HHS Public Access
Author manuscript
Cytotherapy. Author manuscript; available in PMC 2016 February 08.

Published in final edited form as:
Cytotherapy. 2016 February ; 18(2): 151–159. doi:10.1016/j.jcyt.2015.11.008.

International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials

Jacques Galipeau[1,*], **Mauro Krampera**[2,*], **John Barrett**[3], **Francesco Dazzi**[4], **Robert J. Deans**[5], **Joost Debruijn**[6], **Massimo Dominici**[7], **Willem E. Fibbe**[8], **Adrian P. Gee**[9], **Jeffery M. Gimble**[10], **Peiman Hematti**[11], **Mickey B.C. Koh**[12,26], **Katarina Leblanc**[13], **Ivan Martin**[14,*], **Ian K. Mcniece**[15], **Michael Mendicino**[16], **Steve Oh**[17], **Luis Ortiz**[18], **Donald G. Phinney**[19,*], **Valerie Planat**[20], **Yufang Shi**[21,27,*], **David F. Stroncek**[22], **Sowmya Viswanathan**[23], **Daniel J. Weiss**[24], and **Luc Sensebe**[25,*]

[1]Department of Hematology and Medical Oncology, Winship Cancer Institute, and Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA [2]Section of Hematology, Stem Cell Research Laboratory and Cell Factory, Department of Medicine, University of Verona, Verona, Italy [3]Stem Cell Allotransplantation Section, Hematology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA [4]Regenerative and Heamatological Medicine, King's College London, London, UK [5]Regenerative Medicine, Athersys Inc., Cleveland, OH, USA [6]School of Engineering and Materials Science, Queen Mary University of London, London, UK [7]Department of Medical and Surgical Sciences for Children and Adults, Division of Oncology, University-Hospital of Modena and Reggio Emilia, Modena, Italy [8]Department of Immunohematology and Bloodtransfusion, Leiden University Medical Centre, Leiden, Netherlands [9]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston Methodist Hospital, Texas Children's Hospital, Houston, TX, USA [10]Center for Stem Cell Research and Regenerative Medicine, Department of Medicine, and Department of Surgery, Tulane University School of Medicine, New Orleans, LA, USA [11]Department of Medicine, University of Wisconsin—Madison, School of Medicine and Public Health, and University of Wisconsin Carbone Cancer Center, Madison, WI, USA [12]Department of Haematology, St George's Hospital and Medical School, London, UK [13]Division of Clinical Immunology and Transfusion Medicine, Karolinska Institutet, Stockholm, Sweden [14]Department of Biomedicine, University Hospital Basel, Basel, Switzerland [15]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas, MD Anderson Cancer Center, Houston, TX, USA [16]Mesoblast Inc., New York, NY, USA [17]Stem Cell Group, Bioprocessing Technology Institute, Agency for Science Technology and Research (A*STAR), Singapore [18]Division of Occupational and Environmental Health Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA [19]Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, FL, USA [20]IFR150 STROMALab UMR 5273 UPS–CNRS–EFS–INSERM U1031,Toulouse, France [21]Institute of Health Sciences, Chinese Academy of Sciences, Shanghai, China [22]Cell Processing

Correspondence: **Jacques Galipeau**, MD, Winship Cancer Institute, 1365B Clifton Road, Atlanta, GA 30322, USA. jgalipe@emory.edu.
*International Society for Cellular Therapy MSC Committee members.

Section, Department of Transfusion Medicine Clinical Center, NIH, Bethesda, MD, USA [23]Cell Therapy Program, University Health Network, Toronto, Canada [24]Department of Medicine, University of Vermont College of Medicine, Burlington, VT, USA [25]UMR5273 STROMALab CNRS/EFS/UPS—INSERM U1031, Toulouse, France [26]Blood Services Group, Health Sciences Authority, Singapore [27]The First Affiliated Hospital, Soochow University Institutes for Translational Medicine, Suzhou, China

Abstract

Mesenchymal stromal cells (MSCs) as a pharmaceutical for ailments characterized by pathogenic autoimmune, alloimmune and inflammatory processes now cover the spectrum of early- to late-phase clinical trials in both industry and academic sponsored studies. There is a broad consensus that despite different tissue sourcing and varied culture expansion protocols, human MSC-like cell products likely share fundamental mechanisms of action mediating their anti-inflammatory and tissue repair functionalities. Identification of functional markers of potency and reduction to practice of standardized, easily deployable methods of measurements of such would benefit the field. This would satisfy both mechanistic research as well as development of release potency assays to meet Regulatory Authority requirements for conduct of advanced clinical studies and their eventual registration. In response to this unmet need, the International Society for Cellular Therapy (ISCT) addressed the issue at an international workshop in May 2015 as part of the 21st ISCT annual meeting in Las Vegas. The scope of the workshop was focused on discussing potency assays germane to immunomodulation by MSC-like products in clinical indications targeting immune disorders. We here provide consensus perspective arising from this forum. We propose that focused analysis of selected MSC markers robustly deployed by in vitro licensing and metricized with a matrix of assays should be responsive to requirements from Regulatory Authorities. Workshop participants identified three preferred analytic methods that could inform a matrix assay approach: quantitative RNA analysis of selected gene products; flow cytometry analysis of functionally relevant surface markers and protein-based assay of secretome. We also advocate that potency assays acceptable to the Regulatory Authorities be rendered publicly accessible in an "open-access" manner, such as through publication or database collection.

