Claims

1. A method of providing a therapeutically active cell product

wherein the cell product is prepared ex vivo from a tissue donation in the form of a suspension of an original population of cells,

~~wherein optionally the suspension of the original population of cells is washed and re-suspended as a washed and/or single cell suspension prior to the in vitro depletion,~~

wherein the cell product comprises a portion of non-hematopoietic stem cells comprising non-hematopoietic progenitor stem cells, multipotent stem cells and pluripotent stem cells

wherein the method comprises an in vitro immuno-depletion of hematopoietic stem cells and cells of hematopoietic lineage

wherein the immuno-depletion comprises applying an immuno-depletion with a group of at least four selected antibodies, wherein:

- one of said at least four selected antibodies is specific for CD14,
- one of said at least four selected antibodies is specific for CD34, and
- at least two antibodies of said at least four selected antibodies are specific for members of the CD45 family,

with

- one of said at least two antibodies being specific for all members of the CD45 family, and
- one further of said at least two antibodies being specific for the CD45 family member CD45RA.

~~of cells expressing at least one surface antigen of a group of selected surface antigens from the suspension of the original population of cells or from the single cell and/or washed suspension of cells,~~

~~wherein the group of selected surface antigens comprises at least the four surface antigens:~~

~~i. CD14,~~

~~ii. CD34,~~

~~iii. CD45 and~~

~~iv. CD45RA, a further member of the CD45 surface antigen family.~~

2. The method of claim 1,

wherein the immuno-depletion comprises an immuno-labeling procedure labeling cells expressing one or more ~~of the~~ selected surface antigens with specific antibodies comprising a tag

wherein in a direct immuno-labeling procedure the tag is conjugated to the antibodies prior to a specific binding of the antibodies to the surface antigens and/or wherein in an indirect immuno-labeling procedure the tag is conjugated to the antibody after the specific binding of the antibodies to the surface antigens during the immuno-labeling procedure,

wherein the tag in particular is a magnetic bead or a fluorescent tag;

and wherein the immuno-depletion comprises a separation procedure for removal of the cells labeled with antibodies comprising a tag from the suspension in step separate from the labeling procedure or in a combined step.

3. The method of claim 1 or 2,

wherein the immuno-labeling is an immuno-magnetic labeling procedure, and

wherein the tag is a magnetic particle and wherein a magnetic separation device is used in a separation procedure for depleting the immuno-magnetically labeled cells.

4. The method according to one of claims 1 to 3 comprising at least one direct immuno-labeling procedure

wherein the cell suspension is incubated with at least one tag-conjugated surface antigen specific antibody, in particular at least one antibody conjugated to a magnetic particle or to a fluorescent tag.

5. The method of one of claims 1 to 4 comprising at least one indirect immuno-labeling procedure,

wherein in a first step of the method the cell population is incubated with one or more surface antigen specific primary antibodies,

wherein after the first step excess of the one or more unbound primary antibodies are removed by centrifugation followed by re-suspension of the cells;

wherein in a second step of the method the cell population is incubated with at least one tag-conjugated secondary antibody and/or with at least one other tag-conjugated reagent that specifically binds to primary antibodies,

wherein in the first step in particular biotinylated primary antibodies are used and in the second step streptavidin coated tags or tag-conjugated anti-biotin antibodies,

and wherein the tag conjugated to secondary antibodies or to another reagent is in particular a magnetic particle or a fluorescent tag,

wherein the immuno-labeling procedure optionally comprises additional steps before and/or after the first and the second step.

and wherein optionally the number of incubations in the at least one first and the at least one second steps combined comprises a total of up to three or up to four or up to five incubations of the cell suspension with primary and/or secondary antibodies.

6. The method according to claim 5 comprising an indirect immuno-labeling,

wherein in the first step of the immuno-labeling the cell population is incubated with primary antibodies against at least 3 different selected surface antigens at the same time,

wherein optionally each of the antibodies is incubated according to standard incubation conditions, and

wherein in particular standard incubation conditions comprise an antibody concentration for each antibody of 0.1 to 2.5 mg antibody per 100 ml +/- 10 ml incubation volume, in particular of 0.25 to 0.75 mg or 0.5 mg antibody per 100 ml +/- 10 ml incubation volume and wherein optionally the number of cells present does not exceed 10^{10} cells or does not exceed 5×10^9 or 3×10^9 or 2×10^9 or 1.5×10^9 or 1.2×10^9 or 1.0×10^9 cells.

