



HAL
open science

Mesenchymal stromal cell variables influencing clinical potency: the impact of viability, fitness, route of administration and host predisposition

Jacques Galipeau, Mauro Krampera, Katarina Leblanc, Jan A Nolta, Donald G Phinney, Yufang Shi, Karin Tarte, Sowmya Viswanathan, Ivan Martin

► To cite this version:

Jacques Galipeau, Mauro Krampera, Katarina Leblanc, Jan A Nolta, Donald G Phinney, et al.. Mesenchymal stromal cell variables influencing clinical potency: the impact of viability, fitness, route of administration and host predisposition. *Cytotherapy*, 2021, 23 (5), pp.368-372. 10.1016/j.jcyt.2020.11.007 . hal-03229071

HAL Id: hal-03229071

<https://hal.science/hal-03229071>

Submitted on 15 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Title: MSC variables influencing clinical potency: the impact of viability, fitness, route of administration and host predisposition.

Jacques Galipeau^{1*}, Mauro Krampera², Katarina Leblanc³, Jan A. Nolte⁴, Donald G. Phinney⁵, Yufang Shi⁶, Karin Tarte⁷, Sowmya Viswanathan⁸, Ivan Martin⁹.

¹: Univ Wisconsin, Dept Med, Carbone Canc Ctr, Madison, WI USA

²: Univ Verona, Dept Med, Sect Hematol, Verona, Italy

³: Department of Laboratory Medicine, Karolinska Institutet, CAST Karolinska University Hospital, Stockholm, Sweden.

⁴: Univ Calif Davis, Stem Cell Program, 2921 Stockton Blvd, Suite 1300, Sacramento, CA 95817 USA.

⁵: Scripps Res Inst, Dept Mol Therapeut, Jupiter, FL USA

⁶: Soochow Univ, Inst Translat Med, Affiliated Hosp 1, Suzhou, Peoples R China

⁷: Univ Rennes, Etab Francais Sang Bretagne, INSERM, UMR 1236, Rennes, France

⁸: Krembil Research Institute, University Health Network and Institute of Biomedical Engineering and Department of Medicine, University of Toronto, Toronto, ON, Canada

⁹: Univ Hosp Basel, Dept Biomed, Basel, Switzerland

*: corresponding author

jgalipeau@wisc.edu

Abstract: 166 WORD

Body Text (excluding references): 2827 WORDS

References: 77

Tables and Figures: 0

Abstract

The ISCT MSC committee has been an interested observer of community interests in all matters related to MSC identity, mechanism of action, potency assessment and etymology and it has regularly contributed to this conversation through a series of MSC pre-conferences and committee publications dealing with these matters. Arising from these reflections, we propose that an overlooked and potentially disruptive perspective is the impact of *in vivo persistence* on potency that is not predicted by surrogate cellular potency assays performed *in vitro* and how this translates to *in vivo* outcomes. Systemic delivery or extravascular implantation at sites removed from the affected organ system seem to be adequate in affecting clinical outcomes in many pre-clinical murine models of acute tissue injury and inflammatory pathology, including the recently EMA approved use of MSC in Crohn's related fistular disease. We further propose that MSC viability and metabolic fitness likely dominate as a potency quality attribute, especially in recipients poised for salutary benefits as defined by emerging predictive biomarkers of response.

Accepted manuscript

Introduction

Culture adapted mesenchymal stromal cells (hereafter MSC[1]), were first clinically tested in human subjects in the United States in 1995[2]. Nearly a quarter century later, the European Medicines Agency (EMA) approval of darvodstrocel, an adipose-derived MSC product for treatment of Crohn'-related perianal fistular disease, inaugurates the endorsement of MSCs as a *bona fide* pharmaceutical[3-5]. This approval foreshadows the good likelihood that other allogeneic MSC products derived from marrow, adipose and possibly puerperal discards, such as umbilical cord currently undergoing advanced clinical trials internationally, may meet the exacting bar of marketing approval for additional clinical indications. The common thread amongst these platforms is the use of MSC, but substantial divergence exists in practice in handling and delivery of this living cell product. Continuously evolving pre-clinical data in animal systems provide insights on MSC function, fate and host response that may well optimize a pharmaceutical strategy first deployed 25 years ago. This perspective seeks to capture these insights and how they inform the evolving clinical investigational use of MSCs.

Ontogeny of culture adapted MSC

For biologists with an interest in the cellular elements of the marrow hematopoietic stem cell (HSC) niche, the *in vitro* CFU-F assay remains a necessary functional attribute for identifying endogenous mesenchymal stem cells with HSC niche sustaining competency[6]. Murine *in vivo* marrow mesenchymal fate mapping and cell tracking studies have demonstrated that leptin receptor (LepR) marks CXCL12 and stem cell factor (SCF)-expressing HSC-sustaining Prx1⁺ niche cells [eg: CXCL12-abundant reticular (CAR) cells] with robust CFU-F competency[7-10]. LepR⁺ mesodermal progenitor cells represent 0.3% of endogenous cells in adult mouse bone marrow and single cell RNAseq analysis marrow resident LepR⁺ cells demonstrates that this population is not monolithic but rather is constituted by at least fourteen LepR⁺ subsets distinguished by distinct gene signatures. As an aggregate, LepR⁺ mesodermal progenitor cells generate more than 94% of serum-responsive CFU-Fs *in vitro*[11, 12]. Indeed, endogenous LepR⁺ mesodermal progenitor cells constitutively express both PDGFR α and PDGFR β that likely drives their CFU-F competency *in vitro* in response to PDGF-rich, serum containing cell culture media[13]. Interestingly, LepR is dispensable for CFU-F competency since LepR-deficient, but PDGFR⁺ marrow mesodermal progenitor cells maintain the ability to form CFU-F *in vitro*[12]. The pivotal role of PDGF responsiveness to generate CFU-F is further reinforced by the observation that dasatinib (a PDGFR signaling inhibitor) abolishes replicative capacity of CFU-Fs *in vitro*[14]. Therefore, LepR/PDGFR $\alpha\beta$ marks endogenous murine marrow mesodermal progenitor with

CFU-F competency, but PDGFR $\alpha\beta$ is sufficient to initiate and sustain culture adapted mesenchymal stromal cell expansion. Analogous analysis of human marrow derived CD271⁺ MSCs maps CFU-F ontogeny to LepR⁺/PDGFR $\alpha\beta$ ⁺ progenitors which highlights the functional convergence of PDGFR $\alpha\beta$ from a comparative biology perspective between mouse and human CFU-F properties[15, 16].

