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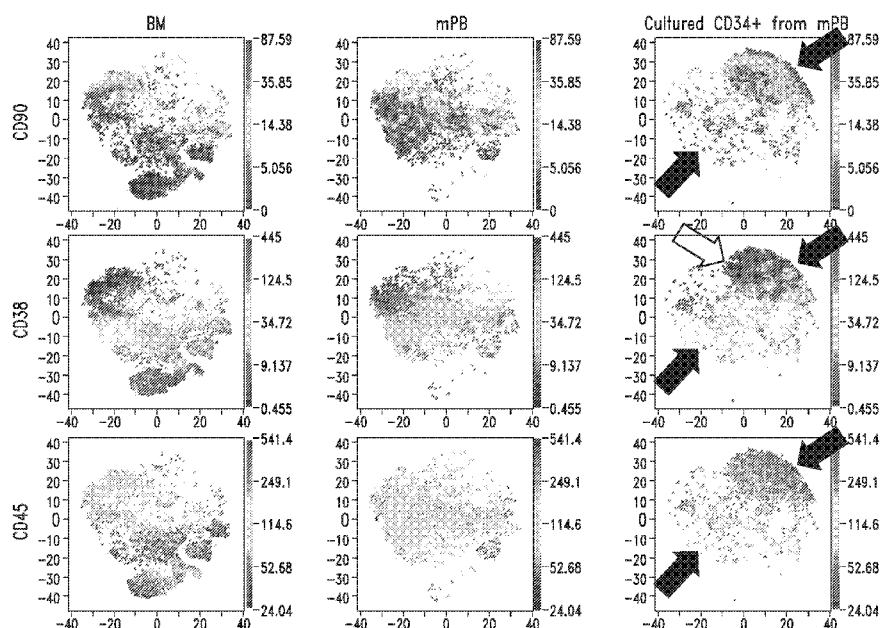


FIG. 1

(57) Abstract: The invention provides improved hematopoietic stem cell compositions and methods of use.

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

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HEMATOPOIETIC STEM CELL COMPOSITIONS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 62/554,479, filed September 5, 2017, which is incorporated by
5 reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with Government support under contract GM104148 awarded by the National Institutes of Health. The Government has certain rights in the invention.

10 BACKGROUND

Technical Field

The present invention generally relates, in part, to hematopoietic stem cell compositions and methods for using the same in gene therapy.

Description of the Related Art

15 Since its original implementation in 1950s, hematopoietic cell transplantation (HCT) has become the most successful and widely used form of stem cell therapy. HCT is currently the standard of care for otherwise incurable hematological malignancies, such as leukemia and lymphoma; genetic diseases; hemoglobinopathies, and immune diseases. More recently, therapeutic applications of HCT have been
20 extended to cell-based gene therapies, where autologous stem cells are modified *ex vivo* in and subsequently re-infused into the patient. However, despite expansion of donor registries and obtaining HSCs from umbilical cord blood (CB), human HSCs are still a very limited, yet vital resource. Moreover, current transplantation of impure HSCs from an unrelated HLA-matched individual can lead to engraftment failure, graft-

versus-host disease (GvHD), and delayed reconstitution, leaving at least 50% of current patients without a cure or with a permanent disability.

BRIEF SUMMARY

Improved human hematopoietic stem cell compositions and methods of making
5 or isolating and using the same to treat, prevent, or ameliorate at least one symptom of a hemoglobinopathy are contemplated herein.

In various embodiments, a population of human hematopoietic cells comprises isolated human CD34+CD84- hematopoietic stem cells.

In particular embodiments, the human CD34+CD84- hematopoietic stem cells
10 are also CD90+.

In further embodiments, the human CD34+CD84- hematopoietic stem cells are also CD45RA-.

In particular embodiments, the human CD34+CD84- hematopoietic stem cells are also CD49f+.

15 In additional embodiments, the human CD34+CD84- hematopoietic stem cells are also CD38Lo/.

In some embodiments, the human CD34+CD84- hematopoietic stem cells are also CD164+.

In particular embodiments, the human CD34+CD84- hematopoietic stem cells
20 are also CD172a+.

In certain embodiments, the human CD34+CD84- hematopoietic stem cells are also CD117+.

In some embodiments, the human CD34+CD84- hematopoietic stem cells are also CD133+.

25 In various embodiments, a population of cultured hematopoietic cells enriched for the human hematopoietic stem cells contemplated herein, is provided.

In various embodiments, a population of cultured hematopoietic cells enriched for human CD34+CD84- hematopoietic stem cells is contemplated.

In various embodiments, a population of cultured hematopoietic cells enriched
30 for human CD34+CD84- CD90+ CD45RA- hematopoietic stem cells is contemplated.

In various embodiments, a population of cultured hematopoietic cells enriched for human CD34+CD84- and one or more of hematopoietic stem cell markers selected from the group consisting of CD90+, CD45RA-, CD49f+, CD38Lo/, CD164+, CD172a+, CD117+, and CD133+ is contemplated.

5 In various embodiments, a human CD34+CD84- hematopoietic stem cell comprises a gene therapy vector.

In particular embodiments, the CD34+CD84- hematopoietic stem cell further comprises one or more of hematopoietic stem cell markers selected from the group consisting of CD90+, CD45RA-, CD49f+, CD38Lo/, CD164+, CD172a+, CD117+, and
10 CD133+.

In various embodiments, a human CD34+CD84- hematopoietic stem cell comprises one or more genome modifications.

In some embodiments, the CD34+CD84- hematopoietic stem cell further comprises one or more of hematopoietic stem cell markers selected from the group consisting of CD90+, CD45RA-, CD49f+, CD38Lo/, CD164+, CD172a+, CD117+, and
15 CD133+.

In various embodiments, a human CD34+CD84- hematopoietic stem cell comprises one or more exogenous polynucleotides.

In certain embodiments, the CD34+CD84- hematopoietic stem cell further
20 comprises one or more of hematopoietic stem cell markers selected from the group consisting of CD90+, CD45RA-, CD49f+, CD38Lo/, CD164+, CD172a+, CD117+, and CD133+.

In various embodiments, a composition comprises at least 50%, at least 75%, at least 85%, at least 90%, or at least 95% of a population of human hematopoietic stem
25 cells, a population of cultured human hematopoietic stem cells, or one or more human hematopoietic stem cells contemplated herein.

In various embodiments, a composition comprises at least 50%, at least 75%, at least 85%, at least 90%, or at least 95% human CD34+CD84- hematopoietic stem cells.

In particular embodiments, the composition comprises a pharmaceutically
30 acceptable carrier.

In some embodiments, the pharmaceutically acceptable carrier comprises a physiologically acceptable cell culture medium.

In various embodiments, a pharmaceutical composition comprises a population of human hematopoietic stem cells, a population of cultured human hematopoietic stem cells, or one or more human hematopoietic stem cells contemplated herein; and a pharmaceutically acceptable cell culture medium.

In various embodiments, a method of treating a subject in need thereof comprises administering an effective amount of a population of human hematopoietic stem cells, a population of cultured human hematopoietic stem cells, one or more human hematopoietic stem cells, or a pharmaceutical composition contemplated herein to the subject.

In various embodiments, a method of treating a subject having a hemoglobinopathy comprises administering an effective amount of a population of human hematopoietic stem cells, a population of cultured human hematopoietic stem cells, one or more human hematopoietic stem cells, or a pharmaceutical composition contemplated herein to the subject.

In various embodiments, a method of treating sickle cell disease in a subject comprises administering an effective amount of a population of human hematopoietic stem cells, a population of cultured human hematopoietic stem cells, one or more human hematopoietic stem cells, or a pharmaceutical composition contemplated herein to the subject.

In various embodiments, a method of treating a β -thalassemia in a subject comprises administering an effective amount of a population of human hematopoietic stem cells, a population of cultured human hematopoietic stem cells, one or more human hematopoietic stem cells, or a pharmaceutical composition contemplated herein to the subject.

In further embodiments, one or more of the human CD34+CD84- - hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector encoding a globin.

In particular embodiments, the retroviral vector or the lentiviral vector is an AnkT9W vector, a T9Ank2W vector, a TNS9 vector, a lentiglobin HPV569 vector, a

lentiglobin BB305 vector, a BG-1 vector, a BGM-1 vector, a d432 β A γ vector, a mLAR β Δ γ V5 vector, a GLOBE vector, a G-GLOBE vector, a β AS3-FB vector, a V5 vector, a V5m3 vector, a V5m3-400 vector, a G9 vector, or a BCL11A shmir vector.

In various embodiments, a method of treating adrenoleukodystrophy or
5 adrenomyeloneuropathy in a subject comprises administering an effective amount of a population of human hematopoietic stem cells, a population of cultured human hematopoietic stem cells, one or more human hematopoietic stem cells, or a pharmaceutical composition contemplated herein to the subject.

In some embodiments, one or more of the human CD34+CD84- hematopoietic
10 stem cells are transduced with a retroviral vector or a lentiviral vector encoding an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide.

In additional embodiments, the retroviral vector or lentiviral vector comprises a myeloproliferative sarcoma virus enhancer, negative control region deleted, d1587rev primer-binding site substituted (MND) promoter or transcriptionally active fragment
15 thereof operably linked to a polynucleotide encoding an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide.

In various embodiments, a method of treating ADA-SCID in a subject comprises administering an effective amount of a population of human hematopoietic stem cells, a population of cultured human hematopoietic stem cells, one or more
20 human hematopoietic stem cells, or a pharmaceutical composition contemplated herein to the subject.

In certain embodiments, one or more of the human CD34+CD84- hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector encoding an adenosine deaminase.

25 In certain embodiments, the retroviral vector or lentiviral vector comprises an elongation factor 1 alpha promoter or and MND promoter operably linked to a polynucleotide encoding adenosine deaminase.

In various embodiments, a method of treating X-SCID in a subject comprises administering an effective amount of a population of human hematopoietic stem cells, a
30 population of cultured human hematopoietic stem cells, one or more human

hematopoietic stem cells, or a pharmaceutical composition contemplated herein to the subject.

In particular embodiments, one or more of the human CD34+CD84- hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector
5 encoding an interleukin 2 receptor gamma.

In some embodiments, the retroviral vector or lentiviral vector comprises an elongation factor 1 alpha promoter operably linked to a polynucleotide encoding interleukin 2 receptor gamma.

In various embodiments, a method of treating Batten's disease in a subject
10 comprises administering an effective amount of a population of human hematopoietic stem cells, a population of cultured human hematopoietic stem cells, one or more human hematopoietic stem cells, or a pharmaceutical composition contemplated herein to the subject.

In certain embodiments, one or more of the human CD34+CD84- hematopoietic
15 stem cells are transduced with a retroviral vector or a lentiviral vector encoding a tripeptidyl peptidase 1.

In particular embodiments, the retroviral vector or lentiviral vector comprises an elongation factor 1 alpha promoter or comprises a myeloproliferative sarcoma virus enhancer, negative control region deleted, d1587rev primer-binding site substituted
20 (MND) promoter operably linked to a polynucleotide encoding tripeptidyl peptidase 1.

In various embodiments, a method of treating MPS I in a subject comprises administering an effective amount of a population of human hematopoietic stem cells, a population of cultured human hematopoietic stem cells, one or more human hematopoietic stem cells, or a pharmaceutical composition contemplated herein to the
25 subject.

In further embodiments, one or more of the human CD34+CD84- hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector encoding an alpha-L iduronidase.

In some embodiments, the retroviral vector or lentiviral vector comprises an
30 elongation factor 1 alpha promoter or comprises a myeloproliferative sarcoma virus

enhancer, negative control region deleted, d1587rev primer-binding site substituted (MND) promoter operably linked to a polynucleotide encoding alpha-L iduronidase.