Keywords

Mesenchymal Stromal cells; potency assays; release assays; matrix assays; immune functional testing; clinical trials; ISCT

Culture-expanded mesenchymal stromal cells (MSCs) meeting minimal core identity for MSCs as defined by International Society for Cellular Therapy (ISCT) in 2006 [1] derived from marrow, adipose tissue, umbilical cord tissue and other sources from either autologous or allogeneic donor sources are being studied in clinical trials across numerous regulatory jurisdictions worldwide. The ailments targeted with this cell pharmaceutical platform fall roughly within two pathophysiological categories: immune/inflammatory and tissue repair/restoration [2]. It is now widely accepted that the pharmaceutical effect of MSC-like cells is predominantly mediated by paracrine and contact factors arising from intrinsic MSC physiological processes that are maintained after culture expansion. It is further accepted that following in vivo delivery, MSCs are further responsive to environmental cues encountered in situ leading to additional cellular functionalities [3]. Culture expanded MSC-like cells are unambiguously classified as a more-than-minimal-manipulated cellular and gene therapy (CGT) product regulated in the United States under section 351 of the Public Health Service Act (PHS Act) (42 U.S.C. 262). As a type of CGT product, MSC-like cells require an Investigational New Drug Application (IND) from the Food & Drug Administration (FDA) for conduct of clinical trials in the USA. The FDA further requires development of tests to measure potency as part of release criteria of advanced clinical trials designed to support marketing approval and registration. Similar requirements are made by the European Medicines Agency (EMA) for Advanced Therapy Medicinal Products (ATMPs), which include cell therapies, as defined by the European Regulation (European Commission [EC]) No. 1394 / 2007,[1] further strengthened on December 30, 2008, and directly enclosed in the legislation of each EU member nation with no need of other implementation. The EU Regulation is in compliance with the 2004/23/EC directive on donation, supply and testing of human cells and tissues and with directive 2002/98/EC on human blood and blood components.[2] The tripartite components of release criteria for MSC-like cellular products in early phase clinical trials—identity, viability and sterility—raise little practical controversy and the consensus on markers for identity of MSC-like cells, considering their intrinsic heterogeneity and phenotype plasticity, is also reasonably well defined [1,4]. However, the issue of potency testing remains largely open-ended and is informed by the putative mechanism of action (MOA) of MSC-like cells in a given indication. Care must be made in distinguishing curiosity-driven research as part of ancillary studies on cell products and release potency assays required to satisfy the Regulatory Authorities. Although pre-clinical MOA studies will necessarily inform the methods and reduction to practice of deployable potency assays, the latter have specific requirements for the following as part of assay validation: accuracy, precision, specificity, linearity and range, system suitability, and robustness.

International regulatory authority guidance on potency tests for cellular therapy products

The FDA has published guidance with recommendations for developing tests to measure potency for CGT products.[3] These recommendations are intended to clarify the potency information that could support an IND or a Biologics License Application. Because potency measurements are designed specifically for a particular product, their guidance does not make recommendations regarding specific types of potency assays nor does it propose acceptance criteria for product release. FDA guidance defines potency (strength is also synonymously used) as the therapeutic activity of the drug product as indicated by appropriate laboratory tests or by adequately developed and controlled clinical data. Regulatory Authorities are not prescriptive in what constitutes a definitive potency assay,

[1]http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:324:0121:0137:EN:PDF.
[2]http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:033:0030:0040:EN:PDF.
[3]http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM243392.pdf.

and FDA regulations allow for considerable flexibility in determining the appropriate measurements of potency for each product. Potency is determined on the basis of individual product attributes; therefore, the adequacy of potency tests is evaluated on a case-by-case basis. The Regulatory Authorities also recognizes the inherent challenges in defining potency assays (Table I). Similar guidance has been published by EMA, which defines potency as the quantitative measure of biological activity based on the attribute of the product, which is linked to the relevant biological properties. Consequently, the assay demonstrating the biological activity should be based on the intended biological effect, which should ideally be related to the clinical response and aimed at investigating major cellular functions by using surrogate markers and appropriate technology.[4] The newly released guidelines of quality control of stem cell products by the Chinese National Health and Family Planning Commission[5] mainly emphasized guidance for facility requirements, biological contaminants, viability and product consistency. No specific criterion was given on the issue of potency assay, and the guidance limits its recommendation to assessment of potency according to disease indications.

There is no single test that can adequately measure product attributes that predict clinical efficacy. Taking into consideration this limitation, the potency assay should represent the product's mechanism of action (i.e., relevant therapeutic activity or intended biological effect). However, many CGT products, including MSC-like cells, have complex (e.g., rely on multiple biological activities) and/or not fully characterized mechanisms of action, making it difficult to determine which product attributes are most relevant to measuring potency. Indeed, it will be extraordinarily challenging to perform reductionist mechanistic experiments in human subjects that will conclusively define substantive MOA of MSC-like cells in vivo and meet modern standards of ethical conduct in clinical trials. Nonetheless, all attempts should be made to develop potency measurements that reflect the product's relevant biological properties and that can also serve as a measure of comparability between production lots [5].Therefore, defining hypothesis-driven MOA based on correlative in vitro experiments and buttressed, where feasible, with comparative biology approach in animal systems will inform the choice of potency assays to be developed. The Regulatory Authorities anticipate that Manufacturers demonstrate clinical effectiveness by correlative "substantial evidence," that is, evidence that the product will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling or proposed labeling thereof (section 505(d) of the FDC Act). The traditional approach for assessing the potency of biological products is to develop a quantitative biological assay (bioassay) that measures the activity of the product related to its specific ability to affect a given result and that also meets the criteria required by Regulatory Authorities (Table II).

