POTENCY ASSAY AND MANUFACTURING METHOD II

FIELD OF THE DISCLOSURE

[0001] The present disclosure relates to improved cellular compositions and potency assays for obtaining the same. Such compositions and assays may be suitable for use in the context of treating disease.

BACKGROUND

[0002] Several cellular therapy products for regenerative or immune therapy applications have advanced to clinical evaluation and market authorization. However, release of these cellular therapy products onto the market is hindered by their complexity and heterogeneity, which makes identification of relevant biologic activities and thus, definition of consistent cellular therapy product quality difficult.

[0003] Physiochemical parameters (for example, characterization of size, morphology, light-scattering properties, tensile strength, cell number, confluence, identification of phenotypic markers) are routinely used for identification and quantification of the active substance, intermediates, impurities and contaminants. However, physiochemical parameters cannot confirm that a product will be biologically active and potent (i.e., elicit the desired effect). In contrast, biologic characterization takes into account the effect of the product on biologic systems, either modelled *in vitro* or *in vivo* in animals and ultimately in the clinic.

[0004] There is a need to develop cell products for treatment of diseases, for example, where immunosuppression is desired. It is also preferable to identify parameters that are critical to the efficacy of cellular therapy products (e.g., via potency testing) so that products of consistent quality can be obtained.

SUMMARY OF THE DISCLOSURE

[0005] The present disclosure encompasses the surprising findings that measuring cell adhesion and proliferation (CAP) via methods disclosed herein provides a standalone potency assay, referred to herein as the CAP assay, for assessing criteria such as

therapeutic efficacy of MLPSC populations, degraded MLPSC product and, manufacturing changes. Surprisingly, the CAP assay is a more sensitive determinant of therapeutic efficacy and/or degraded product compared to individual measures of cell viability, adhesion, and cell growth kinetics (as measured by confluence or nucleus count). In addition, the inventors have discovered that MLPSCs with CAP levels above a particular threshold is indicative of therapeutic efficacy, as determined by improved survival outcomes in patients with graft versus host disease (GvHD) administered various MLPSC drug product lots.

[0006] Accordingly, the present disclosure provides a composition comprising a population of culture expanded mesenchymal lineage precursor or stem cells (MLPSCs), wherein the population of MLPSCs are selected based on a predetermined cell adhesion and proliferation (CAP) level under culture conditions, wherein the predetermined CAP level is greater than or equal to $\geq$ 108%.

[0007] In an example, the predetermined CAP level is $\geq$ 110 %.

[0008] In an example, the population of MLPSCs are also selected based on a predetermined level of IL-2RA inhibition under culture conditions, wherein the predetermined level of IL-2RA inhibition is $\geq$ 60%. In an example, the predetermined level of IL-2RA inhibition is $\geq$ 65%.

[0009] In an example, the population of MLPSCs are mesenchymal stem cells.

[0010] In an example, the population of MLPSCs are a cryopreserved intermediate.

[0011] In an example, the population of MLPSCs are also selected based on expression of one or more or all of CD29, CD54, CD73, CD90, CD102, CD105, CD106, CD166, MHC1.

[0012] In an example, the population MLPSCs comprises $\geq$ 95% CD166+, $\geq$ 95% CD105+, and $\leq$ 0.75% CD45+.

[0013] In an example, the population of MLPSCs expresses low levels of major histocompatibility complex (MHC) class I and minimal levels of human leukocyte antigen (HLA)-DR, CD40, CD80, and CD86.

[0014] In an example, the population of MLPSCs are further characterized by one or more of the following parameters: post-thaw viability of at least 60%; 24 hour nuclei count of at least 100,000; endpoint nuclei confluence of at least 60%; endpoint nuclei count of at least 100,000; less than about 10% annexin V positive cells; and/or IDO-1 protein production of at least 80 pg/µg of total protein.

[0015] In an example, the composition comprises 6.68 x 10^6 /mL viable MLPSCs.

[0016] In an example, the composition comprises 25 x 10^6 MLPSCs.

[0017] In an example, the composition further comprises Plasma-Lyte A, dimethyl sulfoxide (DMSO) and human serum albumin (HSA), preferably wherein the composition comprises Plasma-Lyte A (70%), DMSO (10%), HSA (25%) solution, the HSA solution comprising 5% HSA and 15% buffer.

[0018] The present disclosure also provides a method of selecting a cell population for use in therapy, the method comprising: (i) obtaining a population of mesenchymal lineage precursor or stem cells (MLPSCs); (ii) culturing the MLPSC population in a culture medium; (iii) determining under culture conditions the level of cell adhesion and proliferation (CAP); and, (iv) selecting for use in therapy a MLPSC population that has a CAP level of greater than or equal to (≥) 108% under culture conditions.

[0019] In an example, the method comprises selecting an MLPSC population for use in therapy that has a CAP level of ≥110%.

[0020] In an example, the CAP level is determined on day 3 of culture. In an example, the CAP level is determined between day 2 and day 4 of culture. In an example, the CAP level is determined on 72 ± 12 hours from the start of culture. For example, the CAP level is determined between 60 hours and 84 hours from the start of culture. In an example, the CAP level is determined between 65 hours and 80 hours from the start of culture. In an example, the CAP level is determined between 68 hours and 78 hours from the start of culture. In an example, the CAP level is determined between 70 hours and 74 hours from the start of culture.

[0021] In an example, the method further comprises determining under culture conditions the level of IL-2RA inhibition; and selecting for use in therapy an MLPSC population that inhibits IL-2RA by ≥ 60% under culture conditions.

[0022] In an example, the method comprises selecting an MLPSC population for use in therapy which inhibits IL-2RA by ≥65% under culture conditions.

[0023] In an example, the level of IL-2RA is determined on day 6 of culture. In an example, the level of IL-2RA is determined between day 4 and day 8 of culture. In an example, the level of IL-2RA is determined between day 5 and day 7 of culture.

[0024] In an example, the population is for use in the treatment of graft versus host disease (GvHD).

[0025] The present disclosure also provides a method for determining therapeutic efficacy of a population of culture expanded mesenchymal lineage precursor or stem cells (MLPSCs): (i) obtaining a population of MLPSCs; (ii) culturing the cells in a culture medium; and (iii) determining under culture conditions the level of cell adhesion and proliferation (CAP), wherein a CAP level of greater than or equal to (≥) 108% under culture conditions is indicative of therapeutic efficacy.

[0026] In an example, a CAP level of ≥110% under culture conditions is indicative of therapeutic efficacy.

[0027] In an example, the method further comprises determining under culture conditions the level of IL-2RA inhibition, wherein ≥ 60% IL-2RA inhibition under culture conditions is indicative of therapeutic efficacy.

[0028] In an example, ≥ 65% IL-2RA inhibition under culture conditions is indicative of therapeutic efficacy.

[0029] In an example, the method determines therapeutic efficacy for graft versus host disease (GvHD).

[0030] The present disclosure also provides a method of manufacturing drug product which comprises a population of mesenchymal lineage precursor or stem cells (MLPSCs), the method comprising: acquiring a determination of whether a test population of MLPSCs has a predetermined cell adhesion and proliferation (CAP) level under culture conditions, and processing at least a portion of the test population of MLPSCs as a drug product if the test population of MLPSCs has a predetermined CAP level under culture conditions, thereby manufacturing the drug product; or discarding at least a portion of the test population of MLPSCs if the population of MLPSCs have a CAP level less than the predetermined level under culture conditions, wherein the predetermined level is greater than or equal to (≥) 108%.

[0031] In an example, the predetermined CAP level is ≥110% under culture conditions.

[0032] In an example, the method further comprises acquiring a determination of whether a test population of MLPSCs inhibits IL-2RA at a predetermined IL-2RA inhibition level under culture conditions, and processing at least a portion of the test population of MLPSCs as a drug product if the test population of MLPSCs inhibits IL-2RA by at least the predetermined IL-2RA inhibition level under culture conditions, thereby manufacturing the drug product; or discarding at least a portion of the test population of MLPSCs if the population of MLPSCs inhibits IL-2RA by less than the predetermined

IL-2RA inhibition level under culture conditions, wherein the predetermined IL-2RA inhibition level is ≥ 60%.

[0033] In an example, the predetermined IL-2RA inhibition level is ≥ 65% under culture conditions.

[0034] The present disclosure also provides a method of treating a subject with graft versus host disease (GvHD), the method comprising administering to a subject in need thereof a composition comprising a culture expanded population of mesenchymal lineage precursor or stem cells (MLPSCs), wherein the MLPSCs have a cell adhesion and proliferation (CAP) level of greater than or equal to (≥) 108% under culture conditions.

[0035] In an example, the MLPSCs have a CAP level of ≥ 110% under culture conditions.

[0036] In an example, the MLPSCs inhibit IL-2RA by ≥ 60% under culture conditions.

[0037] In an example, the MLPSCs inhibit IL-2RA by ≥ 65% under culture conditions.

[0038] In an example, the GvHD is acute GvHD. In an example, the GvHD is chronic GvHD. In an example, the GvHD is pediatric GvHD. In an example, the GvHD is adult GvHD. In an example, the GvHD is refractory to steroid therapy. In an example, the GvHD is Grade D GvHD. In an example, the subject has a MAGIC algorithm probability (MAP) score of >0.29.

[0039] In an example, treatment increases 100 day survival to greater than 60%, preferably greater than 70%.

[0040] In an example, the MLPSCs are administered at a dose of 2×10^6 cells/kg body weight. In an example, multiple doses are administered. In an example, MLPSCs are administered at least once a week for four weeks. In an example, MLPSCs are administered at least twice a week for four weeks.

[0041] In an example, the MLPSCs are mesenchymal stem cells.

[0042] In an example, the CAP level is determined by measuring the increase in the number of adherent cells on day 3 in culture relative to freshly seeded cells.

[0043] The present disclosure also provides a method of manufacturing drug product which comprises a population of mesenchymal lineage precursor or stem cells (MLPSCs), the method comprising: (i) acquiring a determination of whether a test population of MLPSCs has a predetermined cell adhesion and proliferation (CAP) level under culture conditions; wherein step (i) comprises:

(i) thawing a population of cryopreserved MLPSCs and culturing the population of MLPSCs under culture conditions for 3 days;

(ii) counting the number of adherent cells;

(iii) measuring the increase in the number of adherent cells on day 3 in culture relative to the thawed population of freshly seeded cells.

[0044] In an example, the increase in the number of adherent cells on day 3 in culture relative to thawed population of freshly seeded cells is expressed as a percentage.

[0045] In an example, the increase in the number of adherent cells on day 3 in culture relative to thawed population of freshly seeded cells is expressed as a fold-change.

[0046] In an example, the increase in the number of adherent cells on day 3 in culture relative to thawed population of freshly seeded cells is expressed as log10.

[0047] In an example, the number of freshly seeded cells is 1.20×10^6.

[0048] In an example, the method further comprises (ii) acquiring a determination of whether a test population of MLPSCs inhibits IL-2RA at a predetermined level under culture conditions, wherein step (ii) comprises co-culturing the test population of MLPSCs with CD3/CD28 activated PBMCs; and determining the level of IL-2RA inhibition relative to a control population of CD3/CD28 activated PBMCs in the absence of MLPSCs; wherein the predetermined level of IL-2RA inhibition is $\geq 60\%$.

[0049] In an example, the control population of CD3/CD28 activated PBMCs express $\geq$ 11,000 pg/ml IL-2RA, preferably $\geq$ 12,000 pg/ml IL-2RA.

[0050] In an example, CD3/CD28 activation of PBMCs comprises stimulating PBMCs with an anti- CD3 antibody and an anti-CD28 antibody.

[0051] In an example, the PBMCs are co-cultured with MLPSCs at a ratio of 5 PBMC:1 MPLSC.

[0052] In an example, $1x10^6$ PBMCs are co-cultured with $2x10^5$ MLPSCs.

[0053] In an example, the PBMCs and MLPSCs are co-cultured for a period of about 3 days.

[0054] In an example, IL-2RA levels are determined on day 6 of culture. In an example, the level of IL-2RA is determined between day 4 and day 8 of culture. In an example, the level of IL-2RA is determined between day 5 and day 7 of culture.

[0055] In an example, the level of IL-2RA inhibition is determined by:

(i) measuring IL-2RA levels in a test population of MLPSCs co-cultured with CD3/CD28-activated PBMCs, a positive control population of CD3/CD28-activated

PBMCs in the absence of MLPSCs, and a negative control population of un-activated PBMCs in the absence of MLPSCs;

(ii) constructing a standard curve to determine IL-2RA levels in the positive control population, negative control population, and test population; and

(iii) calculating % inhibition of IL-2RA levels on the positive control population by the test population of MLPSCs co-cultured with CD3/CD28-activated PBMCs, relative to the positive control CD3/CD28-activated PBMC population.

[0056] In an example, the population of MLPSCs is a culture expanded population.

[0057] In an example, the population of MLPSCs is cryopreserved or has been cryopreserved and thawed.

[0058] In an example, the population of MLPSCs has been culture expanded, cryopreserved and thawed.

[0059] In the above referenced examples, the culture expanded population of mesenchymal lineage precursor or stem cells (MLPSCs) may be culture expanded from a first population of cells comprising about 0.1% to 75% STRO-1+ cells. In this example, the STRO-1+ cells may be obtained from human bone marrow.

BRIEF DESCRIPTION OF ACCOMPANYING FIGURES

[0060] **FIGURE 1:** CAP Assay is a more sensitive determinant of degraded MSC lots after short-term storage at -20°C than it's individual component measures of viability, adhesion, and growth.

[0061] **FIGURE 2:** CAP Assay identifies degraded MSC lots after short-term storage at -20°C, correlates with loss of early IFN Gamma responsiveness and with susceptibility to activation-induced cell death.

[0062] **FIGURE 3:** Log10(CAP) data normal distribution: MSC clinical lots show low degradation and high stability on repeat testing after 8 years.

[0063] **FIGURE 4:** Log10(CAP) data normal distribution: release threshold based on clinical lots in GvHD trial.

[0064] **FIGURE 5:** CAP% relative to IL-2RA inhibition (n=427 lots).

[0065] **FIGURE 6:** Relationship between PBMC stimulation and IL-2RA inhibition by MSC lots.

[0066] **FIGURE 7:** Survivors in Phase 3 GvHD clinical trial received MSC Drug Product with higher mean % IL2RA inhibition.

[0067] **FIGURE 8:** MSC (P3) clinical lots continue to show maintenance of potency as measured by IL-2RA inhibition assay for % IL-2RA inhibition on repeat testing after 8 years.

[0068] **FIGURE 9:** Accurate measurement of % inhibition of IL-2RA requires adequate PBMC stimulation and induced levels of IL-2RA expression.

DETAILED DESCRIPTION

[0069] Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

[0070] Those skilled in the art will appreciate that the disclosure described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

[0071] The present disclosure is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the disclosure, as described herein.

[0072] Unless otherwise indicated, cell culture techniques and assays utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), and F.M. Ausubel et al. (editors), Current Protocols in Molecular Biology, Greene Pub.

Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan et al. (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present).

[0073] Any example disclosed herein shall be taken to apply *mutatis mutandis* to any other example unless specifically stated otherwise.

[0074] Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, stem cell therapy, immunology, immunohistochemistry, protein chemistry, and biochemistry).

[0075] Unless otherwise indicated, the surgical techniques utilized in the present disclosure are standard procedures, well known to those skilled in the art.

[0076] Methods of obtaining and enriching a population of mesenchymal lineage stem or precursor cells are known in the art. For example, enriched populations of mesenchymal lineage stem or precursor cells can be obtained by the use of flow cytometry and cell sorting procedures based on the use of cell surface markers that are expressed on mesenchymal lineage stem or precursor cells.

[0077] All documents cited or referenced herein, and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference in their entirety.

Selected Definitions

[0078] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0079] The term "and/or", e.g., "X and/or Y" shall be understood to mean either "X and Y" or "X or Y" and shall be taken to provide explicit support for both meanings or for either meaning.

[0080] As used herein, the term "about", unless stated to the contrary, refers to +/- 10%, more preferably +/- 5%, of the designated value.

[0081] As used herein, the singular form "a", "an" and "the" include singular and plural references unless the context indicates otherwise.

[0082] Methods of the present disclosure are performed on populations of MLPSCs. Such methods may be performed on a population of MLPSCs that is representative of a larger MLPSC population (e.g. a test population). In an example, methods of the present disclosure are performed on at least a portion of the test population of MLPSCs. In an example, such methods comprise processing at least a portion of a test population of MLPSCs as a drug product if the test population of MLPSCs has a predetermined level of a particular marker, e.g. CAP level, under culture conditions. In an example, such methods can further comprise discarding at least a portion of the test population of MLPSCs if the population of MLPSCs has a CAP level of less than the predetermined level under culture conditions.

[0083] "Cell adhesion and proliferation (CAP)" as used herein refers to the number of adherent cells after a period of time in culture, relative to the starting number of cells (i.e. "freshly seeded cells"). In certain embodiments, methods of the disclosure measure the amount of CAP in a population of cells. In some embodiments, compositions of cells according to the disclosure are characterized by the amount of CAP.

