NATURAL KILLER CELLS AND ILC3 CELLS AND USES THEREOF

[0001] This application claims benefit of U.S. Provisional Patent Application No. 62/242,246, filed October 15, 2015, U.S. Provisional Patent Application No. 62/272,984, filed December 30, 2015, and U.S. Provisional Patent Application No. 62/403,571, filed October 3, 2016, the disclosures of each of which are incorporated by reference herein in its entirety.

1. FIELD

[0002] Provided herein are methods of producing populations of natural killer (NK) cells and/or ILC3 cells from a population of hematopoietic stem or progenitor cells in media comprising stem cell mobilizing factors, *e.g.*, three-stage methods of producing NK cells and/or ILC3 cells in media comprising stem cell mobilizing factors starting with hematopoietic stem or progenitor cells from cells of the placenta, for example, from placental perfusate (*e.g.*, human placental perfusate) or other tissues, for example, umbilical cord blood or peripheral blood. Further provided herein are methods of using the placental perfusate, the NK cells and/or ILC3 cells and/or NK progenitor cells described herein, to, *e.g.*, suppress the proliferation of tumor cells, or to inhibit pathogen infection, *e.g.*, viral infection. In certain embodiments, the NK cells and/or ILC3 cells and/or NK progenitor cells produced by the three-stage methods described herein are used in combination with, and/or treated with, one or more immunomodulatory compounds.

2. BACKGROUND

[0003] Natural killer (NK) cells are cytotoxic lymphocytes that constitute a major component of the innate immune system.

[0004] NK cells are activated in response to interferons or macrophage-derived cytokines. The cytotoxic activity of NK cells is largely regulated by two types of surface receptors, which may be considered "activating receptors" or "inhibitory receptors," although some receptors, *e.g.*, CD94 and 2B4 (CD244), can work either way depending on ligand interactions.

[0005] Among other activities, NK cells play a role in the host rejection of tumors and have been shown capable of killing virus-infected cells. Natural killer cells can become activated by cells lacking, or displaying reduced levels of, major histocompatibility complex (MHC) proteins. Cancer cells with altered or reduced level of self-class I MHC expression result

in induction of NK cell sensitivity. Activated and expanded NK cells, and in some cases LAK cells, from peripheral blood have been used in both *ex vivo* therapy and *in vivo* treatment of patients having advanced cancer, with some success against bone marrow related diseases, such as leukemia; breast cancer; and certain types of lymphoma.

[0006] In spite of the advantageous properties of NK cells in killing tumor cells and virus-infected cells, there remains a need in the art to develop efficient methods to produce and expand natural killer cells that retain tumoricidal functions.

[0007] NK cells are innate lymphoid cells (ILCs). Innate lymphoid cells are related through their dependency on transcription factor ID2 for development. One type of ILC, known as the ILC3 cell, is described in the literature as expressing RORγt and producing IL-22, as well as playing a role in the immune response of adults, without manifesting cytotoxic effectors such as perforin, granzymes, and death receptors (Montaldo *et al.*, 2014, *Immunity* 41:988-1000; Killig *et al.*, 2014, *Front. Immunol.* 5:142; Withers *et al.*, 2012, *J. Immunol.* 189(5):2094-2098).

3. SUMMARY

[0008] Provided herein are methods of expanding and differentiating cells, for example, hematopoietic cells, such as hematopoietic stem cells, *e.g.*, $CD34^+$ hematopoietic stem cells, to produce natural killer (NK) cells and/or ILC3 cells.

[0009] In one aspect, provided herein are methods of producing NK cell populations and/or ILC3 cell populations that comprise three stages as described herein (and referred to herein as the "three-stage method"). Natural killer cells and/or ILC3 cells produced by the three-stage methods provided herein are referred to herein as "NK cells produced by the three-stage method," "ILC3 cells produced by the three-stage method," or "NK cells and/or ILC3 cells produced by the three-stage method." In certain embodiments, said method comprises one or more further or intermediate steps. In certain embodiments, said method does not comprise any fourth or intermediate step in which the cells are contacted (or cultured).

[0010] In one aspect, provided herein is a method of producing NK cells comprising culturing hematopoietic stem cells or progenitor cells, *e.g.*, $CD34^+$ stem cells or progenitor cells, in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells, subsequently culturing said first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells, and subsequently culturing said second population of cells in a third

medium comprising IL-2 and IL-15, and lacking a stem cell mobilizing agent and low-molecular weight heparin (LMWH), to produce a third population of cells, wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and wherein at least 70%, for example 80%, of the natural killer cells are viable. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In certain embodiments, such natural killer cells comprise natural killer cells that are CD16-. In certain embodiments, such natural killer cells comprise natural killer cells that are CD94+. In certain embodiments, such natural killer cells comprise natural killer cells that are CD94+ or CD16+. In certain embodiments, such natural killer cells comprise natural killer cells that are CD94- or CD16-. In certain embodiments, such natural killer cells comprise natural killer cells that are CD94+ and CD16+. In certain embodiments, such natural killer cells comprise natural killer cells that are CD94- and CD16-. In certain embodiments, at least one, two, or all three of said first medium, second medium, and third medium are not the medium GBGM®. In certain embodiments, the third medium lacks added desulphated glycosaminoglycans. In certain embodiments, the third medium lacks desulphated glycosaminoglycans.