MSC form and function

Culture adapted MSC meeting the 2006 ISCT minimal identity definition[17] can be derived from multiple tissue sources. Amongst these, MSC derived bone marrow (M), adipose (A) and umbilical cord (UC) tissue are the most commonly studied in human clinical trials[18]. The CFU-F colony assay used by developmental biologist to define, in part, the stemness of endogenous marrow mesenchymal progenitors is directly analogous to passage 0 (P0) output which seeds the first step in culture-adapting polyclonal MSC expanded in number to form a cellular pharmaceutical. Culture adapted bone MSC maintained in serum-rich media preserve some of the functional attributes of the tissue progenitors from which they are derived[19-21], such as the MSC(M) homeostatic expression of hematopoietic niche maintaining morphogens such as CXCL12 and SCF[13, 22]. However, distinct from their replicative quiescent marrow resident forbearers, post CFU-F MSC will activate the expression of morphogens and leukines not otherwise expressed *in vivo* under homeostatic conditions[23, 24]. An argument can be made that PDGFR expression as a functional marker of MSC CFU-F progenitors is a self-fulfilling prophecy considering that PDGF-rich serum is typically used as a source of mitogens when culture adapting MSC. Whereas endogenous mesodermal progenitors are in replicative quiescence in steady state, mitogen activated MSC deploy a robust replicative activity in response to serum. There is a strong consensus that culture adapted, polyclonal MSCs, are not a clonally pure population[25] but rather cell product with shared mesodermal identity features and replicative fitness of continuously expanding MSCs is associated with the expression of Twist1[26, 27].

Replicating MSC deploy an altered transcriptome reminiscent of their innate response to injurious cues *in vivo* such as the expression of chemokines like CCL2 in addition to their canonical expression of factors like CXCL12 and SCF. An argument can be made that mitogen activated, mitotically active culture adapted MSC adopt a pre-programmed functionality primed for tissue injury response. Indeed, factors comprising the secretome of culture adapted MSCs that convey anti-inflammatory and regenerative effects in murine models of tissue injury include CCL2, HGF, TSGL-1 and COX2, which are not expressed in quiescent endogenous progenitors[28]. A similar profile can be observed in human culture adapted

MSC as well. Interestingly, many of the acquired *in vitro* functionalities of culture adapted MSC(M) mirror the response of endogenous LepR⁺ progenitors to marrow injury or alarmins. The marrow endogenous LepR⁺ mesodermal compartment also will dynamically respond to TLR4 agonists such as LPS by upregulating expression of CCL2 that triggers egress of CCR2⁺ marrow resident myeloid cells and monocytes to the periphery[29] and provides a mechanistic link to the necessary immune modulating role of CCL2 arising from use of culture-adapted MSC[30-33]. Considering that TLR4 agonists also include alarmins such as HMGB1, we can hypothesize that endogenous mesodermal progenitors can be activated by sterile tissue injury as well[34, 35]. These *in vivo* responses of LepR⁺ marrow cells to injurious somatic cues likely foreshadow the functional activation of PDGF-driven culture adapted MSCs and their distinct transcriptome. In essence, CFU-F competency may well mirror the endogenous physiological tissue injury response of marrow-resident LepR⁺ cells. Indeed, close analysis of endogenous LepR responses to injury may provide important mechanistic insights on the pharmacology of MSCs as a cell drug. Of note, the transcriptome of culture adapted MSC(M) can be modulated to optimize functional properties by manipulating culture conditions such as oxygen tension[36], glucose restriction, as well as addition of recombinant morphogens or licensing cytokines[37]. For example, adding interferon- γ alone or in tandem with TNF- α to culture media for a few hours markedly augments the expression of chemokines CCL2, CXCL9/10/11 and induces expression of IDO and PD-L1[38]. Functional tuning of MSC in this manner has been shown to substantially alter the pharmaceutical properties of live MSC administered in preclinical models of disease[39] and are now being evaluated as part of human clinical trials[40]. Conversely, overexuberant culture expansion of MSC morphs the mixed population of mesodermal-sourced cells to become clonally impoverished, with loss of mesodermal tissue plasticity and acquisition of a Twist1^{null} phenotype and senescence that adversely impacts their functionality[41].

Considering that the MSC-dependent mechanism of action is anchored in its secretome[42], a cogent argument can be made that the best cell therapy is done without any cells but rather using elements of the secretome such as components derived from extracellular vesicles (EV) like exosomes[43]. Indeed, characterization and translational development of MSC-derived exosomes[44, 45] and related EVs are of great interest[46, 47]. Whether this biological product can serve as an effective alternative to MSC therapy remains to be determined.