[0084] Interleukin-2 receptor alpha chain ("IL-2RA"; "IL-2Rα" also called CD25) is a validated marker of T-cell activation. In certain embodiments methods of the disclosure measure capability for inhibition of T-cell activation. For the avoidance of doubt, the terms "IL-2RA", "IL-2Rα", "IL-2R Alpha", and variations thereof, are used interchangeably herein.

[0085] The terms "level" and "amount" are used to define the amount of a particular marker in a population of cells, for example, the amount of CAP or the amount of IL-2RA inhibition. In an example, the level is the amount of a particular marker taken from a sample from a subject or in a cell culture media (or sample therefrom). For example, a particular concentration, weight, percentage, ratio, or rate of change between two variables can be used to define the level of a particular marker in a cell population (or sample therefrom). In an example, the level is expressed in terms of how much of a particular marker is expressed by cells of the disclosure under culture conditions. In an example, expression represents cell surface expression. In an example, the level is expressed in terms of how much of a particular marker is inhibited by cells of the disclosure.

[0086] In an example, the level is expressed as a percentage. In an example, the level is expressed as a fold-change. In an example, the level is expressed as log10 of a particular value. In an example, the level is expressed as a percentage or fold-change relative to a control. In some examples, the control is the starting number of cells and the level is the number of cells at a particular endpoint, expressed as a percentage or fold-change from the starting point. In other examples, the control is a second population of cells, e.g. activated PBMCs.

[0087] The term "predetermined" level is used in the context of the present disclosure to refer to a therapeutically effective amount of CAP. In some examples, the predetermined level refers to a therapeutically effective amount of IL-2Rα inhibition. In an example, the predetermined level is a clinically proven effective predetermined level. In an example, the level is clinically proven effective in GvHD. In another example, the predetermined level is predetermined by a regulatory authority such as the US Food and Drug Administration (FDA). In an example, the predetermined level corresponds with increased survival in patients with GvHD. In an example, the predetermined level corresponds with increased survival in patients with grade D GvHD.

[0088] In an example, the predetermined level is a "reference level of CAP". In an example, the reference level is a level of CAP of an FDA approved MLPSC population (e.g., an FDA MSC population approved for treatment of GvHD). In an example, the reference level of CAP provides the criteria for selecting cell populations according to the present disclosure.

[0089] In an example, the level of CAP is determined under culture conditions. The term "culture conditions" is used to refer to cells growing in culture. In an example, culture conditions refers to an actively dividing population of cells. Such cells may, in an example, be in exponential growth phase.

[0090] In an example, the level of IL-2RA inhibition is determined under culture conditions. In an example, culture conditions encompass co-culture of an MLPSC population disclosed herein and a second cell population such as a population which comprises peripheral blood mononuclear cells (PBMC). In an example, co-culture comprises culturing an MLPSC population disclosed herein and a population of activated PBMC. For example, PBMC can be activated using anti-CD3 and anti-CD28 antibodies before co-culture with an MLSPC population disclosed herein.

[0091] In an example, "culture conditions" comprises co-culturing MLPSCs and activated PBMC at a ratio of about 1 MLPSC:2 activated PBMC, or less. For example, 1:3, 1:4, 1:5, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70 1:80, 1:90, or 1 MLPSC:100 activated PBMC, or less. In this example, the level of IL-2RA inhibition is determined after about 30 to 84 hours of cell culture under culture conditions.

[0092] In an example, "culture conditions" comprises co-culturing MLPSCs and T cells at a ratio of about 1 MLPSC:2 T cells, or less. For example, 1:3, 1:4, 1:5, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70 1:80, 1:90, or 1 MLPSC:100 T cells, or less. In this example, the level of IL-2RA inhibition is determined after about 30 to 84 hours of cell culture under culture conditions.

[0093] As discussed further below, "culture conditions" in the context of an IL-2RA inhibition assay may comprise determining the amount of IL-2RA production by PBMCs stimulated with anti-CD3/CD28 antibodies to confirm whether a requisite threshold of IL-2RA production has been achieved. In an example, IL-2RA inhibition is determined based on PBMCs that have received the stimulation necessary to produce a threshold level of IL-2RA, such as >13,000 pg/ml IL-2RA. Although not wishing to be bound by any particular time point, in an example, the level of IL-2RA production by PBMCs is determined around 48 hours post stimulation with anti-CD3/CD28 antibodies.

[0094] In an example, the level of a particular marker, e.g. CAP, can be determined by counting the number of cells in culture. In another example, the level of a particular marker can be determined by taking a sample of cell culture media and measuring the level of marker in the sample. In another example, the level of a particular marker can be determined by taking a sample of cells and measuring the level of the marker in the cell lysate. Those of skill in the art will appreciate that secreted markers can be measured by sampling the culture media while markers expressed on the surface of the cell may be measured by assessing a sample of cell lysate.

[0095] In an example, the sample is taken when the cells are in exponential growth phase. In an example, the sample is taken after at least two or three days in culture. In another example, the sample is taken about 72 hours in culture. In another example, the sample is taken after at least about six days in culture. In another example, the sample is taken after about 30 to 84 hours of co-culture. In the above examples, the term "about" is understood to mean ± 12 hours of the designated time period.

[0096] In an example, the sample is taken from a co-culture of MLPSCs and activated PBMCs. In this example, the cell sample can be lysed and the level of a marker can be determined.

[0097] Culture expanding cells from a cryopreserved intermediate means thawing cells subject to cryogenic freezing and *in vitro* culturing under conditions suitable for growth of the cells.

[0098] In an example, the "level" or "amount" of a particular marker such as IL-2RA is determined before cells have been cryopreserved. In another example, the level or amount of a particular marker is determined after a first cryopreservation of cells. In another example, the level is determined after a second cryopreservation of cells. For example, cells may be culture expanded to provide an intermediate, cryopreserved, defrosted before being re-seeded in culture and further culture expanded before the level of a particular marker can be determined under culture conditions.

[0099] The term "intermediate" is used in the context of the present disclosure to refer to an intermediate population of MLPSCs that have been freshly isolated and subject to early stage culture expansion (e.g. around 2 passages). For example, production of the intermediate population of MLPSCs can include the isolation of nucleated bone marrow cells (NBMC) from Bone Marrow Aspirate (BMA), cell culture and two passages to expand the relevant cells. In an example, cells are then harvested and formulated for analysis according to the methods described herein. In an example the harvested cells are cryopreserved. Accordingly, production of an intermediate population, is the first stage of the process of producing a composition for administration to a subject (i.e. drug product). While the above referenced example refers to isolation of NBMC from BMA, those of skill in the art will appreciate that relevant cells can be obtained from other suitable source(s) as discussed herein (e.g. adipose tissue) and subjected to the necessary culture expansion to intermediate and, drug product.

[0100] In certain examples disclosed herein, intermediates of the disclosure are culture expanded to provide a population of MLPSCs for administration. In an example, such populations can be referred to as a pharmaceutical composition or drug product (DP). For example, culture expansion of the intermediate involves cell culture and more passages (e.g. three more passages) for culture expansion.

[0101] By "isolated" or "purified" it is meant a cell which has been separated from at least some components of its natural environment. This term includes gross physical

separation of the cells from its natural environment (e.g. removal from a donor). The term "isolated" includes alteration of the cell's relationship with the neighboring cells with which it is in direct by, for example, dissociation. The term "isolated" does not refer to a cell which is in a tissue section. When used to refer to the population of cells, the term "isolated" includes populations of cells which result from proliferation of the isolated cells of the disclosure.

[0102] The terms "passage", "passaging" or "sub-culture" are used in the context of the present disclosure to refer to known cell culture techniques that are used to keep cells alive and growing under cultured conditions for extended periods of time so that cell numbers can continually increase. The degree of sub-culturing a cell line has undergone is often expressed as "passage number," which is generally used to refer to the number of times cells have been sub-cultured. In an example, one passage comprises removing non-adherent cells and leaving adherent mesenchymal lineage precursor or stem cells. Such mesenchymal lineage precursor or stem cells can then be dissociated from the substrate or flask (e.g., by using a protease such as trypsin or collagenase), media can be added, optional washing (e.g., by centrifugation) may be performed, and then the mesenchymal lineage precursor or stem cells can be re-plated or reseeded to one or more culture vessels containing a greater surface area in total. The mesenchymal lineage precursor or stem cells can then continue to expand in culture. In another example, methods of removing non-adherent cells include steps of non-enzymatic treatment (e.g., with EDTA). In an example, mesenchymal lineage precursor or stem cells are passaged at or near confluence (e.g., about 75% to about 95% confluence). In an example, the mesenchymal lineage precursor or stem cells are seeded at a concentration of about 10%, about 15%, or about 20% cells/ml of culture medium.

[0103] The term "medium" or "media" as used in the context of the present disclosure, includes the components of the environment surrounding cells in culture. It is envisaged that the media contributes to and/or provides the conditions suitable to allow cells to grow. Media may be solid, liquid, gaseous or a mixture of phases and materials. Media can include liquid growth media as well as liquid media that do not sustain cell growth. Exemplary gaseous media include the gaseous phase that cells growing on a petri dish or other solid or semisolid support are exposed to.

[0104] As used herein, the terms "treating", "treat" or "treatment" include administering a population of mesenchymal lineage stem or precursor cells and/or progeny thereof

and/or soluble factors derived therefrom to thereby reduce or eliminate at least one symptom of GvHD. In an example, treatment includes administering a population of culture expanded mesenchymal lineage stem or precursor cells disclosed herein. In an example, treatment response is determined relative to baseline. In an example, treatment response is determined relative to a control patient population.

[0105] In an example, methods of the present disclosure inhibit disease progression or disease complication in a subject. "Inhibition" of disease progression or disease complication in a subject means preventing or reducing the disease progression and/or disease complication in the subject. Accordingly, in an example, methods of the disclosure inhibit progression of disease severity. For example, such methods can inhibit progression of GvHD severity (i.e. inhibit progression in grading).

[0106] The term “prevent” or “preventing” as used herein include administering a population of mesenchymal lineage stem or precursor cells and/or progeny thereof and/or soluble factors derived therefrom to thereby stop or inhibit the development of at least one symptom of inflammatory disease.

[0107] The term “subject” as used herein refers to a human subject. For example, the subject can be an adult. In another example, the subject is a pediatric subject. Terms such as “subject”, “patient” or “individual” are terms that can, in context, be used interchangeably in the present disclosure. In an example, the subject has GvHD.

[0108] As used herein, the term “genetically unmodified” refers to cells that have not been modified by transfection with a nucleic acid. For the avoidance of doubt, in the context of the present disclosure a mesenchymal lineage precursor or stem cell transfected with a nucleic acid encoding Ang1 would be considered genetically modified.

[0109] The term "clinically proven" (used independently or to modify the term "effective") shall mean that efficacy has been proven by a clinical trial wherein the clinical trial has met the approval standards of U.S. Food and Drug Administration, EMEA or a corresponding national regulatory agency. For example, the clinical study may be an adequately sized, randomized, double-blinded study used to clinically prove the effects of the composition. In an example, a clinically proven effective amount is an amount shown by a clinical trial to meet a specified endpoint. In an example, the end point is protection against death. Put another way, the end point increases survival. For example, 100 day survival may be increased when administering cell populations meeting release criteria according to the present disclosure.

[0110] Accordingly, the terms "clinically proven efficacy" and "clinically proven effective" can be used in the context of the present disclosure to refer to a dose, dosage regimen, treatment or method disclosed herein. Efficacy can be measured based on change in the course of the disease in response to administering a composition disclosed herein. For example, a composition of the disclosure is administered to a subject in an amount and for a time sufficient to induce an improvement, preferably a sustained improvement, in at least one indicator that reflects the severity of inflammatory disease. Various indicators that reflect the severity of the disease can be assessed for determining whether the amount and time of the treatment is sufficient. Such indicators include, for example, clinically recognized indicators of disease severity or symptoms. In an example, the degree of improvement is determined by a physician, who can make this determination based on signs, symptoms, or other test results. In an example, a clinically proven effective amount improves patient survival. In another example, a clinically proven effective amount reduces a subjects risk of mortality. In another example, a clinically proven effective amount increases 100 day survival. In an example, methods of the disclosure administer a clinically proven effective amount of a composition disclosed herein.

[0111] “Therapeutic efficacy” is used in the context of the present disclosure to refer to MLPSCs and compositions disclosed herein that can treat, inhibit and/or prevent disease. For example, therapeutically effective MLPSCs and compositions disclosed herein can treat, inhibit and/or prevent an inflammatory disease such as GvHD. In an example, therapeutically effective MLPSCs and compositions disclosed herein increase 100 day survival in subjects with GvHD.

Cell adhesion and proliferation assay

[0112] The cell adhesion and proliferation (CAP) assay is an integrated measure of healthy cell growth in culture by quantifying the number of viable cells with membrane integrity that are capable of adhering to plastic and proliferating in response to mitogenic signals. According to the present disclosure, the level of CAP is determined based on the increase of adherent cells after about 72 hours in culture over freshly seeded cells. Accordingly, the CAP assay can be characterized as a measurement of cell growth kinetics under culture conditions.

[0113] In an example, MLPSCs are freshly seeded on Day 1 at a particular concentration in a suitable growth medium and under suitable culture conditions. Suitable culture conditions, such as temperature and CO_2 levels, will be apparent to the person skilled in the art and are disclosed herein. In an example, the MLPSCs are incubated at about 37°C and about 5% CO_2. In an example, the cells are seeded at a concentration of 1.20×10^6 cells per flask (e.g. a T175 flask). In an example, the freshly seeded MLPSCs are obtained from a cryopreserved population (i.e. via defrosting the population). In an example, the freshly seeded MLPSCs are obtained from a cryopreserved population of MLPSCs that has been defrosted and culture expanded, i.e. obtained from a cryopreserved intermediate following its expansion in culture.

[0114] After a period of time in culture, the culture medium can be replaced with fresh culture media. In an example, 24 ± 2 hours after the start of incubation on Day 1 the culture medium is removed, discarded, and fresh media is added. The culture is then returned to incubation. In another example, 48 ± 2 hours after the start of incubation on Day 1 the culture medium is removed, discarded, fresh media is added, and the culture is returned to incubation.

[0115] After a period of time in culture, for example about 3 days, the culture medium is removed and discarded, adherent cells are dislodged, washed, and counted. In an example, adherent cells are counted on Day 3 of culture. In an example, adherent cells are counted about 72 ± 12 hours from the start of incubation on Day 1. For example, the cells are counted about 72 hours, 73 hours, 74 hours, 75 hours, 76 hours, 77 hours, 78 hours, 79 hours, 80 hours, 81 hours, 82 hours, 83 hours, or about 84 hours, from the start of incubation on Day 1. For example, the cells are counted between 60 hours and 84 hours from the start of incubation on Day 1. In an example, the cells are counted between 65 hours and 80 hours from the start of incubation on Day 1. In an example, the cells are counted between 68 hours and 78 hours from the start of incubation on Day 1. In an example, the cells are counted between 70 hours and 74 hours from the start of incubation on Day 1.

[0116] In an example, adherent cells are counted between day 2 and day 6 of culture. In an example, adherent cells are counted between day 2 and day 5 of culture. In an example, adherent cells are counted between day 2 and day 4 of culture.

[0117] Adherent cells can be dislodged from the culture flask using standard methods known in the art. In an example, the cells are dislodged using trypsin. In an example, the

cells are dislodged using EDTA or an EDTA-based reagent, such as Versene. In an example, the cells are dislodged by mechanical forces, such as by using a cell scraper.

[0118] Methods for counting cells are known in the art and can include automated or manual methods. For example, cells can be counted using a hemocytometer. Cell suspensions can be loaded onto the hemocytometer and counted in designated squares under a microscope. The average count is then multiplied by a dilution factor and the chamber's specific factor to obtain cells per milliliter. Optionally, cells can be stained with Trypan blue to differentiate live and dead cells.

[0119] The CAP level can be calculated according to the following formula: (total number of cells recovered / total number of freshly seeded cells). This number can then be multiplied by 100 and expressed as a percentage. In an example, the result can be expressed as fold-change. In an example, the result can be log10 transformed and expressed as the log10 value. In an example, the CAP level is calculated according to the following formula: number of recovered cells/mL x 2 mL / flask)/ 1.20 x 10^6 cells/ T175 flask) x 100 = % cells recovered.

[0120] According to the disclosure, MLPSC populations having a particular CAP level are considered to be of a sufficient quality and suitable for use in therapy. In an example, the threshold CAP level is greater than or equal to (≥) 108%, for example ≥ 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 195%, or 200%. In an example, the threshold CAP level is ≥108%. In an example, the threshold CAP level is ≥110%.

[0121] In an example, the threshold CAP level is at least 108%, for example at 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 195%, or 200%. In an example, the threshold CAP level is at least 108%. In an example, the threshold CAP level is at least 110%.

[0122] In an example, the threshold CAP level is between 108% and 500%. In an example, the threshold CAP level is between 108% and 400%. In an example, the threshold CAP level is between 108% and 300%. In an example, the threshold CAP level is between 108% and 280%. In an example, the threshold CAP level is between 108% and 260%. In an example, the threshold CAP level is between 108% and 250%. In an example, the threshold CAP level is between 108% and 230%. In an example, the threshold CAP level is between 108% and 220%. In an example, the threshold CAP level is between 108% and 210%. In an example, the threshold CAP level is between 108%

and 200%. In an example, the threshold CAP level is between 108% and 190%. In an example, the threshold CAP level is between 108% and 180%. In an example, the threshold CAP level is between 108% and 170%. In an example, the threshold CAP level is between 108% and 150%. In an example, the threshold CAP level is between 108% and 140%. In an example, the threshold CAP level is between 108% and 130%. In an example, the threshold CAP level is between 108% and 120%. In an example, the threshold CAP level is between 108% and 110%.

