questions in and suggest cutting-edge experimental avenues to strategically move the field forward.

Systemic delivery of MSCs

Overall, studies that track systemically administered MSCs in animals and humans reveal limited cell persistence due to the rapid

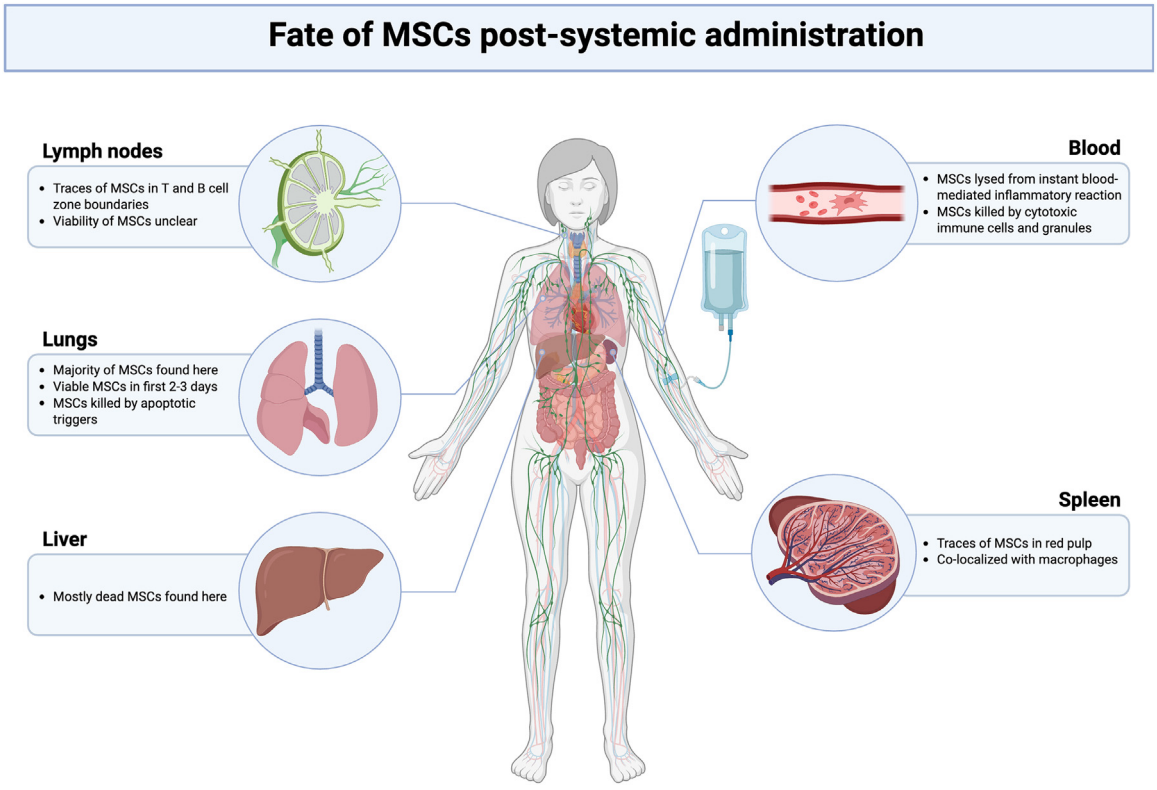

Fig. 1. Fate of MSCs delivered via the intravenous route. Contact with blood appears to be a harsh event, as MSCs can be lysed in a phenomenon known as the instant blood-mediated inflammatory reaction (IBMIR) [9,12,13] or killed by cytotoxic immune cells and granules. MSCs that survive are predominantly found in the pulmonary capillaries, where viable cell signals are detected for an average of 2–3 days. The majority of MSCs undergo apoptosis in the lungs and are largely nonviable after 24 h. After 24 h, dead MSCs or their debris can be found in the liver. Traces of MSCs have also been found in the spleen and lymph nodes, but their colocalization with macrophages suggests that these are cell debris taken up by phagocytes. (Color version of figure is available online.)

clearance of apoptotic and dead MSCs (Fig. 1). MSCs are most commonly delivered via the intravenous (IV) route, where contact with blood induces an **instant** protective response of the innate immune system (termed the IBMIR) toward the infused therapeutic cells [9,12–16]. The adverse triggering of IBMIR is most relevant in the minutes to hours after cell infusion and may compromise MSC survival, efficacy, and safety, all depending on their intrinsic hemocompatibility [7–9]. Any IBMIR-related adverse thromboembolic events are most evident within the first 24 ho of infusion and are an important safety consideration during patient monitoring [9,12,13].

Quantitative assessments have shown that 50–80% of MSCs are immediately rendered nonviable after IV administration as a result of IBMIR, primarily driven by complement-mediated cytotoxicity and natural killer cell (NK) activity, especially for allogeneic or xenogeneic MSCs [14–16]. MSCs that are not immediately killed are predominantly found in the pulmonary capillaries, where they can be detected for only 2–3 days on average, based on tracking studies in rodent models and human patients [9,14,17–23]. However, whether these signals represent viable cells, cell fragments carrying a label, or phagocytosed material is not always clear. MSC signals have also been detected in the liver, although these typically corresponded to dead MSCs [21]. In lymphoid organs such as the spleen and lymph nodes, MSC markers have been found co-localized with macrophages [24], suggestive of uptake of MSC debris.

In human patients who received MSC infusions, low levels of MSC DNA have been found in the lungs, lymph nodes, and intestines [25]. However, MSC DNA is largely undetectable in most tissues beyond 1 week post-infusion, indicating rapid clearance and limited long-term persistence. Thus, the majority of MSCs do not survive IV injection [14,15]; this may not be an undesirable outcome, as the apoptotic death of MSCs and the resulting host immune response have been implicated in anti-inflammatory effects [20,22,26]. Understanding how dying MSCs drive immune suppression *in vivo* could inform more effective treatment strategies and patient outcomes.

As a corollary to this, previous dogma regarding the migration of MSCs upon systemic administration has largely been discarded. There is no evidence, for example, that intravenously delivered MSCs, once lodged in the pulmonary capillaries, subsequently exit the capillaries or migrate across the alveolar-capillary barrier into the alveolar spaces. The prevailing evidence points to a “hit-and-run” mechanism in which most MSCs are rapidly cleared, yet their transient presence delivers a spectrum of immunomodulatory cues, apoptotic bodies, extracellular vesicles, and soluble mediators that are subsequently sampled by resident cells and tissues. Furthermore, infused MSCs can release mitochondrial DNA into the cytosol upon mitochondrial stress or damage and act as damage-associated molecular patterns (mtDAMPs). This immunogenic response is normally inhibited by caspase activity during apoptosis, resulting instead in an anti-inflammatory outcome that contributes to tissue repair and immune regulation [27–29]. Dissecting this early molecular dialogue will help to clarify why short-lived cells can produce durable clinical benefits and guide the rational engineering of MSC products capable of more precisely engaging endogenous repair pathways.