MSC viability and necrobiology

Dead MSCs do not have a functional secretome and fail to inhibit immune cell function using standard in vitro functional assays[48]. Yet intravenous transfusion in mice of dead human MSC will trigger a vigorous host lung tissue transcriptional response leading to expression of a cascade of leukines[49]. The transcriptional response can be mapped to lung resident phagocytic macrophages that engulfed the dead MSCs and thus laden secondarily migrated to liver within 72 hours and promptly vanished thereafter[50]. A similar abbreviated transient persistence occurs following intravenous administration of MSCs with impaired fitness arising from product handling protocols such as cryobanking[51] and thawing at time of infusion. The necrobiological host response is entirely autonomous from any MSC functionality and has to do with cell recycling of necrotic/apoptotic cellular elements. In itself, the necrobiological immune dampening host response may have salutary effects in systemic inflammatory syndromes[52, 53]. However, the effect would be expected to be short lived considering the virtual absence of any MSC functionality or transient engraftment permissive for a MSC-dependent pharmaceutical effect. Indeed, the negative outcome of a placebo-controlled randomized study of IV delivered allogeneic MSC in ARDS was plausibly linked to poor product viability at release[54]. More so than any other quality attribute, MSC product viability and metabolic fitness at the time of administration to recipient most likely influences clinical potency of final formulation[55, 56].

MSC fate following adoptive transfer – the competing forces of transient engraftment and efferocytosis

When culture adapted MSC(M) are transfused intravenously in mice they display a brief period of engraftment predominantly in lung and within days disappear either from apoptosis or efferocytosis. The host lung efferocytotic response is unleashed whether the infused MSC product be viable or deliberately rendered apoptotic. Host phagocytosis of transfused MSC was first considered a biologically silent event, but a mounting body of evidence informs that efferocytosis (phagocytosis of cells) triggers a profound host immune suppressive event driven by IL-10-polarized phagocytic macrophages, let the MSCs be dead or alive[5]. In contrast, live MSCs administered in the extravascular space can persist in vivo for weeks or months and provides a pharmacologically plausible argument that MSC secretome provide over an extended period of time for paracrine factors acting on bystander somatic and immune cells[57]. If the host efferocytotic response was the sole mechanism via which adoptive transfer of MSC lead to clinical effects in vivo, then dead MSCs should be as potent as live cells, which they are not[58]. However, a host efferocytotic response may suffice to gain clinical benefit under certain circumstances such as in acute GVHD. A powerful argument that functional transient

engraftment and metabolic activity of MSCs play a role in their salutary effects in vivo is supported by perfusion experiments of MSC separated from subject blood by permeable membranes that allow for secreted factors to be released yet cloaks MSC from phagocytosis. Indeed, in such a system it was shown that MSC secretome profoundly alters host myeloid cell biology and that live MSCs substantially alter their secretome including massive release of extracellular vesicles and a matrix of leukines[59].

Route of administration impacts MSC persistence in vivo, potency and adverse event risks

Route of administration of MSC for any given ailment follows one of three typical clinical approaches: the most common being intravenous transfusion, followed by directly to afflicted tissue or target organ (eg: intrathecal, intra-articular or arterial inflow to target organ) and as a depot in an extravascular compartment (subdermal, intraperitoneal or intramuscular)[60]. Direct delivery to an afflicted organ is predicated on the premise that pharmaceutical potency is dependent on direct tissue engraftment to maximize paracrine effect. This line of thought rationally informs anatomically targeted tissue engineering where MSC regenerative effect is sought at a specific locale such as bone repair, vascular insufficiency or monoarticular joint degeneration[61]. The successful development of darvodstrocel for Crohn's associated enterocutaneous fistular disease is an example of the successful outcome of this strategy[5]. Along these lines, embedding of MSC in biomaterials and delivered as an implant can have profound effects on persistence and clinical effects[62, 63]. However, where tissue injury and inflammation intersect, organ targeted delivery may not necessarily be the optimal means of MSC delivery to optimize clinical outcome. This theorem has been aggressively pursued in the cardiovascular space where direct myocardial delivery has been examined in an array of clinical trials, including an unsuccessful phase III trial of endomyocardial delivery of autologous MSC [64]. Indeed, tissue targeted delivery can be counterproductive since post hoc analysis suggested an inverted U dose/response relationship [eg: injecting more cells directly in heart made things worse] [65]. A revisionist approach is that intravenous delivery of MSC can be as effective as direct cardiac delivery despite near absence of demonstrable myocardial tropism of transfused cells[66]. A similar paradigm informs most clinical trials for neuroinjury where MSCs are administered IV rather than directly in to brain substance to achieve clinical ends[60], especially considering the effectiveness of IV delivered MSCs to improve experimental rodent neuroinjury[67]. When sought, tropism of MSC to target organ following intravenous delivery can be demonstrated albeit the fraction of input making its way to target is vanishingly small[68, 69]. Indeed, there is no conclusive in vivo evidence that direct tissue tropism is required for the beneficial effects MSCs for improving tissue injury or inflammatory syndromes. For example, subdermal delivery

of MSC – far removed from injured tissue – was the most effective means of improving colitis outcomes in mice[58]. This observation speaks to the likely systemic effect of viable MSCs on triggering a reparative response by injured tissue, and this response is also likely linked to in vivo persistence allowing for prolonged host delivery of MSC secretome[70].

MSC quality attributes and pharmacological disposition inform potency

Culture adapted MSCs that retain replicative fitness will produce a matrix of factors, including extracellular vesicles which as an aggregate drive anti-inflammatory and tissue regenerative functionality. However, these theoretically desirable features are forfeited if at time of administration the cells are dead, dying or damaged defaulting towards a canonical host efferocytotic response[28]. Furthermore, the administration of MSC intravenously – let the product be fit or compromised – does not allow for meaningful in vivo persistence which compromises the window of time for mass action to take place. Tissue targeted delivery of MSCs can address this issue in part but can be clinically unfeasible or counterproductive. The observation that extravascular depot of MSCs is associated with substantially greater cell drug persistence and is associated with meaningful clinical response in organ systems far removed from the depot, speaking the systemic mobilization of host bystander cells that remodel the immune milieu of injured tissue. These observations gleaned from pre-clinical animal model systems inform that undead MSC delivered as an extravascular depot may well provide a potent mechanistically informed alternative to intravenous administration with its attendant limitations on transient engraftment[58]. Furthermore, the avoidance of IBMIR and concerns related to thrombogenesis[71], especially if MSCs are “tuned” in a manner which leads to companion increased surface expression of Tissue Factor or other potentially injurious factors, provides for clinically deployable alternates which can allow for second generation, gene enhanced, MSC products[72, 73].