[0123] In an example, the threshold CAP level is 108%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 195%, or 200%. In an example, the threshold CAP level is 108%.

[0124] In another example, the CAP level can be expressed as fold-change relative to freshly seeded cells. Accordingly, in an example, the threshold CAP level is ≥ a 1-fold increase relative to freshly seeded cells, for example ≥ 1.05, 1.08, 1.1, 1.15, 1.2, 1.25, 1.3, 1.35, 1.4, 1.45, 1.5, 1.55, 1.6, 1.65, 1.7, 1.75, 1.8, 1.85, 1.9, 1.95, or 2.0. In an example, the threshold CAP level is ≥1-fold. In an example, the threshold CAP level is ≥1.08-fold.

[0125] In another example, the CAP level can be expressed as the log10 value. In an example, the threshold log10 CAP level is ≥ 0.035. In an example, the threshold log10 CAP level is ≥ 0.1. In an example, the threshold log10 CAP level is ≥ 0.2. In an example, the threshold log10 CAP level is ≥ 0.26. In an example, the threshold log10 CAP level is ≥ 0.3. In an example, the threshold log10 CAP level is ≥ 0.35.

[0126] As disclosed herein, the CAP assay provides an integrated assessment of multiple attributes of cell health, including confluence over time, endpoint nuclei confluence, endpoint nuclei count, level of apoptosis, and loss off IDO interferon gamma responsiveness. Accordingly, in an example, MLPSC populations characterised by having a threshold CAP level may also be characterised by one or more of the following parameters: post-thaw viability of at least 60%; 24 hour nuclei count of at least 100,000; endpoint nuclei confluence of at least 60%; endpoint nuclei count of at least 100,000; less than about 10% annexin V positive cells; IDO-1 protein production of at least 80 pg/μg of total protein.

[0127] In an example, the population of MLPSCs has a post-thaw viability of at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%.

[0128] In an example, the population of MLPSCs has a 24 hour nuclei count of at least 100,000, at least 110,000, at least 120,000, at least 150,000, at least 160,000, at least 170,000, at least 180,000, or at least 190,000.

[0129] In an example, the population of MLPSCs has an endpoint nuclei confluence of at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%.

[0130] In an example, the population of MLPSCs has an endpoint nuclei count of at least 100,000, at least 110,000, at least 120,000, at least 150,000, at least 160,000, at least 170,000, at least 180,000, or at least 190,000.

[0131] Methods for assessing cell viability and nuclei counts are well known in the art. For example, prior to counting, cells in culture can be washed and stained with Trypan Blue. Trypan Blue penetrates the cell membrane and enters the cytoplasm of cells with compromised membranes (dead cells) to stain them blue. The live cells remain intact and can be distinguished from dead cells by their ability to exclude the blue dye. Accordingly, the number of viable cells can be determined by counting the live cells.

[0132] Confluence is the percentage area covered by adherent cells in a culture dish or flask. Accordingly, confluence is normally determined visually using microscopy. Alternatively, or in addition to determining viability and confluency, nucleated cell counts can also be performed. This can be done by staining cells with a nuclear stain, such as 3% Acetic Acid with Methylene Blue. Acetic acid lyses the cellular membranes, and the methylene blue stains the exposed nuclei. Nucleated cells can then be counted.

[0133] In an example, the population of MLPSCs has less than about 10% annexin V positive cells, less than about 8% annexin V positive cells, less than about 7% annexin V positive cells, less than about 6% annexin V positive cells, less than about 5% annexin V positive cells, less than about 4% annexin V positive cells, less than about 3% annexin V positive cells, less than about 2% annexin V positive cells.

[0134] As the person skilled in the art will appreciate, methods for determining annexin V-positive cells are routine in the art. For example, cell populations of the disclosure can be contacted with fluorescently labelled annexin V and then the number of annexin V-positive cells can be measured using flow cytometry or other fluorescence-based quantification assays.

[0135] In an example, the population of MLPSCs has an IDO-1 protein production level of at least 80 pg/µg of total protein, at least 90 pg/µg of total protein, at least 100 pg/µg of total protein, at least 120 pg/µg of total protein, at least 150 pg/µg of total protein, at least

200 pg/μg of total protein, at least 250 pg/μg of total protein, or at least 300 pg/μg of total protein.

[0136] IDO-1 protein production can be measured using standard techniques for protein measurement. Appropriate methods will be apparent to the person skilled in the art and include, for example, Western blot, immunoassays (e.g. ELISA), or Luminex assays.

IL-2RA inhibition assay

[0137] In certain examples, MLPSC populations of the disclosure are also characterized by their level of IL-2RA inhibition.

[0138] Interleukin-2 receptor alpha chain ("IL-2RA"; "IL-2Rα" also called CD25) is a validated marker of T-cell activation. For the avoidance of doubt, the terms "IL-2RA", "IL-2Rα", "IL-2R Alpha", and variations thereof, are used interchangeably herein.

[0139] In certain embodiments methods of the disclosure measure the inhibition of T-cell activation. In an example, T-cell activation is determined based on the level of T-cell IL-2RA expression following co-culture of activated PBMC with MLPSCs. Inhibition of IL-2RA expression is associated with a suppressive effect on T cell activation.

[0140] In an example, activated PBMC are co-cultured with MLPSCs in a culture medium comprising at least one T cell stimulating agent, preferably at a concentration capable of stimulating and/or activating the T cells. In another embodiment, the T cells are first stimulated and/or activated prior to co-culture with the MLPSCs.

[0141] In an example, IL-2RA inhibition is determined on about day 6 of culture ± 12 hours. In an example, IL-2RA inhibition is determined between day 4 and day 8 of culture. In an example, IL-2RA inhibition is determined between day 5 and day 7 of culture.

[0142] In an exemplary method according to the disclosure, a population of MLPSCs are seeded on day 1 of culture, the CAP level is determined on about day 3 of culture, and level of IL-2RA inhibition is determined on about day 6 of culture. In this example, MLPSCs are co-cultured with PBMCs for 3 days (i.e. beginning on about day 3 of culture).

[0143] In an example, MLPSCs are co-cultured with PBMC in a culture medium comprising an agent that can stimulate CD3 and an agent that can stimulate CD28 on T cells, such as an antibody to CD3 and an antibody to CD28, for example, mouse anti-human CD3 and mouse anti-human CD28. In an example, the antibody to CD3 and/or

the antibody to CD28 is added to the culture medium in soluble form, each at a concentration of about 2µg/ml.

[0144] In an example, CD3/CD28-activated PBMCs express at least 10,000 pg/mL IL-2RA. In an example, CD3/CD28-activated PBMCs express at least 11,000 pg/mL IL-2RA. In an example, CD3/CD28-activated PBMCs express at least 12,000 pg/mL IL-2RA. In an example, CD3/CD28-activated PBMCs express at least 13,000 pg/mL IL-2RA. In an example, CD3/CD28-activated PBMCs express at least 12,811 pg/mL IL-2RA. In an example, CD3/CD28-activated PBMCs express at least 13,000 pg/mL IL-2RA.

[0145] In an example, the PBMC are co-cultured with mesenchymal lineage precursor or stem cells at a ratio of 5 PBMC:1 mesenchymal lineage precursor or stem cell. For example, 1 $x10^6$ PBMCs could be co-cultured with $2x10^5$ MLPSCs. In an example, cells can be co-cultured in a final volume of 1ml.

[0146] Following co-culture of MLPSCs and T cells, the cells can be collected and lysed using well-known methods in the art. The cell lysates can then be assayed for the presence of IL-2RA. The present disclosure contemplates any form of assay, including, for example, Western blot, enzyme-linked immunosorbent assay (ELISA), fluorescence-linked immunosorbent assay (FLISA), competition assay, radioimmunoassay, lateral flow immunoassay, flow-through immunoassay, electrochemiluminescent assay, nephelometric-based assays, turbidometric-based assay, fluorescence activated cell sorting (FACS)-based assays for determining the level of IL-2RA.

[0147] In an example, the level of IL-2RA may be determined using an ELISA-based method. Suitable ELISA kits are known in the art and include, for example, the Quantikine Human CD25/IL-2 Rα ELISA. In an example, the ELISA comprises:

(i) adding sample diluent to each well of a microplate precoated with a monoclonal antibody specific for IL-2RA;

(ii) adding a co-cultured sample to a well of a microplate precoated with a monoclonal antibody specific for IL-2RA;

(iii) incubating the microplate for sufficient time to allow for the monoclonal antibody specific for IL-2RA to specifically bind to any IL-2RA in the sample;

(iv) washing the microplate;

(v) adding IL-2RA conjugate to the well;

(vi) incubating the microplate for sufficient time to allow the conjugate to specifically bind to any captured IL-2RA;

(vii) washing the microplate;

(viii) adding a substrate solution to the well;

(ix) incubating the microplate for sufficient time for colour development;

(x) adding a stop solution to the well;

(xi) reading optical density on a microplate reader set to 450 nm with wavelength correction at 570 nm;

(xii) determining the level of IL-2RA.

[0148] In another example, the level of IL-2RA is determined using fluorescence-activated cell sorting (FACS) using appropriate antibodies such as anti-CD25. Further antibodies may also be employed if required to distinguish CD25+ cell types.

[0149] In an example, level of IL-2Rα inhibition by an MLPSC population disclosed herein is determined relative to a control, such as the level of IL-2Rα expression measured in CD3/CD28 activated PBMCs in the absence of MLPSCs. In an example, the control population of CD3/CD28 activated PBMCs express ≥ 12,000 pg/ml IL-2RA. In an example, the control population of CD3/CD28 activated PBMCs express ≥ 11,000 pg/ml IL-2RA. In an example, the control population of CD3/CD28 activated PBMCs express ≥ 13,000 pg/ml IL-2RA. In an example, the control population of CD3/CD28 activated PBMCs express ≥ 10,000 pg/ml IL-2RA. In an example, the control population of CD3/CD28 activated PBMCs express ≥ 12811 pg/ml IL-2RA. In an example, the control population of CD3/CD28 activated PBMCs express ≥ 13,000 pg/ml IL-2RA.

[0150] In an example, the level of IL-2RA in co-culture is compared with the level of IL-2RA in cultured population of activated PBMC. Levels of IL-2RA are subsequently compared to provide a level of IL-2RA inhibition in co-culture. For example, the level of IL-2RA may be inhibited ≥ 60% in co-culture (i.e. MLPSC:activated PBMC) relative single culture (activated PBMC alone). In another example, the level of IL-2RA may be inhibited ≥ 65% in co-culture.

[0151] In an example, the level of IL-2RA may be inhibited by between 60% and 100% in co-culture. For example, the level of IL-2RA may be inhibited by between 60% and 95%; between 60% and 90%; between 60% and 80%; between 60% and 75%; or between 60% and 70%. In another example, the level of IL-2RA may be inhibited by between 65% and 100% in co-culture. For example, the level of IL-2RA may be inhibited by

between 65% and 95%; between 65% and 90%; between 65% and 80%; between 65% and 75%; or between 65% and 70%.

[0152] Those of skill in the art will appreciate other suitable methods of determining level of IL-2RA under culture conditions.

[0153] In an example, the level of IL-2RA is determined by a standard curve. A "standard curve" refers to a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentrations. In an example, the standard curve is constructed using a four-parameter logistic (4-PL) curve fit. As the person skilled in the art would understand, four parameter logistic (4PL) curve is a regression model often used to analyze bioassays such as ELISA. They follow a sigmoidal, or "s", shaped curve. This type of curve is particularly useful for characterizing bioassays because bioassays are often only linear across a specific range of concentration magnitudes.

[0154] In an example, the inhibition of IL-2RA expression is measured by comparing the level of IL-2RA expression of a population of cells comprising T cells to the level of IL-2RA of a population of cells following co-culture of a population of cells comprising T cells and a population of cells comprising mesenchymal lineage precursor or stem cells, and the difference expressed as "percentage inhibition".

[0155] The assays described above are readily modified to use chemiluminescence or electrochemiluminescence as the basis for detection.

[0156] As will be apparent to the skilled person, other detection methods based on an immunosorbent assay are useful in the performance of the present disclosure. For example, an immunosorbent method based on the description above using a radiolabel for detection, or a gold label (e.g., colloidal gold) for detection, or a liposome, for example, encapsulating NAD+ for detection or an acridinium linked immunosorbent assay.

[0157] In some examples of the disclosure, the level of IL-2RA is determined using a surface plasmon resonance detector (e.g., BIAcore™, GE Healthcare, Piscataway, N.J.), a flow through device (e.g., as described in US patent 7205159), a micro- or nano-immunoassay device (e.g., as described in US patent 7271007), a lateral flow device (e.g., as described in US publication 20040228761 or US publication 20040265926), a fluorescence polarization immunoassay (FPIA, e.g., as described in US patent 4593089 or US patent 4751190), or an immunoturbidimetric assay (e.g., as described in US patent 5571728 or US patent 6248597).

Mesenchymal lineage precursor or stem cells

[0158] As used herein, the term “mesenchymal lineage precursor or stem cell (MLPSC)” refers to undifferentiated multipotent cells that have the capacity to self-renew while maintaining multipotency and the capacity to differentiate into a number of cell types either of mesenchymal origin, for example, osteoblasts, chondrocytes, adipocytes, stromal cells, fibroblasts and tendons, or non-mesodermal origin, for example, hepatocytes, neural cells and epithelial cells. For the avoidance of doubt, a “mesenchymal lineage precursor cell” refers to a cell which can differentiate into a mesenchymal cell such as bone, cartilage, muscle and fat cells, and fibrous connective tissue.

[0159] The term "mesenchymal lineage precursor or stem cells" includes both parent cells and their undifferentiated progeny. The term also includes mesenchymal precursor cells, multipotent stromal cells, mesenchymal stem cells (MSCs), perivascular mesenchymal precursor cells, and their undifferentiated progeny.

[0160] MLPSCs can be autologous, allogeneic, xenogenic, syngenic or isogenic. Autologous cells are isolated from the same individual to which they will be reimplanted. Allogeneic cells are isolated from a donor of the same species. Xenogenic cells are isolated from a donor of another species. Syngenic or isogenic cells are isolated from genetically identical organisms, such as twins, clones, or highly inbred research animal models.

[0161] In an example, the MLPSCs are allogeneic. In an example, the allogeneic mesenchymal lineage precursor or stem cells are culture expanded and cryopreserved.

[0162] MLPSCs reside primarily in the bone marrow, but have also shown to be present in diverse host tissues including, for example, cord blood and umbilical cord, adult peripheral blood, adipose tissue, trabecular bone and dental pulp. They are also found in skin, spleen, pancreas, brain, kidney, liver, heart, retina, brain, hair follicles, intestine, lung, lymph node, thymus, ligament, tendon, skeletal muscle, dermis, and periosteum; and are capable of differentiating into germ lines such as mesoderm and/or endoderm and/or ectoderm. Thus, mesenchymal lineage precursor or stem cells are capable of differentiating into a large number of cell types including, but not limited to, adipose, osseous, cartilaginous, elastic, muscular, and fibrous connective tissues. The specific lineage-commitment and differentiation pathway which these cells enter depends upon various influences from mechanical influences and/or endogenous bioactive factors, such

as growth factors, cytokines, and/or local microenvironmental conditions established by host tissues.

[0163] The terms "enriched", "enrichment" or variations thereof are used herein to describe a population of cells in which the proportion of one particular cell type or the proportion of a number of particular cell types is increased when compared with an untreated population of the cells (e.g., cells in their native environment). In one example, a population enriched for MLPSCs comprises at least about 0.1% or 0.5% or 1% or 2% or 5% or 10% or 15% or 20% or 25% or 30% or 50% or 75% MLPSCs. In this regard, the term "population of cells enriched for MLPSCs" will be taken to provide explicit support for the term "population of cells comprising X% MLPSCs", wherein X% is a percentage as recited herein. The MLPSCs can, in some examples, form clonogenic colonies, e.g. CFU-F (fibroblasts) or a subset thereof (e.g., 50% or 60% or 70% or 70% or 90% or 95%) can have this activity.

[0164] In an example of the present disclosure, the MLPSCs are mesenchymal stem cells (MSCs). The MSCs may be a homogeneous composition or may be a mixed cell population enriched in MSCs. Homogeneous MSC compositions may be obtained by culturing adherent marrow or periosteal cells, and the MSCs may be identified by specific cell surface markers which are identified with unique monoclonal antibodies. A method for obtaining a cell population enriched in MSCs is described, for example, in U.S. Patent No. 5,486,359. Alternative sources for MSCs include, but are not limited to, blood, skin, cord blood, muscle, fat, bone, and perichondrium. In an example, the MSCs are allogeneic. In an example, the MSCs are cryopreserved. In an example, the MSCs are culture expanded and cryopreserved.

[0165] In another example, the mesenchymal lineage precursor or stem cells are CD29+, CD54+, CD73+, CD90+, CD102+, CD105+, CD106+, CD166+, MHC1+ MSCs.