In various embodiments, a method of treating MPS II in a subject comprises administering an effective amount of a population of human hematopoietic stem cells, a
5 population of cultured human hematopoietic stem cells, one or more human hematopoietic stem cells, or a pharmaceutical composition contemplated herein to the subject.

In additional embodiments, one or more of the human CD34+CD84- hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector
10 encoding an iduronate 2-sulfatase.

In certain embodiments, the retroviral vector or lentiviral vector comprises an elongation factor 1 alpha promoter or comprises a myeloproliferative sarcoma virus enhancer, negative control region deleted, d1587rev primer-binding site substituted (MND) promoter operably linked to a polynucleotide encoding iduronate 2-sulfatase.

15 In various embodiments, a method for isolating human CD34+CD84- hematopoietic stem cells from a heterologous cell population comprises contacting a heterologous cell population with one or more binding agents to isolate human CD34+CD84- hematopoietic stem cells from the population.

In particular embodiments, the one or more binding agents are antibodies or
20 antigen binding fragments thereof.

In further embodiments, the one or more binding agents bind CD34 and CD84 and one or more cell surface markers selected from the group consisting of: CD90+, CD45RA-, CD49f+, CD38Lo/, CD164+, CD172a+, CD117+, and CD133+.

In additional embodiments, the isolating the human CD34+CD84-
25 hematopoietic stem cells comprises steps of positive and negative selection.

In some embodiments, the human CD34+CD84- hematopoietic stem cells comprise one or more gene therapy vectors, genome modifications, or exogenous polynucleotides.

In certain embodiments, the heterologous cell population is selected from the
30 group consisting of: bone marrow, mobilized peripheral blood, umbilical cord blood, placenta, or fractions thereof.

In various embodiments, a method for quantitating human CD34+CD84- hematopoietic stem cells from a heterologous cell population comprises contacting a heterologous cell population with one or more binding agents to identify human CD34+CD84- hematopoietic stem cells from the population, and quantitating the
5 identified cells.

In particular embodiments, the one or more binding agents are antibodies or antigen binding fragments thereof.

In particular embodiments, the one or more binding agents bind CD34 and CD84 and one or more cell surface markers selected from the group consisting of:
10 CD90+, CD45RA-, CD49f+, CD38Lo/, CD164+, CD172a+, CD117+, and CD133+.

In certain embodiments, the isolating the human CD34+CD84- hematopoietic stem cells comprises steps of positive and negative selection.

In further embodiments, the human CD34+CD84- hematopoietic stem cells comprise one or more gene therapy vectors, genome modifications, or exogenous
15 polynucleotides.

In some embodiments, the heterologous cell population is selected from the group consisting of: bone marrow, mobilized peripheral blood, umbilical cord blood, placenta, or fractions thereof.

In various embodiments, a kit comprises one or more binding agents to isolate
20 or quantitate human CD34+CD84- hematopoietic stem cells from a heterologous cell population.

In certain embodiments, one or more binding agents are antibodies or antigen binding fragments thereof.

In particular embodiments, the one or more binding agents bind CD34 and
25 CD84 and one or more cell surface markers selected from the group consisting of: CD90+, CD45RA-, CD49f+, CD38Lo/, CD164+, CD172a+, CD117+, and CD133+.

In further embodiments, the human CD34+CD84- hematopoietic stem cells comprise one or more gene therapy vectors, genome modifications, or exogenous polynucleotides.

In some embodiments, the kit is used for isolating or quantitating human CD34⁺CD84⁻ hematopoietic stem cells from bone marrow, mobilized peripheral blood, umbilical cord blood, or placenta.

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

- 5 **Figure 1** shows a viSNE phenotype analysis of hematopoietic cells isolated from bone marrow, mobilized peripheral blood, and cultured hematopoietic cells isolated from mobilized peripheral blood.
- Figure 2** shows the results from cell surface marker expression analysis of CD34⁺ cells isolated from bone marrow.
- 10 **Figure 3** shows the results from a lineage tracing analysis from CD34⁺ cells isolated from bone marrow and mobilized peripheral blood.

DETAILED DESCRIPTION

A. OVERVIEW

- Current methods for hematopoietic cell transplantation (HCT) and
- 15 hematopoietic stem cell (HSC)-based gene therapy rely on suboptimal populations of hematopoietic cells comprising an inadequate number of therapeutically efficacious HSCs.
- There is an urgent an unmet need to develop novel sources of human HSCs and human HSC cellular compositions, expand current sources, and manipulate
- 20 hematopoietic material in order to increase the efficiency of therapeutic applications and minimize adverse effects of HCT. A major limitation of HSC gene therapy isolation of therapeutically relevant cells following the modification and culture of the modified cells. Primary hematopoietic cells, including stem cells, may alter their cell surface marker expression during modification and/or culture; thereby making the stem
- 25 cells difficult to re-isolate post-manipulation.
- One significant problem plaguing the art is the failure to discover novel markers whose expression identifies primary hematopoietic stem cells or hematopoietic stem cells that have been genetically modified and/or cultured. Currently, methods for

identifying and isolating hematopoietic stem cells from various sources includes the concomitant use of antibodies against the surface proteins CD34, CD38, CD90, CD45RA, and CD49ff. Nevertheless, with exception of CD34, the expression levels of the other listed markers change during culture conditions. For example, CD38 is
5 considered a crucial marker to identify CD34⁺ HSCs; HSCs are contained in the CD34⁺CD38⁻ fraction. However, the ability to distinguish between CD34⁺CD38⁻ HSCs and CD34⁺CD38⁺ hematopoietic progenitors following culture conditions is not possible because CD38 expression decreases in all cells following culture. Thus, the variation in cell surface marker expression greatly impairs the ability to identify HSCs
10 after culture.

The present invention offers solutions to this and other problems

Various illustrative embodiments of the invention contemplate improved hematopoietic stem cell compositions with increased therapeutic efficacy and methods of making and using the same. The present inventors have identified novel markers that
15 reliably and reproducibly identify hematopoietic stem cells, whether they have been freshly isolated, genetically modified, and/or cultured. Without wishing to be bound by any particular theory, it is contemplated that cell-based therapies are improved by selecting for HSCs after modification and/or culture and prior to administration to a subject.

20 In particular embodiments, populations of human hematopoietic stem cells are contemplated that express one or more cell surface markers of HSCs and that do not express, or lack expression of, CD84. Surprisingly, the present inventors identified that HSCs lack CD84 expression before and after culture and thus, can be used to distinguish true HSCs from less potent more differentiated hematopoietic progenitor
25 cells.

In particular embodiments, populations of human hematopoietic cells are contemplated that are enriched for HSCs by selecting HSCs that express one or more cell surface HSC markers, *e.g.* CD34, and that do not express, or lack expression of, CD84. In preferred embodiments, the HSC has been modified by introducing genetic
30 material into the cell.

Other preferred embodiments contemplate compositions comprising HSCs that express one or more cell surface HSC markers and that do not express, or lack expression of, CD84.

Methods of treating diseases comprising administering a subject in need thereof
 5 an effective amount of the HSCs or compositions contemplated herein are also provided in particular preferred embodiments.

Various embodiments contemplated herein will employ, unless indicated specifically to the contrary, conventional methods of chemistry, biochemistry, organic chemistry, molecular biology, microbiology, recombinant DNA techniques, genetics,
 10 immunology, and cell biology that are within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual* (3rd Edition, 2001); Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual* (1982);
 15 Ausubel *et al.*, *Current Protocols in Molecular Biology* (John Wiley and Sons, updated July 2008); *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience; Glover, *DNA Cloning: A Practical Approach*, vol. I & II (IRL Press, Oxford, 1985); Anand, *Techniques for the Analysis of Complex Genomes*, (Academic
 20 Press, New York, 1992); *Transcription and Translation* (B. Hames & S. Higgins, Eds., 1984); Perbal, *A Practical Guide to Molecular Cloning* (1984); Harlow and Lane, *Antibodies*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1998) *Current Protocols in Immunology* Q. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, eds., 1991); *Annual Review of Immunology*; as well as
 25 monographs in journals such as *Advances in Immunology*.

B. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to
 30 those described herein can be used in the practice or testing of the present invention,

preferred embodiments of compositions, methods and materials are described herein. For the purposes of the present invention, the following includes terms that are defined below.

The articles “a,” “an,” and “the” are used herein to refer to one or to more than
5 one (*i.e.*, to at least one, or to one or more) of the grammatical object of the article. By way of example, “an element” means one element or one or more elements.

The use of the alternative (*e.g.*, “or”) should be understood to mean either one, both, or any combination thereof of the alternatives.

The term “and/or” should be understood to mean either one, or both of the
10 alternatives.

As used herein, the term “about” or “approximately” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 % to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight
15 or length. In particular embodiments, the terms “about” or “approximately” when preceding a numerical value indicates the value plus or minus a range of 15%, 10%, 5%, or 1%.

As used herein, the term “substantially” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is 80%,
20 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher compared to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In one embodiment, “substantially the same” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that produces an effect, *e.g.*, a physiological effect, that is approximately the
25 same as a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

Throughout this specification, unless the context requires otherwise, the words “comprise”, “comprises” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other
30 step or element or group of steps or elements. As used herein, the terms “include” and “comprise” are used synonymously. By “consisting of” is meant including, and limited

to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present. By “consisting essentially of” is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase “consisting essentially of” indicates that the listed elements are required or mandatory, but that no other elements are present that materially affect the activity or action of the listed elements.

Reference throughout this specification to “one embodiment,” “an embodiment,” “a particular embodiment,” “a related embodiment,” “a certain embodiment,” “an additional embodiment,” or “a further embodiment” or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. It is also understood that the positive recitation of a feature in one embodiment, serves as a basis for excluding the feature in a particular embodiment.

By “enhance” or “promote” or “increase” or “expand” or “potentiate” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is greater than a reference. An “increased” or “enhanced” quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length is a “statistically significant”, and may include an increase that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (*e.g.*, 500, 1000 times) (including all integers and decimal points in between and above 1, *e.g.*, 1.5, 1.6, 1.7, 1.8, *etc.*) a reference.

By “decrease” or “lower” or “lessen” or “reduce” or “abate” or “ablate” or “inhibit” or “dampen” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length) that is less than a reference. A “decreased” or “reduced” quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length is typically “statistically significant”, and may include an

decrease that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (*e.g.*, 500, 1000 times) (including all integers and decimal points in between and above 1, *e.g.*, 1.5, 1.6, 1.7, 1.8, *etc.*) a reference.

By “maintain,” or “preserve,” or “maintenance,” or “no change,” or “no
 5 substantial change,” or “no substantial decrease” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is not significantly different or measurable different from a reference.

Cells contemplated in particular embodiments may be autologous/autogeneic (“self”) or non-autologous (“non-self,” *e.g.*, allogeneic, syngeneic or xenogeneic).

10 “Autologous,” as used herein, refers to cells from the same subject. “Allogeneic,” as used herein, refers to cells of the same species that differ genetically to the cell in comparison. “Syngeneic,” as used herein, refers to cells of a different subject that are genetically identical to the cell in comparison. “Xenogeneic,” as used herein, refers to cells of a different species to the cell in comparison.

15 The term “stem cell” refers to a cell which is an undifferentiated cell capable of (1) long term self -renewal, or the ability to generate at least one identical copy of the original cell, (2) differentiation at the single cell level into multiple, and in some instance only one, specialized cell type and (3) of *in vivo* functional regeneration of tissues. Stem cells are subclassified according to their developmental potential as
 20 totipotent, pluripotent, multipotent and oligo/unipotent. “Self-renewal” refers a cell with a unique capacity to produce unaltered daughter cells and to generate specialized cell types (potency). Self-renewal can be achieved in two ways. Asymmetric cell division produces one daughter cell that is identical to the parental cell and one daughter cell that is different from the parental cell and is a progenitor or differentiated
 25 cell. Symmetric cell division produces two identical daughter cells. “Proliferation” or “expansion” of cells refers to symmetrically dividing cells.