IBMIR upon systemic MSC delivery

Exposure of human MSCs to complement-active human blood, plasma, or normal human serum results in rapid killing of some, but not all, cells, highlighting the underlying issue of MSC heterogeneity. *In vitro*, approximately 57% of freeze-thawed MSCs are lysed within 1 h of normal human serum exposure, while the remaining cells display membrane damage and undergo progressive cell death [14,15]. In

turn, most of the complement-mediated killing of MSCs can be abrogated by the addition of EDTA or citrate to the serum, which both chelate Ca^{2+} and thus render the Ca-dependent convertases of the classical pathway of the complement cascade inactive. While EDTA leads to irreversible inhibition of complement, the addition of Citrate provides reversible inhibition of complement through re-supplementation of Ca^{2+}, to overcome the inhibitory effect of a given dose of Citrate in the blood sampling tube. Some studies also demonstrated that culture-expanded "fresh" MSCs are less susceptible to IBMIR than thawed MSCs that were derived from cryostorage [9,14]. This observation contributes to the ongoing controversies about the relative pros and cons of utilizing freeze-thawed cells [30–32].

Indeed, there are several aspects to the handling of MSCs that can augment the triggering of IBMIR. Conventional MSC products, typically cultivated in FBS-containing medium, have much lower intrinsic regulatory capacity to circumvent IBMIR than the prototypic hemocompatible endothelial cells [9,12,13], but they often possess features that can augment the triggering of IBMIR, such as increased surface expression of highly procoagulant tissue factor (TF/CD142) or surface exposure of phosphatidylserine. In particular, thawed MSCs readily retrieved from cryo-storage before infusion activate IBMIR more strongly than "fresh" MSCs derived from culture, and they often display features that can augment the triggering of IBMIR [30,33,34]. This effect is typically mediated through primary triggering of the complement cascade and concomitant engagement of complement receptor-bearing innate effector cells [30,34]. Furthermore, the uncontrolled release of DNA and other intracellular components from membrane-disrupted MSCs may provoke IBMIR and needs to be further studied. Although interferon-gamma (IFN-γ) licensing can boost the immunomodulatory activity after thawing, it may exacerbate IBMIR, particularly in cultured cells, since IFN-γ licensing increases MSC adherence by upregulation of integrins, which increases the likelihood of cell membrane damage during enzymatic detachment and could lead to a stronger IBMIR [30,34].

To the best of our knowledge, no studies have specifically investigated the traits of MSCs that survive IBMIR using *in vitro* functional assays (e.g., mixed lymphocyte reactions) or *in vivo* injury models. This knowledge gap could be addressed through single-cell omics profiling of MSCs that survive versus those that do not, ideally using cells derived from different primary tissue sources as well as MSCs derived from induced pluripotent stem cells (iMSCs). The extent of IBMIR activation likely depends on the expression of complement regulatory proteins such as CD59, CD46, and CD55, which may protect the cells from IBMIR [16]. This raises the possibility that subsets of MSCs with higher expression of IBMIR-protective receptors are more likely to survive and retain functional competence. Potential strategies to investigate these knowledge gaps are summarized in Table 1.

2) Systemic delivery triggers MSC apoptosis and clearance in the lungs and other organs

Apoptotic MSCs are biologically and functionally distinct from necrotic or dead MSCs [36]. Understanding these differences is essential, as each form of cell death triggers unique responses from host immune cells. Necroptosis and pyroptosis, for example, are forms of lytic cell death that lead to the release of inflammatory proteins. Apoptosis, on the other hand, is "immunologically silent," due in part to the action of activated caspases that suppress type I IFN production and dampen inflammatory signaling pathways [37]. The nature of the host response is therefore shaped by the mode of MSC death, a concept supported by foundational studies showing that apoptotic cells actively induce immunosuppression by inhibiting T cell activation and promoting anti-inflammatory cytokines such as Transforming Growth Factor Beta 1(TGF-β1) [38]. Importantly, the anatomical site at which MSC apoptosis occurs may influence the downstream immune responses. Tissue-resident macrophages exhibit substantial phenotype and functional diversity depending on their environment, and this heterogeneity may determine how apoptotic MSCs are cleared and what immunological signals are generated, which in turn can influence therapeutic outcomes [39]. Thus, both the mechanism of apoptosis and its spatial context warrant careful consideration.

This is all the more important as emerging evidence suggests that apoptosis may play a key role in contributing to the therapeutic effects of MSCs administered systemically, at least in certain disease

Table 1
Experimental opportunities to further clarify the impact of IBMIR on MSC fate and function.

Question	Proposed experiments
What proportion of MSCs survive initial IBMIR, and what features define these surviving MSCs?	Perform high-throughput single-cell omics profiling on MSCs following *in vitro* exposure to plasma or serum to identify survival-associated signatures, using freshly cultured cells that are less susceptible to IBMIR [9,12,15]. Compare surviving vs. non-surviving MSCs to determine defining cell markers, transcriptional states, metabolic features, and *in vivo* efficacy. Differences in *in vivo* efficacy would drive further experimental designs.
Do MSCs that survive initial IBMIR retain functional competence and proliferative capacity?	Isolate surviving MSCs after *in vitro* IBMIR exposure (e.g., whole blood vs. complement-active/inactivated plasma or normal human serum (NHS vs. NHS/EDTA)). Assess proliferation, differentiation, immunomodulation in both *in vitro* assays and *in vivo* models.
Do licensing or other priming methods improve MSC survival during IBMIR?	Evaluate the expression of complement, coagulation, and fibrinolytic cascade regulators and triggers in licensed vs. unlicensed MSCs. Test different priming strategies (e.g., hypoxia, metabolic preconditioning, inflammatory cytokines IFN-γ and TNF) to determine if MSC survival is altered upon IBMIR challenge (e.g., MSC exposure to whole blood vs. NHS or NHS/EDTA).
Does DNase treatment to remove any cell-released DNA reduce IBMIR triggering by freeze-thawed cells compared to fresh cells?	Remove free DNA by DNase treatment and washing of fresh vs. thawed MSCs, and expose them to whole blood, NHS, or NHS/EDTA, to see if IBMIR is reduced in the absence of DNA.
What distinguishes lung-retained MSCs from those rapidly cleared following systemic delivery in terms of cell marker expression, metabolic state, or responsiveness to environmental cues?	Recover MSCs from lung tissue shortly after IV administration (e.g., within 1 h) [35], and characterize them for surface marker profiles, transcriptomic and metabolomic states, and expression of adhesion molecules and chemokine receptors. Compare against rapidly cleared populations to identify determinants of pulmonary retention.
What are the real-time dynamics and spatial interactions between infused MSCs and blood components during IBMIR *in vivo*, and can these be therapeutically modulated?	Use intravital microscopy in a humanized mouse to visualize real-time MSC–immune–coagulation interaction post-infusion. Label MSCs, platelets, neutrophils, and complement components with fluorescent markers. Test IBMIR-modulating agents (e.g., complement and/or tissue factor (TF/CD142) inhibitors) and correlate MSC survival with clotting and cytokine profiles.