[0166] Isolated or enriched MLPSCs can be expanded *in vitro* by culture. Isolated or enriched MLPSCs can be cryopreserved, thawed and subsequently expanded *in vitro* by culture.

[0167] In one example, isolated or enriched MLPSCs are seeded at 50,000 viable cells/cm^2 in culture medium (serum free or serum-supplemented), for example, alpha minimum essential media (αMEM) supplemented with 5% fetal bovine serum (FBS) and glutamine, and allowed to adhere to the culture vessel overnight at 37°C, 20% O_2. The

culture medium is subsequently replaced and/or altered as required and the cells cultured for a further 68 to 72 hours at 37°C, 5% O_2.

[0168] As will be appreciated by those of skill in the art, cultured MLPSCs are phenotypically different to cells *in vivo*. For example, in one embodiment they express one or more of the following markers, CD44, NG2, DC146 and CD140b. Cultured MLPSCs are also biologically different to cells *in vivo*, having a higher rate of proliferation compared to the largely non-cycling (quiescent) cells *in vivo*.

[0169] In one example, the population of cells is enriched from a cell preparation comprising STRO-1+ cells in a selectable form. In this regard, the term "selectable form" will be understood to mean that the cells express a marker (e.g., a cell surface marker) permitting selection of the STRO-1+ cells. The marker can be STRO-1, but need not be. For example, as described and/or exemplified herein, cells (e.g., mesenchymal precursor cells) expressing STRO-2 and/or STRO-3 (TNAP) and/or STRO-4 and/or VCAM-1 and/or CD146 and/or 3G5 also express STRO-1 (and can be STRO-1bright). Accordingly, an indication that cells are STRO-1+ does not mean that the cells are selected solely by STRO-1 expression. In one example, the cells are selected based on at least STRO-3 expression, e.g., they are STRO-3+ (TNAP+). For example, the MPCs can be isolated from bone mononuclear cells with an anti-STRO-3 antibody.

[0170] Reference to selection of a cell or population thereof does not necessarily require selection from a specific tissue source. As described herein STRO-1+ cells can be selected from or isolated from or enriched from a large variety of sources. That said, in some examples, these terms provide support for selection from any tissue comprising STRO-1+ cells (e.g., mesenchymal precursor cells) or vascularized tissue or tissue comprising pericytes (e.g., STRO-1+ pericytes) or any one or more of the tissues recited herein.

[0171] In one example, the cells used in the present disclosure express one or more markers individually or collectively selected from the group consisting of TNAP+, VCAM-1+, THY-1+, STRO-2+, STRO-4+ (HSP-90β), CD45+, CD146+, 3G5+ or any combination thereof.

[0172] By "individually" is meant that the disclosure encompasses the recited markers or groups of markers separately, and that, notwithstanding that individual markers or groups of markers may not be separately listed herein the accompanying claims may define such marker or groups of markers separately and divisibly from each other.

[0173] By "collectively" is meant that the disclosure encompasses any number or combination of the recited markers or groups of markers, and that, notwithstanding that such numbers or combinations of markers or groups of markers may not be specifically listed herein the accompanying claims may define such combinations or sub-combinations separately and divisibly from any other combination of markers or groups of markers.

[0174] As used herein the term "TNAP" is intended to encompass all isoforms of tissue non-specific alkaline phosphatase. For example, the term encompasses the liver isoform (LAP), the bone isoform (BAP) and the kidney isoform (KAP). In one example, the TNAP is BAP. In one example, TNAP as used herein refers to a molecule which can bind the STRO-3 antibody produced by the hybridoma cell line deposited with ATCC on 19 December 2005 under the provisions of the Budapest Treaty under deposit accession number PTA-7282.

[0175] Furthermore, in one example, the STRO-1+ cells are capable of giving rise to clonogenic CFU-F.

[0176] In one example, a significant proportion of the STRO-1+ cells are capable of differentiation into at least two different germ lines. Non-limiting examples of the lineages to which the STRO-1+ cells may be committed include bone precursor cells; hepatocyte progenitors, which are multipotent for bile duct epithelial cells and hepatocytes; neural restricted cells, which can generate glial cell precursors that progress to oligodendrocytes and astrocytes; neuronal precursors that progress to neurons; precursors for cardiac muscle and cardiomyocytes, glucose-responsive insulin secreting pancreatic beta cell lines. Other lineages include, but are not limited to, odontoblasts, dentin-producing cells and chondrocytes, and precursor cells of the following: retinal pigment epithelial cells, fibroblasts, skin cells such as keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, smooth and skeletal muscle cells, testicular progenitors, vascular endothelial cells, tendon, ligament, cartilage, adipocyte, fibroblast, marrow stroma, cardiac muscle, smooth muscle, skeletal muscle, pericyte, vascular, epithelial, glial, neuronal, astrocyte and oligodendrocyte cells.

[0177] In an example, MLPSCs are obtained from a single donor, or multiple donors where the donor samples or MLPSCs are subsequently pooled and then culture expanded.

[0178] MLPSCs encompassed by the present disclosure may also be cryopreserved prior to administration to a subject. In an example, mesenchymal lineage precursor or stem cells are culture expanded and cryopreserved prior to administration to a subject.

[0179] In an example, the present disclosure encompasses MLPSCs as well as progeny thereof, soluble factors derived therefrom, and/or extracellular vesicles isolated therefrom. In another example, the present disclosure encompasses MLPSCs as well as extracellular vesicles isolated therefrom. For example, it is possible to culture expand MLPSCs of the disclosure for a period of time and under conditions suitable for secretion of extracellular vesicles into the cell culture medium. Secreted extracellular vesicles can subsequently be obtained from the culture medium for use in therapy.

[0180] The term "extracellular vesicles" as used herein, refers to lipid particles naturally released from cells and ranging in size from about 30 nm to as a large as 10 microns, although typically they are less than 200 nm in size. They can contain proteins, nucleic acids, lipids, metabolites, or organelles from the releasing cells (e.g., mesenchymal stem cells; STRO-1^+ cells).

[0181] The term "exosomes" as used herein, refers to a type of extracellular vesicle generally ranging in size from about 30 nm to about 150 nm and originating in the endosomal compartment of mammalian cells from which they are trafficked to the cell membrane and released. They may contain nucleic acids (*e.g.*, RNA; microRNAs), proteins, lipids, and metabolites and function in intercellular communication by being secreted from one cell and taken up by other cells to deliver their cargo.

[0182] In an example, compositions of the disclosure comprise cells that induce new blood vessel formation in target tissue. In an example, the target tissue is the heart. In another example, the cells secrete factors that protect at risk or damaged myocardium. In an example, at risk or damaged myocardium has been subject to a lack of blood flow resulting from an ischemic event. In an example, the cells secrete factors that reduce apoptosis in cardiomyocytes.

Culture expansion

[0183] In an example, MLPSCs are culture expanded. "Culture expanded" MLPSCs are distinguished from freshly isolated cells in that they have been cultured in cell culture medium and passaged (i.e. sub-cultured). In an example, freshly isolated cells are culture expanded for about 1 or 2 passages to provide an intermediate population. In an example,

freshly isolated cells are culture expanded for 2 passages to provide an intermediate population. In another example, freshly isolated cells are culture expanded for about 1 to 3 passages to provide an intermediate population.

[0184] Accordingly, in an example, relevant cells are isolated and culture expanded for 2 passages to provide an intermediate MLPSC population. The intermediate MLPSC population is then culture expanded to provide a DP. For example, the intermediate cell population can be cultured for three more passages (i.e. 5 passages total) to provide a DP.

[0185] In an example, MLPSCs are culture expanded for about 4 – 10 passages. In an example, MLPSCs are culture expanded for at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 passages. For example, MLPSCs can be culture expanded for at least 5 passages. In an example, MLPSCs can be culture expanded for at least 5 – 10 passages. In an example, MLPSCs can be culture expanded for at least 5 – 8 passages. In an example, MLPSCs can be culture expanded for at least 5 – 7 passages. In an example, MLPSCs can be culture expanded for more than 7 passages. In these examples, MLPSCs may be culture expanded before being cryopreserved to provide an intermediate cryopreserved MLPSC population and then subject to further culture expansion.

[0186] In an example, DP compositions of the present disclosure are produced by culturing cells from an intermediate cryopreserved MLPSC population or, put another way, a cryopreserved intermediate.

[0187] In an example, compositions of the disclosure comprise MLPSCs that are culture expanded from a cryopreserved intermediate. In an example, the cells culture expanded from a cryopreserved intermediate are culture expanded for at least 3, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 passages. For example, MLPSCs can be culture expanded for at least 3 passages. In an example, MLPSCs can be culture expanded for at least 3 – 10 passages. In an example, MLPSCs can be culture expanded for at least 3 – 8 passages. In an example, MLPSCs can be culture expanded for at least 3 – 7 passages.

[0188] In an example, CAP levels of MLPSCs that are culture expanded from a cryopreserved intermediate disclosed herein are assessed under culture conditions. In an example, these MLPSCs have a CAP level of greater than 108% under culture conditions. In another example, these MLPSCs have a CAP level of greater than 110% under culture conditions.

[0189] In some examples, IL-2RA inhibition by MLPSCs culture expanded from a cryopreserved intermediate disclosed herein are assessed under culture conditions. In an

example, these MLPSCs inhibit IL-2RA greater than 56% under culture conditions. In another example, these MLPSCs inhibit IL-2RA greater than 60% under culture conditions. In another example, these MLPSCs inhibit IL-2RA greater than 65% under culture conditions.

[0190] In an example, culture expanded MLPSCs can be culture expanded in medium free of animal proteins (e.g. in a xeno-free medium). In an example, MLPSCs can be culture expanded in medium that is fetal bovine serum free.

[0191] In an embodiment, MLPSCs can be obtained from a single donor, or multiple donors where the donor samples or MLPSCs are subsequently pooled and then culture expanded as required. In an example, the culture expansion process comprises:

i. expanding by passage expansion the number of viable cells to provide a preparation of at least about 1 billion of the viable cells, wherein the passage expansion comprises establishing a primary culture of isolated MLPSCs and then serially establishing a first non-primary (P1) culture of isolated MLPSCs from the previous culture;

ii. expanding by passage expansion the P1 culture of isolated MLPSCs to a second non-primary (P2) culture of MLPSCs; and,

iii. preparing and cryopreserving an in-process intermediate MLPSC preparation obtained from the P2 culture of MLPSCs; and, optionally

iv. thawing the cryopreserved in-process intermediate MLPSC preparation and expanding by passage expansion the in-process intermediate MLPSC preparation.

[0192] In the context of the present disclosure, certain assays may be performed between steps iii and iv. For example, the CAP level may be determined under culture conditions after step iii. In an example, step iv is only performed if a desired level of CAP is observed under culture conditions. In this example, the cell population is selected for culture expansion on the basis of CAP under culture conditions.

[0193] In some examples, IL-2RA inhibition may also be determined under culture conditions after step iii. In an example, step iv is only performed if a desired level of CAP and IL-2RA inhibition is observed under culture conditions. In this example, the cell population is selected for culture expansion on the basis of CAP and IL-2RA inhibition under culture conditions.

[0194] In an example, the expanded MLPSC preparation has an antigen profile and an activity profile comprising:

i. less than about 0.75% CD45+ cells;

ii. at least about 95% CD105+ cells;

iii. at least about 95% CD166+ cells.

[0195] In an example, the expanded MLPSC population is expanded from an intermediate MLPSC population and has a CAP level of ≥ 108%. In an example, the expanded MLPSC population is expanded from an intermediate MLPSC population and has a CAP level of ≥ 110%.

[0196] In an example, the expanded MLPSC population is expanded from an intermediate MLPSC population and is capable of inhibiting IL-2RA expression by CD3/CD28-activated PBMCs by at least 56% relative to a control. In another example, the expanded MLPSC population is expanded from an intermediate MLPSC population and is capable of inhibiting IL-2RA expression by CD3/CD28-activated PBMCs by at least 60% relative to a control. In another example, the expanded MLPSC population is expanded from an intermediate MLPSC population and is capable of inhibiting IL-2RA expression by CD3/CD28-activated PBMCs by at least 65% relative to a control.

[0197] In an example, the expanded MLPSC population is expanded from an intermediate MLPSC population and (i) has a CAP level of ≥ 108%, and (ii) is capable of inhibiting IL-2RA expression by CD3/CD28-activated PBMCs by at least 65% relative to a control.

[0198] The process of MLPSC isolation and *ex vivo* expansion can be performed using any equipment and cell handing methods known in the art. Various culture expansion embodiments of the present disclosure employ steps that require manipulation of cells, for example, steps of seeding, feeding, dissociating an adherent culture, or washing. Any step of manipulating cells has the potential to insult the cells. Although MLPSC can generally withstand a certain amount of insult during preparation, cells are preferably manipulated by handling procedures and/or equipment that adequately performs the given step(s) while minimizing insult to the cells.

[0199] In an example, MLPSCs are washed in an apparatus that includes a cell source bag, a wash solution bag, a recirculation wash bag, a spinning membrane filter having inlet and outlet ports, a filtrate bag, a mixing zone, an end product bag for the washed cells, and appropriate tubing, for example, as described in US 6,251,295, which is hereby incorporated by reference.

[0200] In an example, a MLPSC composition cultured according to the present disclosure is 95% homogeneous with respect to being CD105 positive and CD166 positive and being CD45 negative. In an example, this homogeneity persists through *ex vivo* expansion; i.e. though multiple population doublings.

[0201] In an example, MLPSCs of the disclosure are culture expanded in 2D culture. For example, MLPSCs of the disclosure can be culture expanded in a cell factory. In certain examples, 3D culture of intermediates disclosed herein may follow using, for example, a bioreactor. In an example, MLPSCs of the disclosure are initially culture expanded in 2D culture prior to being further expanded in 3D culture. In an example, intermediate cell populations of the disclosure have not been culture expanded in 3D culture. In an example, CAP levels and IL-2RA inhibition is assessed after culture expansion in 3D culture.

[0202] In an example, MLPSCs of the disclosure are culture expanded from an intermediate population. In an example, MLPSCs of the disclosure are culture expanded from the intermediate in 2D culture before seeding in 3D culture.

[0203] In the context of both intermediate populations and therapeutic compositions expanded from the same, in an example, MLPSCs of the disclosure are culture expanded in 2D culture for at least 3 days before seeding in a further culture system such as cell factory or 3D culture in a bioreactor. In an example, MLPSCs of the disclosure are culture expanded in 2D culture for at least 4 days before seeding in a further culture system. In an example, MLPSCs of the disclosure are culture expanded in 2D culture for between 3 and 5 days before seeding in a further culture system. In these examples, 2D culture can be performed in a cell factory. Various cell factory products are available commercially (e.g. Thermofisher, Sigma, Corning). In an example, the cell factory has at least 5 layers. In an example, the cell factory has at least 10 layers. In an example, the cell factory has at least 20 layers. 3D culture may be performed in various bioreactor types such as stirred tank, wave bag, and vertical wheel.

[0204] In an example, CO_2 is provided during culture expansion of MLPSCs. In an example, MLPSCs are culture expanded in less than 9% CO_2. In an example, MLPSCs are culture expanded in less than 8% CO_2. In an example, MLPSCs are culture expanded in 5% CO_2. For example, MLPSCs can be culture expanded in 5% +/- 2% CO_2. In an example, the MLPSCs are culture expanded with passive priming of CO_2. For example, cell factories can be passively primed with 5% CO_2.

[0205] Priming cell factories maintains the CO_2 tension between the cell factory and incubator and stabilizes the pH level of the growth medium. Active priming involves actively passing CO_2 gas through a bacterial vent air filter into each culture vessel (e.g. cell factory) for a defined period of time (e.g. around 10 minutes). However, active priming has the potential to introduce contamination into culture as it requires an open port to provide gas. Passive priming involves placing a closed culture system into an incubator at appropriate CO_2 concentration prior to cell seeding (e.g. around 12 to 72 hours).

[0206] In an example, cells of the disclosure are STRO-3+ before they are culture expanded to provide an intermediate cell population.

Cell Culture Medium

[0207] MLPSCs disclosed herein can be culture expanded in various suitable growth mediums.

[0208] The term "medium" or "media" as used in the context of the present disclosure, includes the components of the environment surrounding the cells. The media contributes to and/or provides the conditions suitable to allow cells to grow. Media may be solid, liquid, gaseous or a mixture of phases and materials. Media can include liquid growth media as well as liquid media that do not sustain cell growth. Media also include gelatinous media such as agar, agarose, gelatin and collagen matrices. Exemplary gaseous media include the gaseous phase that cells growing on a petri dish or other solid or semisolid support are exposed to.

[0209] The cell culture media used for culture expansion contains all essential amino acids and may also contain non-essential amino acids. In general, amino acids are classified into essential amino acids (Thr, Met, Val, Leu, Ile, Phe, Trp, Lys, His) and non-essential amino acids (Gly, Ala, Ser, Cys, Gln, Asn, Asp, Tyr, Arg, Pro).

[0210] Those of skill in the art will appreciate that for optimal results, the basal medium must be appropriate for the cell line of interest. For example, it may be necessary to increase the level of glucose (or other energy source) in the basal medium, or to add glucose (or other energy source) during the course of culture, if this energy source is found to be depleted and to thus limit growth. In an example, dissolved oxygen (DO) levels can also be controlled.