As used herein, the term “progenitor” or “progenitor cells” refers to cells have the capacity to self-renew and to differentiate into more mature cells. Many progenitor cells differentiate along a single lineage, but may have quite extensive proliferative
 30 capacity.

Hematopoietic stem cells (HSCs) give rise to committed hematopoietic progenitor cells (HPCs) that are capable of generating the entire repertoire of mature blood cells over the lifetime of an organism. The term “hematopoietic stem cell” or “HSC” refers to multipotent stem cells that give rise to the all the blood cell types of an organism, including myeloid (e.g., monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (e.g., T-cells, B-cells, NK-cells), and others known in the art (See Fei, R., et al., U.S. Patent No. 5,635,387; McGlave, et al., U.S. Patent No. 5,460,964; Simmons, P., et al., U.S. Patent No. 5,677,136; Tsukamoto, et al., U.S. Patent No. 5,750,397; Schwartz, et al., U.S. Patent No. 5,759,793; DiGuisto, et al., U.S. Patent No. 5,681,599; Tsukamoto, et al., U.S. Patent No. 5,716,827). When transplanted into lethally irradiated animals or humans, hematopoietic stem cells can reconstitute the blood cell lineages.

As used herein, the term “population of cells” refers to a plurality of cells that may be made up of any number and/or combination of homogenous or heterogeneous cell types, as described elsewhere herein. For example, a population of hematopoietic cells may be isolated or obtained from umbilical cord blood, placental blood, bone marrow, or peripheral blood. A population of cells may comprise about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 100% or any intervening percentage of hematopoietic stem cells.

The term “*ex vivo*” refers generally to activities that take place outside an organism, such as modification and/or culture done in or on living tissue in an artificial environment outside the organism, preferably with minimum alteration of the natural conditions. Tissue culture experiments or procedures lasting longer than a few days using living cells or tissue are typically considered to be “*in vitro*,” though in certain embodiments, this term can be used interchangeably with *ex vivo*. In one embodiment, HSCs are modified and/or cultured *ex vivo*.

The term “*in vivo*” refers generally to activities that take place inside an organism.

As used herein, the term “genetically engineered” or “genetically modified” refers to the chromosomal or extrachromosomal addition of extra genetic material in the

form of DNA or RNA to the total genetic material in a cell. Genetic modifications may be targeted or non-targeted to a particular site in a cell's genome.

As used herein, the term "gene therapy" refers to the introduction of extra genetic material into the total genetic material in a cell that restores, corrects, or
5 modifies expression of a gene or gene product, or for the purpose of expressing a therapeutic polypeptide.

As used herein, the term "genome editing" refers to the substitution, deletion, and/or introduction of genetic material at a target site in the cell's genome, which restores, corrects, disrupts, and/or modifies expression of a gene or gene product.

10 The terms "reprogrammed nuclease," "engineered nuclease," or "nuclease variant" are used interchangeably and refer to a nuclease comprising one or more DNA binding domains and one or more DNA cleavage domains, wherein the nuclease has been designed and/or modified from a parental or naturally occurring nuclease, to bind and cleave a double-stranded DNA target sequence.

15 A "target site" or "target sequence" is a chromosomal or extrachromosomal nucleic acid sequence that defines a portion of a nucleic acid to which a binding molecule will bind and/or cleave, provided sufficient conditions for binding and/or cleavage exist.

An "exogenous" molecule is a molecule that is not normally present in a cell,
20 but that is introduced into a cell by one or more genetic, biochemical or other methods. Exemplary exogenous molecules include, but are not limited to small organic molecules, protein, nucleic acid, carbohydrate, lipid, glycoprotein, lipoprotein, polysaccharide, any modified derivative of the above molecules, or any complex comprising one or more of the above molecules. Methods for the introduction of
25 exogenous molecules into cells are known to those of skill in the art and include, but are not limited to, lipid-mediated transfer (i.e., liposomes, including neutral and cationic lipids), electroporation, direct injection, cell fusion, particle bombardment, biopolymer nanoparticle, calcium phosphate co-precipitation, DEAE-dextran-mediated transfer and viral vector-mediated transfer.

30 An "endogenous" molecule is one that is normally present in a particular cell at a particular developmental stage under particular environmental conditions.

As used herein, the term “isolated” means material, *e.g.*, a polynucleotide, a polypeptide, a cell, that is substantially or essentially free from components that normally accompany it in its native state. In particular embodiments, the term “obtained” or “derived” is used synonymously with isolated.

5 As used herein, the terms “polynucleotide” or “nucleic acid” refers to genomic DNA (gDNA), complementary DNA (cDNA) or DNA. Polynucleotides include single and double stranded polynucleotides, either recombinant, synthetic, or isolated. In some embodiments, polynucleotide refers to messenger RNA (mRNA), RNA, genomic RNA (gRNA), plus strand RNA (RNA(+)), minus strand RNA (RNA(-)). As used here,
10 the terms “polyribonucleotide” or “ribonucleic acid” also refer to messenger RNA (mRNA), RNA, genomic RNA (gRNA), plus strand RNA (RNA(+)), minus strand RNA (RNA(-)) and inhibitory RNAs, including but not limited to siRNA, shRNA, piRNA, miRNA or microRNA, and shRNAs embedded in a microRNA backbone (shmir). Preferably, polynucleotides of the invention include polynucleotides or
15 variants having at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any of reference sequence, typically where the variant maintains at least one biological activity of the reference sequence. Exemplary polynucleotides include expression control sequences and polynucleotides encoding a therapeutic polypeptide including, but not limited to, a
20 globin polypeptide, an antisickling globin polypeptide, an adenosine deaminase polypeptide, an interleukin 2 receptor gamma polypeptide, a tripeptidyl peptidase 1 polypeptide, an alpha-L iduronidase polypeptide, an iduronate 2-sulfatase polypeptide, or an ATP-binding cassette, sub-family D (ALD), member 1 (ABCD1) polypeptide, as discussed elsewhere herein are contemplated.

25 Polynucleotides, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters and/or enhancers, untranslated regions (UTRs), Kozak sequences, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, internal ribosomal entry sites (IRES), recombinase recognition sites (*e.g.*, LoxP, FRT, and Att sites), termination codons, transcriptional
30 termination signals, and polynucleotides encoding self-cleaving polypeptides, epitope

tags, as disclosed elsewhere herein or as known in the art, such that their overall length may vary considerably.

The term “expression control sequence” refers to a polynucleotide sequence that comprises one or more promoters, enhancers, or other transcriptional control elements
5 or combinations thereof that are capable of directing, increasing, regulating, or controlling the transcription or expression of an operatively linked polynucleotide.

The term “operably linked”, refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. In one embodiment, the term refers to a functional linkage between a nucleic acid
10 expression control sequence (such as a promoter, and/or enhancer or other expression control sequence) and a second polynucleotide sequence, *e.g.*, a polynucleotide encoding a therapeutic polypeptide, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

The terms “polypeptide” and “protein” are used interchangeably herein to refer
15 to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues are synthetic non-naturally occurring amino acids, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers. Illustrative examples of polypeptides include, but are
20 not limited to globin polypeptides, suitable for use in the compositions and methods of particular embodiments. Also, see, *e.g.*, US Patents 6,051,402; 7,901,671; and 9,068,199, the full disclosure and claims of which are specifically incorporated herein by reference in their entirety.

Illustrative examples of polypeptides include but are not limited to a globin
25 polypeptide, an antisickling globin polypeptide, an adenosine deaminase polypeptide, an interleukin 2 receptor gamma polypeptide, a tripeptidyl peptidase 1 polypeptide, an alpha-L iduronidase polypeptide, an iduronate 2-sulfatase polypeptide, or an ATP-binding cassette, sub-family D (ALD), member 1 (ABCD1) polypeptide.

The term “vector” is used herein to refer to a molecule capable transferring or
30 transporting another nucleic acid molecule. Useful vectors include, but are not limited to, plasmids (*e.g.*, DNA plasmids or RNA plasmids), transposons, cosmids, bacterial

artificial chromosomes, and viral vectors. Useful viral vectors include, but are not limited to, replication defective retroviruses and lentiviruses, adenoviruses, adeno-associated viruses, herpes simplex viruses, and vaccinia viruses.

As will be evident to one of skill in the art, the term “viral vector” is widely used to refer either to a nucleic acid molecule that includes virus-derived nucleic acid elements that typically facilitate transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. The term viral vector may refer either to a virus or viral particle capable of transferring a nucleic acid into a cell or to the transferred nucleic acid itself. In one embodiment, the viral vector is episomal.

As used herein, the term “retrovirus” refers an RNA virus that reverse transcribes its genomic RNA into a linear double-stranded DNA copy and subsequently covalently integrates its genomic DNA into a host genome. Illustrative retroviruses include, but are not limited to: Moloney murine leukemia virus (M-MuLV), Moloney murine sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV)) and lentivirus.

As used herein, the term “lentivirus” refers to a group (or genus) of complex retroviruses. Illustrative lentiviruses include, but are not limited to: HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi virus (VMV) virus; the caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). In one embodiment, HIV based vector backbones (*i.e.*, HIV cis-acting sequence elements) are preferred. A variety of lentiviral vectors are known in the art, *see* Naldini *et al.*, (1996a, 1996b, and 1998); Zufferey *et al.*, (1997); Dull *et al.*, 1998, U.S. Pat. Nos. 6,013,516; and 5,994,136, many of which may be adapted to produce a viral vector or transfer plasmid of the present invention.

As used herein, the term “adeno-associated virus” or “AAV” refers to a small (~26 nm) replication-defective, primarily episomal, non-enveloped virus. AAV can infect both

dividing and non-dividing cells and may incorporate its genome into that of the host cell. Recombinant AAV (rAAV) are typically composed of, at a minimum, a transgene and its regulatory sequences, and 5' and 3' AAV inverted terminal repeats (ITRs). The ITR sequences are about 145 bp in length. In particular embodiments, the rAAV comprises
5 ITRs and capsid sequences isolated from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10. Construction of rAAV vectors, production, and purification thereof have been disclosed, e.g., in U.S. Patent Nos. 9,169,494; 9,169,492; 9,012,224; 8,889,641; 8,809,058; and 8,784,799, each of which is incorporated by reference herein, in its entirety.

10 As used herein, the term "adenovirus" refers to adenoviral-based vectors capable of very high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and high levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. Most adenovirus vectors are engineered such that a transgene replaces the Ad E1a, E1b, and/or E3 genes; subsequently
15 the replication defective vector is propagated in human 293 cells that supply deleted gene function in trans. Ad vectors can transduce multiple types of tissues *in vivo*, including non-dividing, differentiated cells such as those found in liver, kidney and muscle. Conventional Ad vectors have a large carrying capacity. Adenovirus vectors have been described in Graham *et al.*, 1977, Jones & Shenk, 1978, Graham & Prevec, 1991, Levrero *et al.*, 1991
20 and Gomez-Foix *et al.*, 1992, for example.

As used herein, the terms "herpes simplex virus" or "HSV" refer to a modified HSV vector deficient in one or more essential or non-essential HSV genes. The mature HSV virion consists of an enveloped icosahedral capsid with a viral genome consisting of a linear double-stranded DNA molecule that is 152 kb. In one embodiment, the HSV based
25 viral vector is replication deficient. Most replication deficient HSV vectors contain a deletion to remove one or more intermediate-early, early, or late HSV genes to prevent replication. For example, the HSV vector may be deficient in an immediate early gene selected from the group consisting of: ICP4, ICP22, ICP27, ICP47, and a combination thereof. Advantages of the HSV vector are its ability to enter a latent stage that can result
30 in long-term DNA expression and its large viral DNA genome that can accommodate exogenous DNA inserts of up to 25 kb. HSV-based vectors are described in, for example, U.S. Pat. Nos. 5,837,532, 5,846,782, and 5,804,413, and International Patent Applications

WO 91/02788, WO 96/04394, WO 98/15637, and WO 99/06583, each of which are incorporated by reference herein in its entirety.