conditions [22,26]. While cells can undergo various forms of regulated death, susceptibility to these pathways is highly cell-type dependent. MSCs exhibit relative resistance to necroptosis and pyroptosis [40], both of which are inflammatory forms of cell death. Inhibitor of apoptosis proteins also limit their sensitivity to death receptor-mediated apoptosis. However, inhibition of the pro-survival molecules, B-cell lymphoma-extra-large (BCL-XL) and myeloid cell leukemia-1 (MCL-1), efficiently triggers mitochondrial apoptosis in MSCs, a process accelerated by tumor necrosis factor (TNF) and IFN-γ, cytokines commonly used to "license" MSCs [35]. This raises an important question: do TNF/IFN-γ-licensed MSCs exert their immunomodulatory effects because they are primed to die? Alternatively, does priming of any type increase MSC survival, particularly in inflammatory environments, as shown by some [41]?

Systemically administered MSCs are predominantly trapped in the lungs, where many undergo apoptosis. In disease settings that involve an influx of allogeneic T cells into the lungs, for instance, in a murine model of graft-versus-host disease (GvHD), host cytotoxic cells were found to induce MSC apoptosis via granzyme- and perforin-mediated mechanisms [20]. Yet, substantial MSC death is observed even in severely immunodeficient mouse models such as NOD/SCID/*Il2rγc*$^{-/-}$ (NSG) and BALB/c NOD.*Sirpa Rag2*$^{-/-}$*Il2rγc*$^{-/-}$ (BRGS) mice [22], which lack key adaptive and innate immune cells, suggesting that MSC death can be driven by nonimmune factors and residual innate responses. However, the specific features of the lung environment that trigger MSC apoptosis remain unclear but likely involves physical entrapment and environmental stressors. *Ex vivo* exposure of MSCs to bronchoalveolar lavage fluid (BALF) offers a useful model for simulating the lung extracellular microenvironment. MSCs exposed to BALF from healthy lungs upregulate genes associated with an acute innate immune response that may promote their clearance, whereas BALF from patients with acute respiratory distress syndrome blunts this response. Further, the presence of toxins produced by infectious organisms in the lung, for example, gliotoxin produced by *Aspergillus*-infected lungs in cystic fibrosis patients, rapidly kills MSCs through mitochondrial poisoning [42]. This suggests that the lung environment shapes MSC survival and function [42]. Potential approaches to address these gaps are summarized in Table 2.

Table 2
Experimental opportunities to further clarify what happens to MSCs in the lungs and other organs following systemic administration.

Questions	Proposed experiments
What specific factors in the healthy or diseased lung microenvironment promote or inhibit MSC apoptosis in different disease settings?	Characterize the lung microenvironment, including soluble factors in BALF, cellular composition, ligands and the extracellular matrix components, to identify potential ligand-receptor interactions with systemically administered MSCs that may influence survival.
Using patient-derived BALF and/or serum as surrogates for the *in vivo* environment, how do they affect MSC survival and other behaviors? This includes assessment of whether apoptotic MSCs can respond to the inflammatory lung microenvironment by upregulation of complement/coagulation regulatory genes/factors, e.g., CD46, CD55, CD59, tissue factor pathway inhibitor (TFPI) [46]. Can these surrogates be used to pre-screen MSC for desirable attributes?	Expose naïve or apoptotic MSCs *in vitro* to BALF and/or serum samples from healthy individuals and patients with respiratory or systemic inflammatory conditions (e.g., sepsis/septic shock, acute graft-versus-host disease (GvHD)) and assess MSC survival, transcriptomic changes and secretory profiles. Assess the downstream effects of BALF- or serum-exposed MSCs on inflammatory cells such as macrophages following administration into different disease models.
Do alternative administration routes that bypass pulmonary circulation result in similar levels of apoptosis and efferocytosis by tissue-resident macrophages? (This is further explored in sections below.)	Administer MSCs via nonintravenous routes (e.g., intra-arterial, intranasal, intratracheal) and assess retention, apoptosis including immune- and nonimmune-mediated mechanisms, and clearance in the lungs and distal organs. Evaluate whether the type and degree of MSC cell death correlate with therapeutic efficacy.
Which subsets of lung macrophages (alveolar, interstitial, intravascular) and/or other phagocytes are responsible for MSC clearance and therapeutic effects?	Utilize immunofluorescence and spatial/single-cell transcriptomics to map MSC interactions with phagocytes in the lung tissue after systemic administration in animal models. Using cell-specific genetic knockouts, selective depletion of cell populations, or targeted blockade of specific phagocytic receptors, the functional roles of specific phagocytes in MSC clearance and therapeutic effects can be further elucidated.
How do MSCs interact with pulmonary capillary endothelial cells?	Investigate *in vitro* and *in vivo* MSC-endothelial interactions, focusing on adhesion molecules and integrins that may regulate retention and transmigration.
What is the adhesion molecule profile that distinguishes MSCs that accumulate in the lung from those that do not? Do retained MSCs require a specific integrin signature?	Use targeted inhibition of integrin-ligand interactions to modulate MSC retention or biodistribution. Assess the effect using intravital imaging in the pulmonary vasculature.
To what extent does cell size influence MSC retention and apoptosis in the lung vasculature?	Compare primary MSCs obtained from various sources and from different donors, cultured under different preconditioning regimens and population doublings or passage numbers (to increase size), with iMSCs (which are reportedly smaller), using similar delivery protocols and analytical tools (e.g., imaging, recovery, omics) [47]. Evaluate size-related differences in physical entrapment, fate and functional performance postadministration. This acknowledges that iMSCs can be heterogeneous even if derived from the same donor.
In addition to entrapment in the pulmonary capillary system, do other mechanical cues in the lung (e.g., shear stress, cyclic stretch) accelerate or attenuate MSC apoptosis?	Seed MSCs in a breathing "lung-on-a-chip" or other devices that apply programmable airflow, stretch and capillary-level shear. Vary the mechanical parameters and assess cellular and molecular indices of apoptosis. Compare with static Transwell and *ex vivo* lung-slice cultures. Recreate the mechanical microenvironment that perfused cells encounter; chip models now routinely achieve human-relevant compliance and gas–liquid interface [48].
How do different apoptotic stimuli affect MSC–immune cell interactions and efficacy?	Induce MSC apoptosis using different methods (e.g., inhibition of pro-survival BCL-2-family proteins, ultraviolet exposure, Fas ligation, growth factor withdrawal, hypoxia or chemical agents), then evaluate immune effectors *in vitro*. Assess the therapeutic effects of differently primed apoptotic MSCs in relevant disease models.
Does the site of apoptosis (e.g., lung capillaries vs. other locations) influence immunomodulatory outcomes?	Following systemic MSC delivery, perform high-resolution or high-throughput profiling of immune cells in situ (e.g., lung vs. local tissues) at defined time points to capture local responses.
Can MSCs be engineered to undergo apoptosis in a controlled, time-specific or site-specific manner to enhance therapeutic effects?	Develop drug-inducible suicide gene constructs that allow for precise spatial and temporal control of MSC apoptosis. Assess the impact on local and circulating immune cell modulation and therapeutic outcome *in vivo*.