[0211] In an example, the cell culture medium contains human derived additives. For example, human serum and human platelet cell lysate can be added to the cell culture media.

[0212] In an example, the cell culture medium contains only human derived additives. Thus, in an example, the cell culture media is xeno-free. For avoidance of doubt, in these examples, the culture medium is free of animal proteins. In an example, cell culture medium used in the methods of the disclosure is free of animal components.

[0213] In an example, the culture medium comprises serum. For example, fetal calf serum and/or new born calf serum. In other examples the culture medium is fetal bovine serum free culture medium comprising growth factors that promote MLPSC proliferation. In an embodiment, the culture medium is serum free stem cell culture medium. In an example, the cell culture medium comprises:

a basal medium;

platelet derived growth factor (PDGF);

fibroblast growth factor 2 (FGF2).

[0214] In an example, the culture medium comprises platelet derived growth factor (PDGF) and fibroblast growth factor 2 (FGF2), wherein the level of FGF2 is less than about 6 ng/ml. For example, the FGF2 level may be less than about 5 ng/ml, less than about 4 ng/ml, less than about 3 ng/ml, less than about 2 ng/ml, less than about 1 ng/ml. In an example, the FGF2 level is about 1 ng/ml.

[0215] In an example, the PDGF is PDGF-BB. In an example, the level of PDGF-BB is between about 1 ng/ml and 150 ng/ml. In another example, the level of PDGF-BB is between about 7.5 ng/ml and 120 ng/ml. In another example, the level of PDGF-BB is between about 15 ng/ml and 60 ng/ml. In another example, the level of PDGF-BB is about 10 ng/ml. In another example, the level of PDGF-BB is at least about 10 ng/ml or less.

[0216] In other examples, additional factors can be added to the cell culture medium. In an example, the culture medium further comprising EGF. EGF is a growth factor that stimulates cell proliferation by binding to its receptor EGFR. In an example, the method of the present disclosure comprises culturing a population of stem cells in a fetal bovine serum free cell culture medium further comprising EGF. In an example, the level of EGF is between about 0.1 and 7 ng/ml. For example, the level of EGF can be at least about 5 ng/ml.

[0217] In another example, the level of EGF is between about 1 ng/ml and 8 ng/ml. In another example, the level of EGF is between about 3 ng/ml and 6 ng/ml. In another example, the level of EGF is about 5 ng/ml. In another example, the level of EGF is about 5 ng/ml or less.

[0218] In the above examples, basal medium such as Alpha MEM or StemSpan™ can be supplemented with the referenced quantity of growth factor. In an example, the culture medium comprises Alpha MEM or StemSpan™ supplemented with 10 ng/ml PDGF-BB, 5 ng/ml EGF and 1 ng/ml FGF.

[0219] In other examples, additional factors can be added to the cell culture medium. For example, the cell culture media can be supplemented with one or more stimulatory factors selected from the group consisting of epidermal growth factor (EGF), lα,25-dihydroxyvitamin D3 (1,25D), tumor necrosis factor α (TNF- α), interleukin -lβ (IL-lβ) and stromal derived factor lα (SDF-lα). In another embodiment, cells may also be cultured in the presence of at least one cytokine in an amount adequate to support growth of the cells. In another embodiment, cells can be cultured in the presence of heparin or a derivative thereof. In an example, the heparin derivative is a sulphate). Various forms of heparin sulphate are known in the art and include heparin sulphate 2 (HS2). HS2 can be derived from various sources including for example, the liver of male and/or female mammals. Thus, an exemplary heparin sulphate includes male liver *heparin sulphate (MML HS)* and female liver heparin sulphate (FML HS).

[0220] In another example, the cell culture medium of the present disclosure maintain stem cells in an undifferentiated state. Stem cells are considered to be undifferentiated when they have not committed to a specific differentiation lineage. As discussed above, stem cells display morphological characteristics that distinguish them from differentiated cells. Furthermore, undifferentiated stem cells express genes that may be used as markers to detect differentiation status. The polypeptide products may also be used as markers to detect differentiation status. Accordingly, one of skill in the art could readily determine whether the methods of the present disclosure maintain stem cells in an undifferentiated state using routine morphological, genetic and/or proteomic analysis.

Method of manufacturing drug product

[0221] In an example, the present disclosure relates to a method of manufacturing drug product which comprises a population of mesenchymal lineage precursor or stem cells

(MLPSCs), the method comprising: acquiring a determination of whether a test population of MLPSCs has a predetermined cell adhesion and proliferation (CAP) level under culture conditions, and processing at least a portion of the test population of MLPSCs as a drug product if the test population of MLPSCs has a predetermined CAP level under culture conditions, thereby manufacturing the drug product; or discarding at least a portion of the test population of MLPSCs if the population of MLPSCs have a CAP level less than the predetermined level under culture conditions, wherein the predetermined level is 108%. In an example, the predetermined level is ≥ 110% under culture conditions.

[0222] In an example, acquiring a determination of whether a test population of MLPSCs has a predetermined CAP level under culture conditions comprises measuring the increase in the number of adherent cells in culture relative to the number of freshly seeded cells according to the methods disclosed herein.

[0223] In an example, the method further comprises: acquiring a determination of whether a test population of MLPSCs inhibits IL-2RA at a predetermined level under culture conditions, and processing at least a portion of the test population of MLPSCs as a drug product if the test population of MLPSCs inhibits IL-2RA by at least the predetermined level under culture conditions, thereby manufacturing the drug product; or discarding at least a portion of the test population of MLPSCs if the population of mesenchymal stem cells inhibits IL-2RA by less than the predetermined level under culture conditions, wherein the predetermined level is 56%. In an example, the predetermined level is ≥ 60% under culture conditions. In an example, the predetermined level is ≥ 65% under culture conditions.

[0224] In an example, acquiring a determination of whether a test population of MLPSCs inhibits IL-2RA at a predetermined level under culture conditions comprises co-culturing a representative amount of the MLPSCs according to the methods disclosed herein.

[0225] In an example, the test population is obtained from a population of MLPSCs in 3D culture. For example, the MLPCSs can be in a bioreactor culture. In an example, the test population is obtained from cryopreserved population of MLPSCs. In an example, the test population is representative of a larger population of MLPSCs such as multiple cryopreserved populations of MLPSCs. In an example, the multiple cryopreserved populations of MLPSCs have been culture expanded from the same intermediate population of MLPSCs.

[0226] In an example, drug product comprises a composition disclosed herein. In an example, the drug product comprise 25 x 10^6 MLPSCs.

[0227] In an example, the present disclosure provides methods of manufacturing MSC drug product, such methods include a first step of providing (e.g., culture expanding (e.g., in small scale or large scale cell culture) or manufacturing) or obtaining (e.g., receiving and/or purchasing from a third party (including a contractually related third party or a non-contractually-related (e.g., an independent) third party) a test MSC population (e.g., a sample of a test MSC population), a second step of acquiring (e.g., detecting, measuring, receiving, or obtaining) at least one value (e.g., 1, 2, 3 and/or 4) for an MSC parameter listed in Table A for the test MSC population, and a third step of processing at least a portion of the test MSC population (e.g., processing a portion of a manufacturing lot, culture, or run, an entire manufacturing lot, culture, or run, or multiple manufacturing lots, cultures, or runs) as MSC drug product (e.g., in a form or packaging intended for administration as described subsequently herein; optionally cryopreserved) if the at least one value for the test MSC population meets a reference criterion shown in Table A for the parameter, thereby manufacturing MSC drug product. In an example, the value(s) comprise parameter number 1. In another example, the value(s) comprise parameter number 2. In another example, the value(s) comprise parameter number 3. In another example, the value(s) comprise parameter number 4. In another example, the value(s) comprise parameter number 1 and 2. In another example, the value(s) comprise parameter number 1 and 3. In another example, the value(s) comprise parameter number 1, 2 and 4.

[0228] In an example, such methods comprise a second step which includes acquiring values for any combination of two or more MSC parameters listed in Table A, and the third step of such methods includes processing at least a portion of the test MSC population as MSC drug product if the values for the any combination of two or more MSC parameters meet the corresponding reference criterion shown in Table A for the parameters.

Table A: MSC Parameters

Parameter #	Description	Reference Criterion
1	IL-2RA inhibition under culture conditions	≥60%
2	CD29, CD54, CD73, CD90, CD102, CD105, CD106, CD166, MHC1	CD29+, CD54+, CD73+, CD90+, CD102+, CD105+, CD106+, CD166+, MHC1+
3	CD45, CD105, CD166	CD45-, CD105+, CD166+
4	CD45, CD105, CD166	<0.75% CD45+, >95% CD105+; >95% CD166+

Selecting cells for use in treatment

[0229] In an embodiment, the present disclosure encompasses selecting cells for use in treatment. In an example, the MLPSC population is assessed for the requisite criteria, e.g. CAP level, and, when met, said population is selected for use in treatment of a disease. As discussed herein, the CAP level of a population of MLPSCs is indicative of the populations overall viability, quality, health and/or clinical potency. Accordingly, the person skilled in the art will appreciate that MLPSCs meeting the requisite CAP level can be selected for use in any disease or condition that MLPSCs are known to be useful for treating.

[0230] In an example the present disclosure encompasses selecting cells for use in treatment of graft versus host disease (GvHD), multiple sclerosis, Type 1 diabetes, rheumatoid arthritis, osteoarthritis, uveitis, autoimmune thyroid disease, inflammatory bowel disease (IBD), scleroderma, Graves' Disease, lupus, Crohn's disease, autoimmune lymphoproliferative disease (ALPS), demyelinating disease, autoimmune encephalomyelitis, autoimmune gastritis (AIG), and autoimmune glomerular diseases, allergy, psoriasis, Alzheimer's Disease, Parkinson's Disease, stroke, Acute Respiratory Distress Syndrome (ARDS), Chronic Obstructive Pulmonary Disease (COPD), Idiopathic Pulmonary Fibrosis (IPF), asthma, and pulmonary hypertension, hyperinflammation, chronic pain, low back pain, retinopathies, or heart failure.

[0231] In an example, an MLPSC population is selected for use in treatment if it has a CAP level of at least 108% under culture conditions. In an example, the MLPSC population is selected for use in treatment if it has a CAP level of ≥ 110% under culture conditions.

[0232] In an example the present disclosure encompasses selecting cells for use in treatment of GvHD. In an example, an MLPSC population is selected for use in treatment of GvHD if it has a CAP level of ≥ 108% under culture conditions. In an example, an MLPSC population is selected for use in treatment of GvHD if it has a CAP level of ≥ 110% under culture conditions .

[0233] In an example, an MLPSC population is selected for use in treatment of GvHD if it has a CAP level of ≥ 108% and inhibits IL-2RA by ≥ 60% under culture conditions. In an example, an MLPSC population is selected for use in treatment of GvHD if it has a CAP level of ≥ 108% and inhibits IL-2RA by ≥ 65% under culture conditions. In an example, an MLPSC population is selected for use in treatment of GvHD if it has a CAP level of ≥ 110% and inhibits IL-2RA by ≥ 60% under culture conditions. In an example, an MLPSC population is selected for use in treatment of GvHD if it has a CAP level of ≥ 110% and inhibits IL-2RA by ≥ 65% under culture conditions.

[0234] Selection processes are not particularly limited so long as they are able to select cell populations characterized by the relevant criteria such as CAP or %IL-2RA inhibition. . In an example, a series of intermediate MLPSC populations are assessed for CAP under culture conditions and those populations which have a CAP level of ≥108% under culture conditions are selected for further expansion. In an example, a series of intermediate MLPSC populations are assessed for CAP and IL-2RA inhibition under culture conditions and those populations which have a CAP level of ≥108% and inhibit IL-2RA by at least 60% under culture conditions are selected for further expansion. In an example, a series of intermediate MLPSC populations are assessed for CAP and IL-2RA inhibition under culture conditions and those populations which have a CAP level of ≥108% and inhibit IL-2RA by at least 65% under culture conditions are selected for further expansion. In an example, a series of intermediate MLPSC populations are assessed for CAP under culture conditions and those populations which have a CAP level of ≥110% under culture conditions are selected for further expansion. In an example, a series of intermediate MLPSC populations are assessed for CAP and IL-2RA inhibition under culture conditions and those populations which have a CAP level of ≥110% and inhibit IL-2RA by at least 60% under culture conditions are selected for further expansion. In an example, a series of intermediate MLPSC populations are assessed for CAP and IL-2RA inhibition under culture conditions and those populations which have a

CAP level of ≥110% and inhibit IL-2RA by at least 65% under culture conditions are selected for further expansion.

[0235] In some examples, selected MLPSC populations are capable of inhibiting IL-2RA expression by CD3/CD28-activated PBMCs by at least 56% relative to a control. In another example, selected MLPSC populations are capable of inhibiting IL-2RA expression by CD3/CD28-activated PBMCs by at least 60% relative to a control. In another example, selected MLPSC populations are capable of inhibiting IL-2RA expression by CD3/CD28-activated PBMCs between 56% and 70% relative to a control.

[0236] In an example, the obtained population of MLPSCs is representative of a larger MLPSC population. For example, the obtained population of MLPSCs may be representative of a series of cryopreserved MLPSC populations such as a series of doses. For example, the doses may be originate from the same population and one or more of the doses is obtained and assessed for therapeutic efficacy according to methods disclosed herein. In this example, the obtained dose(s) are representative of the remaining doses originating from the same MLPSC population. In an example, the obtained population of MLPSCs is representative of a population of MLPSCs in 3D culture. In these examples, cells from an alternative population of MLPSCs can be selected based on an assessment of a representative test population of MLPSCs.

[0237] In an example, selected cell populations are cryopreserved to allow administration at a later stage.

[0238] In another example, the present disclosure relates to compositions comprising population(s) of selected MLPSCs. In an example, the composition comprises a population of culture expanded MLPSCs, wherein the population of MLPSCs are selected based on a predetermined CAP level under culture conditions, wherein the predetermined CAP level is ≥108%. In an example, the predetermined CAP level is ≥110%.

[0239] In another example, the composition comprises a population of culture expanded MLPSCs, wherein the population of MLPSCs are selected based on a predetermined level of CAP and a predetermined level of IL-2RA inhibition under culture conditions, wherein the predetermined level of CAP is ≥108% and the pre-determined level of IL-2RA inhibition is ≥ 60%. In an example, the predetermined level of CAP is ≥108% and the pre-determined level of IL-2RA inhibition is ≥ 65%. In an example, the predetermined level of CAP is ≥110% and the pre-determined level of IL-2RA inhibition is ≥ 60%. In an

example, the predetermined level of CAP is ≥110% and the pre-determined level of IL-2RA inhibition is ≥ 65%.

[0240] In another example, the MLPSCs are also selected based on expression of one or more or all of CD29, CD54, CD73, CD90, CD102, CD105, CD106, CD166, MHC1. In an example, the MLPSC populations that are ≥ 95% CD166+, ≥ 95% CD105+, and ≤ 0.75% CD45+ are selected.

[0241] In another example, MLPSC populations that express low levels of major histocompatibility complex (MHC) class I and minimal levels of human leukocyte antigen (HLA)-DR, CD40, CD80, and CD86 are selected.

[0242] In an example, MLPSCs populations comprising compositions of the disclosure are selected based on one or more parameters described in Table A.

Methods of treatment

[0243] In an example, the methods of the present disclosure relate to the provision of cell populations effective for treating graft versus host disease (GvHD). In an example, treatment involves defrosting a composition of the present disclosure and assessing CAP and capability for IL-2RA inhibition before administration. In an example, cells in the composition are defrosted and established in cell culture. In this example, CAP may be assessed after 3 days in culture and IL-2RA inhibition may be assessed after 6 days in culture. Compositions meeting the "release criteria" from CAP and IL-2RA inhibition are subsequently administered to the subject.

[0244] "Graft versus Host Disease (GvHD)" is an immunological disorder that is the major factor that limits the success and availability of allogeneic bone marrow or stem cell transplantation. GvHD occurs in acute (aGvHD) or chronic (cGvHD) forms. Acute GvHD usually manifests within 100 days following bone marrow or stem cell transplantation. Chronic GvHD generally manifests later than aGvHD (>100 days post transplantation) and has some features of autoimmune diseases. It may develop either *de novo*, following resolution of aGvHD or as an extension of aGvHD. Chronic GvHD can cause multiple, often debilitating symptoms, including widespread skin rashes, painful mouth ulcers, shortness of breath, and limb and joint pain. In an example, patients with cGvHD have impaired reconstitution of CD5+ B cells. In an example, cGvHD is refractory to steroid therapy. In an example, cGvHD is refractory to a biologic therapy. In an example cGvHD is refractory to steroid therapy and a biologic therapy.

[0245] GvHD severity can be graded by patterns of organ involvement and clinical performance status. Multi-organ involvement includes skin rash, liver involvement, and/or gastrointestinal (GI) involvement. Examples of skin rash, liver involvement, and GI involvement are provided in Table 1 and Table 2. In an example, the subject has GvHD with multi-organ involvement. In an example, subjects treated according to the disclosure have severe GvHD. In an example, severe GvHD is graded according to the Glucksberg scale (Glucksberg et al, 1974; Thomas et al, 1975) (Table 1). For example, the subject can have Grade II GvHD or Grade III/IV GvHD according to the Glucksberg scale. In one example, the subject has Grade II GvHD. In another example, the subject has Grade III/IV GvHD.