A “subject,” as used herein, includes any animal that exhibits a symptom of a monogenic disease, disorder, or condition that can be treated with the gene therapy
5 vectors, cell-based therapeutics, and methods disclosed elsewhere herein. In preferred embodiments, a subject includes any animal that exhibits symptoms of a disease, disorder, or condition of the hematopoietic system. Suitable subjects (e.g., patients) include laboratory animals (such as mouse, rat, rabbit, or guinea pig), farm animals, and domestic animals or pets (such as a cat or dog). Non-human primates and, preferably,
10 human patients, are subjects. Typical subjects include animals that exhibit aberrant amounts (lower or higher amounts than a “normal” or “healthy” subject) of one or more physiological activities that can be modulated by gene therapy.

As used herein, the term “patient” refers to a subject that has been diagnosed with a particular disease, disorder, or condition that can be treated with the
15 compositions and methods contemplated herein.

As used herein “treatment” or “treating,” includes any beneficial or desirable effect on the symptoms or pathology of a disease or pathological condition, and may include even minimal reductions in one or more measurable markers of the disease or condition being treated. Treatment can involve optionally either the reduction or
20 amelioration of symptoms of the disease or condition, or the delaying of the progression of the disease or condition. “Treatment” does not necessarily indicate complete eradication or cure of the disease or condition, or associated symptoms thereof.

As used herein, “prevent,” and similar words such as “prevented,” “preventing” etc., indicate an approach for preventing, inhibiting, or reducing the likelihood of the
25 occurrence or recurrence of, a disease or condition. It also refers to delaying the onset or recurrence of a disease or condition or delaying the occurrence or recurrence of the symptoms of a disease or condition. As used herein, “prevention” and similar words also includes reducing the intensity, effect, symptoms and/or burden of a disease or condition prior to onset or recurrence of the disease or condition.

30 As used herein, the term “amount” refers to “an amount effective” or “an effective amount” of therapeutic cells to achieve a beneficial or desired prophylactic or therapeutic result, including clinical results.

A “prophylactically effective amount” refers to an amount of therapeutic cells effective to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount is less than the therapeutically effective amount.

5 A “therapeutically effective amount” of therapeutic cells may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the stem and progenitor cells to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the therapeutic cells are outweighed by the therapeutically beneficial effects. The term
10 “therapeutically effective amount” includes an amount that is effective to “treat” a subject (e.g., a patient).

In the following description, certain specific details are set forth in order to provide a thorough understanding of various illustrative embodiments of the invention contemplated herein. However, one skilled in the art will understand that particular
15 illustrative embodiments may be practiced without these details. In addition, it should be understood that the individual vectors, or groups of vectors, derived from the various combinations of the structures and substituents described herein, are disclosed by the present application to the same extent as if each vector or group of vectors was set forth individually. Thus, selection of particular vector structures or particular substituents is
20 within the scope of the present disclosure.

C. HEMATOPOIETIC STEM CELLS

Populations of hematopoietic cells contemplated in various embodiments comprise human hematopoietic stem cells (HSCs). The HSCs contemplated by the present disclosure express one or more cell surface markers of HSCs and that do not
25 express, or lack expression of, CD84. As used herein, the terms “HSC marker” or “marker of HSC” refers to a protein that is expressed in an HSC and/or whose expression distinguishes between an HSC and another hematopoietic and/or non-hematopoietic cell. HSC markers are preferably cell surface expressed proteins, referred to as “cell surface markers.” Cell surface markers are advantageous in
30 particular embodiments, because expression of cell surface proteins may be determined

by antibodies that bind the proteins. Cells expressing certain combinations of cell surface markers may be distinguished by positively and negatively selection based on binding to labeled binding agents such as antibodies. In one non-limiting example, cells may be labeled with one or more labeled antibodies that bind to an HSC marker
 5 and be positively selected for using flow cytometry or antibody conjugated beads, *e.g.*, antibody conjugated magnetic beads. In another non-limiting example, hematopoietic cells that are not HSCs can be “panned” from a populations of cells based on negative selection by being labeled with one or more labeled antibodies that bind to a marker of differentiated or lineage committed hematopoietic cells. In preferred embodiments,
 10 HSCs are selected with a combination of positive and negative selection.

In preferred embodiments, a population of cells comprising one or more isolated hematopoietic stem cells that are CD34⁺CD84⁻ hematopoietic stem cells are contemplated.

In preferred embodiments, a population of cells comprising one or more isolated
 15 hematopoietic stem cells that are CD34⁺CD84⁻ and comprise one or more of hematopoietic stem cell markers selected from the group consisting of CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/-}, CD164⁺, CD172a⁺, CD117⁺, and CD133⁺ are contemplated.

In particular embodiments, a population of hematopoietic cells comprises one or
 20 more isolated CD34⁺CD84⁻ hematopoietic stem cells. Populations of cells contemplated herein, may further comprise CD34⁺CD84⁻CD90⁺ hematopoietic stem cells, CD34⁺CD84⁻CD45RA⁻ hematopoietic stem cells, CD34⁺CD84⁻CD49f⁺ hematopoietic stem cells, CD34⁺CD84⁻CD38^{Lo/-} hematopoietic stem cells, CD34⁺CD84⁻CD164⁺ hematopoietic stem cells, CD34⁺CD84⁻CD172a⁺ hematopoietic stem cells,
 25 CD34⁺CD84⁻CD133⁺ hematopoietic stem cells, CD34⁺CD84⁻CD117⁺ hematopoietic stem cells, CD34⁺CD84⁻Lin⁻ hematopoietic stem cells, or CD34⁺CD84⁻ hematopoietic stem cells comprising any one or more of the cell surface marker expression patterns selected from the group consisting of: CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/-}, CD164⁺, CD172a⁺, CD133⁺, CD117⁺, and Lin⁻.

30 In particular embodiments, a population of hematopoietic cells comprises one or more isolated CD34⁺CD84⁻ hematopoietic stem cells modified by introducing genetic

material into the cell. CD34⁺CD84⁻ hematopoietic stem cells may be modified by introducing one or more exogenous polynucleotides into the cells. The exogenous polynucleotides may comprise mRNA or DNA. In particular embodiments, a polynucleotide comprising one or more expression control sequences operably linked to a polynucleotide encoding a therapeutic polypeptide is introduced into the cell. In other embodiments, an mRNA encoding a therapeutic polypeptide is introduced into the cell.

In particular embodiments, a population of hematopoietic cells comprises one or more isolated CD34⁺CD84⁻ hematopoietic stem cells modified by introducing a vector, *e.g.*, viral vector into the cell. The viral vector may be a retroviral vector, such as a lentiviral vector, an AAV, a vaccinia viral vector, and adenoviral vector, or an HSV vector comprising one or more exogenous polynucleotides. The exogenous polynucleotides may encode one or more therapeutic polypeptides or one or more expression control sequences operably linked to a polynucleotide encoding a therapeutic polypeptide. In particular embodiments, the vector comprises one or more expression control sequences operably linked to a polynucleotide encoding a globin, a β -globin, a γ -globin, and anti-sickling globin, an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide, an adenosine deaminase polypeptide, an interleukin 2 receptor gamma polypeptide, a tripeptidyl peptidase 1 polypeptide, an alpha-L iduronidase polypeptide, or an iduronate 2-sulfatase polypeptide.

In particular embodiments, a population of hematopoietic cells comprises one or more isolated CD34⁺CD84⁻ hematopoietic stem cells modified by introducing one or more engineered nucleases that bind and cleave a target site in the cell's genome. Illustrative examples of engineered nucleases that can be introduced into the cells to effect gene editing include, but are not limited to, homing endonuclease variants, megaTALs, TALENs, zinc finger nucleases, CRISPR/CAS9 nuclease systems, and ARCUS nucleases. In particular embodiments, the engineered nuclease is introduced into the cells and disrupts gene function and/or expression by creating a double-strand break in the genome that is repaired by non-homologous end joining (NHEJ). In particular embodiments, a homology repair template and an engineered nuclease are introduced into the cells and disrupts or restores gene function and/or expression by

creating a double-strand break in the genome that is repaired by homology directed repair (HDR) and insertion of the homology repair template into the genome at the site of the double-strand break.

In particular embodiments, a population of hematopoietic cells comprises one or more isolated CD34⁺CD84⁻ hematopoietic stem cells cultured *ex vivo*.

In particular embodiments, a population of hematopoietic cells comprises one or more isolated CD34⁺CD84⁻ hematopoietic stem cells modified by introducing genetic material into the cell and cultured *ex vivo*.

D. COMPOSITIONS AND FORMULATIONS

Compositions comprising HSCs that express one or more cell surface HSC markers and that do not express, or lack expression of, CD84 are contemplated in various embodiments. In particular embodiments, compositions comprise one or more isolated CD34⁺CD84⁻ hematopoietic stem cells. In particular embodiments, compositions comprise one or more isolated CD34⁺CD84⁻ hematopoietic stem cells that comprise one or more hematopoietic stem cell markers selected from the group consisting of CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/-}, CD164⁺, CD172a⁺, CD117⁺, and CD133⁺. Compositions may comprise one or more isolated CD34⁺CD84⁻ hematopoietic stem cells, CD34⁺CD84⁻CD90⁺ hematopoietic stem cells, CD34⁺CD84⁻CD45RA⁻ hematopoietic stem cells, CD34⁺CD84⁻CD49f⁺ hematopoietic stem cells, CD34⁺CD84⁻CD38^{Lo/-} hematopoietic stem cells, CD34⁺CD84⁻CD164⁺ hematopoietic stem cells, CD34⁺CD84⁻CD172a⁺ hematopoietic stem cells, CD34⁺CD84⁻CD133⁺ hematopoietic stem cells, CD34⁺CD84⁻CD117⁺ hematopoietic stem cells, CD34⁺CD84⁻Lin⁻ hematopoietic stem cells, or CD34⁺CD84⁻ hematopoietic stem cells comprising any one or more of the cell surface marker expression patterns selected from the group consisting of: CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/-}, CD164⁺, CD172a⁺, CD133⁺, CD117⁺, and Lin⁻.

The formulations and compositions contemplated in particular embodiments may comprise genetically modified and/or cultured HSCs and/or HSCs comprising one or more, viral vectors, polypeptides, or polynucleotides, formulated in pharmaceutically-acceptable or physiologically-acceptable solutions (*e.g.*, culture

medium) for administration to a cell, tissue, organ, or an animal, either alone, or in combination with one or more other modalities of therapy. Compositions include, without limitation, cultures contemplated herein, and in particular embodiments, the terms composition and culture may be used synonymously.

5 Particular *ex vivo* and *in vitro* formulations and compositions contemplated herein may comprise a population of hematopoietic cells that has been enriched for CD34⁺CD84⁻ hematopoietic stem cells formulated in pharmaceutically-acceptable or physiologically-acceptable solutions (*e.g.*, culture medium) for administration to a cell, tissue, organ, or an animal, either alone, or in combination with one or more other
10 modalities of therapy.

In certain embodiments, compositions contemplated herein comprise a population of cells comprising a therapeutically-effective amount of CD34⁺CD84⁻ hematopoietic stem cells, as described herein, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents (*e.g.*, pharmaceutically
15 acceptable cell culture medium).

In particular embodiments, a composition comprises a population of hematopoietic cells and a therapeutically-effective amount of CD34⁺CD84⁻ hematopoietic stem cells. In particular illustrative embodiments, the population of hematopoietic cells comprises at least about 30%, at least about 40%, at least about
20 50%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% CD34⁺CD84⁻ hematopoietic stem cells, including any intervening percentages.