Table 3
Experimental opportunities for studying apoptotic MSC interactions and effects on host immune effector mechanisms.

Questions	Proposed experiments
What effects does exposure to apoptotic MSCs have on immune cells?	Expose macrophages, T cells or other immune effectors (alone or in combination) to apoptotic vs. nonapoptotic MSCs *ex vivo*, and characterize the resulting immune responses using omics/cytometry by time of flight (CyTOF) profiling.
Can immune cells conditioned by apoptotic MSCs mediate therapeutic effects when adoptively transferred? Do different immune cell types respond differently to MSC licensing?	Expose macrophages, T cells or other immune effectors (alone or in combination) to apoptotic MSCs *ex vivo* and administer them into various animal disease models. Assess whether MSC licensing influences the outcomes?
What cellular interactions, or secreted factors, mediate the *in vivo* effects of apoptotic MSCs, particularly in the lung?	Use functional blockade (e.g., targeting known immunomodulatory ligands) or CRISPR/Cas9 knockouts in MSCs to disrupt key immunomodulatory molecules (e.g., PD-L1, cyclooxygenase-2 [COX-2], etc.). Assess changes in immune modulation and efficacy.
Can immune effector cells conditioned or educated by apoptotic MSCs (e.g., monocytes, macrophages) secrete extracellular vesicles or soluble factors that independently mediate therapeutic effects? While prior studies have highlighted MSC-derived EVs that modulate macrophage phenotype [55,56], it remains unclear whether MSC-educated immune cells subsequently release EVs with distinct immunomodulatory properties, even though MSCs cultured under different stimuli can secrete functionally and molecularly distinct EVs, even from the same donor. Future investigations should explore whether such vesicles can be tracked and functionally assessed *in vivo* (*see also EV Section 4 for broader discussion on EV tracking and function*).	Isolate macrophages/monocytes and other immune effector cells postcoculture with apoptotic MSCs. Collect immune cell secretome and EVs, and assess immunomodulatory activity through *in vitro* assays (e.g., T-cell suppression, cytokine profiling). Administer immune-cell-isolated EVs or secretome into specific preclinical disease models to evaluate therapeutic efficacy. *See also Section 4 (EV) for further discussion on EVs originating from the MSCs themselves.*
Which specific apoptotic pathways (e.g., caspase-dependent vs. independent) and death-associated molecular patterns (DAMPs) from MSCs drive distinct immunomodulatory responses in recipient immune cells?	Induce apoptosis in MSCs via different mechanisms (e.g., caspase-3 activation, necroptosis via receptor-interacting protein kinase 3 [RIPK3]/mixed lineage kinase domain-like pseudokinase [MLKL], ferroptosis), and co-culture with monocytes, macrophages or dendritic cells.

In rodent models, MSCs that reach the pulmonary capillary beds appear to be retained primarily due to size-based obstruction [43], with pulmonary retention correlating with cell size [44]. Once trapped, MSCs deform and release microvesicles (~1 μm in diameter) within 40–60 min of intravascular delivery, with cellular breakdown occurring around 2 h and complete disintegration by 24 h [43]. Despite the physical obstruction of the pulmonary vessels, no ischemia or embolism has been observed, likely due to the rapid degradation of cells. In human studies, there is little evidence of clinically significant emboli, supporting a favorable safety profile even in procoagulant conditions such as COVID-19–associated acute respiratory distress syndrome [45]. Studies addressing these and the other related issues are summarized in Table 2.

3) Apoptotic MSCs and host immune effector interactions

Like viable MSCs, apoptotic MSCs also interact with host immune cells in ways that are critical for determining their fate and function. However, the nature of these interactions, whether involving live or apoptotic MSCs, may influence immune effector function differently. This distinction points to the importance of active signaling by MSCs [49], versus their passive recognition and engulfment by phagocytes [50], and highlights the need to dissect the relative contributions of the two mechanisms.

Interestingly, apoptotic MSCs have exhibited efficacy in improving disease outcomes in animal models of allergic asthma [22], sepsis [51], steroid-refractory GvHD [20], and acute lung injury [52]. A key host response mechanism involves the phagocytosis of apoptotic MSCs by host macrophages, which adopt a more anti-inflammatory phenotype after engulfing them. These apoptotic MSC-educated macrophages exhibit reduced nitric oxide production, suppressed pro-inflammatory cytokine expression, and increased levels of anti-inflammatory mediators such as interleukin (IL-10), programmed death ligand 1, and prostaglandin E2 [53,54]. Similar immunosuppressive effects have been observed when apoptotic cells are taken up by dendritic cells, where IFN-γ–induced nitric oxide production plays a central role in dampening immune responses [36]. While the immunomodulatory actions of viable MSCs have been extensively studied, the unique and possibly complementary roles of apoptotic MSCs remain less defined. Dissecting the immune responses elicited by apoptotic MSCs and how these differ from those triggered by live MSCs is crucial for refining and optimizing MSC-based therapeutic strategies. Potential approaches to study these interactions are summarized in Table 3.

4) MSC membrane fragments, mitochondria, apoptotic bodies, EVs from live or apoptotic MSCs

A central question in the field is whether the immunomodulatory effects of MSCs require intact, viable cells or whether cell fragments, apoptotic bodies, EVs, or mitochondria are sufficient to replicate these effects. Phosphatidylserine exposure on apoptotic bodies and MSC-derived membrane fragments can serve as eat me" signals that promote host macrophage responses, such as anti-inflammatory polarization [20,57]. In addition, growing evidence shows that MSCs can donate intact, respiration-competent mitochondria to recipient immune cells via tunnelling nanotubes or mitochondria-laden EVs [58–60]. Moreover, other extracellular mechanisms, such as CD73-mediated conversion of adenosine monophosphate to adenosine [61], which occurs on the surface of apoptotic bodies and EVs [62], can also drive anti-inflammatory signaling. This organelle hand-off reprograms macrophages and other phagocytes toward an anti-inflammatory, pro-resolving phenotype, providing a complementary mechanism of immunoregulation alongside soluble factors and vesicles. What specific features of MSCs make them uniquely capable of this, or can other cell types elicit similar host responses? Furthermore, can discrete cellular components, such as membrane fragments, mitochondria or EVs, independently or coordinately mediate therapeutic effects?