Predictive biomarkers of MSC response – the next frontier

Notwithstanding the quality attributes of MSC defining their potency in pre-clinical animal systems, the human condition entails genetic and acquired diversity of subjects that muddies the predictable MSC responsiveness seen in inbred animal systems. These animal systems are not well adapted to identify host biological genetic or functional features predictive of response to an otherwise identical cell pharmaceutical. It is therefore not surprising, despite use of MSCs bearing identical identity and functional features, that human subject clinical response will lack uniformity. Hence, the value in defining demographic, clinical and biological features of human subjects that are predictive of MSC

response prior to initiation of therapy[74]. The utility of such biomarkers is critical for reasoned attribution of targeted therapies in many clinical conditions. The same line of thought applies for MSC therapies where the pharmacoeconomic and opportunity costs of delivering costly treatments to subjects unlikely to respond may forfeit the value proposition of MSC[75]. A patient unlikely to respond can pivot to alternates rather than be subjected to futility. The MSC in GVHD experience highlights the reasoned restriction to pediatric subjects due to their observed higher rate of response than their elders as a simple demographically defined biomarker[76]. The identification of subject functional properties, such as the ability of patient blood lymphocytes to lyse MSCs in vitro as a potential predictor of response of adults with GVHD is also showing promise[77]. The identification and prospective validation of host predictive markers of response to MSC may well pave the way to targeted testing and deployment of MSC cell technologies in clinical trials that complement the parallel efforts in optimizing their cell intrinsic potency potential.

Conclusion

MSC have graduated from investigational to regulatory approved cell drug treatments in major jurisdictions. This development foreshadows likely positive outcomes for treatment of an expanding pool of inflammatory and tissue injury syndromes. Evolving understanding of mechanism of action and empirical clinical experience is informing upon heretofore poorly understood quality attributes as well as pharmacological disposition that markedly affect clinical potency. This knowledge base will inform the ISCT espoused ethical and scientifically sound clinical development of “tuned” MSC and their byproducts as useful tools in addressing disorders poorly responsive to conventional medicinal chemistry by soliciting host intrinsic repair mechanisms.

Acknowledgements

This paper is written in memory of Luc Sensébé, former Chair of ISCT MSC committee and long standing member. All the authors are current and active ISCT MSC Committee members. JG is a recipient of NIDDK grant R01DK109508.

Author contributions

JG wrote the manuscript. MK, KL, JAN, DGP, YS, KT, SW, IM edited contents.

Conflict of Interest Disclosures

The authors declare no competing financial interests.

Accepted manuscript

References

1. Viswanathan, S., Shi, Y., Galipeau, J., Krampera, M., Leblanc, K., Martin, I., Nolte, J., Phinney, D. G. & Sensebe, L. (2019) Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT(R)) Mesenchymal Stromal Cell committee position statement on nomenclature, *Cytotherapy*. **21**, 1019-1024.
2. Lazarus, H. M., Haynesworth, S. E., Gerson, S. L., Rosenthal, N. S. & Caplan, A. I. (1995) Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use, *Bone Marrow Transplant*. **16**, 557-64.
3. Panes, J., Garcia-Olmo, D., Van Assche, G., Colombel, J. F., Reinisch, W., Baumgart, D. C., Dignass, A., Nachury, M., Ferrante, M., Kazemi-Shirazi, L., Grimaud, J. C., de la Portilla, F., Goldin, E., Richard, M. P., Leselbaum, A., Danese, S. & Collaborators, A. C. S. G. (2016) Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial, *Lancet*. **388**, 1281-90.
4. Panes, J., Garcia-Olmo, D., Van Assche, G., Colombel, J. F., Reinisch, W., Baumgart, D. C., Dignass, A., Nachury, M., Ferrante, M., Kazemi-Shirazi, L., Grimaud, J. C., de la Portilla, F., Goldin, E., Richard, M. P., Diez, M. C., Tagarro, I., Leselbaum, A., Danese, S., Collaborators, A. C. S. G., Panes, J., Baumgart, D. C., Colombel, J. F., Danese, S., Van Assche, G., Reinisch, W., Garcia-Olmo, D., Stift, A., Tschmelitsch, J., Mrak, K., Tilg, H., Kroberger, I., D'Hoore, A., De Looze, D., Baert, F., Pattyn, P., Zerbib, P., Zerbib, F., Viennot, S., Dupas, J.-L., Orsoni, P.-C., Hebuterne, X., Rahili, A., Allez, M., Panis, Y., Reinshagen, M., Scherer, R., Sturm, A., Kruis, W., Duek, D.-S., Waterman, M., Lahat-Zok, A., Zmora, O., Tulchinsky, H., Edden, Y., Spinelli, A., Annese, V., Angriman, I., Riegler, G., Selvaggi, F., Oldenburg, B., Gilissen, L., Van Montfort, G., Lowenberg, M., Bemelman, A. W., Almenara, R., Martin Arranz, M. D., Garcia-Arranz, M., Perez Gisbert, J., Palasi, R., Samso, C. T., Herrera Justiniano, J. M., Rada, R., Butron, M. T., Lopez, D. C., Lopez-Sanroman, A., Hinojosa de Val, J., Solana, A., Gonzalez Argente, F. X., Pastor, C. & Guadalajara, H. (2018) Long-term Efficacy and Safety of Stem Cell Therapy (Cx601) for Complex Perianal Fistulas in Patients With Crohn's Disease, *Gastroenterology*. **154**, 1334-1342.e4.
5. Hoogduijn, M. J. & Lombardo, E. (2019) Mesenchymal Stromal Cells Anno 2019: Dawn of the Therapeutic Era? Concise Review, *Stem Cells Transl Med*. **8**, 1126-1134.
6. Crane, G. M., Jeffery, E. & Morrison, S. J. (2017) Adult haematopoietic stem cell niches, *Nat Rev Immunol*. **17**, 573-590.
7. Farahani, R. M. & Xaymardan, M. (2015) Platelet-Derived Growth Factor Receptor Alpha as a Marker of Mesenchymal Stem Cells in Development and Stem Cell Biology, *Stem Cells Int*. **2015**, 362753.
8. Mabuchi, Y. & Matsuzaki, Y. (2016) Prospective isolation of resident adult human mesenchymal stem cell population from multiple organs, *Int J Hematol*. **103**, 138-44.
9. Wei, Q. & Frenette, P. S. (2018) Niches for Hematopoietic Stem Cells and Their Progeny, *Immunity*. **48**, 632-648.
10. Kfoury, Y. & Scadden, D. T. (2015) Mesenchymal cell contributions to the stem cell niche, *Cell Stem Cell*. **16**, 239-53.
11. Tikhonova, A. N., Dolgalev, I., Hu, H., Sivaraj, K. K., Hoxha, E., Cuesta-Dominguez, A., Pinho, S., Akhmetzyanova, I., Gao, J., Witkowski, M., Guillamot, M., Gutkin, M. C., Zhang, Y., Marier, C., Diefenbach, C., Kousteni, S., Heguy, A., Zhong, H., Fooksman, D. R., Butler, J. M., Economides, A., Frenette, P. S., Adams, R. H., Satija, R., Tsigos, A. & Aifantis, I. (2019) The bone marrow microenvironment at single-cell resolution, *Nature*. **569**, 222-228.