Analytical methods to measure potency

Bioassays can provide a measure of potency by evaluating a product's active ingredients within a living biological system. Bioassays can include in vivo animal studies, in vitro

[4]http://www.ema.europa.eu/docs/en_GB/document_library/Presentation/2015/05/WC500187352.pdf.
[5]http://www.nhfpc.gov.cn/qjjys/s3581/201508/15d0dcf66b734f338c31f67477136cef.shtml.

organ, tissue or cell culture systems, or any combination of these. Development of a quantitative bioassay for MSC-like products may be complicated by properties of the product and/or technical limitations of certain assays (see Table I). In cases in which development of a suitable bioassay is not feasible, it may be necessary to identify a surrogate measurement of biological activity. For example, the use of non-biological analytical assays performed outside of a living system that is practical and demonstrates adequate performance characteristics for lot release. Examples of such analytical assays provided by the FDA include methods that measure immunochemical (e.g., quantitative flow cytometry, enzyme-linked immunosorbent assay), molecular (e.g., reverse transcription polymerase chain reaction, quantitative polymerase chain reaction, microarray) or biochemical (e.g., protein binding, enzymatic reactions) properties of the product outside of a living system. Analytical assays can provide extensive product characterization data by evaluating immunochemical, biochemical and/or molecular attributes of the product. These attributes may be used to demonstrate potency if the surrogate measurement(s) can be substantiated by correlation to a relevant product-specific biological activity(s).

Assay matrix

The FDA states that a single biological or analytical assay may not provide an adequate measure of potency. The following are some potential reasons: (i) product has complex and/or not fully characterized mechanism of action, (ii) product has multiple active ingredients and/or multiple biological activities, (iii) limited product stability, or (iv) biological assay is not quantitative, not sufficiently robust or lacks precision. If one assay is not sufficient to measure the product attributes that indicates potency, then an alternative approach could be used, such as developing multiple complementary assays that measure different product attributes associated with quality, consistency and stability. When used together and when results are correlated with a relevant biological activity, these complementary assays should provide an adequate measure of potency. Such a collection of assays (referred to as an assay matrix) might consist of a combination of biological assays, biological and analytical assays or analytical assays alone. The assay matrix may include assays that give a quantitative readout (e.g., units of activity) and/or qualitative readout (e.g., pass–fail). If qualitative assays are used as part of an assay matrix to determine potency for lot release, stability or comparability studies, they should be accompanied by one or more quantitative assays. A concrete example of an assay matrix for MSC-like cells recognized by the FDA is detailed in a published report by Athersys Inc. (Cleveland, OH) outlining their effort in establishing an angiogenic potency assay for their MultiStem product based on measure of CXCL5, interleukin (IL)-8 and vascular endothelial growth factor (VEGF) production coupled to an in vitro cell-based angiogenic assay [6]. The correlative relationship between the surrogate measurement and biological activity may be established using various approaches, including comparison to preclinical/proof of concept data, in vivo data (animal or clinical) or in vitro cellular or biochemical data. The suitability of data used to support the correlative relationship between the surrogate assay and the biological activity of a MSC-like product will be evaluated on a case-by-case basis by the Regulatory Authorities and depends on or is influenced by the following: (i) type and relevance of the correlations being made, (ii) the amount of product information accumulated, (iii) how well the biological activity of the product is understood, and (iv) how well the surrogate measurements reflects biological activity.

Defining release potency assays for MSC-like cells developed for immunomodulation

The open-ended guidance from the Regulatory Authorities and the published precedent of a matrix potency release assay approach used by Athersys Inc. for Multistem in support of their advanced clinical studies informs a path forward on how to characterize a MSC-like product coupling an in vitro bioassay interrogating the cellular secretome by enzyme-linked immunosorbent assay (ELISA) and a functional cell-based assay. Another industry sponsored example is that of Prochymal (Osiris Therapeutics, Inc.), which is an industrial-scale expanded MSC-like product derived from marrow collected from random donors that was studied as part of prospective randomized clinical trials. As a surrogate measure of potency, soluble TNFR1 was defined as a release criterion [7]. There are likely other potency release assays strategies that have been considered by the Regulatory Authorities for MSC-like ATMPs for use in immunomodulation, but these are unpublished and are not available for public consultation because Regulatory Authorities are not at liberty to publicly disclose otherwise confidential IND disclosure made by Manufacturers (academic or industrial). Nonetheless, these precedents inform us that a minimal set of assay components to be taken into consideration will likely require direct assay(s) of cell functionalities of MSC-like products and possibly a companion cell physiology assay on a responder lymphomyeloid cell population.

Functional in vitro assays with responder immune cells

Allogeneic random donor human peripheral blood mononuclear cell (PBMCs) serve as a useful tool to decipher MOA of human MSCs. However, there are serious limitations to their use as robust and reproducible potency release assay for human clinical trials [8]. Activated $CD3^+$ T cells provide the opportunity to measure inhibition of proliferation and cytokine production in vitro, yet it is unknown whether this assay accurately reflects the MOA of MSCs in vivo. Indeed, there are published pre-clinical data demonstrating that MSCs and other stem cell types can influence the cell physiology of monocytes, B-cells, natural killer cells and granulocytes, none of which is reflected in a classic mixed lymphocyte reaction (MLR)-like assay [9].The use of unfractionated PBMCs collected from consenting normal human volunteers contains a mixture of lymphomyeloid cells that varies among human subjects and further complicates the reproducibility and interpretation of such assays. Finally, if the goal of the immunopotency assay is to highlight the MSC MOA that will be effective in a specific clinical setting, the use of purified immune effector cells that are involved in the disease pathogenesis rather than unfractionated responder PBMCs could be more informative. Considering the inherent shortcomings of using PBMCs in cell responder release assays, a reductionist perspective may provide guidance to robust and predictive MSC potency assays. One may rationally argue that if the mechanism by which MSCs suppress T-cell proliferation in vitro is defined, direct analysis of expression and induction of such as outlined earlier by a matrix assay approach may avoid the intrinsic variance in using random donor PBMC responder cells as part of descriptive release assay.