25 In particular illustrative embodiments, the population of hematopoietic cells comprises about 30%, about 40%, about 50%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% CD34⁺CD84⁻ hematopoietic stem cells, including any intervening percentages.

30 In other illustrative embodiments, the population of hematopoietic cells comprises at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least

70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% CD34⁺CD84⁻ hematopoietic stem cells, including any intervening percentages.

- 5 Compositions contemplated in particular embodiments, comprise a population of hematopoietic cells comprising CD34⁺CD84⁻ hematopoietic stem cells that have been cultured for about 6, about 12, about 24, about 36, about 48, about 60, or about 72 hours or more.

- In some embodiments, a composition comprises a population of hematopoietic
10 cells comprising CD34⁺CD84⁻ hematopoietic stem cells that have one of the following β -globin alleles: β^E/β^0 , β^C/β^0 , β^0/β^0 , β^E/β^E , β^C/β^+ , β^E/β^+ , β^0/β^+ , β^+/ β^+ , β^C/β^C , β^E/β^S , β^0/β^S , β^C/β^S , β^+/ β^S or β^S/β^S .

- Pharmaceutical compositions contemplated herein comprise a pharmaceutically acceptable carrier and one or more CD34⁺CD84⁻ hematopoietic stem cells produced,
15 genetically modified, cultured, and/or isolated according to methods described herein.

- The phrase “pharmaceutically acceptable” refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state
20 government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

- The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic cells are administered. Illustrative examples of pharmaceutical carriers can be sterile liquids, such as cell culture media, water and oils, including those of
25 petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients in particular embodiments, include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol
30 monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Except insofar as any conventional media or agent is incompatible

with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

In one embodiment, a composition comprising a carrier is suitable for parenteral administration, *e.g.*, intravascular (intravenous or intraarterial), intraperitoneal or
5 intramuscular administration. Pharmaceutically acceptable carriers include sterile aqueous solutions, cell culture media, or dispersions. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the transduced cells, use thereof in the pharmaceutical compositions is contemplated.

10 In particular embodiments, compositions contemplated herein comprise one or more CD34⁺CD84⁻ hematopoietic stem cells and a pharmaceutically acceptable carrier, *e.g.*, pharmaceutically acceptable cell culture medium. In particular embodiments, compositions contemplated herein comprise one or more genetically modified CD34⁺CD84⁻ hematopoietic stem cells and a pharmaceutically acceptable carrier, *e.g.*,
15 pharmaceutically acceptable cell culture medium.

A composition comprising a cell-based composition contemplated herein can be administered separately by enteral or parenteral administration methods or in combination with other suitable compounds to effect the desired treatment goals.

The pharmaceutically acceptable carrier must be of sufficiently high purity and
20 of sufficiently low toxicity to render it suitable for administration to the human subject being treated. It further should maintain or increase the stability of the composition. The pharmaceutically acceptable carrier can be liquid or solid and is selected, with the planned manner of administration in mind, to provide for the desired bulk, consistency, *etc.*, when combined with other components of the composition. For example, the
25 pharmaceutically acceptable carrier can be, without limitation, a binding agent (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, *etc.*), a filler (*e.g.*, lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates, calcium hydrogen phosphate, *etc.*), a lubricant (*e.g.*, magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid,
30 metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, *etc.*), a disintegrant (*e.g.*, starch, sodium starch

glycolate, *etc.*), or a wetting agent (*e.g.*, sodium lauryl sulfate, *etc.*). Other suitable pharmaceutically acceptable carriers for the compositions contemplated herein include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatins, amyloses, magnesium stearates, talcs, silicic acids, viscous paraffins,

5 hydroxymethylcelluloses, polyvinylpyrrolidones and the like.

Such carrier solutions also can contain buffers, diluents and other suitable additives. The term “buffer” as used herein refers to a solution or liquid whose chemical makeup neutralizes acids or bases without a significant change in pH.

Examples of buffers contemplated herein include, but are not limited to, Dulbecco’s
10 phosphate buffered saline (PBS), Ringer’s solution, 5% dextrose in water (D5W), normal/physiologic saline (0.9% NaCl).

The pharmaceutically acceptable carriers and/or diluents may be present in amounts sufficient to maintain a pH of the therapeutic composition of about 7.

Alternatively, the therapeutic composition has a pH in a range from about 6.8 to about
15 7.4, *e.g.*, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, and 7.4. In still another embodiment, the therapeutic composition has a pH of about 7.4.

Compositions contemplated herein may comprise a nontoxic pharmaceutically acceptable medium. The compositions may be a suspension. The term “suspension” as used herein refers to non-adherent conditions in which cells are not attached to a solid
20 support. For example, cells maintained as a suspension may be stirred or agitated and are not adhered to a support, such as a culture dish.

In particular embodiments, compositions contemplated herein are formulated in a suspension, where the hematopoietic stem cells are dispersed within an acceptable liquid medium or solution, *e.g.*, saline or serum-free medium, in an intravenous (IV)
25 bag or the like. Acceptable diluents include, but are not limited to water, PlasmaLyte, Ringer’s solution, isotonic sodium chloride (saline) solution, serum-free cell culture medium, and medium suitable for cryogenic storage, *e.g.*, Cryostor® medium.

In certain embodiments, a pharmaceutically acceptable carrier is substantially free of natural proteins of human or animal origin, and suitable for storing a
30 composition comprising a population of hematopoietic stem cells. The composition is intended to be administered into a human patient, and thus is substantially free of cell

culture components such as bovine serum albumin, horse serum, and fetal bovine serum.

In some embodiments, compositions are formulated in a pharmaceutically acceptable cell culture medium. Such compositions are suitable for administration to human subjects. In particular embodiments, the pharmaceutically acceptable cell culture medium is a serum free medium.

Serum-free medium has several advantages over serum containing medium, including a simplified and better defined composition, a reduced degree of contaminants, elimination of a potential source of infectious agents, and lower cost. In various embodiments, the serum-free medium is animal-free, and may optionally be protein-free. Optionally, the medium may contain biopharmaceutically acceptable recombinant proteins. "Animal-free" medium refers to medium wherein the components are derived from non-animal sources. Recombinant proteins replace native animal proteins in animal-free medium and the nutrients are obtained from synthetic, plant or microbial sources. "Protein-free" medium, in contrast, is defined as substantially free of protein.

Illustrative examples of serum-free media used in particular compositions includes, but is not limited to QBSF-60 (Quality Biological, Inc.), StemPro-34 (Life Technologies), and X-VIVO 10.

In a preferred embodiment, the compositions comprising hematopoietic stem and/or progenitor cells are formulated in PlasmaLyte.

In various embodiments, compositions comprising hematopoietic stem and/or progenitor cells are formulated in a cryopreservation medium. For example, cryopreservation media with cryopreservation agents may be used to maintain a high cell viability outcome post-thaw. Illustrative examples of cryopreservation media used in particular compositions includes, but is not limited to, CryoStor CS10, CryoStor CS5, and CryoStor CS2.

In one embodiment, the compositions are formulated in a solution comprising 50:50 PlasmaLyte A to CryoStor CS10.

In particular embodiments, the composition is substantially free of mycoplasma, endotoxin, and microbial contamination. By "substantially free" with respect to

endotoxin is meant that there is less endotoxin per dose of cells than is allowed by the FDA for a biologic, which is a total endotoxin of 5 EU/kg body weight per day, which for an average 70 kg person is 350 EU per total dose of cells. In particular embodiments, compositions comprising hematopoietic stem or progenitor cells transduced with a retroviral vector contemplated herein contains about 0.5 EU/mL to about 5.0 EU/mL, or about 0.5 EU/mL, 1.0 EU/mL, 1.5 EU/mL, 2.0 EU/mL, 2.5 EU/mL, 3.0 EU/mL, 3.5 EU/mL, 4.0 EU/mL, 4.5 EU/mL, or 5.0 EU/mL.

In various embodiments, compositions comprising one or more CD34⁺CD84⁻ hematopoietic stem cells are useful for *ex vivo* and *in vivo* cell-based therapies. In particular embodiments, compositions may comprise a purified or isolated population of CD34⁺CD84⁻ cells in culture, *i.e.*, a cell culture composition. A cell culture composition may comprise a population of cells comprising one or more CD34⁺CD84⁻ and a suitable cell culture medium. Cell culture compositions contemplated in particular embodiments may be formulated for administration to a subject.

It would be understood by the skilled artisan that particular embodiments contemplated herein may comprise other formulations, such as those that are well known in the pharmaceutical art, and are described, for example, in *Remington: The Science and Practice of Pharmacy*, 20th Edition. Baltimore, MD: Lippincott Williams & Wilkins, 2005, which is incorporated by reference herein, in its entirety.

E. METHODS

Hematopoietic stem cells contemplated in particular embodiments are suitable for administration to a subject in need thereof. In various embodiments, a therapeutically effective amount of CD34⁺CD84⁻CD90⁺CD45RA⁻ HSCs is administered to a subject in need thereof to provide curative, preventative, or ameliorative benefits to a subject diagnosed with or that is suspected of having monogenic disease, disorder, or condition or a disease, disorder, or condition that is amenable to hematopoietic stem cell therapy.

In particular embodiments, CD34⁺CD84⁻CD90⁺CD45RA⁻ HSCs are genetically modified to express a therapeutic polypeptide and administered to an individual in need of therapy for a lysosomal storage disorder, an adrenoleukodystrophy or adrenomyeloneuropathy.

In particular embodiments, CD34⁺CD84⁺CD90⁺CD45RA⁻ HSCs are genetically modified to express a therapeutic polypeptide and administered to an individual to treat, prevent, and/or ameliorate a monogenic disease, disorder, or condition or a disease, disorder, or condition of the hematopoietic system in a subject, *e.g.*, a

5 hemoglobinopathy.

Illustrative examples of monogenic disorders and their corresponding therapeutic gene(s)/polypeptides that are suitable for use in particular embodiments contemplated herein include, but are not limited to: X-SCID (IL2R γ), ADA-SCID (adenosine deaminase), Batten's disease (tripeptidyl peptidase 1),

10 Mucopolysaccharidosis type 1 - Hurler Syndrome (alpha-L iduronidase), Mucopolysaccharidosis type 2 - Hunter Syndrome (iduronate 2-sulfatase), Mucopolysaccharidosis type 3 - Sanfilippo Syndrome (N-sulfoglucosamine sulfohydrolase), Mucopolysaccharidosis type 4A - Morquio A Syndrome (galactosamine-6 sulfatase), Mucopolysaccharidosis type 4B - Morquio B Syndrome
15 (beta-galactosidase), Mucopolysaccharidosis type 6 - Maroteaux-Lamy Syndrome (N-acetylgalactosamine- 4-sulphatase), Gaucher disease (glucocerebrosidase), Metachromatic Leukodystrophy (arylsulfatase A), chronic granulomatous diseases (cytochrome b-245 alpha chain, cytochrome b-245 beta chain, neutrophil cytosolic factor 1, neutrophil cytosolic factor 2, neutrophil cytosolic factor 4), Wiskott Aldrich Syndrome (wiskott
20 aldrich syndrome protein), perforin deficiency (PRF1), Fanconi Anemia (FANC genes, *e.g.*, FANCA, FANCC, FANCG), X-linked lymphoproliferative syndrome (SH2D1A), RAG deficiency (RAG1/2), Artemis protein deficiency (DCLRE1C), Hemophilia A (Factor VIII), Hemophilia B (Factor IX), and Leukocyte adhesion deficiency (CD18).

As used herein, the term "hemoglobinopathy" refers to a diverse group of
25 inherited blood disorders that involve the presence of abnormal hemoglobin molecules resulting from alterations in the structure and/or synthesis of hemoglobin. The most common hemoglobinopathies include sickle cell disease, β -thalassemia, and α -thalassemia.