The EV field is complex and still ill-defined, as there are a broad range of microparticles that are labeled as "EVs." EVs are typically prepared and analyzed as pan-EV preparations. For example, EVs are commonly classified by the presence of cell surface markers CD9, CD63, and CD81 and the presence of RNAs. However, EVs with the requisite surface profiles do not necessarily contain RNA, and RNA-containing EVs are not necessarily positive for these markers [63]. Cogently, only a proportion of EVs mediate therapeutic effects, while many have other functions, such as transferring cell waste products to the liver for further processing. Notably, there are currently no specific dyes available to track individual EV populations. Thus, the field should consider that only a minor proportion of EVs or apoptotic material has therapeutic relevance. Due to the lack of EV identity and

Table 4
Experimental opportunities for studying potential roles and mechanisms of action of MSC membrane fragments, mitochondria, apoptotic bodies, EVs from live or apoptotic MSCs.

Questions	Proposed experiments
Can apoptotic bodies or cell membrane fragments reproduce the full immunomodulatory effects of MSCs? Are these effects unique to MSCs or are they shared by other stromal cells (e.g., fibroblasts)?	Isolate membrane fragments from MSCs using osmotic shock or differential centrifugation and compare their immunomodulatory effects to those of intact or apoptotic MSCs, fibroblasts and their respective EVs.
What are the biodistribution patterns, persistence and functional outcomes of EVs from live vs. apoptotic MSCs? Can MSC-EV uptake be tracked in specific immune or stromal cell subsets *in vivo* using labeling or barcoding technologies? What distinct molecular cargo (e.g., microRNAs, proteins) are enriched in EVs from apoptotic vs. viable MSCs, and how does this impact recipient cell behavior? Based on this, can EVs be engineered or enriched to selectively replicate specific MSC functions (e.g., immunosuppression, angiogenesis, wound healing)?	Tag parental MSCs or EVs from live or apoptotic MSCs and other control cell types to track their biodistribution *in vivo* following systemic administration in specific disease models. Persistence of MSCs or EVs can also be measured by flow cytometry of tagged MSCs or EVs.
Are there optimal administration routes or dose thresholds that enhance EV delivery to inflamed or lymphoid tissues? Do EVs reach these target tissues directly, or are they primarily taken up by circulating or tissue-resident immune/inflammatory cells en route?	Label EVs from live and apoptotic MSCs using membrane dyes or RNA barcodes. Administer via intravenous (IV), intraperitoneal (IP) or intranasal routes at varying doses in inflamed mouse models. Utilize imaging and flow cytometry to monitor the biodistribution, tissue accumulation and uptake of EVs by specific immune/stromal cells (e.g., macrophages, T-cells). Compare delivery efficiency across routes, doses and EV sources.
Do MSC-donated mitochondria make a distinct, non-redundant contribution to immunoregulation?	Engineer MSCs to express a mitochondria-targeted, photoconvertible reporter (e.g., mito-Dendra2) or mitochondrial DNA (mtDNA) barcodes. Track transfer into lung macrophages and dendritic cells after IV infusion. Disrupt transfer with Miro-1 knockdown, cytochalasin D (TNT inhibitor) or mitophagy induction, and compare IL-10/TNF ratios, macrophage metabolic profiles and disease read-outs vs. controls.
Can isolatedmitochondrial EVs (MitoEVs) or purified mitochondria reproduce MSC immunomodulation *in vivo* [65]?	Collect mitochondria-enriched EV fractions from licensed or apoptotic MSCs using density-gradient + TOMM20-immunocapture [65]. Administer equivalent total-protein doses of (i) whole MSC-EVs, (ii) MitoEVs and (iii) mitochondria-depleted EVs in ARDS or septic-shock models, then assess cytokine profiles, survival and tissue histology. Include intact MSCs as a benchmark to quantify relative immunomodulatory potency and determine whether mitochondrial cargo directly contributes to therapeutic effects or merely correlates with them.

functional markers, any conclusions about their site of action, biodistribution, and stability may be inaccurate. We propose that the following EV studies be completed using specific EV populations prepared and characterized according to the MISEV guidelines [64].

These proposed experiments are summarized in Table 4.

Nonsystemic delivery

Nonsystemic delivery of MSCs is a useful strategy to administer a concentrated dose of cells directly to an injured or diseased site, and to potentially prolong the survival of the administered cells and effector mechanisms. MSC persistence facilitates two therapeutic mechanisms: (i) MSCs as living pharmacies, and (ii) MSCs as living bioreactors. As living pharmacies, persistent MSCs can maintain synthesis of soluble factors, including their secretome and EVs, and tune this response to feedback from the evolving inflammatory environment, thereby establishing a bespoke, dynamic treatment modality. As living bioreactors, MSCs can be gene-modified to synthesize transgenic cargo, including antibodies [66–69], oncolytic viruses [70–73], biologics [74–80], and more. This approach is particularly useful to facilitate single-dose treatment of transgene products with truncated half-lives and/or genetic modifications that synergize with or enhance the MSC secretome.

Studies have shown that local MSC delivery can have superior outcomes compared to IV infusion in certain applications. In a preclinical colitis model, magnetic particle imaging demonstrated that IP delivery achieved more sustained targeting and efficacy than IV infusion [81]. In a chronic kidney disease model of unilateral ureteral obstruction, local subcapsular injection of licensed AT MSCs (MSC(AT)) embedded in a collagen hydrogel reduced fibrosis, whereas IV delivery had no such benefit [82]. A preclinical study of osteoarthritis demonstrated that intra-articular injection of CB-derived MSCs (MSC(CB)) provided superior benefits compared to IV-infused MSCs [83]. However, the field remains complex, as some studies have demonstrated that IV is preferable to nonsystemic delivery. For example, in a rat model, local MSC injection was less effective than systemic MSC injection at enhancing peri-implant epithelial sealing around titanium implants [84]. Ahn *et al.* [85] found that while IV application had lower delivery efficiency of MSCs into the brain, IV and intracranial methods provided similar protective effects against severe intraventricular hemorrhage in animal models. Essawy *et al.* [86] also reported comparable outcomes using intracranial and IV routes to deliver MSCs in a rat model of Parkinson's disease. These outcomes suggest that for critically ill patients, including neonates, the minimally invasive IV route could be a suitable alternative for certain indications. Further research is needed to evaluate the potential benefits of local MSC delivery in other contexts, particularly since the safety and feasibility of alternative routes, such as intrabronchial infusion, have been demonstrated in Phase I clinical trials [87,88].