The key will be defining functionalities that are theoretically causative of suppressor function in vivo so as to best inform on comparability of MSC-like platforms and also provide guidance on selection of clinical trial subjects which are poised to best respond based on hypothesis-driven mechanistic predictors of response.

Assay matrix for MSC-like cells to assay immunomodulation functionalities

The FDA provides guidance on assay systems that can serve as methods to ascertain potency/strength of cell therapy such as MSC-like cells. As an aggregate, these assays measure specific functionalities that can be correlated with in vitro cell-based systems. From a Manufacturer's perspective (academic or industrial), the assay matrix needs to comply with criteria required by the FDA (Table II) and possess certain elements of robustness, reproducibility and economy. Taking these in to consideration, workshop participants identified three preferred analytic methods that could inform a matrix assay approach: (i) quantitative RNA analysis of selected gene products, (ii) flow cytometry analysis of functionally relevant surface markers and (iii) protein-based assay of secretome. The analysis of MSC-like cells at time of lot release typically is performed on cells that are in log phase of growth within the boundaries of manufacturing parameters under study. In essence, this is a snapshot of steady-state functionalities. The presence of static cell markers (e.g., $CD73^+$, $CD90^+$, $CD105^+$, $CD45^-$) are useful to validate identity of MSC-like products, but they have not been demonstrated to act directly or predict for immune modulating function [9].Within this context, the use of a "cell ruler" against which released lots are compared gains nominal importance [10,11].

Immune plasticity

The MSC committee of the ISCT released a position statement paper in 2013 that proposed immunological characterization of Multipotent Mesenchymal Stromal Cells [9]. Relative to their homeostatic resting state, MSCs undergo polarization toward inhibitory functionality on exposure to various pro-inflammatory cytokines such as interferon (IFN)γ, tumor necrosis factor (TNF)-α, IL-1α or IL-1β. In vitro MSC inflammatory "licensing" better recapitulates what likely happens in vivo once MSCs are transfused into patients with dysregulated immune responses or with systemic inflammation [12]. Measurable immunological features of MSCs, both at phenotypic and functional levels, depend on their activation status at the time of interaction with effector cells, although variability may be observed among different donors [13].Thus, if these functional assays aim to assess the immune regulatory functions of MSCs for clinical purposes, comparing the results with both resting and licensed MSCs would be most informative, regardless of the tissue origin. Different protocols of MSC licensing are available from the literature [14], but normally the addition of IFNγ for 12–48 h is adequate to obtain MSC activation that allows for their analysis as part of an assay matrix. IFNγ-activated MSC-like cells readily increase expression of surface markers relevant to functional immune modulation and are amenable to routine clinical flow cytometric analysis (Table III). Similarly, quantitative RNA analysis of genes identified in the literature as likely related to MOA (Table IV) before and after IFNγ activation MSCs leads to increased transcription of an array of immune relevant genes among which a significant subset are increased more than a thousand-fold (e.g., IDO, CXCL9, CXCL10, CXCL11 and CIITA) [15]. Protein-based assays such as ELISA can further measure secreted factors produced directly by MSC-like cells, especially those amplified by in vitro IFNγ licensing, which can be reasonably postulated to play a role in immune modulation. The approach here high-lighted does not exclude the use of alternate methods of in vitro MSC licensing such as the use of TLR agonists or distinct cytokines (TNF-α, IL-1, etc.), inasmuch as these deploy immune modulation functionalities linked to effector function. Further, open-mindedness toward emerging concepts in MSC cell physiology and function including, but not restricted to, the influence of exosomes and micro RNA, for example, may play a part of release potency assays as their mechanistic role becomes better defined.

Controls

As with all well-designed experiments, developing a potency assay must include appropriate assay controls and a comparison to an appropriate product-specific reference material, when available. Running a product-specific reference material and/or control samples in parallel with the product helps ensure that the assay is performing as expected. In addition, controls help establish that the equipment and reagents are working within established limits. A well-designed set of control samples can substantially increase confidence that results are meaningful and reliable. The Manufacturer is expected to develop "in-house" reference material(s) as part of product development when feasible. These may include well-characterized clinical lots or other well-characterized materials prepared by the Manufacturer or another resource (e.g., a well-characterized cell line with a profile similar to MSC-like cells) that has been appropriately qualified. Taking this perspective in consideration, the objective of developing a universal MSC "cell ruler" has been addressed [16] and in itself represents a challenge almost as great as developing the potency assays for which they are to serve as a reference [10]. A complementary approach that may address the regulatory requirement for a reference material could include using "resting" MSCs as an internal "cell-ruler" control and their contemporaneous "activated" MSCs counterparts as the test [12]. In essence, each MSC lot serves as its own "control" when assaying immune plasticity.