[0011] In one aspect, provided herein is a method of producing NK cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments,

said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[0012] In one aspect, provided herein is a method of producing NK cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of stem cell factor (SCF) and LMWH, to produce a third population of cells; wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[0013] In one aspect, provided herein is a method of producing NK cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of SCF, a stem cell mobilizing agent, and LMWH, to produce a third population of cells; wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments,

none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[0014] In one aspect, provided herein is a method of producing NK cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of a stem cell mobilizing agent and LMWH, to produce a third population of cells; and (d) isolating CD11a+ cells from the third population of cells to produce a fourth population of cells; wherein the fourth population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[0015] In certain embodiments, said natural killer cells express perforin and eomesodermin (EOMES). In certain embodiments, said natural killer cells do not express either RAR-related orphan receptor gamma (RORγt) or interleukin-1 receptor 1 (IL1R1).

[0016] In one aspect, provided herein is a method of producing ILC3 cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first

medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[0017] In one aspect, provided herein is a method of producing ILC3 cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising a stem cell mobilizing agent, IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[0018] In one aspect, provided herein is a method of producing ILC3 cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising SCF, IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[0019] In one aspect, provided herein is a method of producing ILC3 cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising a stem cell mobilizing agent, SCF, IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[0020] In one aspect, provided herein is a method of producing ILC3 cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of a stem cell mobilizing agent and LMWH, to produce a third population of cells; and (d) isolating CD11a- cells from the third population of cells to produce a fourth population of cells; wherein the fourth population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[0021] In certain embodiments, said ILC3 cells express RORγt and IL1R1. In certain embodiments, said ILC3 cells do not express either perforin or EOMES. In certain embodiments,

said third medium lacks added desulphated glycosaminoglycans. In certain embodiments, said third medium lacks desulphated glycosaminoglycans.

[0022] In certain embodiments, said hematopoietic stem or progenitor cells are mammalian cells. In specific embodiments, said hematopoietic stem or progenitor cells are human cells. In specific embodiments, said hematopoietic stem or progenitor cells are primate cells. In specific embodiments, said hematopoietic stem or progenitor cells are canine cells. In specific embodiments, said hematopoietic stem or progenitor cells are rodent cells. In specific embodiments, said hematopoietic stem or progenitor cells are cells from a mammal other than a human, primate, canine or rodent.

[0023] In certain aspects, the hematopoietic stem cells or progenitor cells cultured in the first medium are $CD34^+$ stem cells or progenitor cells. In certain aspects, the hematopoietic stem cells or progenitor cells are placental hematopoietic stem cells or progenitor cells. In certain aspects, the placental hematopoietic stem cells or progenitor cells are obtained from, or obtainable from placental perfusate (*e.g.* obtained from or obtainable from isolated nucleated cells from placental perfusate). In certain aspects, said hematopoietic stem or progenitor cells are obtained from, or obtainable from, umbilical cord blood. In certain aspects, said hematopoietic stem or progenitor cells are fetal liver cells. In certain aspects, said hematopoietic stem or progenitor cells are mobilized peripheral blood cells. In certain aspects, said hematopoietic stem or progenitor cells are bone marrow cells.

[0024] In certain aspects, said first medium used in the three-stage method comprises a stem cell mobilizing agent and thrombopoietin (Tpo). In certain aspects, the first medium used in the three-stage method comprises, in addition to a stem cell mobilizing agent and Tpo, one or more of Low Molecular Weight Heparin (LMWH), Flt-3 Ligand (Flt-3L), stem cell factor (SCF), IL-6, IL-7, granulocyte colony-stimulating factor (G-CSF), or granulocyte-macrophage-stimulating factor (GM-CSF). In certain aspects, said first medium does not comprise added LMWH. In certain aspects, said first medium does not comprise added desulphated glycosaminoglycans. In certain aspects, said first medium does not comprise LMWH. In certain aspects, said first medium does not comprise desulphated glycosaminoglycans. In certain aspects, the first medium used in the three-stage method comprises, in addition to a stem cell mobilizing agent and Tpo, each of LMWH, Flt-3L, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In certain aspects, the first medium used in the three-stage method comprises, in addition to a stem cell mobilizing