12. Zhou, B. O., Yue, R., Murphy, M. M., Peyer, J. G. & Morrison, S. J. (2014) Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow, *Cell Stem Cell*. **15**, 154-68.
13. Matsuzaki, Y., Mabuchi, Y. & Okano, H. (2014) Leptin receptor makes its mark on MSCs, *Cell Stem Cell*. **15**, 112-4.
14. Id Boufker, H., Lagneaux, L., Najjar, M., Piccart, M., Ghanem, G., Body, J. J. & Journe, F. (2010) The Src inhibitor dasatinib accelerates the differentiation of human bone marrow-derived mesenchymal stromal cells into osteoblasts, *BMC Cancer*. **10**, 298.
15. Ghazanfari, R., Li, H., Zacharaki, D., Lim, H. C. & Scheduling, S. (2016) Human Non-Hematopoietic CD271pos/CD140alow/neg Bone Marrow Stroma Cells Fulfill Stringent Stem Cell Criteria in Serial Transplantations, *Stem Cells Dev*. **25**, 1652-1658.
16. Lo Furno, D., Mannino, G., Cardile, V., Parenti, R. & Giuffrida, R. (2016) Potential Therapeutic Applications of Adipose-Derived Mesenchymal Stem Cells, *Stem Cells Dev*. **25**, 1615-1628.
17. Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prockop, D. & Horwitz, E. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, *Cytotherapy*. **8**, 315-7.
18. Phinney, D. G., Galipeau, J., Msc Committee Of The International Society Of, C. & Gene, T. (2019) Manufacturing mesenchymal stromal cells for clinical applications: A survey of Good Manufacturing Practices at U.S. academic centers, *Cytotherapy*. **21**, 782-792.
19. Menard, C., Dulong, J., Roulois, D., Hebraud, B., Verdier, L., Pangault, C., Sibut, V., Bezier, I., Bescher, N., Monvoisin, C., Gadelorge, M., Bertheuil, N., Flecher, E., Casteilla, L., Collas, P., Sensebe, L., Bourin, P., Espagnol, N. & Tarte, K. (2020) Integrated transcriptomic, phenotypic, and functional study reveals tissue-specific immune properties of mesenchymal stromal cells, *Stem Cells*. **38**, 146-159.
20. Ho, Y. T., Shimbo, T., Wijaya, E., Ouchi, Y., Takaki, E., Yamamoto, R., Kikuchi, Y., Kaneda, Y. & Tamai, K. (2018) Chromatin accessibility identifies diversity in mesenchymal stem cells from different tissue origins, *Sci Rep*. **8**, 17765.
21. Cho, K. A., Park, M., Kim, Y. H., Woo, S. Y. & Ryu, K. H. (2017) RNA sequencing reveals a transcriptomic portrait of human mesenchymal stem cells from bone marrow, adipose tissue, and palatine tonsils, *Sci Rep*. **7**, 17114.
22. Farahani, R. M. & Xaymardan, M. (2015) Platelet-Derived Growth Factor Receptor Alpha as a Marker of Mesenchymal Stem Cells in Development and Stem Cell Biology, *Stem Cells Int*. **2015**, 362753.
23. Bara, J. J., Richards, R. G., Alini, M. & Stoddart, M. J. (2014) Concise review: Bone marrow-derived mesenchymal stem cells change phenotype following in vitro culture: implications for basic research and the clinic, *Stem Cells*. **32**, 1713-23.
24. Shi, Y., Wang, Y., Li, Q., Liu, K., Hou, J., Shao, C. & Wang, Y. (2018) Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases, *Nat Rev Nephrol*. **14**, 493-507.
25. Selich, A., Daudert, J., Hass, R., Philipp, F., von Kaisenberg, C., Paul, G., Cornils, K., Fehse, B., Rittinghausen, S., Schambach, A. & Rothe, M. (2016) Massive Clonal Selection and Transiently Contributing Clones During Expansion of Mesenchymal Stem Cell Cultures Revealed by Lentiviral RGB-Barcode Technology, *Stem Cells Transl Med*. **5**, 591-601.
26. Phinney, D. G. (2012) Functional heterogeneity of mesenchymal stem cells: implications for cell therapy, *J Cell Biochem*. **113**, 2806-12.
27. Boregowda, S. V., Krishnappa, V., Haga, C. L., Ortiz, L. A. & Phinney, D. G. (2016) A Clinical Indications Prediction Scale Based on TWIST1 for Human Mesenchymal Stem Cells, *EBioMedicine*. **4**, 62-73.
28. Galipeau, J. & Sensebe, L. (2018) Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities, *Cell Stem Cell*. **22**, 824-833.