Perspective statement: A focused analysis of selected markers robustly deployed by in vitro cytokine licensing (e.g., IFNγ) of MSCs and metricized with a complementary matrix of assays (fluorescence-activated cell sorting, quantitative RNA and proteomic [ELISA]) using "resting" MSCs as controls (from the same lot) should be responsive to requirements from Regulator Authorities regarding release potency assay (Table II). Candidate MOA surrogates outlined in Tables III and IV could serve as potential "universal" markers of strength for MSCs developed for their suppressive functionalities. It is also advisable to discuss potency development with the Regulatory Authorities early on and before initiation of phase 3 trials when potency becomes a Regulatory Authority requirement.

Conclusion

The key deliverable for the translational community invested in developing MSC-like cells to their full clinical potential will be defining functionalities which are predictive of their tissue repair and immune and inflammation modulatory functions in vivo to best inform on comparability of MSC-like platforms and also provide guidance on selection of clinical trial subjects that are poised to best respond based on hypothesis-driven mechanistic predictors of response. Although this guidance is focused on release potency assays, these remain tightly coupled to companion identity assays that themselves likely require refinement from the original 2006 ISCT position paper [1] as new knowledge on MSC-like cells from various tissue sources is gained by the field at large. Furthermore, the concept of economy is of material importance. Although the cost of release testing of large industrial lots can be easily amortized on a per-unit cost, the same is not applicable to one patient/one product setting of autologous cell manufacturing or low passage allogeneic MSC doses (usually <10 doses) typically manufactured by academic health centers in support of clinical trials. Indeed, complex matrix assays using cutting-edge—and expensive—technologies can add substantial costs to manufacturing and release of personalized MSC units. Thus, an open mind-set by Regulatory Authorities in allowing for use of assays systems that are economical, especially in the setting of autologous cell therapies (e.g., one product per patient) or for allogeneic products with small number of cell does per lot (as is often done in academic health center sponsored allogeneic MSC clinical trials) would be useful. It is also desirable that unfettered open access of Regulatory Authority–approved release potency assay systems for MSC-like cells by Manufacturers engaged in clinical trials be encouraged and embraced by the cell therapy community. Public access of these elements can only be achieved by voluntary public disclosure by Manufacturers, and this shared regulatory data will further advance the field by allowing stakeholders to adopt rationally developed and validated common standards and assays.

Acknowledgments

The workshop on potency release assays was sponsored by an unrestricted grant from the International Society for Cell Therapy (ISCT) as part of the 21st ISCT annual meeting held in Las Vegas, Nevada, in May 2015. This ISCT guidance article is not necessarily the position/perspective of the authors' respective companies or institutions.

References

1. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8:315–317. PMID 16923606; 150. [PubMed: 16923606]
2. Phinney DG, Galipeau J, Krampera M, Martin I, Shi Y, Sensebe L. MSCs: science and trials. Nat Med. 2013; 19:812. PMID 23836216; 432. [PubMed: 23836216]
3. Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol. 2014; 15:1009–1016. PMID 25329189; 446. [PubMed: 25329189]
4. Mendicino M, Bailey AM, Wonnacott K, Puri RK, Bauer SR. MSC-based product characterization for clinical trials: an FDA perspective. Cell Stem Cell. 2014; 14:141–145. PMID 24506881; 439. [PubMed: 24506881]
5. Salmikangas P, Menezes-Ferreira M, Reischl I, Tsiftsoglou A, Kyselovic J, Borg JJ, et al. Manufacturing, characterization and control of cell-based medicinal products: challenging paradigms toward commercial use. Regen Med. 2015; 10:65–78. PMID 25562353; 447. [PubMed: 25562353]

