

Claims

1. An *in vitro* method for selecting a stem cell (SC) population suitable for allogeneic therapy, in particular for treatment of presensitized patients or
5 retreatment of patients with allogeneic therapy, comprising the following steps:
 - a) culturing a sample of an SC population in the presence of an IFN- γ concentration capable of inducing maximal HLA-Class I expression in said SC population (test sample) and separately culturing a sample of the SC population in the absence of IFN- γ (control sample);
 - 10 b) contacting the test sample and the control sample with a range of different concentrations of an HLA-Class I antibody under conditions such that the HLA-Class I antibody binds to HLA-Class I expressed in the test sample and the control sample;
 - c) adding complement to the test sample and the control sample such that
15 the bound HLA-Class I antibody is saturated with complement and complement-dependent cytotoxicity (CDC) is induced;
 - d) determining the CDC for the range of different concentrations of the HLA-Class I antibody by measuring the cell lysis induced in the test sample and the control sample;
 - 20 e) determining the concentration of HLA-Class I antibody that induces 50% of the maximal CDC (EC_{50} value) in the test sample and the control sample; and
 - f1) selecting the SC population for allogeneic therapy if the ratio of the EC_{50} value of the control sample to the EC_{50} value of the test sample is less
25 than 1.25, preferably less than 1.0, more preferably less than 0.5; particularly preferably less than 0.25; or
 - f2) selecting the SC population for allogeneic therapy if the EC_{50} value of the test sample is at least 3.5 ng/ml, preferably at least 9 ng/ml, more preferably at least 15 ng/ml, particularly preferably at least 20 ng/ml of
30 the HLA-Class I antibody.

2. The *in vitro* method of claim 1, wherein the method further comprises the steps of determining the CD46 expression level in the test sample and in the control sample; and selecting the SC population for allogeneic therapy if the ratio of
5 CD46 expression in the test sample to the CD46 expression in the control sample is more than 2.0, preferably more than 2.5, particularly preferably more than 3.0.

3. The *in vitro* method of claim 1 or 2, wherein the SC population is a mesenchymal stem cell (MSC) population, preferably a human MSC population,
10 more preferably the mesenchymal stem cell population is a bone-marrow-derived stem cell (BM-MSC) population or the mesenchymal stem cell population is an adipose tissue-derived stem cell (ASC) population, even more preferably the ASC population expresses CD29, CD73, CD90 and/or CD105.

15 4. The *in vitro* method of any one of claims 1 to 3, wherein the IFN- γ concentration capable of inducing maximal HLA-class I expression in said SC population is from about 0.5 to about 30 ng/ml, preferably from about 1 to about 15 ng/ml, more preferred from about 2 to about 4 ng/ml, preferably the IFN- γ concentration capable of inducing maximal HLA-class I expression in said SC
20 population is 3 ng IFN- γ /ml, preferably applied over a time period of 48 hours.

5. The *in vitro* method of any one of claims 1 to 4, wherein the HLA-Class I antibody specifically binds to HLA-A, HLA-B and/or HLA-C, preferably the HLA-Class I antibody specifically binds to HLA-A, HLA-B and HLA-C, more
25 preferably the HLA-Class I antibody is a murine monoclonal antibody, even more preferably the HLA-Class I antibody has essentially the same binding affinity for HLA-A as the antibody produced by the hybridoma clone w6/32 obtainable from ATCC (designation: HB-95) or ECACC (No.: 84112003), most preferred the antibody is produced by the hybridoma clone w6/32 obtainable from ATCC
30 (designation: HB-95) or ECACC (No.: 84112003).

6. The *in vitro* method of any one of claims 1 to 5, wherein two or three different concentrations of the HLA-Class I antibody within the range of from about 1 to about 50 ng/ml are used, preferably four or five different concentrations of the HLA-Class I antibody within the range of from about 1 to about 50 ng/ml are used, more preferably six or seven different concentrations of the HLA-Class I antibody within the range of from about 1 to about 50 ng/ml are used, even more preferably eight or nine different concentrations of the HLA-Class I antibody within the range of from about 1 to about 50 ng/ml are used and , most preferably the range of different concentrations of the HLA-Class I antibody is 1 ng/ml, 3 ng/ml, 5 ng/ml, 10 ng/ml, 15 ng/ml, 20 ng/ml, 30 ng/ml, 40 ng/ml and 50 ng/ml.