7. The method according to one of claims 2 to 6, wherein one or more steps of the immuno-labeling procedure and/or the separation procedure of the immuno-depletion is performed under limiting conditions

wherein the limiting conditions favor the depletion of cells from the original population of cells which have a higher number of selected surface-antigen-

specific antibodies bound per cell compared to cells which have a lower number of such selected antibodies bound per cell and
wherein one or more of the following conditions is applied:

- wherein in the direct immuno-labeling procedure limiting incubation conditions are allowing for only a partial saturation of the antigenic binding sites of the selected surface antigens on the cells by the tag-conjugated antibodies and/or

- wherein in the first step of the indirect immuno-labeling procedure limiting incubation conditions are allowing for only a partial saturation of the antigenic binding sites of the selected surface antigens on the cells by the primary antibodies and/or

- wherein in the second step of the indirect immuno-labeling procedure limiting incubation conditions are allowing for only a partial saturation of the antigenic binding sites of the primary antibodies by the tag-conjugated secondary antibodies and/or

- wherein in the first step of the indirect immuno-labeling standard incubation conditions are applied to allow for a maximized saturation of the selected surface antigenic binding sites while minimizing unspecific binding of the primary antibodies to the cells and wherein in the second step limiting incubation conditions are allowing for only a partial saturation of the antigenic binding sites on the primary antibodies by the tag-conjugated secondary antibodies and/or

- wherein in the first step of the indirect immuno-labeling limiting incubation conditions are adjusted to allow for only a partial saturation of the selected surface antigenic binding sites on the cells, while in the second step standard incubation conditions are allowing for a maximized saturation of the antigenic binding sites on the primary antibodies and/or

- wherein in the separation procedure conditions are adjusted such that cells which are labeled with two or more tags, in particular with at least three or four or more than four tags, are removed from the original cell population whereas cells comprising fewer tags remain in the original cell population, and wherein the tags are in particular magnetic particles.

8. The method according to one of claims 2 to 7,

wherein incubation conditions of one or more steps of the immuno-depletion are adjusted to allow for only a partial saturation of antigenic binding sites of at least one of the selected surface antigens, said selected surface antigens comprising ~~at least one of CD14, CD19, CD34, CD45 surface antigen family, CD117, and ICAM-1, preferably~~ at least one of CD14, and/or CD34 and/or at least one member of CD45 surface antigen family.

9. The method according to claim 8,

wherein the partial saturation of the antigenic binding sites is achieved by reducing the contact efficiency between antibody and antigen or by reducing the binding probability or by reducing the binding strength between the antibody and antigen, in particular by selecting one or more of the following options compared to standard incubation conditions,

- by lowering the ratio of amount of antibodies to number of cells and/or
- by increasing the incubation volume by a factor of 1.5 to 4 like 1.5 to 3.5 or 2 to 3 and/or
- by reducing the incubation time and/or
- by adapting the incubation temperature and/or
- by adapting the moving conditions like the moving speed,

wherein the binding probability or the contact efficiency or the binding strength in standard incubation conditions are optimized for maximal saturation with specifically bound antibodies to corresponding antigenic binding sites of selected surface antigens while keeping non-specific binding of antibodies to cells lacking the specific selected surface antigen at a level of less than 30%, in particular less than 20% or 10% or 5% of the level of specific binding of antibodies to cells expressing the corresponding surface antigen and/or wherein the standard incubation conditions comply with the specifications by the manufacturer of the antibodies.