agent and Tpo, each of Flt-3L, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In certain aspects, said Tpo is present in the first medium at a concentration of from 1 ng/mL to 100 ng/mL, from 1 ng/mL to 50 ng/mL, from 20 ng/mL to 30 ng/mL, or about 25 ng/mL. In certain aspects, in the first medium, the LMWH is present at a concentration of from 1U/mL to 10U/mL; the Flt-3L is present at a concentration of from 1 ng/mL to 50 ng/mL; the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL. In certain aspects, in the first medium, the Flt-3L is present at a concentration of from 1 ng/mL to 50 ng/mL; the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL. In certain aspects, in the first medium, the LMWH is present at a concentration of from 4U/mL to 5U/mL; the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the first medium, the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the first medium, the LMWH is present at a concentration of about 4.5U/mL; the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about .25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL. In certain aspects, in the first medium, the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL;

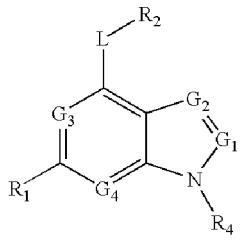
the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about .25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL. In certain embodiments, said first medium is not GBGM®.

[0025] In certain aspects, said second medium used in the three-stage method comprises a stem cell mobilizing agent and interleukin-15 (IL-15), and lacks Tpo. In certain aspects, the second medium used in the three-stage method comprises, in addition to a stem cell mobilizing agent and IL-15, one or more of LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In certain aspects, the second medium does not comprise added LMWH. In certain aspects, the second medium does not comprise added desulphated glycosaminoglycans. In certain aspects, the second medium does not comprise LMWH. In certain aspects, the second medium does not comprise desulphated glycosaminoglycans. In certain aspects, the second medium used in the three-stage method comprises, in addition to a stem cell mobilizing agent and IL-15, each of LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In certain aspects, the second medium used in the three-stage method comprises, in addition to a stem cell mobilizing agent and IL-15, each of Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In certain aspects, said IL-15 is present in said second medium at a concentration of from 1 ng/mL to 50 ng/mL, from 10 ng/mL to 30 ng/mL, or about 20 ng/mL. In certain aspects, in said second medium, the LMWH is present at a concentration of from 1U/mL to 10U/mL; the Flt-3L is present at a concentration of from 1 ng/mL to 50 ng/mL; the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL. In certain aspects, in the second medium, the LMWH is present in the second medium at a concentration of from 4U/mL to 5U/mL; the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the second medium, the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration

of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the second medium, the LMWH is present in the second medium at a concentration of from 4U/mL to 5U/mL; the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the second medium, the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the second medium, the LMWH is present in the second medium at a concentration of about 4.5U/mL; the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about 0.25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL. In certain aspects, in the second medium, the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about 0.25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL. In certain embodiments, said second medium is not GBGM®.

[0026] In certain aspects, the stem cell mobilizing factor present in said first medium, said second medium, or said first and second media, is an aryl hydrocarbon receptor inhibitor, *e.g.*, an aryl hydrocarbon receptor antagonist. In certain aspects, said aryl hydrocarbon receptor inhibitor is resveratrol. Is certain aspects, said aryl hydrocarbon receptor inhibitor is compound of the formula

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in which:

G_1 is selected from N and CR_3;

G_2, G_3 and G_4 are independently selected from CH and N; with the proviso that at least 1 of G_3 and G_4 is N; with the proviso that G_1 and G_2 are not both N;

L is selected from --$NR_{5a}(CH_2)_{0-3}$--, --$NR_{5a}CH(C(O)OCH_3)CH_2$--, --$NR_{5a}(CH_2)_2NR_{5b}$--, --$NR_{5a}(CH_2)_2S$--, --$NR_{5a}CH_2CH(CH_3)CH_2$--, --$NR_{5a}CH_2CH(OH)$-- and --$NR_{5a}CH(CH_3)CH_2$--; wherein R_{5a} and R_{5b} are independently selected from hydrogen and C_{1-4}alkyl;

R_1 is selected from hydrogen, phenyl, thiophenyl, furanyl, 1H-benzoimidazolyl, isoquinolinyl, 1H-imidazopyridinyl, benzothiophenyl, pyrimidinyl, 1H-pyrazolyl, pyridinyl, 1H-imidazolyl, pyrrolidinyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl and thiazolyl; wherein said phenyl, thiophenyl, furanyl, 1H-benzoimidazolyl, isoquinolinyl, 1H-imidazopyridinyl, benzothiophenyl, pyrimidinyl, 1H-pyrazolyl, pyridinyl, 1H-imidazolyl, pyrrolidinyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl or thiazolyl of R_1 can be optionally substituted by 1 to 3 radicals independently selected from cyano, hydroxy, C_{1-4}alkyl, C_{1-4}alkoxy, halo, halo-substituted-C_{1-4}alkyl, halo-substituted-C_{1-4}alkoxy, hydroxy, amino, --$C(O)R_{8a}$, --$S(O)_{0-2}R_{8a}$, --$C(O)OR_{8a}$ and --$C(O)NR_{8a}R_{8b}$; wherein R_{8a} and R_{8b} are independently selected from hydrogen and C_{1-4}alkyl; with the proviso that R_1 and R_3 are not both hydrogen;