[0246] In another example, severe GvHD is graded according to IBMTR Severity Index (Table 2) (Rowlings et al., 1997). In one example, the subject has Grade B, Grade C, or Grade D GvHD according to the IBMTR severity scale. In an example, subject has Grade D GvHD.

[0247] In another example, the subject has Minnesota high risk GvHD. Minnesota high risk acute GvHD is defined as either skin stage 4; lower gastrointestinal (GI) stage 3–4 or liver stage 3–4; or skin stage 3+ and either lower GI 2–4 or liver stage 2–4 GvHD (MacMillan et al., 2015).

[0248] In another example, GvHD severity is assessed according to the Mount Sinai Acute GVHD International Consortium (MAGIC) algorithm probability (MAP) score. MAP is a validated analysis that combines the serum concentrations of two biomarkers, Regenerating islet-derived protein 3 alpha (Reg3α) and soluble interleukin 1 receptor-like 1 (ST2), into a single value that predicts long-term outcomes such as response to therapy after 28 days and 6-month non-relapse mortality (NRM). MAP is calculated by: $\log10[-\log10(1-\hat{p})] = -11.263 + 1.844(\log10ST2) + 0.577(\log10REG3\alpha)$, where $\hat{p}$ is the predicted probability of 6-month NRM (Hartwell et al.; Major-Monfried et al.).

[0249] Accordingly, in the above examples, the subject can also have a high MAGIC algorithm probability (MAP) score. For example, the subject can have a MAP score $\geq$ 0.29.

Table 1: Glucksberg clinical stage and grade of acute GvHD (Rowlings et al., 1997).

Stage	**Skin**	**Liver**	**Intestinal tract**
1	Maculopapular rash <25% of body surface	Bilirubin 34-50 µmol/l	>500 ml diarrhoea/d
2	Maculopapular rash 25-50% of body surface	Bilirubin 51-102 µmol/l	>1000 ml diarrhoea/d
3	Generalised erythroderma	Bilirubin 103-225 µmol/l	>1500 ml diarrhoea/d
4	Generalised erythroderma with bullous formation and desquamation	Bilirubin >225 µmol/l	Severe abdominal pain, with or without ileus
Grade	**Degree of organ involvement**		
I	Stage 1-2 skin rash: no gut involvement: no liver involvement: no decrease in clinical performance		
II	Stage 1-3 skin rash: stage 1 gut involvement or stage 1 liver involvement (or both): mild decrease in clinical performance		
III	Stage 2-3 skin rash: stage 2-3 gut involvement or stage 2-4 liver involvement (or both): marked decrease in clinical performance		
IV	Similar to Grade III with stage-4 organ involvement and extreme decrease in clinical performance		

Table 2: Criteria for IBMTR Severity Index for acute GvHD (Rowlings et al., 1997).

	Skin involvement			Liver involvement			Gastrointestinal involvement	
Index*	Stage (max)	Extent of rash		Stage (max)	Total bilirubin (µmol/l)		Stage (max)	Volume of diarrhoea (ml/d)
A	1	<25%		0	<34		0	<500
B	2	25-50%	or	1-2	34-102	or	1-2	550-1500
C	3	>50%	or	3	103-255	or	3	>1500
D	4	Bullae	or	4	>255	or	4	Severe pain and ileus

* Assign Index based on maximum in an individual organ system

[0250] In an example, subjects with severe GvHD do not respond to treatment with primary therapy. In an example, the subject has worsened within 3 days of primary therapy. In this example, the subject has worsened if their GvHD has increased in severity. For example, the subject's GvHD severity has increased according to MAP. In another example, the subject's GvHD severity has increased according to the Glucksberg scale. In another example, the subject's GvHD severity has increased according to the IMBTR scale. In another example, the subject's GvHD severity has increased according to organ involvement.

[0251] In another example, the subject has not responded within 7 days of a primary therapy. For example, the subject is refractory to primary therapy. In one example, primary therapy is systemic steroids. In one example, the subject has severe GvHD and is refractory to a steroid. In an example, the steroid is a corticosteroid. In another example, the steroid is a glucocorticoid. In another example, the steroid is prednisone. In another example, the subject has severe GvHD and is refractory to steroids and a second line therapy. For example, a second line therapy can include extracorporeal photopheresis, etanercept, infliximab, ruxolitinib, anti-thymocyte globulin, mycophenolate, alemtuzumab, basiliximab, or tocilizumab. In an example, the subject is refractory to steroids, an anti-TNFα antibody and, ruxolitinib.

[0252] Methods of the present disclosure relate to the treatment of GvHD. As used herein, the terms "treating", "treat", "treatment", "reducing progression" include administering a population of mesenchymal lineage stem or precursor cells and/or progeny thereof and/or soluble factors derived therefrom and/or extracellular vesicles derived therefrom to thereby reduce or eliminate at least one symptom of the GvHD.

[0253] As used herein, the term "response" means response to therapy. In an example, a subject is considered to have had a response if they have an improvement in at least one organ without progression in any other organs and if additional therapy was not required. In another example, a subject is considered not to have had a response if they had stable or progressive GvHD or if the subsequent addition of secondary therapy is required. In this example, a subject who does not have a response is a non-responder.

[0254] In an example, treatment induces a partial response. In an example, the partial response is induced at least 28 after treatment is initiated. In an example, the partial response is induced 28 days after treatment is initiated. In an example, the partial

response is induced at least 30 days after treatment is initiated. In an example, the partial response is induced at least 2 months after treatment is initiated. In another example, the partial response is induced at least 3 months after treatment is initiated. In another example, the partial response is induced within 3 months. In another example, the partial response is induced 28 to 56 days after treatment is initiated. In another example, the partial response is induced 100 days after treatment is initiated. In another example, the partial response is induced 160 days after treatment is initiated. In another example, the partial response is induced 180 days after treatment is initiated.

[0255] In another example, the partial response is induced after two doses. In another example, the partial response is induced after two doses administered once weekly. In another example, the partial response is induced after two doses administered once weekly every two weeks. In another example, the partial response is induced after three doses or more. In an example, in the context of GvHD, a partial response is characterized by one or more or all of:

- Reduction in Skin % BSA score of at least one point;
- Reduction in mouth score of at least one point;
- Reduction in eye score of at least one point;
- Reduction in skin features score of at least one point;
- Reduction in gastrointestinal tract score of at least one point;
- Reduction in liver score of at least one point;
- Reduction in lung symptom score of at least one point;
- Reduction in lung FEV1 score of at least one point;
- Reduction in joints and fascia score of at least one point;
- Reduction in genital tract score of at least one point.

[0256] In an example, a partial response is characterized by a reduction in Skin % BSA score of at least one point. In another example, a partial response is characterized by a reduction in mouth score of at least one point. In another example, a partial response is characterized by a reduction in eye score of at least one point. In these examples, scores can be obtained using the NIH Consensus Criteria 2014 for GvHD.

[0257] In another example, a partial response is characterized by one or more or all of:

- Reduction in Skin % BSA score of at least one point;
- Reduction in mouth score of at least one point;
- Reduction in eye score of at least one point.

[0258] There are various classification systems for characterizing GvHD (Lee, S., (2017) Blood., 129(1): 30–37). In an example, the NIH Consensus Criteria 2014 can be used for scoring outcomes disclosed herein (Jagasia et al., (2015) *Biol Blood Marrow Transplant.*, 21:389-401). The components of the NIH Consensus Criteria 2014 are shown in the following table:

Table 3: Organ Scoring of GvHD

Organ	Score 0	Score 1	Score 2	Score 3
Skin % BSA[1]	No BSA involved	1-18% BSA	19-50% BSA	>50% BSA
Skin Features	No sclerotic features	N/A	Superficial sclerotic features, but not "hidebound"	Deep sclerotic features; "hidebound"; impaired mobility; ulceration
Mouth	No symptoms	Mild symptoms **with** disease signs but not limiting oral intake significantly	Moderate symptoms with disease signs **with** partial limitation of oral intake	Severe symptoms with disease signs **with** major limitation of oral intake
Eyes	No symptoms	Mild dry eye symptoms not affecting ADL (requirement of lubricant drops ≤3x/day)	Moderate dry eye symptoms partially affecting ADL (requiring lubricant drops >3x/day or punctal plugs) **WITHOUT** new vision impairment due to keratoconjunctivitis sicca (KCS)	Severe dry eye symptoms significantly affecting ADL (special eyewear to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to keratoconjunctivitis sicca (KCS)
GI Tract	No symptoms	Symptoms without significant weight loss (<5%)	Symptoms associated with mild to moderate weight loss (5-15%) within 3 months **OR** moderate diarrhea without significant	Symptoms associated with significant weight loss (>15%) within 3 months, requires nutritional supplement for most calorie needs

			interference with daily living	**OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
Liver	Normal total bilirubin and ALT or AP <3 x ULN	Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥3 x ULN	Elevated total bilirubin but ≤3 mg/dL or ALT >5 x ULN	Elevated total bilirubin but >3 mg/dL
Lungs Symptom Score	No symptoms	Mild symptoms (SOB after climbing one flight of steps)	Moderate symptoms (SOB after walking on flat ground)	Severe symptoms (SOB at rests; requires O2)
Lungs Lung Score	FEV1 ≥80%	FEV1 60-79%	FEV1 40-59%	FEV1 ≤39%
Joints and Fascia	No symptoms	Mild tightness of arms and legs, normal or mild decreased range of motion **AND** not affecting ADL	Tightness of arms or legs **OR** joint contractures, erythema thought to be due to fasciitis, moderate decrease of range of motion **AND** mild to moderate limitation of ADL	Contractures **WITH** significant decrease of range of motion **AND** significant limitation of ADL (unable to tie shoes, button shirts, dress shelf, etc.)
Genital Tract	No signs	Mild signs and females with or without discomfort on exam	Moderate signs and may have signs of discomfort on exam	Severe signs with or without symptoms
Other features	No GvHD	Mild	Moderate	Severe

[0259] In an example, a partial response is a decrease of ≥ 1 point on the organ-specific NIH Consensus Criteria 2014 score from the Table above. Accordingly, in an example, treatment induces ≥1 point decrease in Skin % BSA score. In another example, treatment induces ≥1 point decrease in mouth score. In another example, treatment induces ≥1 point decrease in eye score. In another example, treatment induces ≥1 point decrease in skin features score. In another example, treatment induces ≥1 point decrease in gastrointestinal tract score. In another example, treatment induces ≥1 point decrease in liver score. In another example, treatment induces ≥1 point decrease in lung symptom score. In another example, treatment induces ≥1 point decrease in lung FEV1 score. In another example, treatment induces ≥1 point decrease in joints and fascia score. In another example, treatment induces ≥1 point decrease in genital tract score.

[0260] In an example, the treatment induces a complete response after treatment is initiated. In an example, a complete response is the complete resolution of GvHD symptoms in all organs. In an example, the complete response is induced 28 days after treatment is initiated. In an example, the complete response is induced at least 28 after treatment is initiated. In an example, the complete response is induced at least 30 after treatment is initiated. In an example, the complete response is induced at least 2 months after treatment is initiated. In another example, the complete response is induced at least 3 months after treatment is initiated. In another example, the complete response is induced 28 to 56 days after treatment is initiated. In another example, the complete response is induced 100 days after treatment is initiated. In another example, the complete response is induced 160 days after treatment is initiated. In another example, the complete response is induced 180 days after treatment is initiated.

[0261] In another example, the complete response is induced after two doses. In another example, the complete response is induced after two doses administered once weekly. In another example, the complete response is induced after two doses administered once weekly every two weeks. In another example, the complete response is induced after three doses or more.

[0262] In another example, again in the context of GvHD, the treatment increases the probability of the subject's survival. For example, treatment increases the probability of the subject surviving for at least 20 days to 200 days after initiation of treatment. In one example, treatment increases the probability of the subject surviving for at least 180 days after initiation of treatment. In another example, treatment increases the probability of the

subject surviving at least 100 days. In an example, the increased probability is determined relative to a subject that is not treated with a composition of the disclosure. In an example, treatment increases 100 day survival greater than 60%. In another example, treatment increases 100 day survival greater than 60%. In another example, treatment increases 100 day survival greater than 65%. In another example, treatment increases 100 day survival greater than 68%. In another example, treatment increases 100 day survival greater than 70%. In another example, treatment increases 100 day survival greater than 71%. In another example, treatment increases 100 day survival greater than 72%. In another example, treatment increases 100 day survival between 60% and 75%. In another example, treatment increases 100 day survival between 65% and 75%. In another example, treatment increases 100 day survival between 70% and 75%. In an example, 100 day survival is determined across a population of patients with the average 100 day survival observed in the population providing the measure of 100 day survival for each patient in the population. In an example, this measure is compared relative to a subject (or population of subjects) that is not treated with a composition of the disclosure.

[0263] Methods of treatment according to the disclosure comprise administering an effective amount or a therapeutically or prophylactically effective amount of a population MLPSCs characterized as being of a sufficient clinical potency according to the methods disclosed herein.

[0264] The exact amount of cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the subject, and the extent and severity of the disorder being treated.

[0265] In an example, a dose of 2×10^6 MLPSCs/kg body weight is administered to a subject.

[0266] In an example, about $1x10^5$ to about $1x10^9$ MLPSCs, or about $1.25x10^3$ to about $1.25x10^7$ MLPSCs/kg (80 kg subject) is administered to a subject. In another example, 50×10^6 to 200×10^7 MLPSCs are administered. In other examples, 60×10^6 to 200×10^6 cells or 75×10^6 to 150×10^6 MLPSCs are administered. In an example, 75×10^6 MLPSCs are administered. In another example, 150×10^6 cells are administered.

[0267] In an example, MLPSCs may be administered to a wall of a subjects gastrointestinal tract. In an example, MLPSCs can be administered to a site of inflammation in a subjects gastrointestinal tract wall. For example, MLPSCs can be administered into a site of inflammation in a subjects gastrointestinal tract wall. In these

examples, the site of inflammation may be endoscopically confirmed prior to administration. For example, endoscopic confirmation can be based on visual inspection by a trained physician and/or histological analysis of endoscopic biopsy. In another example, compositions of the disclosure are administered intravenously.

[0268] In an example, MLPSCs may be administered as a single dose.

[0269] In some examples, MLPSCs may be administered over multiple doses. For example, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 doses.

[0270] In an example, MLPSCs may be administered intravenously. In another example, MLPSCs are administered once weekly. For example, MLPSCs can be administered once weekly every two weeks. In an example, MLPSCs can be administered once monthly. In an example, two doses of MLPSCs are administered once weekly. In another example, two doses of MLPSCs are administered once weekly every two weeks. For example, two doses of MLPSCs can be administered once weekly every two weeks before subsequent doses are administered once monthly. In an embodiment of this example, doses are administered monthly for a further one, two, three, four, five, six, seven or more months.

Compositions

[0271] The present disclosure encompasses compositions comprising a population of MLPSCs selected according to methods disclosed herein. In example, compositions of the present disclosure comprise a therapeutically effective amount of MLPSCs, wherein therapeutic efficacy is determined based on a method disclosed herein. In an example, the composition is a drug product manufactured according to the present disclosure, thereby having a predetermined CAP level.

[0272] In certain examples, compositions of the disclosure comprise a pharmaceutically acceptable carrier and/or excipient.

[0273] The terms "carrier" and "excipient" refer to compositions of matter that are conventionally used in the art to facilitate the storage, administration, and/or the biological activity of an active compound (see, e.g., Remington's Pharmaceutical Sciences, 16th Ed., Mac Publishing Company (1980). A carrier may also reduce any undesirable side effects of the active compound. A suitable carrier is, for example, stable, e.g., incapable of reacting with other ingredients in the carrier. In one example, the carrier

does not produce significant local or systemic adverse effect in recipients at the dosages and concentrations employed for treatment.

[0274] Suitable carriers for the present disclosure include those conventionally used, e.g., water, saline, aqueous dextrose, lactose, Ringer's solution, a buffered solution, hyaluronan and glycols are exemplary liquid carriers, particularly (when isotonic) for solutions. Suitable pharmaceutical carriers and excipients include starch, cellulose, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, glycerol, propylene glycol, water, ethanol, and the like.

[0275] In another example, a carrier is a media composition, e.g., in which a cell is grown or suspended. For example, such a media composition does not induce any adverse effects in a subject to whom it is administered.

[0276] Exemplary carriers and excipients do not adversely affect the viability of a cell and/or the ability of a cell to reduce, prevent or delay metabolic syndrome and/or obesity.

[0277] In one example, the carrier or excipient provides a buffering activity to maintain the cells and/or soluble factors at a suitable pH to thereby exert a biological activity, e.g., the carrier or excipient is phosphate buffered saline (PBS). PBS represents an attractive carrier or excipient because it interacts with cells and factors minimally and permits rapid release of the cells and factors, in such a case, the composition of the disclosure may be produced as a liquid for direct application to the blood stream or into a tissue or a region surrounding or adjacent to a tissue, e.g., by injection.