The site of local MSC administration plays a crucial role in determining therapeutic outcomes, as the local tissue environments, including immune, stromal, and extracellular components, can influence MSC survival, retention, and function [89,90]. For example, studies have shown that the responsiveness of MSCs to oxygen saturation at the recipient site can affect their performance, and that various preconditioning strategies can overcome this limitation. MSCs show promise for treating myocardial infarction; however, the elevated reactive oxygen species in ischemic tissue impair their adhesion and survival [91]. This oxidative stress can thus lead to anoikis, a form of programmed cell death triggered by the loss of cell-matrix interactions, which may be overcome by pretreatment with antioxidants [83] or by the addition of adhesion-promoting graphene oxide flakes [92]. Enhanced functional potency has also been reported for MSCs in hypoxic environments, predominantly mediated by the upregulation of hypoxia-inducible factor 1-α. A recent study demonstrated that loading MSCs with the nanoparticle ferumoxytol upregulated hypoxia-inducible factor 1-α, providing a strategic approach to ensure the high performance of MSCs, irrespective of oxygen saturation at the recipient site. Ferumoxytol has the dual benefit of also being trackable by magnetic resonance imaging (MRI) [93,94].

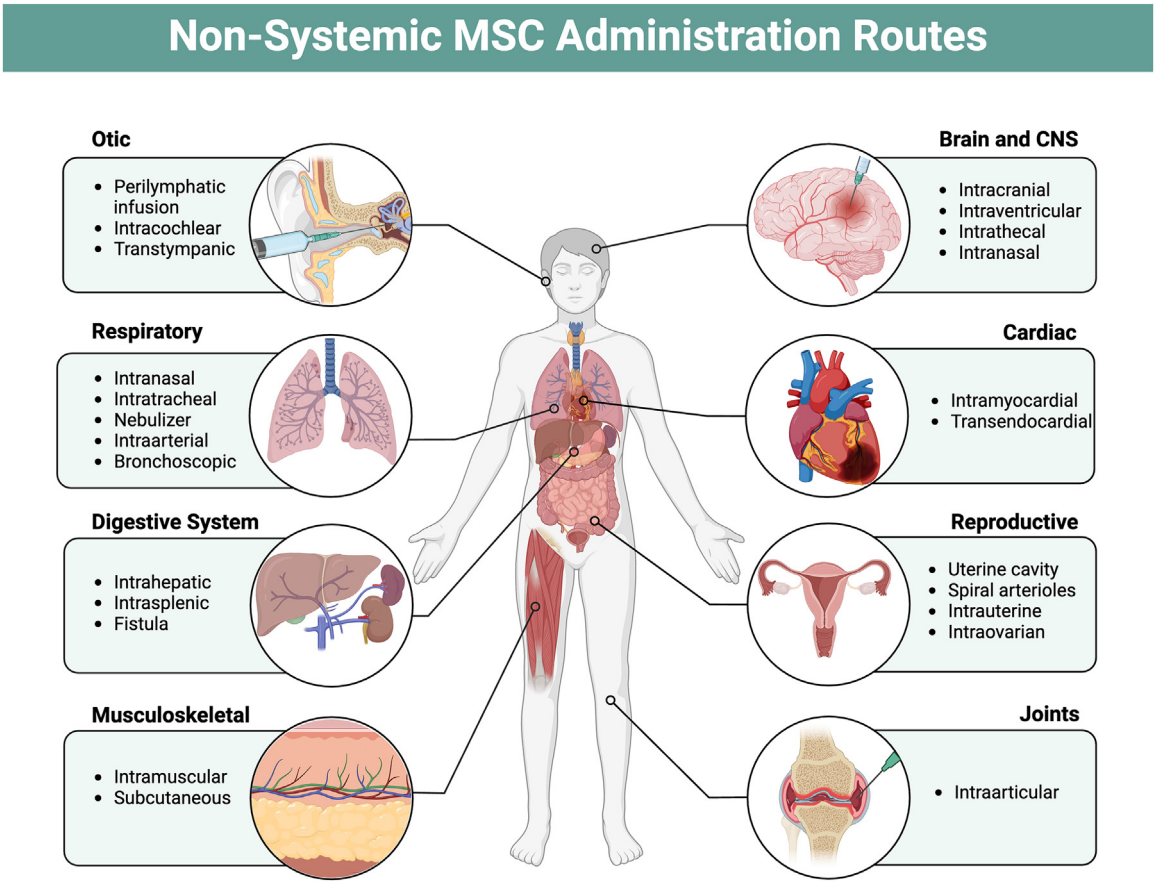

Fig. 2. Different routes of nonsystemic MSC administration. MSC can be administered through a wide range of nonsystemic routes that target specific tissues or organs. These include local delivery methods to the central nervous system (intracranial, intraventricular, intrathecal, intranasal), heart (intramyocardial, transendocardial), joints (intraarticular), reproductive tract (intrauterine, intraovarian) and liver or spleen (intrahepatic, intrasplenic). Other routes include perilymphatic or intracochlear infusion for the ear, intramuscular (IM) or subcutaneous injection (SC); respiratory delivery includes intranasal, intratracheal, nebulized, intra-arterial and bronchoscopic administration. These approaches aim to bypass systemic circulation and enhance MSC retention, viability, and local effects at targeted sites. (Color version of figure is available online.)

Although a plethora of routes have been employed in preclinical and clinical studies (Figure 2), our understanding of the survival and fate of locally delivered MSCs [81,84,87,94–97] leaves many open questions. It is not yet known whether clearance of MSCs by induced apoptosis or efferocytosis contributes to immune modulation as it does in IV infusion, as shown in preclinical models of osteoarthritis [94]. In addition, the rate and extent of MSC death may differ within a local site of inflammation, injury, or disease, altering not only how long the MSCs persist, but potentially the mechanisms by which they impact immunomodulation. A recent study provided compelling evidence that the anti-inflammatory mechanisms of IV and locally SC administered MSCs in an LPS-provoked model of skin inflammation are distinct. In this study, SC-delivered MSCs persisted for several days and exerted immunoregulatory effects both locally and at the draining lymph node before their apoptosis, effects that were not observed in mice treated with IV-infused MSCs, which also reduced acute inflammation [98]. Studies examining the delivery of MSCs into physiologically normal (noninflamed) tissues, distant unaffected sites [68,99] such as IP, SC, or IM injection into healthy, noninjured animal tissue sites, show significant attrition over the first 3–7 days after injection [100], indicating that MSC death may occur irrespective of the inflammatory status of the recipient site, although in some preclinical models, there is a slower loss of MSC in a disease versus nondisease setting [94]. These MSCs were also retained significantly longer than IV-infused MSCs [100], suggesting that persistence does not solely depend on MSC activation or response to inflammation when delivered nonsystemically.

Several studies have also shown that implanted MSCs can have therapeutic effects at distal sites. In a carrageenan-induced murine model of hind-limb inflammation, IM injection of MSCs in the contralateral limb reduced the acute inflammation, while SC- and IP-administered MSCs reduced acute colon inflammation in murine experimental colitis models [49,99]. A subsequent independent study further improved the survival and efficacy of IP-injected MSCs for colitis by delivering the cells as 3D spheroids rather than as single-cell suspensions [101]. In contrast, SC injection of MSCs directly into inflamed murine skin had an anti-inflammatory effect, while MSCs delivered SC at a remote site had no benefit [98]. These observations raise important questions that remain to be investigated, and potential experimental approaches are summarized in Table 5.