6. Lehman N, Cutrone R, Raber A, Perry R, Van't Hof W, Deans R, et al. Development of a surrogate angiogenic potency assay for clinical-grade stem cell production. Cytotherapy. 2012; 14:994–1004. PMID 22687190; 440. [PubMed: 22687190]
7. Kebriaei P, Isola L, Bahceci E, Holland K, Rowley S, McGuirk J, et al. Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. Biol Blood Marrow Transplant. 2009; 15:804–811. PMID 19539211; 149. [PubMed: 19539211]
8. Galipeau J, Krampera M. The challenge of defining mesenchymal stromal cell potency assays and their potential use as release criteria. Cytotherapy. 2015; 17:125–127. PMID 25593076; 445. [PubMed: 25593076]
9. Krampera M, Galipeau J, Shi Y, Tarte K, Sensebe L. Immunological characterization of multipotent mesenchymal stromal cells—the International Society for Cellular Therapy (ISCT) working proposal. Cytotherapy. 2013; 15:1054–1061. PMID 23602578; 250. [PubMed: 23602578]
10. Deans R. Towards the creation of a standard MSC line as a calibration tool. Cytotherapy. 2015; 17:1167–1168. PMID 26276000; 443. [PubMed: 26276000]
11. Salem B, Miner S, Hensel NF, Battiwalla M, Keyvanfar K, Stroncek DF, et al. Quantitative activation suppression assay to evaluate human bone marrow-derived mesenchymal stromal cell potency. Cytotherapy. 2015; 17:1675. PMID 26422657; 448. [PubMed: 26422657]
12. Krampera M. Mesenchymal stromal cell "licensing": a multistep process. Leukemia. 2011; 25:1408–1414. PMID 21617697; 138. [PubMed: 21617697]
13. Francois M, Romieu-Mourez R, Li M, Galipeau J. Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation. Mol Ther. 2012; 20:187–195. PMID 21934657; 15. [PubMed: 21934657]
14. Menard C, Pacelli L, Bassi G, Dulong J, Bifari F, Bezier I, et al. Clinical-grade mesenchymal stromal cells produced under various good manufacturing practice processes differ in their immunomodulatory properties: standardization of immune quality controls. Stem Cells Dev. 2013; 22:1789–1801. PMID 23339531; 4443668498. [PubMed: 23339531]
15. Chinnadurai R, Copland IB, Ng S, Garcia M, Prasad M, Arafat D, et al. Mesenchymal stromal cells derived from Crohn's patients deploy indoleamine 2,3-dioxygenase mediated immune suppression, independent of autophagy. Mol Ther. 2015; 23:1248–1261. PMID 25899824; 416. [PubMed: 25899824]
16. Viswanathan S, Keating A, Deans R, Hematti P, Prockop D, Stroncek DF, et al. Soliciting strategies for developing cell-based reference materials to advance mesenchymal stromal cell research and clinical translation. Stem Cells Dev. 2014; 23:1157–1167. PMID 24422625; 4414027980. [PubMed: 24422625]
17. Croitoru-Lamoury J, Lamoury FM, Zaunders JJ, Veas LA, Brew BJ. Human mesenchymal stem cells constitutively express chemokines and chemokine receptors that can be upregulated by cytokines, IFN-beta, and Copaxone. J Interferon Cytokine Res. 2007; 27:53–64. PMID 17266444; 344. [PubMed: 17266444]
18. Chamberlain G, Smith H, Rainger GE, Middleton J. Mesenchymal stem cells exhibit firm adhesion, crawling, spreading and transmigration across aortic endothelial cells: effects of chemokines and shear. PLoS ONE. 2011; 6:e25663. PMID 21980522; 3453182247. [PubMed: 21980522]
19. Feng Y, Yu HM, Shang DS, Fang WG, He ZY, Chen YH. The involvement of CXCL11 in bone marrow-derived mesenchymal stem cell migration through human brain microvascular endothelial cells. Neurochem Res. 2014; 39:700–706. PMID 24526602; 346. [PubMed: 24526602]
20. Tang KC, Trzaska KA, Smirnov SV, Kotenko SV, Schwander SK, Ellner JJ, et al. Down-regulation of MHC II in mesenchymal stem cells at high IFN-gamma can be partly explained by cytoplasmic retention of CIITA. J Immunol. 2008; 180:1826–1833. PMID 18209080; 347. [PubMed: 18209080]
21. Ren G, Zhao X, Zhang L, Zhang J, L'Huillier A, Ling W, et al. Inflammatory cytokine-induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in mesenchymal stem cells are critical for immunosuppression. J Immunol. 2010; 184:2321–2328. PMID 20130212; 3532881946. [PubMed: 20130212]

22. Kimura K, Nagano M, Salazar G, Yamashita T, Tsuboi I, Mishima H, et al. The role of CCL5 in the ability of adipose tissue-derived mesenchymal stem cells to support repair of ischemic regions. Stem Cells Dev. 2014; 23:488–501. PMID 24171667; 3493928761. [PubMed: 24171667]
23. Yang X, Du J, Xu X, Xu C, Song W. IFN-gamma-secreting-mesenchymal stem cells exert an antitumor effect in vivo via the TRAIL pathway. J Immunol Res. 2014; 2014:318098. PMID 24971369; 3504058226. [PubMed: 24971369]
24. Romieu-Mourez R, Francois M, Boivin MN, Bouchentouf M, Spaner DE, Galipeau J. Cytokine modulation of TLR expression and activation in mesenchymal stromal cells leads to a proinflammatory phenotype. J Immunol. 2009; 182:7963–7973. PMID 19494321; 351. [PubMed: 19494321]
25. Schenk S, Mal N, Finan A, Zhang M, Kiedrowski M, Popovic Z, et al. Monocyte chemotactic protein-3 is a myocardial mesenchymal stem cell homing factor. Stem Cells. 2007; 25:245–251. PMID 17053210; 352. [PubMed: 17053210]
26. Chinnadurai R, Copland IB, Patel SR, Galipeau J. IDO-independent suppression of T cell effector function by IFN-gamma-licensed human mesenchymal stromal cells. J Immunol. 2014; 192:1491–1501. PMID 24403533; 217. [PubMed: 24403533]
27. Bai L, Lennon DP, Caplan AI, DeChant A, Hecker J, Kranso J, et al. Hepatocyte growth factor mediates mesenchymal stem cell-induced recovery in multiple sclerosis models. Nat Neurosci. 2012; 15:862–870. PMID 22610068; 3563427471. [PubMed: 22610068]
28. Francois M, Birman E, Forner KA, Gaboury L, Galipeau J. Adoptive transfer of mesenchymal stromal cells accelerates intestinal epithelium recovery of irradiated mice in an interleukin-6-dependent manner. Cytotherapy. 2012; 14:1164–1170. PMID 22574720; 4. [PubMed: 22574720]
29. Naji A, Rouas-Freiss N, Durrbach A, Carosella ED, Sensebe L, Deschaseaux F. Concise review: combining human leukocyte antigen G and mesenchymal stem cells for immunosuppressant biotherapy. Stem Cells. 2013; 31:2296–2303. PMID 23922260; 357. [PubMed: 23922260]
30. Rafei M, Hsieh J, Fortier S, Li M, Yuan S, Birman E, et al. Mesenchymal stromal cell-derived CCL2 suppresses plasma cell immunoglobulin production via STAT3 inactivation and PAX5 induction. Blood. 2008; 112:4991–4998. PMID 18812467; 57. [PubMed: 18812467]
31. Bladergroen BA, Meijer CJ, ten Berge RL, Hack CE, Muris JJ, Dukers DF, et al. Expression of the granzyme B inhibitor, protease inhibitor 9, by tumor cells in patients with non-Hodgkin and Hodgkin lymphoma: a novel protective mechanism for tumor cells to circumvent the immune system? Blood. 2002; 99:232–237. PMID 11756176; 386. [PubMed: 11756176]
32. Li H, Jiang Y, Jiang X, Guo X, Ning H, Li Y, et al. CCR7 guides migration of mesenchymal stem cell to secondary lymphoid organs: a novel approach to separate GvHD from GvL effect. Stem Cells. 2014; 32:1890–1903. PMID 24496849; 364. [PubMed: 24496849]
33. Kagiwada H, Yashiki T, Ohshima A, Tadokoro M, Nagaya N, Ohgushi H. Human mesenchymal stem cells as a stable source of VEGF-producing cells. J Tissue Eng Regen Med. 2008; 2:184–189. PMID 18452238; 365. [PubMed: 18452238]
34. Hung SC, Pochampally RR, Hsu SC, Sanchez C, Chen SC, Spees J, et al. Short-term exposure of multipotent stromal cells to low oxygen increases their expression of CX3CR1 and CXCR4 and their engraftment in vivo. PLoS ONE. 2007; 2:e416. PMID 17476338; 3661855077. [PubMed: 17476338]
35. Campeau PM, Rafei M, Boivin MN, Sun Y, Grabowski GA, Galipeau J. Characterization of Gaucher disease bone marrow mesenchymal stromal cells reveals an altered inflammatory secretome. Blood. 2009; 114:3181–390. PMID 19587377; 3682925728. [PubMed: 19587377]
36. Podechard N, Fardel O, Corolleur M, Bernard M, Lecureur V. Inhibition of human mesenchymal stem cell-derived adipogenesis by the environmental contaminant benzo(a)pyrene. Toxicol in Vitro. 2009; 23:1139–1144. PMID 19486938; 384. [PubMed: 19486938]
37. Qi Y, Jiang D, Sindrilaru A, Stegemann A, Schatz S, Treiber N, et al. TSG-6 released from intradermally injected mesenchymal stem cells accelerates wound healing and reduces tissue fibrosis in murine full-thickness skin wounds. J Invest Dermatol. 2014; 134:526–537. PMID 23921952; 369. [PubMed: 23921952]