[0278] In an example, a composition of the disclosure comprises between 1.5 million and 3 million cells/kg. In an example, a composition of the disclosure comprises 2 million cells/kg.

[0279] In an example, the composition comprises greater than $5.00x10^6$ viable cells/mL. In another example, the composition comprises greater than $5.50x10^6$ viable cells/mL. In another example, the composition comprises greater than $6.00x10^6$ viable cells/mL. In another example, the composition comprises greater than $6.50x10^6$ viable cells/mL. In another example, the composition comprises greater than $6.68x10^6$ viable cells/mL.

[0280] In an example, the composition is provided in a 3.8 mL vial. In an example, the composition comprises 25×10^6 cells.

[0281] Compositions of the disclosure may be washed and subsequently cryopreserved. Such compositions may be characterized based on residual levels of component(s) present

in the cell culture media used to prepare them. In an example, compositions of the disclosure comprise < 10 µg /mL bovine serum albumin. In another example, the composition comprises < 5 µg /mL bovine serum albumin. In another example, the composition comprises < 10 µg /mL recombinant trypsin. In another example, the composition comprises < 6 µg /mL recombinant trypsin.

[0282] Compositions of the disclosure may be cryopreserved. Cryopreservation of mesenchymal lineage precursor or stem cells can be carried out using slow-rate cooling methods or 'fast' freezing protocols known in the art. Preferably, the method of cryopreservation maintains similar phenotypes, cell surface markers and growth rates of cryopreserved cells in comparison with unfrozen cells.

[0283] The cryopreserved composition may comprise a cryopreservation solution. The pH of the cryopreservation solution is typically 6.5 to 8, preferably 7.4.

[0284] The cryopreservation solution may comprise a sterile, non-pyrogenic isotonic solution such as, for example, PlasmaLyte A™. 100 mL of PlasmaLyte A™ contains 526 mg of sodium chloride, USP (NaCl); 502 mg of sodium gluconate ($C_6H_{11}NaO_7$); 368 mg of sodium acetate trihydrate, USP ($C_2H_3NaO_2 \cdot 3H_2O$); 37 mg of potassium chloride, USP (KCl); and 30 mg of magnesium chloride, USP ($MgCl_2 \cdot 6H_2O$). It contains no antimicrobial agents. The pH is adjusted with sodium hydroxide. The pH is 7.4 (6.5 to 8.0).

[0285] The cryopreservation solution may comprise Profreeze™. The cryopreservation solution may additionally or alternatively comprise culture medium, for example, αMEM.

[0286] To facilitate freezing, a cryoprotectant such as, for example, dimethylsulfoxide (DMSO), is usually added to the cryopreservation solution. Ideally, the cryoprotectant should be nontoxic for cells and patients, nonantigenic, chemically inert, provide high survival rate after thawing and allow transplantation without washing. However, the most commonly used cryoprotector, DMSO, shows some cytotoxicity . Hydroxylethyl starch (HES) may be used as a substitute or in combination with DMSO to reduce cytotoxicity of the cryopreservation solution.

[0287] The cryopreservation solution may comprise one or more of DMSO, hydroxyethyl starch, human serum components and other protein bulking agents. In one example, the cryopreserved solution comprises about 5% human serum albumin (HSA) and about 10% DMSO. The cryopreservation solution may further comprise one or more of methycellulose, polyvinyl pyrrolidone (PVP) and trehalose.

[0288] In one embodiment, cells are suspended in 42.5% Profreeze™/50% αMEM/7.5% DMSO and cooled in a controlled-rate freezer.

[0289] The cryopreserved composition may be thawed and administered directly to the subject or added to another solution, for example, comprising hyaluronic acid (HA). Alternatively, the cryopreserved composition may be thawed and the MLPSCs resuspended in an alternate carrier prior to administration.

[0290] In an example, cellular compositions of the disclosure can comprise Plasma-Lyte A, dimethyl sulfoxide (DMSO) and human serum albumin (HSA). For example, compositions of the disclosure may comprise Plasma-Lyte A (70%), DMSO (10%), HSA (25%) solution, the HSA solution comprising 5% HSA and 15% buffer.

[0291] It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the above-described embodiments, without departing from the broad general scope of the present disclosure. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

[0292] The following specific examples are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent.

EXAMPLES

Example 1: Background

[0293] Potency of MSC drug products were previously evaluated using a 6-day IL-2Rα inhibition assay. Adequate peripheral blood mononuclear cells (PBMC) activation is a fundamental requirement for this assay which depends on release from activated T cells and macrophages of pro-inflammatory cytokines such as IFN-γ, IL-1 and TNF-α, mimicking the *in vivo* scenario in patients with aGVHD. These inflammatory cytokines are necessary for stimulation of mesenchymal stromal cells following receptor engagement, such that MSCs can exert their suppressive effects on the activated T cells via induced expression of immunomodulatory proteins such as IDO1 and by release of paracrine factors such as PGE2 and PD-L1.

[0294] However, the IL-2Rα inhibition assay can be associated with variability in results. In extensive studies to identify the root cause of that variability, it was determined that the

primary cause was inadequate activation of PBMC by anti-CD3 and anti-CD28 monoclonal antibodies, as detected by a minimum threshold level of IL-2Rα production from activated T cells.

[0295] Whilst investigating ways to reduce IL-2Rα inhibition assay variability, quality control of PBMC lots and reagents were introduced and the IL-2Rα inhibition assay was modified by streamlining the procedure and reducing the culture period preceding a 3-day co-incubation with PBMC from 3 days to just 1 day. While controlling PBMC quality resulted in acceptably low variability for measurement of IL-2Rα inhibition using either the shorter 4-day assay or the original 6-day assay, the two assays did not show concordance, which prompted a further analytic comparison of the two assays.

[0296] On closer examination, it became evident that the initial 3 days of MSC culture in the original IL-2Rα inhibition assay prior to a 3-day co-incubation with PBMC constituted a critical period during which measures of viability, adherence, and proliferation provided an integrated evaluation of the state of cell health of the lot being tested in addition to the subsequent measurement of the lot's ability to inhibit IL-2Rα expression. These parameters were formally measured quantitatively and prospectively at day 3, prior to the subsequent measure of IL-2Rα inhibition at day 6 following 3 days of co-culture with PBMC. The present inventors surprisingly identified that these parameters underpin an important standalone potency assay, referred to herein as the CAP assay, for assessing therapeutic efficacy of MLPSC populations. As discussed further below, the CAP assay can then be combined with other assays such as IL-2RA to further characterize therapeutic efficacy.

[0297] The cell adhesion and proliferation (CAP) assay described in the following Examples is the day 3 measures of cell viability, adherence and proliferation in response to mitogenic signals, expressed as % viable adherent cells recovered at day 3 relative to time 0. Described in the following Examples are extensive data showing that the CAP assay is an integrated measure of the state of health of the MSC lot being tested, reflective of inherent functional properties of MSC cells in short-term culture including cell adherence, resistance to activation induced cellular apoptosis, cell growth kinetics, and induction of IDO-1 in response to interferon-gamma. The assay is stability indicating (both short-term and long-term) and is more sensitive for detection of a degraded product than other assays that measure related attributes.

Example 2: Methods

Cell Adhesion and Proliferation (CAP) assay

[0298] The CAP assay is an integrated assessment of healthy cell growth over 72 hours in culture by quantifying the number of viable cells with membrane integrity that are capable of adhering to plastic and proliferating in response to mitogenic signals (cell growth kinetics). The CAP Assay consists of the following steps:

[0299] On Day 1 one vial of cryopreserved culture expanded (ce)-MSC is thawed, washed and then cultured in T175 flasks at a concentration of 1.20 x 10^6 cells per flask in growth medium. The flasks are then incubated at 37°C±2°C, 5%±2% CO_2.

[0300] 24 ± 2 hours after the start of incubation on Day 1 the culture medium is removed, discarded, and fresh media is added. The culture is then returned to incubation at 37°C±2°C, 5%±2% CO_2.

[0301] 72 ± 12 hours from the start of incubation on Day 1 (i.e. "Day 3"), medium is removed and discarded, adherent cells are dislodged using trypsin, washed and counted.

[0302] The % of cells recovered on day 3 (i.e. CAP level) is calculated as follows: ((Recovered cells/mL x 2 mL / flask)/ 1.20 x 10^6 cells/ T175 flask) x 100 = % cells recovered.

IL-2Rα Inhibition assay

[0303] The expression of Interleukin-2 receptor α (IL-2Rα) on the cell surface is an early marker T-cell activation. A major function of IL-2/IL-2Rα is to promote proliferation of CD4+ (helper) and CD8+ (cytotoxic) T-cells.

[0304] MSCs (2.00 x 10^5 cells/well) are co-cultured with mouse anti-human CD3/CD28 stimulated PBMCs (1.00 x 10^6 cells/well) and incubated for 72±2 hours at 37°C±2°C, 5%±2% CO_2.

[0305] Unstimulated (without CD3/CD28) and stimulated PBMC (with CD3/CD28, but without ceMSCs) are included as negative and positive controls, respectively.

[0306] At the end of the incubation period, cells are collected for lysate preparation. The cell lysates are then aliquoted in sterile tubes for storage at ≤-60°C up to 29 days until use in IL-2Rα ELISA.

[0307] The IL-2Rα levels in lysates of unstimulated and stimulated PBMC alone as well as co-culture are measured at one dilution in triplicate using Quantikine Human CD25/IL-2 Rα ELISA kit.

[0308] A standard curve constructed using a four-parameter logistic (4-PL) curve fit is used to determine IL-2Rα levels in controls and samples. Final ceMSC potency is evaluated by the % inhibition of IL-2Rα levels on CD3/CD28-stimulated PBMC by ceMSC relative to the control CD3/CD28-stimulated PBMC cultured without ceMSC.

Example 3: MLPSC characteristics under stress conditions

[0309] To evaluate the ability of the CAP assay to detect degraded MSC DP lots following short-term temperature excursions, studies were performed on MSC lots held in long-term cryostorage at -140°C compared to lots held at -20°C for 3, 12, 24, 48 and 72 hours. Lots were examined for individual assessment of post-thaw viability, adhesion, and growth kinetics (confluence over time, endpoint nuclei confluence and endpoint nuclei count), as well as by the formal CAP assay integrating these attributes into one assay (Figure 1).

[0310] As can be seen in Figure 1, the CAP assay was able to detect unstable product from two different MSC lots stored at -20°C for as little as 3-12 hours, and was a more sensitive determinant of each degraded MSC lot than each of the individual related assays measuring viability, adhesion and cell growth kinetics (as measured by confluence or nucleus counts), since none of these individual assays on their own could detect product degradation at earlier timepoints than 24-48 hours at -20°C.

[0311] Figure 2 shows that the CAP assay measure of product degradation correlates closely with two other product characteristics, increase in activation-induced apoptosis and loss of IDO interferon gamma responsiveness as measured by induced IDO-1 production. These data indicate that the integrated measurement of viability, adhesion, and cell growth by the CAP assay reflects important functional attributes of cell health and bioactivity related additionally to cell survival and ability to respond to cytokine signalling.

[0312] It was also investigated whether the CAP assay could detect long-term lot stability by comparing values of cryopreserved clinical lots of MSC manufactured and tested by the CAP assay in 2015 and tested again by the CAP assay in 2023. As seen in Figure 3, the slope of degradation in log(CAP) is -0.012, which is equivalent to <1% per year

decline in CAP on a linear scale, indicating that MSC lots remains highly stable over 8 years with respect to CAP. Comparing the regression lines with actual assay values provides a highly sensitive, real-world test of CAP assay precision, as assessed in the 19 lots used in this study. The ability of the linear stability modeling using a common slope to predict actual test values was used to calculate the assay standard deviation based on the residuals in the linear regression. This calculation indicates a standard deviation for the CAP assay of 0.06 on a log scale, corresponding to 15.2% on a linear scale, indicating sufficient precision for use of this assay to measure a critical quality attribute.

Example 4: Clinical evaluation of the CAP assay

[0313] The CAP assay was evaluated in lots administered in a Phase 3 clinical trial in pediatric patients with GVHD. MSC lot potency was measured by both the CAP assay and IL-2Rα inhibition.

[0314] Since CAP values using % or fold increase in cell recovery over freshly-seeded cells was not normally distributed, the data were log10 transformed to calculate mean and SD values for the CAP assay, Figure 4. The value of 0.035, 3SD below the log10 transformed mean of 0.26 for all lots (equivalent to 108% or 1.08 fold increase at day 3 over freshly-seeded cells) was found to be a threshold value for clinical potency.

[0315] The mean CAP for the 40 lots used in the clinical trial was 176%, median 170% and the lowest CAP value was 106%. Analysis of day 28 overall response and day 100 survival was performed in patients who received lots with CAP levels above 106%, comparing outcomes at every 10% increments through 200%. No relationship was identified between these additional thresholds and clinical outcomes.

[0316] Across all lots tested with both CAP assay and IL-2Ra inhibition assay, no concordance was seen between CAP values measured at day 4 and IL-2Rα inhibition activity measured at day 7 by the same drug product lot (Figure 5). Taken together with the clinical trial analysis regarding the IL-2Rα inhibition assay discussed further below, these results indicate that CAP value $\geq$108% reflects an attribute of the final product, cell health integrity, that is separate and distinct from IL-2Rα inhibition by the lot and any further potential clinical benefit that may be associated with this attribute. In other words, there is an important role for CAP alone and also in conjunction with an IL-2RA inhibition assay.

Example 5: Establishment Of Acceptance Criteria For CAP Assay And IL-2Rα Inhibition Assay Based On Relationship Between Potency Of MSC Lots And Clinical Outcomes.

[0317] To establish acceptance criteria for CAP and IL-2Ra inhibition that would assure that MSC lots will have similar clinical performance, relationships between clinical outcomes and MSC lots were evaluated. The clinical trial was a Phase 3 clinical trial in pediatric patients with GVHD. MSC lot potency was measured by both the CAP assay and IL-2Rα inhibition.

[0318] The IL-2Rα inhibition assay at day 6 in co-culture with PBMC was used to measure IL-2Rα inhibition by MSC lots as a measure of potency targeting the GVHD disease mechanism. This critical quality attribute is a measure of the effect of MSCs on the key mechanism by which the immune system mediates disease severity in SR-aGVHD, namely alloreactive T cell activation, proliferation and inflammatory cytokine production.

[0319] The degree of PBMC stimulation and subsequent inflammatory cytokine release when measuring IL-2Ra inhibition is a critical component in the ability to induce MSC lot release of immunomodulatory factors necessary for IL-2Rα inhibitory activity, such as IDO-1, PGE2 and PD-L1. To assess the minimum level of PBMC stimulation by anti-CD3 and anti-CD28 antibodies necessary to result in reliable measurement of lot potency, we performed regression analyses to assess the relationship between IL2R alpha produced by stimulated PBMC and degree of induced suppressive activity by MSC lots.

[0320] As shown in Figure 6-1, using the IL-2Rα inhibition assay to measure potency of lots used in the pediatric Phase 3 trial there was an observed correlation between the amount of IL2R alpha produced by stimulated PBMC and % inhibition of IL2R alpha across all 40 lots (p=0.012). This association remained significant at post-stimulation PBMC IL2R alpha expression thresholds of 10000, 11000 and 12000 pg/ml, but was no longer evident when the amount of IL2R alpha produced by stimulated PBMC exceeded 13,000 pg/ml. Mean IL2R alpha inhibition by the 30 lots tested with PBMC producing >13,000 pg/ml IL2R alpha was 83% (range 71-91%) compared with 77% inhibition (range 63-86) for 10 lots tested with PBMC producing <13,000 IL2R alpha (p=0.005; Figure 6-2).

[0321] These results indicate that IL2R alpha production of 12,000-13,000 pg/ml reflects the minimum threshold of PBMC stimulation necessary to ensure that there is adequate activation of MSC lots to induce maximum IL2R alpha inhibitory activity. While the

mean IL2R alpha inhibitory activity for the lots tested with PBMC producing <13,000 IL2R alpha was apparently lower than for those tested with PBMC producing >13,000 pg/ml IL2R alpha, these data indicate that potency of MSC lots tested with inadequately stimulated PBMC is not reliable and likely underestimates the true potency values for these lots.

[0322] Next, Reverse Cumulative Distribution curves were used to assess the relationship between product potency and clinical outcomes in those patients (n=34) who received lots with potency values measured with adequately stimulated PBMC (n=30 lots, >12811 pg/ml IL2R alpha). 12811 was chosen as an appropriate threshold for PBMC stimulation.

[0323] As can be seen in Figure 7, there was a significant relationship between weighted mean value of % IL2Ralpha inhibition of lots received and day 100 survivorship by Reverse Cumulative Distribution curves (p=0.03). Since mean IL2R alpha inhibition was 83.1% $\pm$ 5.8% for these 30 lots, 65.7% can be used as the minimum threshold IL2R alpha inhibition required for lot release by the IL-2Rα inhibition assay with adequately stimulated PBMC ($\geq$12811 pg/ml IL2R alpha). The 65.7% threshold represents 3SD below the measured 83.1% mean inhibition value for these lots used in the Phase 3 trial.