7. The *in vitro* method of any one of claims 1 to 6, wherein the complement used in step (c) of claim 1 is from serum, preferably the serum has not been treated with heat, more preferably the serum is from rabbit, goat or sheep, most preferably, the serum is from rabbit.

8. The *in vitro* method of any one of claims 1 to 7, wherein the complement is from serum and the resulting serum concentration is from 50 to 83.33 % (v/v), preferably the resulting serum concentration is from 66.66 to 83.33 % (v/v).

9. The *in vitro* method of any one of claims 1 to 8, wherein the cell lysis is determined by measuring a chemiluminescent or fluorescent dye selective for viable or lysed cells or a radioactive agent released from lysed cells.

10. An SC population suitable for allogeneic therapy, in particular for the treatment of presensitized patients or retreatment with allogeneic therapy, having any of the following properties:

i) a ratio of the EC₅₀ value of the control sample to the EC₅₀ value of the test sample of less than 1.25, preferably less than 1.0, more preferably less than 0.5 and particularly preferably less than 0.25, wherein the EC₅₀ value is determined as set forth in claim 1;

ii) an EC₅₀ value of the test sample of at least 3.5 ng/ml HLA-Class I antibody, preferably at least 9 ng/ml, more preferably at least 15 ng/ml, particularly preferably at least 20 ng/ml HLA-Class I antibody, wherein the EC₅₀ value is determined as set forth in claim 1; and/or

5 iii) a ratio of CD46 expression in the test sample to the CD46 expression in the control sample is more than 2.0, preferably more than 2.5, particularly preferably more than 3.0, wherein the CD46 expression is determined as set forth in claim 2;

10 ~~11. The SC population according to claim 10,~~ wherein the SC population is selected by the method of any one of claims 1 to 9, preferably the SC population is a mesenchymal stem cell (MSC) population, more preferably the SC population is a BM-MSC population or an ASC population, even more preferably the SC population is a human BM-MSC population or a human ASC population.

15 ~~11~~2. A pharmaceutical composition comprising the SC population according to claim 10 ~~or 11~~ and ~~optionally~~ a pharmaceutically acceptable carrier.

20 ~~12~~3. A method for preparing a pharmaceutical composition comprising the steps:
 a) performing the method of any one of claims 1 to 9;
 b) formulating the selected SC population with at least one pharmaceutically acceptable carrier.

25 ~~13~~4. Use of the The SC population according to claim 10 ~~or 11 for use in the manufacture of a medicament for~~ allogeneic stem cell therapy in a patient in need thereof, ~~preferably in a presensitized patient or a patient undergoing retreatment, preferably wherein~~ the patient in need suffers from a disease selected from fistulas, leukemia, lymphoma, neurodegenerative diseases, brain and spinal cord injury, heart
 30 diseases, blindness and vision impairment, pancreatic beta cell loss of function,

cartilage repair, osteoarthritis, musculoskeletal diseases, wounds, infertility, autoimmune diseases and inflammatory diseases such as inflammatory bowel disease, more preferably the disease is a fistula, even more preferably the disease is a complex perianal fistula.

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~~15. — An allogeneic stem cell therapy method comprising administering the SC population according to claim 10 or 11 to a patient in need thereof, preferably in a presensitized patient or a patient undergoing retreatment, preferably the patient in need suffers from a disease selected from leukemia, lymphoma, neurodegenerative diseases, brain and spinal cord injury, heart diseases, blindness and vision impairment, pancreatic beta cell loss of function, cartilage repair, osteoarthritis, musculoskeletal diseases, wounds, infertility, autoimmune diseases and inflammatory diseases such as inflammatory bowel disease, more preferably the disease is a fistula, even more preferably the disease is a complex perianal fistula.~~

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