In particular embodiments, genetically modified CD34⁺CD84⁺CD90⁺CD45RA⁻
30 HSCs are used to treat, prevent, or ameliorate a hemoglobinopathy is selected from the group consisting of: hemoglobin C disease, hemoglobin E disease, sickle cell anemia, sickle cell disease (SCD), thalassemia, β -thalassemia, thalassemia major, thalassemia

intermedia, α -thalassemia, hemoglobin Bart syndrome and hemoglobin H disease. In some embodiments, the genetically modified $CD34^+CD84^-CD90^+CD45RA^-$ HSCs have a β -globin genotype selected from the group consisting of: β^E/β^0 , β^C/β^0 , β^0/β^0 , β^E/β^E , β^C/β^+ , β^E/β^+ , β^0/β^+ , β^+/ β^+ , β^C/β^C , β^E/β^S , β^0/β^S , β^C/β^S , β^+/ β^S or β^S/β^S .

5 In one embodiment, a dose of $CD34^+CD84^-CD90^+CD45RA^-$ HSCs is delivered to a subject intravenously.

In particular embodiments, a population of $CD34^+CD84^-CD90^+CD45RA^-$ HSCs is transduced with a vector and the transduced cells are administered to an individual in need of therapy for an adrenoleukodystrophy or an adrenomyeloneuropathy.

10 In particular embodiments, a population of $CD34^+CD84^-CD90^+CD45RA^-$ HSCs is transduced with a vector and the transduced cells are administered to an individual in need of therapy for ADA-SCID, X-SCID, Batten's Disease, MPSI, or MPSII.

In particular embodiments, a method for isolating and/or quantitating human $CD34^+CD84^-$ hematopoietic stem cells from a heterologous cell population is provided.

15 The method may comprise contacting a heterologous cell population, including but not limited to bone marrow, mobilized peripheral blood, umbilical cord blood, and placenta, with one or more binding agents for a time sufficient to isolate human $CD34^+CD84^-$ hematopoietic stem cells from the population.

In certain embodiments, any suitable binding agent may be used to bind and isolate
20 the $CD34^+CD84^-$ hematopoietic stem cells from the population.

In preferred embodiments, the binding agents used to bind and isolate the $CD34^+CD84^-$ hematopoietic stem cells from the population comprise antibodies or antigen binding fragments thereof. The binding agents may be directed against CD34 and CD84 and one or more cell surface markers selected from the group consisting of: $CD90^+$,
25 $CD45RA^-$, $CD49f^+$, $CD38^{Lo/}$, $CD164^+$, $CD172a^+$, $CD117^+$, and $CD133^+$. It would be understood that both positive and negative antibody-based selection methods may be employed. For example, a CD34 antibody may be used to positively select for hematopoietic stem cells expressing this marker ($CD34^+$) and a CD84 antibody may be used to negatively select undesired cells that express CD84; the resulting population would
30 then comprise $CD34^+CD84^-$ hematopoietic stem cells.

In methods directed to quantitating cells, the isolated cells may then be counted.

F. KITS

In particular embodiments, a kit for isolating and/or quantitating human CD34⁺CD84⁻ hematopoietic stem cells from a heterologous cell population is provided. The kit comprise one or more binding agents and instructions for contacting a heterologous
5 cell population, including but not limited to bone marrow, mobilized peripheral blood, umbilical cord blood, and placenta, with the one or more binding agents for a time sufficient to isolate human CD34⁺CD84⁻ hematopoietic stem cells from the population.

In certain embodiments, any suitable binding agent may be used to bind and isolate the CD34⁺CD84⁻ hematopoietic stem cells from the population.

10 In preferred embodiments, the binding agents used to bind and isolate the CD34⁺CD84⁻ hematopoietic stem cells from the population comprise antibodies or antigen binding fragments thereof. The binding agents may be directed against CD34 and CD84 and one or more cell surface markers selected from the group consisting of: CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/}, CD164⁺, CD172a⁺, CD117⁺, and CD133⁺. It would be
15 understood that both positive and negative antibody-based selection methods may be employed. For example, a CD34 antibody may be used to positively select for hematopoietic stem cells expressing this marker (CD34⁺) and a CD84 antibody may be used to negatively select undesired cells that express CD84; the resulting population would then comprise CD34⁺CD84⁻ hematopoietic stem cells.

20

All publications, patent applications, and issued patents cited in this specification are herein incorporated by reference as if each individual publication, patent application, or issued patent were specifically and individually indicated to be incorporated by reference.

25 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims. The following examples are provided by way of
30 illustration only and not by way of limitation. Those of skill in the art will readily

recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

EXAMPLES

EXAMPLE 1ViSNE MAPPING OF THE CD34⁺ CELL COMPARTMENT

Healthy donor bone marrow (BM) and GCSF-mobilized peripheral blood (mPB) were collected by apheresis. Bone marrow mononuclear cells (MNCs) were isolated by density gradient separation (Ficoll-Paque). CD34⁺ cells were then isolated from the BM MNCs using the CD34 MicroBead Kit and MACS cell separation columns (Miltenyi Biotec).

mPB was collected by apheresis, washed to remove platelets and incubated with CliniMACS CD34 reagent. Labeled CD34⁺ cells were then enriched using the CliniMACS Plus instrument (Miltenyi Biotec). Enriched cells were aliquoted and cryopreserved in CryoStor CS10.

To generate cultured cells, mPB CD34⁺ cells were thawed and cultured in SCGM (CellGenix) supplemented with 100 ng/ml Flt3, Scf, and TPO (CellGenix) for 72 hrs at 37°C and 5% CO₂.

For CyTOF, cells were stained for BMI-1, CD117, CD123, CD2, CD34, CD38, CD45, CD71, CD90, CEBPa, FoxO1, FoxP1, HLA-DR, PBX1, SATB1, TCRab, TdT, DNA (iridium), and viability (CisPlatin), using lanthanide-conjugated antibodies and Cell-ID reagents (Fluidigm, Inc). Mass cytometry data was acquired using CyTOF 2 instrument (Fluidigm), normalized, and DNA⁺ viable CD34⁺ single cells were analyzed using ViSNE algorithm (Cytobank). BMI-1, CD117, CD123, CD2, CD38, CD71, CD90, CEBPa, FoxO1, FoxP1, HLA-DR, PBX1, SATB1, TCRab, and TdT were used for ViSNE analysis.

A phenotypic tSNE map of single CD34⁺ cells from human bone marrow (Figure 1; BM; left panels) and mobilized peripheral blood (Figure 1; mPB; middle panels) show similar but distinct distribution of cell types. However, CD34⁺ cells substantially alter their phenotypes after culture (Figure 1; cultured CD34⁺ from mPB; right panels; dark arrows). CD38 expression is downregulated in culture and consequently most cultured cells are CD38 negative (Figure 1; cultured CD34⁺ from

mPB; right center panel; white arrow), making it impossible the detection of hematopoietic stem cells post culture.

EXAMPLE 2

ANALYZING CELL SURFACE MARKER EXPRESSION OF CD34⁺ BONE MARROW CELLS

5 Bone marrow was collected from 8 healthy donors and was pooled, volume reduced and RBC-depleted using centrifugation (Optia). Post processing on the Optia device BM MNCs were incubated with CliniMACS CD34 reagent. Labeled CD34⁺ cells were then enriched using the CliniMACS Plus instrument (Miltenyi Biotec). Enriched cells were aliquoted and cryopreserved in CryoStor CS10. 5 million cells
10 were stained with CD7-, CD33, CD34, CD38, and 7-AAD and aliquoted into LEGENDSCREEN 96-well plates (Biolegend) containing 348 PE-labeled antibodies for surface markers. FACS data was acquired for each well and analyzed in Cytobank. Potential stem cell markers, including CD84, were identified (Figure 2, boxed markers).

EXAMPLE 3

15 LINEAGE TRACING OF CD34⁺ CELLS FROM BONE MARROW AND MOBILIZED PERIPHERAL BLOOD

Pooled CD34⁺ cells isolated from bone marrow and mobilized peripheral blood were thawed, resuspended in SCGM, and labeled with Cell-Trace CFSE (ThermoFisher). Labeled cells were stained with CD34, CD38, CD45RA, and CD90
20 antibodies and at least 10,000 CD34⁺CD38^{lo/-}CD90⁺CD45RA⁻ long-term hematopoietic stem cells (LT-HSCs) were isolated by FACS and combined with at least 1×10^6 reserved, CFSE⁻ CD34⁺ cells from the same sample such that the final CFSE⁺ LT-HSC content was at least 1%. The cell mixture was cultured in SCGM supplemented with 100 ng/ml Flt3, Scf, and TPO for 72 hrs at 37°C and 5% CO₂ and frozen in CryoStor
25 CS10.

For CyTOF, CD34⁺ cells were stained for Fluorescein (CFSE), CD34, CD38, CD84, CD90, CD117, CD164, and CD172a, DNA (iridium), and viability (CisPlatin), using lanthanide-conjugated antibodies and Cell-ID reagents (Fluidigm, Inc). Mass

cytometry data was acquired using CyTOF 2 instrument (Fluidigm), normalized, and DNA⁺ viable CD34⁺ single cells were analyzed using Cytobank. Figure 3.

Analysis of traced HSCs showed that CD84 is a candidate stem cell marker when used in combination with other hematopoietic stem cell markers, serving as a
5 surrogate for CD38 which expression is downregulated *in vitro* (Figure 3, arrows).

In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full
10 scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

1. A population of human hematopoietic cells comprising isolated human CD34⁺CD84⁻ hematopoietic stem cells.
2. The population of human hematopoietic cells of claim 1, wherein the human CD34⁺CD84⁻ hematopoietic stem cells are also CD90⁺.
3. The population of human hematopoietic cells of claim 1 or claim 2, wherein the human CD34⁺CD84⁻ hematopoietic stem cells are also CD45RA⁺.
4. The population of human hematopoietic cells of any one of claims 1-3, wherein the human CD34⁺CD84⁻ hematopoietic stem cells are also CD49f⁺.
5. The population of human hematopoietic cells of any one of claims 1-4, wherein the human CD34⁺CD84⁻ hematopoietic stem cells are also CD38^{Lo/}.
6. The population of human hematopoietic cells any one of claims 1-3, wherein the human CD34⁺CD84⁻ hematopoietic stem cells are also CD164⁺.
7. The population of human hematopoietic cells any one of claims 1-4, wherein the human CD34⁺CD84⁻ hematopoietic stem cells are also CD172a⁺.
8. The population of human hematopoietic cells any one of claims 1-4, wherein the human CD34⁺CD84⁻ hematopoietic stem cells are also CD117⁺.
9. The population of human hematopoietic cells any one of claims 1-4, wherein the human CD34⁺CD84⁻ hematopoietic stem cells are also CD133⁺.
10. A population of cultured hematopoietic cells enriched for the human hematopoietic stem cells of any one of claims 1-9.

11. A population of cultured hematopoietic cells enriched for human CD34⁺CD84⁻ hematopoietic stem cells.
12. A population of cultured hematopoietic cells enriched for human CD34⁺CD84⁻CD90⁺ CD45RA⁻ hematopoietic stem cells.
13. A population of cultured hematopoietic cells enriched for human CD34⁺CD84⁻ and one or more of hematopoietic stem cell markers selected from the group consisting of CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/}, CD164⁺, CD172a⁺, CD117⁺, and CD133⁺.
14. A human CD34⁺CD84⁻ hematopoietic stem cell comprising a gene therapy vector.
15. The CD34⁺CD84⁻ hematopoietic stem cell of claim 14, further comprising one or more of hematopoietic stem cell markers selected from the group consisting of CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/}, CD164⁺, CD172a⁺, CD117⁺, and CD133⁺.
16. A human CD34⁺CD84⁻ hematopoietic stem cell comprising one or more genome modifications.
17. The CD34⁺CD84⁻ hematopoietic stem cell of claim 16, further comprising one or more of hematopoietic stem cell markers selected from the group consisting of CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/}, CD164⁺, CD172a⁺, CD117⁺, and CD133⁺.
18. A human CD34⁺CD84⁻ hematopoietic stem cell comprising one or more exogenous polynucleotides.
19. The CD34⁺CD84⁻ hematopoietic stem cell of claim 18, further comprising one or more of hematopoietic stem cell markers selected from the group consisting of CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/}, CD164⁺, CD172a⁺, CD117⁺, and CD133⁺.