Is MSC persistence necessary for beneficial clinical effects?

MSCs have long been investigated for their regenerative potential, but the question of whether their persistence *in vivo* is necessary for observed therapeutic efficacy remains an open question [89,102,103]. Potential differences in MSC mechanisms related to persistence are summarized in Table 6.

A growing body of preclinical evidence shows that locally delivered MSCs encapsulated within natural or synthetic scaffolds exhibit prolonged persistence and deliver enhanced therapeutic benefits, including improved viability, engraftment, immune protection, and functional outcomes across various disease models [104–106]. For example, MSCs encapsulated in licensing hydrogels exerted systemic

Table 5
Experimental opportunities for studying fate and mechanisms of MSC actions following nonsystemic administration.

Questions	Proposed experiment
Are there distinct populations of MSCs that preferentially survive at different sites of local administration? Are the nonsurviving cells biologically distinct from the persistent MSCs, and are they predestined to be short-lived in this environment? Does the microenvironment at the recipient site influence which MSC sub-populations are most likely to persist?	Conduct *in vivo* administration of DNA-barcoded or tagged MSCs for tracking, followed by tissue recovery at defined time points for single-cell RNA sequencing to identify the transcriptional signature of persistence vs. short-lived MSCs. DNA-barcoding is less likely to produce false positives compared with other labeling strategies, which might remain detectable in cell fragments or in cells that have phagocytosed the MSCs.
Is the primary phase of MSC attrition due to inadequate resources (oxygen, nutrients, survival factors, adhesion sites) or due to targeted elimination by the recipient immune system?	Conduct flow cytometry or single-cell RNA sequencing of tagged MSCs to quantify local cytotoxic killing and/or efferocytosis at defined time points after administration.
How do immune effector cell phenotypes and distributions differ between unaffected, diseased, and injured tissue sites?	Conduct immunofluorescence or flow cytometry analysis of immune effector cells at local sites, considering that these sites may be unaffected, diseased, or injured.
Local delivery includes direct administration to the target tissue vs. depot delivery. What are the mechanisms of immune modulation in both? Do EVs play a role in distal targeting after local/depot injections?	Compare MSCs delivered either as a single bolus into the target tissue (direct) or encapsulated in a depot (e.g., PLGA microspheres, hydrogel) that releases the same total dose over time. Monitor local, draining-node and systemic immune responses over time (hours → weeks). Characterize EV release from the injection site and test whether EVs carry payload/signals that reach distal tissues and alter immune cells. Use loss-of-function perturbations (block EV biogenesis, deplete macrophages/DCs, block lymphatic drainage) to test causality.

Experimental endpoints for these studies will parallel those in Tables 1–4.

Table 6
Comparison of proposed mechanisms of MSC action in relation to delivery strategy and persistence.

MSC delivery strategies	Humoral activities	Mechanical and differentiation activities	"Die to heal"
Local injection with biomaterials	++	+++	+
Local injection without biomaterials	+++	+/–	++
Systemic injection	+++	+/–	+++

+++ = highly desirable; ++ = desirable; + = minor or negligible; – = absent. Different MSC delivery strategies (local injection with or without biomaterials, and systemic injection) are associated with varying contributions from humoral activities, mechanical and differentiation activities, and the "die to heal" phenomenon. The relative desirability of each mechanism depends on the persistence of MSCs in the target environment.

protection in a murine model of colitis [107]; single-cell microgel encapsulation improved MSC survival and efficacy in intervertebral disc degeneration by suppressing pyroptosis [108]; and poly(I:C)-primed MSC spheroids demonstrated enhanced survival and immunomodulatory function [101]. MSCs derived from bone marrow (MSC(M)), when combined with biomaterials such as calcium phosphate scaffolds, have demonstrated efficacy in promoting bone healing via localized administration [109,110]. Clinical protocols enabling local point-of-care use, with fresh MSCs embedded in grafts shortly before implantation, show promise [111,112]. Similar regenerative benefits are observed in preclinical and clinical models using MSC(AT), which support vascularization, preserve graft integrity, and attenuate necrosis in transplanted fat tissue [113], resulting in persistence for 60 days after subcutaneous implantation into the nape of xenotransplant immunodeficient mice [114,115]. Recent studies have also demonstrated that MSCs spatially organized into temperature-responsive, aligned cell sheets demonstrate enhanced cytokine secretion and preserved multipotency [116]. These findings underscore that local persistence, mediated via biomaterials and integration of MSCs at the injury site, can facilitate tissue recovery.

MSCs have also been genetically engineered to act as delivery vehicles in oncology, transporting antitumor agents such as TNF-related apoptosis-inducing ligand directly into tumors [117]. In a preclinical model of pancreatic cancer, intratumoral administration of TNF-related apoptosis-inducing ligand-expressing MSCs maintained local activity for at least 7 days, inducing tumor cell apoptosis and augmenting the efficacy of chemotherapy. Intratumoral persistence of engineered MSCs was associated with their therapeutic efficacy and is thus a desirable attribute. Similarly, chimeric antigen receptor-engineered MSCs targeting E-cadherin, which show a clearance time of around 30 days, are also being explored to ameliorate acute GvHD symptoms and survival [118]. The persistence of chimeric antigen receptor-MSCs, despite intraperitoneal delivery, further speaks to the importance of the persistence of genetically modified MSCs for the delivery of their payloads.

This leads to a provocative reframing: can "persistence" be reconceptualized as long-term effects on host immune and other effector cells? As discussed above, apoptotic MSCs can exert potent effects either for immunoregulatory action [22,111] or even for tissue regeneration [119]. The interaction of dying MSCs with innate immune cells may thus initiate reparative cascades, shifting the paradigm from cellular persistence to functional impact, whether living or dying.

Repeat dosing of MSCs has sometimes been shown to be more effective than a single MSC injection, particularly in clinical settings of GvHD [120], Crohn's disease [121], and osteoarthritis [122]. It is not yet clear whether this outcome is related to the renewed persistence of MSCs or whether subsequent doses act to train the host immune response. While repeat dosing may have beneficial outcomes, this modality must be balanced against the eventual triggering of an adaptive immune response in recipients that could reduce therapeutic efficacy through accelerated clearance, as reviewed elsewhere [123]. Taken together, these contrasting findings highlight the unresolved question of whether MSC persistence is truly necessary for therapeutic benefit, and potential approaches to address this are summarized in Table 7.