38. Casey ML, MacDonald PC. Keratinocyte growth factor expression in the mesenchymal cells of human amnion. J Clin Endocrinol Metab. 1997; 82:3319–3323. PMID 9329361; 370. [PubMed: 9329361]

39. Greenbaum A, Hsu YM, Day RB, Schuettpelz LG, Christopher MJ, Borgerding JN, et al. CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. Nature. 2013; 495:227–230. PMID 23434756; 3823600148. [PubMed: 23434756]

40. Soland MA, Bego M, Colletti E, Zanjani ED, St Jeor S, Porada CD, et al. Mesenchymal stem cells engineered to inhibit complement-mediated damage. PLoS ONE. 2013; 8:e60461. PMID 23555976; 3813608620. [PubMed: 23555976]

41. Yoo SW, Chang DY, Lee HS, Kim GH, Park JS, Ryu BY, et al. Immune following suppression mesenchymal stem cell transplantation in the ischemic brain is mediated by TGF-beta. Neurobiol Dis. 2013; 58:249–257. PMID 23759293; 385. [PubMed: 23759293]

42. Brooke G, Tong H, Levesque JP, Atkinson K. Molecular trafficking mechanisms of multipotent mesenchymal stem cells derived from human bone marrow and placenta. Stem Cells Dev. 2008; 17:929–940. PMID 18564033; 378. [PubMed: 18564033]

43. Ries C, Egea V, Karow M, Kolb H, Jochum M, Neth P. MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines. Blood. 2007; 109:4055–4063. PMID 17197427; 377. [PubMed: 17197427]

44. Li Y, Lin F. Mesenchymal stem cells are injured by complement after their contact with serum. Blood. 2012; 120:3436–3443. PMID 22966167; 3763482856. [PubMed: 22966167]

45. Yang K, Wang J, Xiang AP, Zhan X, Wang Y, Wu M, et al. like receptors control the survival of mesenchymal stem cells. Cell Death Dis. 2013; 4:e967. PMID 24336087; 3753877571. [PubMed: 24336087]

46. Schweizer R, Kamat P, Schweizer D, Dennler C, Zhang S, Schnider JT, et al. Bone marrow-derived mesenchymal stromal cells improve vascular regeneration and reduce leukocyte-endothelium activation in critical ischemic murine skin in a dose-dependent manner. Cytotherapy. 2014; 16:1345–1360. PMID 24972742; 374. [PubMed: 24972742]

47. Catrysse L, Vereecke L, Beyaert R, van Loo G. A20 in inflammation and autoimmunity. Trends Immunol. 2014; 35:22–31. PMID 24246475; 373. [PubMed: 24246475]

48. Francois M, Copland IB, Yuan S, Romieu-Mourez R, Waller EK, Galipeau J. Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-gamma licensing. Cytotherapy. 2012; 14:147–152. PMID 22029655; 113279133. [PubMed: 22029655]

49. Giuliani M, Bennaceur-Griscelli A, Nanbakhsh A, Oudrhiri N, Chouaib S, Azzarone B, et al. TLR ligands stimulation protects MSC from NK killing. Stem Cells. 2014; 32:290–300. PMID 24123639; 372. [PubMed: 24123639]