[0324] Clinical lots from the Phase 3 trial were initially tested on release prior to use IL-2Rα inhibition assay with adequately stimulated PBMC ($\geq$12811 pg/ml IL2R alpha). These lots were retested to evaluate whether potency was maintained on cryostorage over several years. As seen in Figure 8, IL-2Rα inhibition measured at two timepoints with the same clinical lot demonstrated a high degree of clinical lot stability for at least 8 years in cryostorage.

[0325] The slope of degradation for IL-2Rα inhibition value is 1.2% per year, indicating that MSC lots remain highly stable over time with respect to IL-2Rα inhibition. Comparing the regression lines with actual assay values provides a highly sensitive, real-world test of IL-2Rα inhibition assay precision, as assessed in the 10 lots for which adequately stimulated PBMCs were used at both time points. The ability of the linear stability modeling using a common slope to predict actual test values was used to calculate the assay standard deviation based on the residuals in the linear regression. This calculation indicates a standard deviation for the IL-2Rα inhibition assay of less than 7%, indicating sufficient precision for use of this assay to measure a critical quality attribute.

[0326] Correlation between PBMC production of IL2R alpha and IL2R alpha inhibition by co-cultured MSC lots was only observed up to stimulated PBMC producing 12000-13000 pg/ml IL2R alpha, indicating that induction of MSC suppressive potency was maximal above these levels of PBMC stimulation. While 95% of commercial inventory lots tested with PBMC stimulated to produce >13000 pg/ml IL2R alpha showed >60% inhibition of IL2R alpha, only 70% of lots tested with PBMC stimulated suboptimally to produce lesser amounts of IL2Ralpha achieved this level of inhibition, Figure 9.

[0327] These results confirm that accurate measurement of MSC lot potency for inhibition of IL2R alpha requires optimal PBMC stimulation and inflammatory cytokine secretion, and these can be achieved by quality control and strict acceptance criteria for PBMC stimulation with anti-CD3/CD28 antibodies.

Example 6: Proposed Acceptance Criteria for CAP and IL2R Alpha Inhibition Assays As An Assay Matrix Providing Complementary And Distinct Determinants Of Product Potency.

[0328] CAP Assay: The value of 108% or 1.08 fold increase at day 4 over freshly-seeded cells is equivalent to the log10 transformed value of 0.035, 3SD below the log10 transformed mean of 0.26; this value provides a minimum threshold level met by 95% of all lots.

[0329] IL2R Alpha Inhibition Assay: The value of 65.7% also represents the minimum threshold of IL2R alpha inhibition met by 95% of all lots as measured by the IL-2RA Inhibition assay when PBMC stimulation achieved IL2R alpha levels ≥12811 pg/ml.

[0330] The proposed Critical Quality Attributes (CQA) for batch release of MSC investigational DP is provided in Table 4.

Table 4: Proposed CQA for MSC Investigational Drug Product

Test Name	Purpose	Specification
In-Process Sterility	Safety	Negative
Mycoplasma	Safety	Negative
Phenotype (FACS) CD166	Identity	≥ 95% CD166+
Phenotype (FACS) CD105	Identity	≥ 95% CD105+

Test Name	Purpose	Specification
Phenotype (FACS) CD45	Purity	≤ 0.75% CD45+
Sterility	Purity / Safety	Negative
Endotoxin	Purity / Safety	≤ 1.67 EU/mL
CAP	Potency	≥108%
IL-2Rα inhibition	Potency	≥65.7%
Appearance Test	Appearance	Opalescent cell suspension, GY4-GY6 (greenish-yellow) in color, free of visible extrinsic particulates
Cell Viability	Potency	≥ 70%
Cell concentration	Potency	≥ 6.68 x 10^6 cells/mL
Residual Bovine Serum Albumin	Purity	≤ 5 µg /mL
Residual rTrypsin	Purity	≤ 6 µg/mL
Visual Inspection and ANSI Sampling	Appearance	≤ 0.65% for major defects 0.01 – 0.10% for critical container integrity defects

Example 7: Summary

[0331] Accordingly, the present inventors have discovered that CAP provides independent information directly reflective of the overall health of MSCs, integrating elements of cell viability, adherence, and growth. In accelerated stability studies, CAP loss correlates with loss of cellular response to inflammatory cytokines and with increased apoptosis. CAP is also stability-indicating over 8 years of product storage.

[0332] CAP correlates independently of IL-2Ra inhibition with changes in manufacturing process and with clinical outcomes. The IL-2Ra inhibition assay recapitulates the immunomodulatory mechanism of action of mesenchymal stromal cells with respect to responsiveness to inflammatory cytokines produced by activated T cells, and subsequent inhibition of T cell activation and proliferation, elements central to acute GVHD disease pathogenesis. Accordingly, the IL-2Ra inhibition assay provides information that is complementary to that provided by the CAP assay.

[0333] Additional study of the IL-2Ra inhibition assay revealed that when PBMCs used in the assay are not adequately stimulated, a necessary requirement for inflammatory cytokine release, the assay underestimates IL2Ra inhibition capability by responding lots. Thus, an analysis of survival in patients in the clinical trial who received MSC lots with assays using adequately stimulated PBMC showed a significant correlation between lot potency measuring IL2Ra inhibition using the IL-2Ra inhibition assay and survivorship.

[0334] Accordingly, the assay matrix of CAP and IL-2Ra inhibition provides substantial standardization and characterization of stem cell potency. Each assay independently measures attributes that are essential for product quality and are related to product clinical efficacy, and this combination thus provides the most complete potency characterization of MSCs.

[0335] The present application claims priority from 63/567,343 filed on 19 March 2024, the disclosures of which are incorporated herein by reference.

[0336] It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

[0337] All publications discussed above are incorporated herein in their entirety.

[0338] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

CLAIMS:

1. A composition comprising a population of culture expanded mesenchymal lineage precursor or stem cells (MLPSCs), wherein the population of MLPSCs are selected based on a predetermined cell adhesion and proliferation (CAP) level under culture conditions, wherein the predetermined CAP level is greater than or equal to (≥) 108%.

2. The composition of claim 1, wherein the population of MLPSCs are also selected based on a predetermined level of IL-2RA inhibition under culture conditions, wherein the predetermined level of IL-2RA inhibition is ≥ 60%.

3. The composition of claim 2, wherein the predetermined level of IL-2RA inhibition is ≥ 65%.

4. The composition according to any one of claims 1 to 3, wherein the population of MLPSCs are mesenchymal stem cells.

5. The composition according to any one of claims 1 to 4, wherein the population of MLPSCs are a cryopreserved intermediate.

6. The composition according to any one of claims 1 to 5, wherein the composition comprises < 5 µg /mL bovine serum albumin and/or < 6 µg /mL recombinant trypsin.

7. The composition according to any one of claims 1 to 6, wherein the population of MLPSCs are also selected based on expression of one or more or all of CD29, CD54, CD73, CD90, CD102, CD105, CD106, CD166, MHC1.

8. The composition of claim 7, wherein the population MLPSCs comprises ≥ 95% CD166+, ≥ 95% CD105+, and ≤ 0.75% CD45+.

9. The composition according to any one of claims 5 to 8, wherein the population of MLPSCs expresses low levels of major histocompatibility complex (MHC) class I and minimal levels of human leukocyte antigen (HLA)-DR, CD40, CD80, and CD86.

10. The composition according to any one of claims 1 to 9, wherein the population of MLPSCs are further characterized by one or more of the following parameters:
- post-thaw viability of at least 60%;
- 24 hour nuclei count of at least 100,000;
- endpoint nuclei confluence of at least 60%;
- endpoint nuclei count of at least 100,000;
- less than about 10% annexin V positive cells; and/or
- IDO-1 protein production of at least 80 pg/µg of total protein.

11. The composition according to any one of claims 1 to 10, wherein the composition comprises at least 6.68 x 10^6 /mL viable MLPSCs.

12. The composition according to any one of claims 1 to 10, wherein the composition comprises 25 x 10^6 MLPSCs.

13. The composition according to any one of claims 1 to 12, wherein the composition further comprises Plasma-Lyte A, dimethyl sulfoxide (DMSO) and human serum albumin (HSA), preferably wherein the composition comprises Plasma-Lyte A (70%), DMSO (10%), HSA (25%) solution, the HSA solution comprising 5% HSA and 15% buffer.

14. A method of selecting a cell population for use in therapy, the method comprising: (i) obtaining a population of mesenchymal lineage precursor or stem cells (MLPSCs); (ii) culturing the MLPSC population in a culture medium; (iii) determining under culture conditions the level of cell adhesion and proliferation (CAP); and, (iv) selecting for use in therapy a MLPSC population that has a CAP level of greater than or equal to (≥) 108% under culture conditions.

15. The method of claim 14, which comprises selecting an MLPSC population for use in therapy that has a CAP level of ≥110%.

16. The method of claim 14 or 15, wherein the CAP level is determined on day 3 of culture.

17. The method according to any one of claims 14 to 16, further comprising determining under culture conditions the level of IL-2RA inhibition; and selecting for use in therapy an MLPSC population that inhibits IL-2RA by ≥ 60% under culture conditions.

18. The method of claim 17, which comprises selecting an MLPSC population for use in therapy which inhibits IL-2RA by ≥65% under culture conditions.

19. The method of claim 18, wherein the level of IL-2RA is determined on day 6 of culture.

20. The method of any one of claims 14 to 19, wherein the population is for use in the treatment of graft versus host disease (GvHD).

21. A method for determining therapeutic efficacy of a population of culture expanded mesenchymal lineage precursor or stem cells (MLPSCs): (i) obtaining a population of MLPSCs; (ii) culturing the cells in a culture medium; and (iii) determining under culture conditions the level of cell adhesion and proliferation (CAP), wherein a CAP level of greater than or equal to (≥) 108% under culture conditions is indicative of therapeutic efficacy.

22. The method of claim 21, wherein a CAP level of ≥ 110% under culture conditions is indicative of therapeutic efficacy.

23. The method of claim 22 or 22, further comprising determining under culture conditions the level of IL-2RA inhibition, wherein ≥ 60% IL-2RA inhibition under culture conditions is indicative of therapeutic efficacy.

24. The method of claim 23, wherein ≥ 65% IL-2RA inhibition under culture conditions is indicative of therapeutic efficacy.

25. The method according to any one of claims 21 to 24, wherein the method determines therapeutic efficacy for graft versus host disease (GvHD).

26. A method of manufacturing drug product which comprises a population of mesenchymal lineage precursor or stem cells (MLPSCs), the method comprising: acquiring a determination of whether a test population of MLPSCs has a predetermined cell adhesion and proliferation (CAP) level under culture conditions, and processing at least a portion of the test population of MLPSCs as a drug product if the test population of MLPSCs has a predetermined CAP level under culture conditions, thereby manufacturing the drug product; or discarding at least a portion of the test population of MLPSCs if the population of MLPSCs have a CAP level less than the predetermined level under culture conditions, wherein the predetermined level is greater than or equal to (≥) 108%.

27. The method of claim 26, wherein the predetermined CAP level is ≥ 110% under culture conditions.

28. The method of claim 26 or 27, further comprising acquiring a determination of whether a test population of MLPSCs inhibits IL-2RA at a predetermined IL-2RA inhibition level under culture conditions, and processing at least a portion of the test population of MLPSCs as a drug product if the test population of MLPSCs inhibits IL-2RA by at least the predetermined IL-2RA inhibition level under culture conditions, thereby manufacturing the drug product; or discarding at least a portion of the test population of MLPSCs if the population of MLPSCs inhibits IL-2RA by less than the predetermined IL-2RA inhibition level under culture conditions, wherein the predetermined IL-2RA inhibition level is ≥ 60%.

29. The method of claim 28, wherein the predetermined IL-2RA inhibition level is ≥ 65% under culture conditions.

30. A method of treating a subject with graft versus host disease (GvHD), the method comprising administering to a subject in need thereof a composition comprising a culture expanded population of mesenchymal lineage precursor or stem cells (MLPSCs), wherein the MLPSCs have a cell adhesion and proliferation (CAP) level of greater than or equal to (≥) 108% under culture conditions.

31. The method of claim 30, wherein the MLPSCs have a CAP level of ≥ 110% under culture conditions.

32. The method of claim 30 or 31, wherein the MLPSCs inhibit IL-2RA by ≥ 60% under culture conditions.

33. The method of claim 32, wherein the MLPSCs inhibit IL-2RA by ≥ 65% under culture conditions.

34. The method according to any one of claims 30 to 33, wherein the GvHD is acute GvHD.

35. The method according to any one of claims 30 to 33, wherein the GvHD is chronic GvHD.

36. The method according to any one of claims 30 to 35, wherein the GvHD is pediatric GvHD.

37. The method according to any one of claims 30 to 36, wherein the GvHD is adult GvHD.

38. The method according to any one of claims 30 to 37, wherein the GvHD is refractory to steroid therapy.

39. The method according to any one of claims 30 to 38, wherein the GvHD is Grade D GvHD.

40. The method according to any one of claims 30 to 39, wherein the subject has a MAGIC algorithm probability (MAP) score of >0.29.

41. The method according to any one of claims 30 to 40, wherein treatment increases 100 day survival to greater than 60%, preferably greater than 70%.

42. The method according to any one of claims 30 to 41, wherein the MLPSCs are administered at a dose of 2×10^6 cells/kg body weight.

43. The method according to any one of claims 30 to 42, wherein multiple doses are administered.

44. The method according to any one of claims 30 to 43, wherein MLPSCs are administered at least once a week for four weeks.

45. The method according to any one of claims 30 to 43, wherein MLPSCs are administered at least twice a week for four weeks.

46. The method according to any one of claims 14 to 45, wherein the MLPSCs are mesenchymal stem cells.

47. The composition according to any one of claims 1 to 13, or the method according to any one of claims 14 to 46, wherein the CAP level is determined by measuring the increase in the number of adherent cells on day 3 in culture relative to freshly seeded cells.

48. A method of manufacturing drug product which comprises a population of mesenchymal lineage precursor or stem cells (MLPSCs), the method comprising: (i) acquiring a determination of whether a test population of MLPSCs has a predetermined cell adhesion and proliferation (CAP) level under culture conditions; wherein step (i) comprises:

(i) thawing a population of cryopreserved MLPSCs and culturing the population of MLPSCs under culture conditions for 3 days;

(ii) counting the number of adherent cells;

(iii) measuring the increase in the number of adherent cells on day 3 in culture relative to the thawed population of freshly seeded cells.

49. The method of claim 48, wherein the increase in the number of adherent cells on day 3 in culture relative to thawed population of freshly seeded cells is expressed as a percentage.

50. The method of claim 48, wherein the increase in the number of adherent cells on day 3 in culture relative to thawed population of freshly seeded cells is expressed as a fold-change.

51. The method of claim 48, wherein the increase in the number of adherent cells on day 3 in culture relative to thawed population of freshly seeded cells is expressed as log10.

52. The method of claim 48 or 49, wherein the number of freshly seeded cells is 1.20×10^6.

53. The method according to any one of claims 48 to 52, further comprising (ii)acquiring a determination of whether a test population of MLPSCs inhibits IL-2RA at a predetermined level under culture conditions, wherein step (ii) comprises co-culturing the test population of MLPSCs with CD3/CD28 activated PBMCs; and determining the level of IL-2RA inhibition relative to a control population of CD3/CD28 activated PBMCs in the absence of MLPSCs; wherein the predetermined level of IL-2RA inhibition is $\geq 60\%$.

54. The method of claim 53, wherein the control population of CD3/CD28 activated PBMCs express $\geq$ 11,000 pg/ml IL-2RA, preferably $\geq$ 12,000 pg/ml IL-2RA.

55. The method of claim 53 or 54, wherein CD3/CD28 activation of PBMCs comprises stimulating PBMCs with an anti- CD3 antibody and an anti-CD28 antibody.

56. The method according to any one of claims 53 to 55, wherein the PBMCs are co-cultured with MLPSCs at a ratio of 5 PBMC:1 MPLSC.

57. The method according to claim 56, wherein $1x10^6$ PBMCs are co-cultured with $2x10^5$ MLPSCs.

58. The method according to any one of claims 53 to 57, wherein the PBMCs and MLPSCs are co-cultured for a period of about 3 days.

59. The method according to any one of claims 53 to 58, wherein IL-2RA levels are determined on day 6 of culture.

60. 60. The method according to any one of claims 53 to 59, wherein the level of IL-2RA inhibition is determined by:

(i) measuring IL-2RA levels in a test population of MLPSCs co-cultured with CD3/CD28-activated PBMCs, a positive control population of CD3/CD28-activated PBMCs in the absence of MLPSCs, and a negative control population of un-activated PBMCs in the absence of MLPSCs;

(ii) constructing a standard curve to determine IL-2RA levels in the positive control population, negative control population, and test population; and

(iii) calculating % inhibition of IL-2RA levels on the positive control population by the test population of MLPSCs co-cultured with CD3/CD28-activated PBMCs, relative to the positive control CD3/CD28-activated PBMC population.

61. The composition according to any one of claims 1 to 13, or the method according to any one of claims 14 to 60, wherein the population of MLPSCs is a culture expanded population.

62. The composition according to any one of claims 1 to 13, or the method according to any one of claims 14 to 60, wherein the population of MLPSCs is cryopreserved or has been cryopreserved and thawed.

63. The composition according to any one of claims 1 to 13, or the method according to any one of claims 14 to 60, wherein the population of MLPSCs has been culture expanded, cryopreserved and thawed.