Summary and future directions

The *in vivo* fate of MSCs, including their clearance, interaction with host tissues, and persistence following systemic or local clinical administration to patients, remains incompletely understood. As the majority of mechanistic, preclinical, and clinical MSC studies have investigated systemic administration, it is critical to continue and expand studies using this mode of administration. The prevailing thinking is that IV infusion involves transient MSC persistence with effects on local host immune effector systems as the major mechanism(s) of MSC action. In parallel, for selected applications involving local administration, for example, in orthopedic injuries or diseases, it remains unclear whether prolonged MSC survival is necessary or

Table 7
Experimental opportunities for determining whether MSC persistence following *in vivo* administration is necessary for beneficial clinical effects.

Questions	Proposed experiment
Is the effect of repeated MSC dosing due to immune "training," replenishment of effector molecules, or both?	Investigate the trained phenotype of both circulating and bone marrow-derived immune cells (e.g., MSCs from bone marrow [MSC(M)]) following single or repeated doses of MSC. Could perform an adoptive transfer of trained immune cells to investigate if the protection can be transferred.
Can repeated apoptotic MSC doses more effectively "educate" host immunity over time?	Similar experiments to those outlined above.
What animal models could be used to systematically test memory/recall immune responses to MSC or EV exposure?	Use mouse models of inflammatory disease or allergy (e.g., OVA-induced asthma, contact hypersensitivity) to evaluate recall immune responses following repeated exposure to MSCs or MSC-derived EVs. Assess immune priming, tolerance, or modulation by tracking cytokine profiles, antibody titers, or T cell responses upon re-challenge.

even required for therapeutic efficacy. Adjunct approaches, such as embedding the MSC in scaffolds and/or genetically modifying them to deliver payloads as living bioreactors with accompanying increased persistence, may have utility in certain applications. In fact, the plurality of evidence and approaches in different disease settings confounds our ability to answer these questions simply, and the answer is most certainly context dependent.

In addition to established analytical methodologies, the emergence of new tools and technologies, including multi-omics measurements driven by powerful machine learning approaches, offers an exciting avenue to answer these fundamental questions about MSC delivery, persistence, and correlations to efficacy. Pharmacokinetic modeling is emerging as a valuable tool to integrate cell tracking data and predict MSC distribution and persistence, helping to guide dosing strategies and optimize therapeutic outcomes [124,125]. Machine learning can effectively query multiple, merged datasets to correlate therapeutic efficacy of MSCs with multiple parameters, including systemic delivery versus local or depot injection, licensed or preconditioned versus naïve, fresh versus cryopreserved, affected versus normal recipient site, correlations with baseline systemic and/or local inflammatory biomarkers, correlation with baseline systemic and/or local immunophenotype, etc. Such parametric and systematic analysis will provide a framework to assess and rank the multiple variables that influence MSC efficacy, several of which have already been described and partially characterized in preclinical and clinical studies. Availability, curation, and sharing of datasets, at least at the preclinical level, can facilitate such an undertaking, but this is not an easy task given institutional and ethical barriers.

While animal studies are necessary to begin probing these questions, it is important to remain mindful that their ability to predict the fate of MSCs in human recipients is limited [126]. Human MSCs administered to immunecompetent animals are targeted by a xenogeneic response that does not occur in patients. Conversely, the use of immunecompromised animal models to circumvent targeted elimination of human MSCs eliminates potential inflammatory and immune responses against, and from MSCs in these trials. Humanized mice offer a valuable platform for studying human immune responses under controlled experimental conditions. However, these models exhibit inherent limitations, including costs, that require careful consideration and contextual interpretation [127].

Ultimately, there is a critical need for clinical trials that incorporate labeled MSCs and systematically track their biodistribution and persistence in parallel with their safety and efficacy. There are already multiple options for passively loaded nanoparticles to enable surveillance of post-implant MSCs using common clinical imagers, including computed tomography, positron emission tomography and MRI [103,127–130]. Recent advances in label-free imaging, such as mannose-weighted Chemical Exchange Saturation Transfer MRI [131], which detects high-mannose *N*-linked glycans, offer new opportunities to track MSCs *in vivo* without extrinsic modifications that could alter their behavior.

Equally crucial is to identify the patients who are most likely to benefit from MSC therapies. Growing clinical data suggest that patients who experience durable responses after MSC infusion often share distinctive baseline signatures [132], e.g., a low neutrophil-to-lymphocyte ratio, a metabolically flexible macrophage profile, or a "poised-to-resolve" cytokine milieu. Systematically mining pre- and post-treatment multi-omics layers (genome, epigenome, proteome, lipidome, metabolome, glycome, and immunophenome) with machine-learning pipelines may reveal composite responder indices that outperform any single biomarker. These predictive fingerprints would enable prospective stratification of candidates, personalized dosing schedules, and prevent futile treatments in non-responders.

Complementing in-patient analytics, advanced organ-on-a-chip platforms can replicate the dynamic mechanical, biochemical, and immune landscapes of human lung, gut, bone marrow, or joint tissue with increasing fidelity [133]. By seeding chips with primary cells from "responder" versus "non-responder" donors and introducing MSCs in distinct physiological states (fresh vs. cryopreserved, naïve vs. IFN-γ–licensed, live vs. apoptosis-primed), researchers can observe real-time trafficking, mitochondrial transfer, vesicle release, and efferocytosis under tightly controlled flow and stretch conditions [134,135]. Coupled with high-content imaging and single-cell read-outs, these microphysiological systems provide a scalable, ethically low-barrier test-bed to validate the computationally derived responder profiles and to fine-tune MSC products before they ever enter a patient. The integration of patient-stratification analytics with chip-based functional assays creates a reciprocal cycle, with each informing the other to advance more personalized MSC-based approaches.

The complexity and heterogeneity of MSC therapeutic mechanisms, shaped by variables such as delivery route, cell state, microenvironment, and host immune context, demand advanced computational approaches. Advances in systems biology and AI now make it possible to integrate large-scale omics and clinical datasets to improve translational predictability and accelerate clinical implementation [2,3]. Developing integrative algorithms and machine-learning models that analyze multi-omics, imaging, and clinical data will be pivotal in predicting MSC fate and therapeutic outcomes. In particular, AI-driven potency assessment strategies are emerging as powerful tools to define unified critical quality attributes and connect them with clinical efficacy [4]. Such predictive tools can help identify key variables driving efficacy, and safety, optimize MSC manufacturing and delivery protocols, and enable personalized MSC therapies tailored to individual patient profiles.

The therapeutic value of MSCs may ultimately not rely solely on their persistence, but on their context-dependent fate. Persistence can also be thought of as sustained effects on local immune and other effector systems even after MSCs have been cleared. The future of MSC therapies depends on understanding and harnessing these multiple fates: stemness, stromal support, immunomodulation, and apoptosis in a manner tailored to the specific clinical indication.

We have listed a series of questions and experiments that need to be systematically addressed in various models and disease systems. Thus, a better understanding and manipulation of MSC behaviors will enable us to strategically design next-generation MSC-based therapies, ensuring that these versatile cells achieve their full clinical potential.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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