29. Shi, C., Jia, T., Mendez-Ferrer, S., Hohl, T. M., Serbina, N. V., Lipuma, L., Leiner, I., Li, M. O., Frenette, P. S. & Pamer, E. G. (2011) Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating toll-like receptor ligands, *Immunity*. **34**, 590-601.
30. Rafei, M., Hsieh, J., Fortier, S., Li, M., Yuan, S., Birman, E., Forner, K., Boivin, M.-N., Doody, K., Tremblay, M., Annabi, B. & Galipeau, J. (2008) Mesenchymal stromal cell-derived CCL2 suppresses plasma cell immunoglobulin production via STAT3 inactivation and PAX5 induction, *Blood*. **112**, 4991-8.
31. Takeda, K., Webb, T. L., Ning, F., Shiraishi, Y., Regan, D. P., Chow, L., Smith, M. J., Ashino, S., Guth, A. M., Hopkins, S., Gelfand, E. W. & Dow, S. (2018) Mesenchymal Stem Cells Recruit CCR2 + Monocytes To Suppress Allergic Airway Inflammation, *J Immunol*. **200**, 1261-1269.
32. Lee, H. K., Kim, H. S., Kim, J. S., Kim, Y. G., Park, K. H., Lee, J. H., Kim, K. H., Chang, I. Y., Bae, S.-C., Kim, Y., Hong, J. T., Kehrl, J. H. & Han, S.-B. (2017) CCL2 deficient mesenchymal stem cells fail to establish long-lasting contact with T cells and no longer ameliorate lupus symptoms, *Sci Rep*. **7**, 41258.
33. Giri, J., Das, R., Nylen, E., Chinnadurai, R. & Galipeau, J. (2020) CCL2 and CXCL12 Derived from Mesenchymal Stromal Cells Cooperatively Polarize IL-10+ Tissue Macrophages to Mitigate Gut Injury, *Cell Rep*. **30**, 1923-1934 e4.
34. Chan, J. K., Roth, J., Oppenheim, J. J., Tracey, K. J., Vogl, T., Feldmann, M., Horwood, N. & Nanchahal, J. (2012) Alarmins: awaiting a clinical response, *J Clin Invest*. **122**, 2711-9.
35. Lee, G., Espirito Santo, A. I., Zwingenberger, S., Cai, L., Vogl, T., Feldmann, M., Horwood, N. J., Chan, J. K. & Nanchahal, J. (2018) Fully reduced HMGB1 accelerates the regeneration of multiple tissues by transitioning stem cells to GAlert, *Proc Natl Acad Sci U S A*. **115**, E4463-E4472.
36. Beegle, J., Lakatos, K., Kalomoiris, S., Stewart, H., Isseroff, R. R., Nolte, J. A. & Fierro, F. A. (2015) Hypoxic preconditioning of mesenchymal stromal cells induces metabolic changes, enhances survival, and promotes cell retention in vivo, *Stem Cells*. **33**, 1818-28.
37. Yin, J. Q., Zhu, J. & Ankrum, J. A. (2019) Manufacturing of primed mesenchymal stromal cells for therapy, *Nat Biomed Eng*. **3**, 90-104.
38. Chinnadurai, R., Rajan, D., Qayed, M., Arafat, D., Garcia, M., Liu, Y., Kugathasan, S., Anderson, L. J., Gibson, G. & Galipeau, J. (2018) Potency Analysis of Mesenchymal Stromal Cells Using a Combinatorial Assay Matrix Approach, *Cell Rep*. **22**, 2504-2517.
39. Pittenger, M. F., Discher, D. E., Peault, B. M., Phinney, D. G., Hare, J. M. & Caplan, A. I. (2019) Mesenchymal stem cell perspective: cell biology to clinical progress, *NPJ Regen Med*. **4**, 22.
40. Carvalho, A. E. S., Sousa, M. R. R., Alencar-Silva, T., Carvalho, J. L. & Saldanha-Araujo, F. (2019) Mesenchymal stem cells immunomodulation: The road to IFN-gamma licensing and the path ahead, *Cytokine Growth Factor Rev*. **47**, 32-42.
41. Gnani, D., Crippa, S., Della Volpe, L., Rossella, V., Conti, A., Lettera, E., Ravis, S., Ometti, M., Fraschini, G., Bernardo, M. E. & Di Micco, R. (2019) An early-senescence state in aged mesenchymal stromal cells contributes to hematopoietic stem and progenitor cell clonogenic impairment through the activation of a pro-inflammatory program, *Aging Cell*. **18**, e12933.
42. Spees, J. L., Lee, R. H. & Gregory, C. A. (2016) Mechanisms of mesenchymal stem/stromal cell function, *Stem Cell Res Ther*. **7**, 125.
43. Phinney, D. G. & Pittenger, M. F. (2017) Concise Review: MSC-Derived Exosomes for Cell-Free Therapy, *Stem Cells*. **35**, 851-858.
44. Elahi, F. M., Farwell, D. G., Nolte, J. A. & Anderson, J. D. (2020) Preclinical translation of exosomes derived from mesenchymal stem/stromal cells, *Stem Cells*. **38**, 15-21.
45. Dal Collo, G., Adamo, A., Gatti, A., Tamellini, E., Bazzoni, R., Takam Kamga, P., Tecchio, C., Quaglia, F. M. & Krampera, M. (2020) Functional dosing of mesenchymal stromal cell-derived extracellular vesicles for the prevention of acute graft-versus-host-disease, *Stem Cells*. **38**, 698-711.
46. Mendt, M., Rezvani, K. & Shpall, E. (2019) Mesenchymal stem cell-derived exosomes for clinical use, *Bone Marrow Transplant*. **54**, 789-792.