20. A composition comprising at least 50%, at least 75%, at least 85%, at least 90%, or at least 95% of the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, or one or more human hematopoietic stem cells of any one of claims 14-19.

21. A composition comprising at least 50%, at least 75%, at least 85%, at least 90%, or at least 95% human CD34⁺CD84⁻ hematopoietic stem cells.

22. The composition of claim 20 or claim 21, comprising a pharmaceutically acceptable carrier.

23. The composition of any one of claims 20-22, wherein the pharmaceutically acceptable carrier comprises a physiologically acceptable cell culture medium.

24. A pharmaceutical composition comprising the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, or one or more human hematopoietic stem cells of any one of claims 14-19; and a pharmaceutically acceptable cell culture medium.

25. A method of treating a subject in need thereof comprising administering an effective amount of the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, one or more human hematopoietic stem cells of any one of claims 14-19, a composition of any one of claims 20-23, or a pharmaceutical composition of claim 24 to the subject.

26. A method of treating a subject having a hemoglobinopathy comprising administering an effective amount of the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, one or more human hematopoietic stem cells of any one of claims 14-19, a

composition of any one of claims 20-23, or a pharmaceutical composition of claim 24 to the subject.

27. A method of treating sickle cell disease in a subject comprising administering an effective amount of the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, one or more human hematopoietic stem cells of any one of claims 14-19, a composition of any one of claims 20-23, or a pharmaceutical composition of claim 24 to the subject.

28. A method of treating a β -thalassemia in a subject comprising administering an effective amount of the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, one or more human hematopoietic stem cells of any one of claims 14-19, a composition of any one of claims 20-23, or a pharmaceutical composition of claim 24 to the subject.

29. The method of any one of claims 25-28, wherein one or more of the human $CD34^+CD84^-$ hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector encoding a globin.

30. The method of claim 29, wherein the retroviral vector or the lentiviral vector is an AnkT9W vector, a T9Ank2W vector, a TNS9 vector, a lentiglobin HPV569 vector, a lentiglobin BB305 vector, a BG-1 vector, a BGM-1 vector, a d432 β A γ vector, a mLAR β A γ V5 vector, a GLOBE vector, a G-GLOBE vector, a β AS3-FB vector, a V5 vector, a V5m3 vector, a V5m3-400 vector, a G9 vector, or a BCL11A shmir vector.

31. A method of treating adrenoleukodystrophy or adrenomyeloneuropathy in a subject comprising administering an effective amount of the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, one or more human hematopoietic stem

cells of any one of claims 14-19, a composition of any one of claims 20-23, or a pharmaceutical composition of claim 24 to the subject.

32. The method of claim 31, wherein one or more of the human CD34⁺CD84⁻ hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector encoding an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide.

33. The method of claim 31, wherein the retroviral vector or lentiviral vector comprises a myeloproliferative sarcoma virus enhancer, negative control region deleted, d1587rev primer-binding site substituted (MND) promoter or transcriptionally active fragment thereof operably linked to a polynucleotide encoding an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide.

34. A method of treating ADA-SCID in a subject comprising administering an effective amount of the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, one or more human hematopoietic stem cells of any one of claims 14-19, a composition of any one of claims 20-23, or a pharmaceutical composition of claim 24 to the subject.

35. The method of claim 34, wherein one or more of the human CD34⁺CD84⁻ hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector encoding an adenosine deaminase.

36. The method of claim 34, wherein the retroviral vector or lentiviral vector comprises an elongation factor 1 alpha promoter or and MND promoter operably linked to a polynucleotide encoding adenosine deaminase.

37. A method of treating X-SCID in a subject comprising administering an effective amount of the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, one

or more human hematopoietic stem cells of any one of claims 14-19, a composition of any one of claims 20-23, or a pharmaceutical composition of claim 24 to the subject.

38. The method of claim 37, wherein one or more of the human CD34⁺CD84⁻ hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector encoding an interleukin 2 receptor gamma.

39. The method of claim 37, wherein the retroviral vector or lentiviral vector comprises an elongation factor 1 alpha promoter operably linked to a polynucleotide encoding interleukin 2 receptor gamma.

40. A method of treating Batten's disease in a subject comprising administering an effective amount of the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, one or more human hematopoietic stem cells of any one of claims 14-19, a composition of any one of claims 20-23, or a pharmaceutical composition of claim 24 to the subject.

41. The method of claim 40, wherein one or more of the human CD34⁺CD84⁻ hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector encoding a tripeptidyl peptidase 1.

42. The method of claim 40, wherein the retroviral vector or lentiviral vector comprises an elongation factor 1 alpha promoter or comprises a myeloproliferative sarcoma virus enhancer, negative control region deleted, d1587rev primer-binding site substituted (MND) promoter operably linked to a polynucleotide encoding tripeptidyl peptidase 1.

43. A method of treating MPS I in a subject comprising administering an effective amount of the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, one or more human hematopoietic stem cells of any one of claims 14-19, a composition of any one of claims 20-23, or a pharmaceutical composition of claim 24 to the subject.

44. The method of claim 43, wherein one or more of the human CD34⁺CD84⁻ hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector encoding an alpha-L iduronidase.

45. The method of claim 43, wherein the retroviral vector or lentiviral vector comprises an elongation factor 1 alpha promoter or comprises a myeloproliferative sarcoma virus enhancer, negative control region deleted, d1587rev primer-binding site substituted (MND) promoter operably linked to a polynucleotide encoding alpha-L iduronidase.

46. A method of treating MPS II in a subject comprising administering an effective amount of the population of human hematopoietic stem cells of any one of claims 1-9, the population of cultured human hematopoietic stem cells of any one of claims 10-13, one or more human hematopoietic stem cells of any one of claims 14-19, a composition of any one of claims 20-23, or a pharmaceutical composition of claim 24 to the subject.

47. The method of claim 46, wherein one or more of the human CD34⁺CD84⁻ hematopoietic stem cells are transduced with a retroviral vector or a lentiviral vector encoding an iduronate 2-sulfatase.

48. The method of claim 46, wherein the retroviral vector or lentiviral vector comprises an elongation factor 1 alpha promoter or comprises a myeloproliferative sarcoma virus enhancer, negative control region deleted, d1587rev primer-binding site substituted (MND) promoter operably linked to a polynucleotide encoding iduronate 2-sulfatase.

49. A method for isolating human CD34⁺CD84⁻ hematopoietic stem cells from a heterologous cell population comprising: contacting a heterologous cell population with one or more binding agents to isolate human CD34⁺CD84⁻ hematopoietic stem cells from the population.

50. The method of claim 49, wherein the one or more binding agents are antibodies or antigen binding fragments thereof.

51. The method of claim 48 or claim 49, wherein the one or more binding agents bind CD34 and CD84 and one or more cell surface markers selected from the group consisting of: CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/}, CD164⁺, CD172a⁺, CD117⁺, and CD133⁺.

52. The method of any one of claims 49-51, wherein the isolating the human CD34⁺CD84⁻ hematopoietic stem cells comprises steps of positive and negative selection.

53. The method of any one of claims 48-52, wherein the human CD34⁺CD84⁻ hematopoietic stem cells comprise one or more gene therapy vectors, genome modifications, or exogenous polynucleotides.

54. The method of any one of claims 48-53, wherein the heterologous cell population is selected from the group consisting of: bone marrow, mobilized peripheral blood, umbilical cord blood, placenta, or fractions thereof.

55. A method for quantitating human CD34⁺CD84⁻ hematopoietic stem cells from a heterologous cell population comprising: contacting a heterologous cell population with one or more binding agents to identify human CD34⁺CD84⁻ hematopoietic stem cells from the population, and quantitating the identified cells.

56. The method of claim 55, wherein the one or more binding agents are antibodies or antigen binding fragments thereof.

57. The method of claim 55 or claim 56, wherein the one or more binding agents bind CD34 and CD84 and one or more cell surface markers selected from the group consisting of: CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/}, CD164⁺, CD172a⁺, CD117⁺, and CD133⁺.

58. The method of any one of claims 55-57, wherein the isolating the human CD34⁺CD84⁻ hematopoietic stem cells comprises steps of positive and negative selection.

59. The method of any one of claims 55-58, wherein the human CD34⁺CD84⁻ hematopoietic stem cells comprise one or more gene therapy vectors, genome modifications, or exogenous polynucleotides.

60. The method of any one of claims 55-59, wherein the heterologous cell population is selected from the group consisting of: bone marrow, mobilized peripheral blood, umbilical cord blood, placenta, or fractions thereof.

61. A kit comprising one or more binding agents to isolate or quantitate human CD34⁺CD84⁻ hematopoietic stem cells from a heterologous cell population.

62. The kit of claim 61, wherein the one or more binding agents are antibodies or antigen binding fragments thereof.

63. The kit of claim 61 or claim 62, wherein the one or more binding agents bind CD34 and CD84 and one or more cell surface markers selected from the group consisting of: CD90⁺, CD45RA⁻, CD49f⁺, CD38^{Lo/}, CD164⁺, CD172a⁺, CD117⁺, and CD133⁺.

64. The kit of any one of claims 61-63, wherein the human CD34⁺CD84⁻ hematopoietic stem cells comprise one or more gene therapy vectors, genome modifications, or exogenous polynucleotides.

65. The kit of any one of claims 61-64, wherein the kit is used for isolating or quantitating human CD34⁺CD84⁻ hematopoietic stem cells from bone marrow, mobilized peripheral blood, umbilical cord blood, or placenta.