50. Ringe J, Strassburg S, Neumann K, Endres M, Notter M, Burmester GR, et al. Towards in situ tissue repair: human mesenchymal stem cells express chemokine receptors CXCR1, CXCR2 and CCR2, and migrate upon stimulation with CXCL8 but not CCL2. J Cell Biochem. 2007; 101:135–146. PMID 17295203; 362. [PubMed: 17295203]

51. Gieseke F, Bohringer J, Bussolari R, Dominici M, Handgretinger R, Muller I. Human multipotent mesenchymal stromal cells use galectin-1 to inhibit immune effector cells. Blood. 2010; 116:3770–3779. PMID 20644118; 361. [PubMed: 20644118]

52. Wei FY, Leung KS, Li G, Qin J, Chow SK, Huang S, et al. Low intensity pulsed ultrasound enhanced mesenchymal stem cell recruitment through stromal derived factor-1 signaling in fracture healing. PLoS ONE. 2014; 9:e106722. PMID 25181476; 3604152330. [PubMed: 25181476]

53. Hall SR, Tsoyi K, Ith B, Padera RF Jr, Lederer JA, Wang Z, et al. Mesenchymal stromal cells improve survival during sepsis in the absence of heme oxygenase-1: the importance of neutrophils. Stem Cells. 2013; 31:397–407. PMID 23132816; 3593572335. [PubMed: 23132816]

54. Egea V, Zahler S, Rieth N, Neth P, Popp T, Kehe K, et al. Tissue inhibitor of metalloproteinase-1 (TIMP-1) regulates mesenchymal stem cells through let-7f microRNA and Wnt/beta-catenin

signaling. Proc Natl Acad Sci U S A. 2012; 109:E309–E316. PMID 22223664; 3583277529. [PubMed: 22223664]

55. Ortiz LA, Dutreil M, Fattman C, Pandey AC, Torres G, Go K, et al. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proc Natl Acad Sci U S A. 2007; 104:11002–11007. PMID 17569781; 4491891813. [PubMed: 17569781]

Table I

Challenges to potency assay development for MSC-like products.

Challenges	Examples
Inherent variability for starting materials	Autologous and allogeneic MSC donor variabiliy Tissue source for MSCs (adipose, marrow, puerperal products)
Limited lot size and limited material for testing	Single-dose therapy using autologous cells suspended in a small volume
Limited stability	Viability of cell products Functionality of cell products at time of administration relative to banking (thawing)
Lack of appropriate reference standards	Autologous cell material
Complex MOA	Multiple potential effector functions of cells Multiple steps required for function
In vivo fate of product	Migration from site of administration Half-life of cellular product post administration Cellular differentiation or activation in to the desired cell type

Adapted from http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM243392.pdf.

Table II

Release testing of licensed biological product.

Release testing	Applicable FDA biologics and cGMP regulations
Indicate potency (biological activity/activities) specific to the product	21 CFR 600.3(s) and 610.10; and 21 CFR 210.3(b)(16)(ii)
Provide quantitative data	21 CFR 211.194; see also 21 CFR 600.3(kk); 21 CFR 211.165(d); 211.165(e)
Meet pre-defined acceptance and/or rejection criteria	21 CFR 211.165(d); see also 21 CFR 600.3(kk); and 21 CFR 210.3(b)(20)
Include appropriate reference materials, standards, and/or controls	21 CFR 210.3(b)(16)(ii) and 211.160
Establish and document the accuracy, sensitivity, specificity and reproducibility of the test methods used through validation	21 CFR 211.165(e) and 211.194(a)(2))
Measure identity and strength (activity) of all active ingredients	21 CFR 211.165(a); see also 21 CFR 210.3(b)(7))
Provide data to establish dating periods	21 CFR 600.3(l) and 610.53(a)
Meet labeling requirements	21 CFR 610.61(g)(3) and 610.61(r)

Adapted from http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM243392.pdf.

Table III

FACS phenotype of resting and IFNγ-primed MSCs.

FACS phenotype	Resting	IFNγ activated
CD40	−	++
CD80	−	−
CD86	−	−
HLA-ABC	++	++++
HLA-DR	−	+++
CD274 (PD-L1)	+	++++
CD54 (ICAM-1)	+	++++

Adapted from Krampera et al [9].

FACS, fluorescence-activated cell sorting.

Author Manuscript

Author Manuscript

Author Manuscript

Table IV

Genes significant to MSC immune-biology amenable to Fluidigm nanoscale qPCR array and/or ELISA.

IDO [13], CXCL10 [17], CXCL9 [18], CXCL11 [19], CIITA [20], ICAM-1 [21], CCL5 [22],TRAIL [23],TLR3 [24], CCL7 [25],VCAM-1 [21], HLADR [26], HGF [27], IL-6 [28], HLA-G 5 [29], CCL2 [30], PI9 [31], CCR7 [32],VEGF [33], PDL1 [26], CX3CR1 [34], COX-2 [35], AHR [36], TSG-6 [37], KGF [38],TLR4 [24], CXCL12 [39], CD46 [40], PDL2 [26],TGF-β [41], CXCR6 [18], CCR10 [42], TIMP-2 [43], CD55 [44], BCL-2 [45], ANGPT2 [46], A20 [47], HSP70A [48], IL-8 [24], ULBP-3 [49], HSP70B [48], CXCR1 [50], GAL-1 [51], CXCR4 [52], HO-1 [53],TIMP-1 [54], IL-1RA [55]

Adapted from Chinnadurai et al [15]. qPCR, quantitative polymerase chain reaction.