47. Tieu, A., Slobodian, M., Fergusson, D. A., Montroy, J., Burger, D., Stewart, D. J., Shorr, R., Allan, D. S. & Lalu, M. M. (2019) Methods and efficacy of extracellular vesicles derived from mesenchymal stromal cells in animal models of disease: a preclinical systematic review protocol, *Syst Rev.* **8**, 322.
48. Luk, F., de Witte, S. F., Korevaar, S. S., Roemeling-van Rhijn, M., Franquesa, M., Strini, T., van den Engel, S., Gargasha, M., Roy, D., Dor, F. J., Horwitz, E. M., de Bruin, R. W., Betjes, M. G., Baan, C. C. & Hoogduijn, M. J. (2016) Inactivated Mesenchymal Stem Cells Maintain Immunomodulatory Capacity, *Stem Cells Dev.* **25**, 1342-54.
49. Lee, O. J., Luk, F., Korevaar, S. S., Koch, T. G., Baan, C. C., Merino, A. & Hoogduijn, M. J. (2020) The Importance of Dosing, Timing, and (in)Activation of Adipose Tissue-Derived Mesenchymal Stromal Cells on Their Immunomodulatory Effects, *Stem Cells Dev.* **29**, 38-48.
50. de Witte, S. F. H., Luk, F., Sierra Parraga, J. M., Gargasha, M., Merino, A., Korevaar, S. S., Shankar, A. S., O'Flynn, L., Elliman, S. J., Roy, D., Betjes, M. G. H., Newsome, P. N., Baan, C. C. & Hoogduijn, M. J. (2018) Immunomodulation By Therapeutic Mesenchymal Stromal Cells (MSC) Is Triggered Through Phagocytosis of MSC By Monocytic Cells, *Stem Cells.* **36**, 602-615.
51. Oja, S., Kaartinen, T., Ahti, M., Korhonen, M., Laitinen, A. & Nystedt, J. (2019) The Utilization of Freezing Steps in Mesenchymal Stromal Cell (MSC) Manufacturing: Potential Impact on Quality and Cell Functionality Attributes, *Front Immunol.* **10**, 1627.
52. Weiss, D. J., English, K., Krasnodembskaya, A., Isaza-Correa, J. M., Hawthorne, I. J. & Mahon, B. P. (2019) The Necrobiology of Mesenchymal Stromal Cells Affects Therapeutic Efficacy, *Front Immunol.* **10**, 1228.
53. Galleu, A., Riffo-Vasquez, Y., Trento, C., Lomas, C., Dolcetti, L., Cheung, T. S., von Bonin, M., Barbieri, L., Halai, K., Ward, S., Weng, L., Chakraverty, R., Lombardi, G., Watt, F. M., Orchard, K., Marks, D. I., Apperley, J., Bornhauser, M., Walczak, H., Bennett, C. & Dazzi, F. (2017) Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation, *Sci Transl Med.* **9**.
54. Matthay, M. A., Calfee, C. S., Zhuo, H., Thompson, B. T., Wilson, J. G., Levitt, J. E., Rogers, A. J., Gotts, J. E., Wiener-Kronish, J. P., Bajwa, E. K., Donahoe, M. P., McVerry, B. J., Ortiz, L. A., Exline, M., Christman, J. W., Abbott, J., Delucchi, K. L., Caballero, L., McMillan, M., McKenna, D. H. & Liu, K. D. (2019) Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial, *Lancet Respir Med.* **7**, 154-162.
55. Moll, G., Geissler, S., Catar, R., Ignatowicz, L., Hoogduijn, M. J., Strunk, D., Bieback, K. & Ringden, O. (2016) Cryopreserved or Fresh Mesenchymal Stromal Cells: Only a Matter of Taste or Key to Unleash the Full Clinical Potential of MSC Therapy?, *Adv Exp Med Biol.* **951**, 77-98.
56. Chinnadurai, R., Copland, I. B., Garcia, M. A., Petersen, C. T., Lewis, C. N., Waller, E. K., Kirk, A. D. & Galipeau, J. (2016) Cryopreserved Mesenchymal Stromal Cells Are Susceptible to T-Cell Mediated Apoptosis Which Is Partly Rescued by IFN γ Licensing, *Stem Cells.* **34**, 2429-42.
57. Braid, L. R., Wood, C. A., Wiese, D. M. & Ford, B. N. (2018) Intramuscular administration potentiates extended dwell time of mesenchymal stromal cells compared to other routes, *Cytotherapy.* **20**, 232-244.
58. Giri, J. & Galipeau, J. (2020) Mesenchymal stromal cell therapeutic potency is dependent upon viability, route of delivery, and immune match, *Blood Adv.* **4**, 1987-1997.
59. Allen, A., Vaninov, N., Li, M., Nguyen, S., Igo, P., Tilles, A. W., O'Rourke, B., Miller, B. L. K., Parekkadan, B. & Barcia, R. N. (2020) Mesenchymal Stromal Cell Bioreactor for Ex Vivo Reprogramming of Human Immune Cells, *Sci Rep.* **10**, 10142.
60. Kabat, M., Bobkov, I., Kumar, S. & Grumet, M. (2020) Trends in mesenchymal stem cell clinical trials 2004-2018: Is efficacy optimal in a narrow dose range?, *Stem Cells Transl Med.* **9**, 17-27.
61. Meyerrose, T., Olson, S., Pontow, S., Kalomoiris, S., Jung, Y., Annett, G., Bauer, G. & Nolte, J. A. (2010) Mesenchymal stem cells for the sustained in vivo delivery of bioactive factors, *Adv Drug Deliv Rev.* **62**, 1167-74.