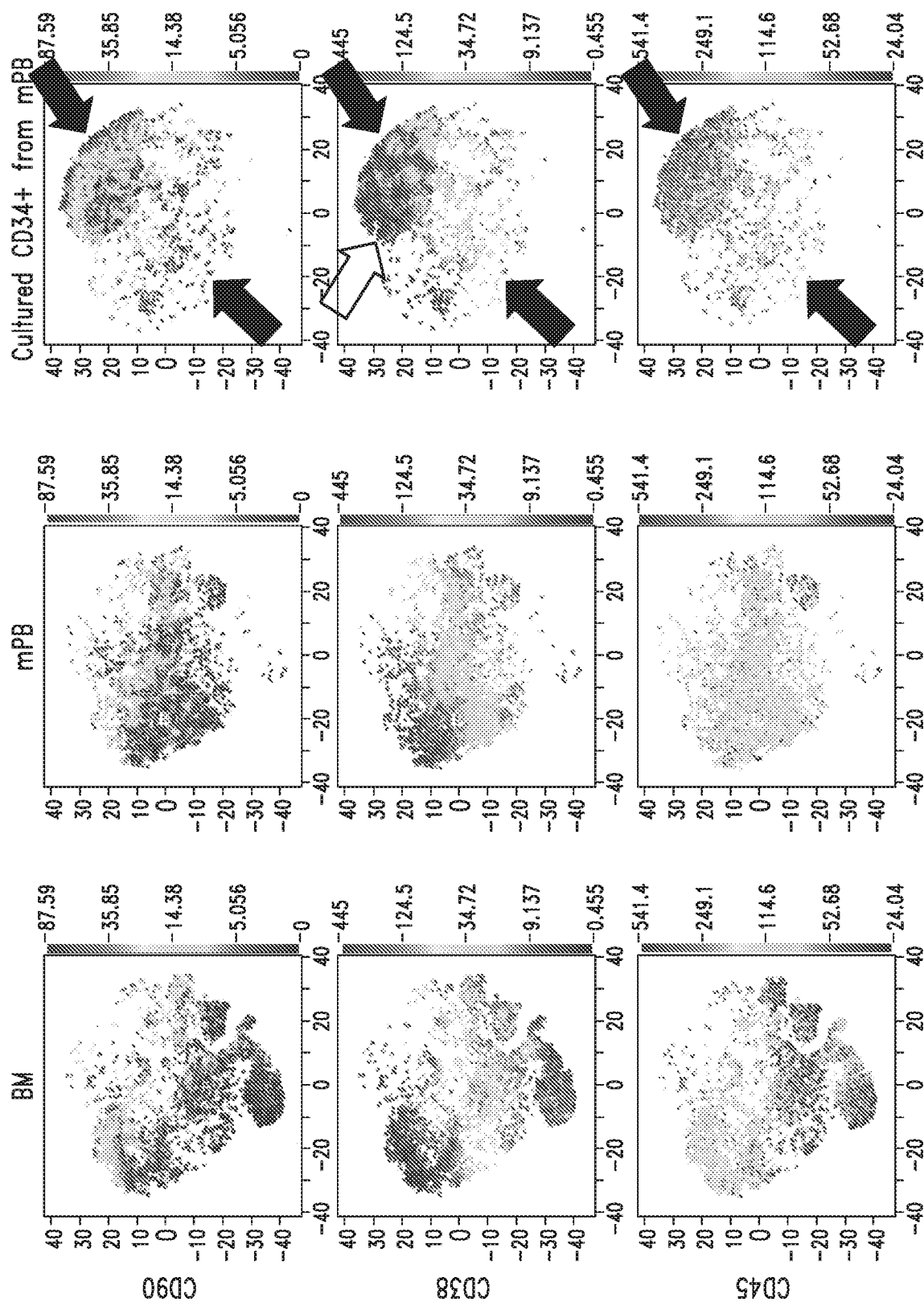


FIG. 1

2/3

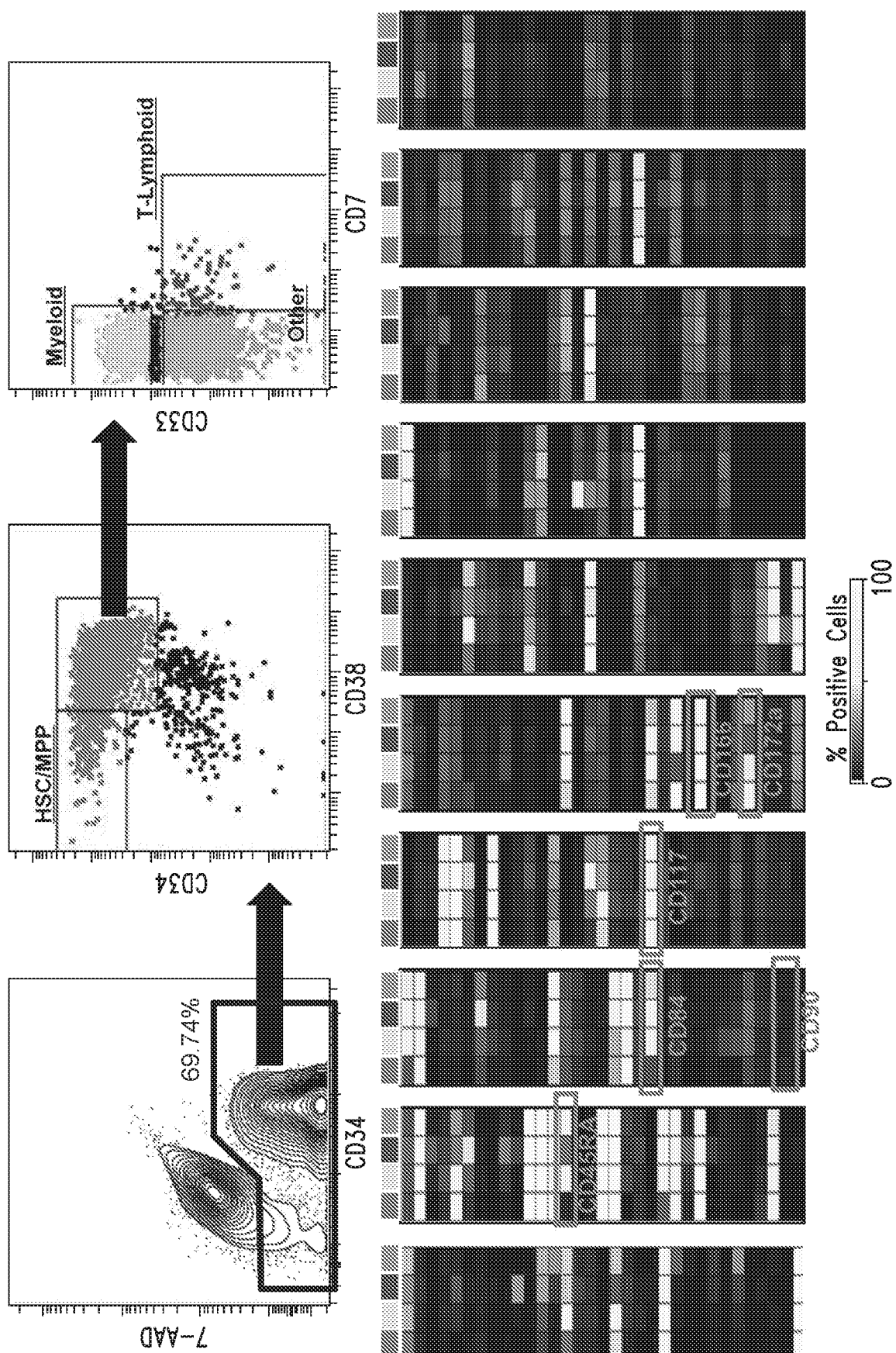


FIG. 2

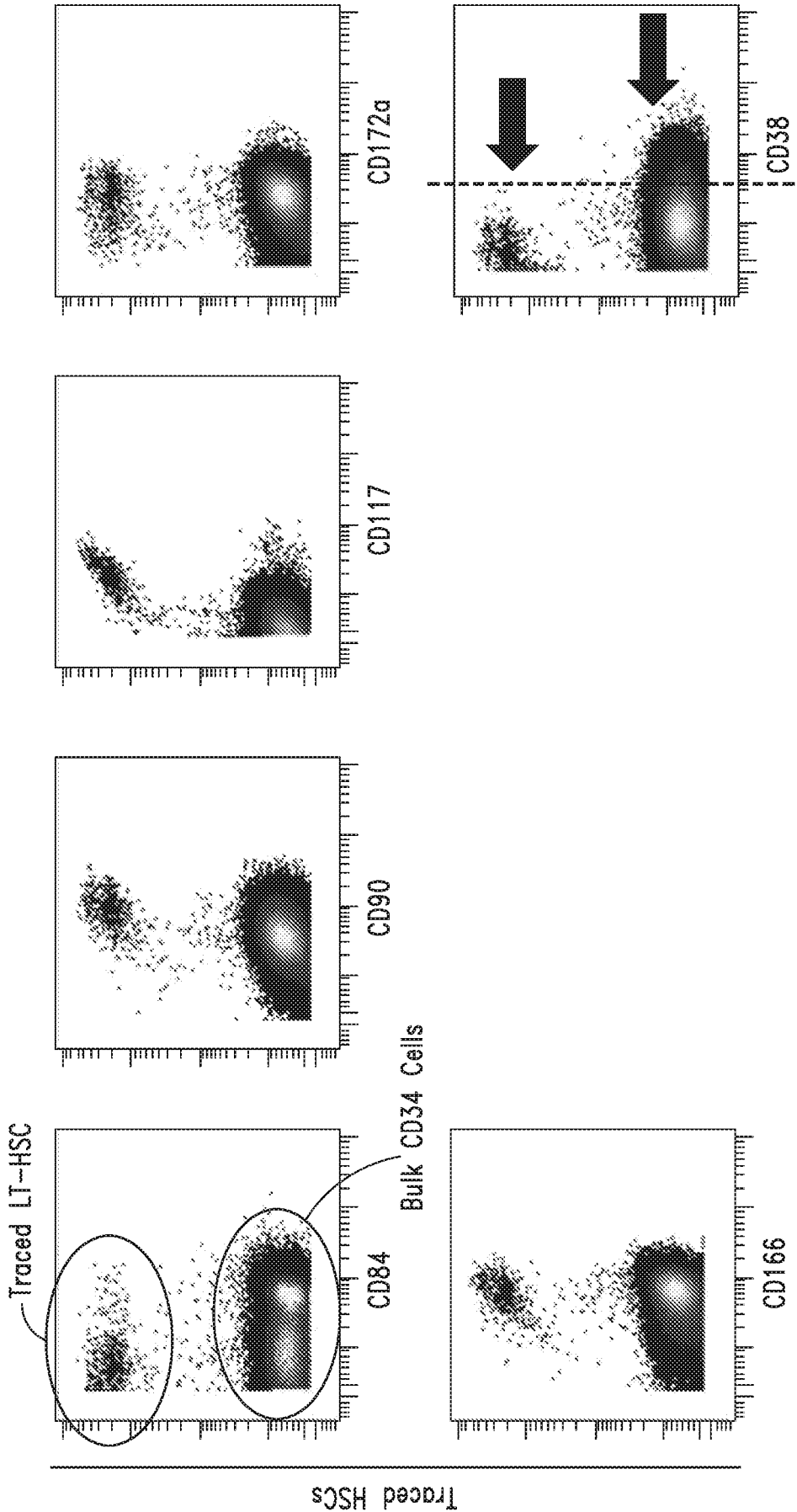


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/049355

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/537; A61K 31/7088; A61K 35/28; A61K 38/02; A61K 38/22; A61K 39/00 (2018.01)

CPC - A61K 35/28; A61K 38/164; A61K 38/168; A61K 39/3955; A61K 39/44; A61K 47/6415; A61K 47/6825; A61K 47/6849; A61K 47/6898; A61K 2039/505; C07K 4/04; C07K 16/2866; C07K 16/289; C12N 5/0647; G01N 33/5073; G01N 33/56966; G01N 33/57407; G01N 33/57426; G01N 33/57434 (2018.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 435/366; 435/370; 435/372 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZAISS et al. "CD84 Expression on Human Hematopoietic Progenitor Cells," <i>Experimental Hematology</i> , 01 September 2003 (01.09.2003), Vol. 31, Iss. 9, Pgs. 798-805. entire document	1, 11, 13, 21, 49, 50, 55-57
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Y		2, 3, 12, 14-19, 63
X	US 2005/0158857 A1 (YILMAZ et al) 21 July 2005 (21.07.2005) entire document	61, 62
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Y		63
Y	WO 2016/210292 A1 (CHILDREN'S MEDICAL CENTER CORPORATION et al) 29 December 2016 (29.12.2016) entire document	2, 3, 12
Y	US 5,681,559 A (DIGIUSTO et al) 28 October 1997 (28.10.1997) entire document	14-19
P, X	WO 2018/071871 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE et al) 19 April 2018 (19.04.2018) entire document	1-3, 11-19, 21, 49, 50, 55-57, 61-63
A	US 2016/0151422 A1 (COGNATE BIOSERVICES, INC) 02 June 2016 (02.06.2016) entire document	1-3, 11-19, 21, 49, 50, 55-57, 61-63
A	US 2016/0324982 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE et al) 10 November 2016 (10.11.2016) entire document	1-3, 11-19, 21, 49, 50, 55-57, 61-63

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

15 October 2018

Date of mailing of the international search report

08 NOV 2018

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
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Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/049355

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-10, 20, 22-48, 51-54, 58-60, 64, 65
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.