10. The method according to one of the previous claims,

wherein the in vitro immuno-depletion comprises an immuno-labeling procedure for labeling the cells expressing at least one surface antigen of the group of selected surface antigens,

wherein the immuno-labeling is performed in at least two stages,

wherein in a first stage cells are labeled with antibodies against selected surface antigens except for antibodies against CD34 and optionally except for antibodies against one or both of CD133 and CD117 and

wherein in a second stage performed after the first stage cells are labeled with antibodies

against CD34 and optionally with antibodies against one or both of CD133 and CD117 surface antigen and optionally with antibodies against further selected antigens and

wherein optionally both the first and the second stage comprise one or more individual incubation steps, each step for immuno-labeling with antibodies against one selected antibody separately, or for immuno-labeling with an antibody cocktail comprising several antibodies against several selected surface antigens.

11. The method according to one of the previous claims, wherein the duration of the ex vivo manipulations comprising or consisting of the in vitro depletion and optionally including an ex vivo washing and/or filtering step of the suspension of the original population of cells is limited to less than 10 or 8 or 6 or 5 hours.

~~12. The method according to one of the previous claims, wherein the group of selected surface antigens additionally comprises at least one of surface antigens which are~~

~~- characteristic for one or more cell types of hematopoietic stem cells and/or cells of hematopoietic lineage, including e.g. CD2, CD3, CD10, CD11b, CD15 (SSEA-1), CD16, CD19, CD44, CD56, CD123, CD235a, CD326, CD49f, ICAM-1, and/or~~

~~- absent or essentially absent on mesenchymal stem cells or on cells which have been reported to promote tissue regeneration, e.g. CD11a/LFA-1, CD31, CD80, CD86, und CD40 and CD144 and/or~~

~~- characteristic for tumor cells or cells prone to transform into tumor cells, in particular CD9, CD15, CD20, CD24, CD31, CD38, CD44, CD117, CD146,~~

~~CD166, CD171, CD184, CD324, CD325, CD326, CD338, ERb2 or HER2/neu.~~

~~13. The method according to one of the previous claims, comprising at least a further member of the CD45 family selected from CD45R, CD45RB, CD45RC, CD45RAB, CD45RAC, CD45RBC, CD45RO, CD45R(ABC).~~

~~14. The method according to one of the previous claims, wherein the group of selected surface antigens comprises CD45RO.~~

12~~5~~. The method according to one of the previous claims wherein the percentage ratio of portions of CD44 positive cells in the cell product versus the original population of cells is at least 7%.

13~~6~~. A therapeutically active cell product obtained by the method according to one of the previous claims.

14~~7~~. A therapeutically active cell product comprising non-hematopoietic progenitor stem cells, multipotent stem cells and pluripotent stem cells obtainable ex vivo from an original population of cells derived from a tissue probe, in particular from bone marrow, by in vitro depletion of hematopoietic cells for a group of surface antigens characteristic of hematopoietic stem cells comprising CD14, CD34, and CD45, and further comprising CD45RA and/or CD45RO, wherein the portion of cells in the cell product expressing at least one surface antigen of said~~a~~ group of surface antigens characteristic of hematopoietic cells is reduced by a factor of at least 2, or at least 3, or least 5, least 10, or at least 50 compared to the original population of cells~~, and wherein the group of surface antigens~~

~~indicative of hematopoietic surface antigens comprises at least the four surface antigens:~~

~~CD14,~~

~~CD34,~~

~~CD45 and~~

~~CD45RA, a further member of the CD45 surface antigen family~~.

15~~8~~. A therapeutically active cell product according to one of claims 16~~3~~ to 17~~4~~ for medical therapy, more particularly for regeneration of lost or damaged tissue and in particular for the treatment of degenerative neurological disease and/or the treatment of autoimmune diseases and in particular for the treatment of multiple sclerosis, diabetes mellitus type I, diabetes mellitus type II, rheumatoid arthritis, myocardial infarction, ischemic stroke.

19~~6~~. A pharmaceutical formulation comprising the therapeutically active cell product according to one of claims 13~~6~~ to 18~~5~~.

17~~20~~. The pharmaceutical formulation according to claim 19~~6~~ for systemic administration, in particular for intravenous administration, more particularly between 1 and 10 x 10^6 cells per kg body weight of the patient or in particular between 2 and 6 x 10^6 cells per kg body weight or between 2 and 8 x 10^6 cells per kg body weight, or the pharmaceutical formulation according to claim 16~~8~~ for site-specific or organ-specific administration.