62. Mao, A. S., Ozkale, B., Shah, N. J., Vining, K. H., Descombes, T., Zhang, L., Tringides, C. M., Wong, S. W., Shin, J. W., Scadden, D. T., Weitz, D. A. & Mooney, D. J. (2019) Programmable microencapsulation for enhanced mesenchymal stem cell persistence and immunomodulation, *Proc Natl Acad Sci U S A*. **116**, 15392-15397.
63. Daga, A., Muraglia, A., Quarto, R., Cancedda, R. & Corte, G. (2002) Enhanced engraftment of EPO-transduced human bone marrow stromal cells transplanted in a 3D matrix in non-conditioned NOD/SCID mice, *Gene Ther*. **9**, 915-21.
64. Bartunek, J., Davison, B., Sherman, W., Povsic, T., Henry, T. D., Gersh, B., Metra, M., Filippatos, G., Hajjar, R., Behfar, A., Homsy, C., Cotter, G., Wijns, W., Tendera, M. & Terzic, A. (2016) Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) trial design, *Eur J Heart Fail*. **18**, 160-8.
65. Teerlink, J. R., Metra, M., Filippatos, G. S., Davison, B. A., Bartunek, J., Terzic, A., Gersh, B. J., Povsic, T. J., Henry, T. D., Alexandre, B., Homsy, C., Edwards, C., Seron, A., Wijns, W., Cotter, G. & Investigators, C. (2017) Benefit of cardiopoietic mesenchymal stem cell therapy on left ventricular remodelling: results from the Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) study, *Eur J Heart Fail*. **19**, 1520-1529.
66. Bartolucci, J., Verdugo, F. J., Gonzalez, P. L., Larrea, R. E., Abarzua, E., Goset, C., Rojo, P., Palma, I., Lamich, R., Pedreros, P. A., Valdivia, G., Lopez, V. M., Nazzari, C., Alcayaga-Miranda, F., Cuenca, J., Brobeck, M. J., Patel, A. N., Figueroa, F. E. & Khoury, M. (2017) Safety and Efficacy of the Intravenous Infusion of Umbilical Cord Mesenchymal Stem Cells in Patients With Heart Failure: A Phase 1/2 Randomized Controlled Trial (RIMECARD Trial [Randomized Clinical Trial of Intravenous Infusion Umbilical Cord Mesenchymal Stem Cells on Cardiopathy]), *Circ Res*. **121**, 1192-1204.
67. Lankford, K. L., Arroyo, E. J., Nazimek, K., Bryniarski, K., Askenase, P. W. & Kocsis, J. D. (2018) Intravenously delivered mesenchymal stem cell-derived exosomes target M2-type macrophages in the injured spinal cord, *PLoS One*. **13**, e0190358.
68. Sensebe, L. & Fleury-Cappellesso, S. (2013) Biodistribution of mesenchymal stem/stromal cells in a preclinical setting, *Stem Cells Int*. **2013**, 678063.
69. von Bahr, L., Batsis, I., Moll, G., Hagg, M., Szakos, A., Sundberg, B., Uzunel, M., Ringden, O. & Le Blanc, K. (2012) Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation, *Stem Cells*. **30**, 1575-8.
70. Caplan, H., Olson, S. D., Kumar, A., George, M., Prabhakara, K. S., Wenzel, P., Bedi, S., Toledano-Furman, N. E., Triolo, F., Kamhieh-Milz, J., Moll, G. & Cox, C. S., Jr. (2019) Mesenchymal Stromal Cell Therapeutic Delivery: Translational Challenges to Clinical Application, *Front Immunol*. **10**, 1645.
71. Moll, G., Ankrum, J. A., Kamhieh-Milz, J., Bieback, K., Ringden, O., Volk, H. D., Geissler, S. & Reinke, P. (2019) Intravascular Mesenchymal Stromal/Stem Cell Therapy Product Diversification: Time for New Clinical Guidelines, *Trends Mol Med*. **25**, 149-163.
72. Guess, A. J., Daneault, B., Wang, R., Bradbury, H., La Perle, K. M. D., Fitch, J., Hedrick, S. L., Hamelberg, E., Astbury, C., White, P., Overolt, K., Rangarajan, H., Abu-Arja, R., Devine, S. M., Otsuru, S., Dominici, M., O'Donnell, L. & Horwitz, E. M. (2017) Safety Profile of Good Manufacturing Practice Manufactured Interferon gamma-Primed Mesenchymal Stem/Stromal Cells for Clinical Trials, *Stem Cells Transl Med*. **6**, 1868-1879.
73. Kimbrel, E. A. & Lanza, R. (2020) Next-generation stem cells - ushering in a new era of cell-based therapies, *Nat Rev Drug Discov*. **19**, 463-479.
74. Nolte, J. A., Galipeau, J. & Phinney, D. G. (2020) Improving mesenchymal stem/stromal cell potency and survival: Proceedings from the International Society of Cell Therapy (ISCT) MSC preconference held in May 2018, Palais des Congres de Montreal, Organized by the ISCT MSC Scientific Committee, *Cytotherapy*. **22**, 123-126.
75. Martin, I., Galipeau, J., Kessler, C., Le Blanc, K. & Dazzi, F. (2019) Challenges for mesenchymal stromal cell therapies, *Sci Transl Med*. **11**.

76. Kurtzberg, J., Prasad, V., Grimley, M., Horn, B., Carpenter, P., Jacobsohn, D. & Prockop, S. (2010) Allogeneic human mesenchymal stem cell therapy (Prochymal®) as a rescue agent for severe treatment resistant GVHD in pediatric patients, *Biology of Blood and Marrow Transplantation*. **16**, S169.
77. Cheung, T. S., Bertolino, G. M., Giacomini, C., Bornhauser, M., Dazzi, F. & Galleu, A. (2020) Mesenchymal Stromal Cells for Graft Versus Host Disease: Mechanism-Based Biomarkers, *Front Immunol*. **11**, 1338.

Accepted manuscript