R_2 is selected from --$S(O)_2NR_{6a}R_{6b}$, --$NR_{9a}C(O)R_{9b}$, --$NR_{6a}C(O)NR_{6b}R_{6c}$, phenyl, 1H-pyrrolopyridin-3-yl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl and 1H-indazolyl; wherein R_{6a}, R_{6b} and R_{6c} are independently selected from hydrogen and C_{1-4}alkyl; wherein said phenyl, 1H-pyrrolopyridin-3-yl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl or 1H-indazolyl of R_2 is optionally substituted with 1 to 3 radicals

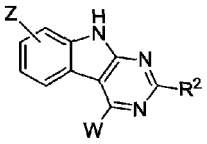
independently selected from hydroxy, halo, methyl, methoxy, amino, --$O(CH_2)_nNR_{7a}R_{7b}$, --$S(O)_2NR_{7a}R_{7b}$, --$OS(O)_2NR_{7a}R_{7b}$ and --$NR_{7a}S(O)_2R_{7b}$; wherein R_{7a} and R_{7b} are independently selected from hydrogen and C_{1-4}alkyl;

R_3 is selected from hydrogen, C_{1-4}alkyl and biphenyl; and

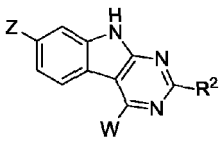
R_4 is selected from C_{1-10}alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-3-yl, tetrahydro-2H-pyran-4-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl; wherein said alkyl, cyclopropyl, cyclohexyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-3-yl, oxetan-2-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, tetrahydro-2H-pyran-4-yl, phenyl, tetrahydrofuran-3-yl, tetrahydrofuran-2-yl, benzyl, (4-pentylphenyl)(phenyl)methyl or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl can be optionally substituted with 1 to 3 radicals independently selected from hydroxy, C_{1-4}alkyl and halo-substituted-C_{1-4}alkyl; or a salt thereof.

[0027] In certain aspects, said aryl hydrocarbon receptor inhibitor is StemRegenin-1 (SR-1) (4-(2-(2-(benzo[b]thiophen-3-yl)-9-isopropyl-9H-purin- 6-ylamino)ethyl)phenol). In certain aspects, said aryl hydrocarbon receptor inhibitor is the compound CH223191 (1-Methyl-N-[2-methyl-4-[2-(2-methylphenyl)diazenyl]phenyl-1H-pyrazole-5-carboxamide].

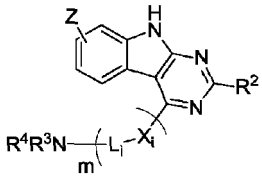
[0028] In certain aspects, the stem cell mobilizing factor present in said first medium, said second medium, or said first and second mediums is a pyrimido(4,5-b)indole derivative. In certain aspects, said pyrimido(4,5-b)indole derivative is one or more of:



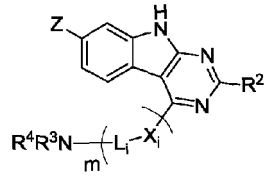
I



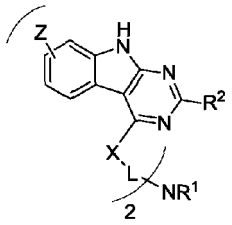
II



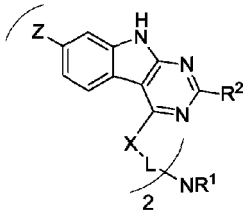
III



IV



V



VI

or a salt or a prodrug thereof, wherein:

Z is

1) -P(O) (OR<1>) (OR<1>),

2) -C(0)OR<1>,

3) -C(0)NHR<1>,

4) -C(0)N(R)R<1>,

5) -C(0)R<1>,

6) -CN,

7) -SR ,

8) -S(0)2NH2,

9) -S(0)2NHR<1>,

10) -S(0)2N(R)R<1>,

1 1) -S(0)R<1>,

12) -S(0)2R<1>,

13) -L,

14) -benzyl optionally substituted with 1 , 2 or 3 R<A> or R<1> substituents,

15) -L-heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and the heteroaryl groups,

16) -L-heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either one or both the L and the heterocyclyl groups, 17) -L-aryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and the heteroaryl groups,

18) -heteroaryl optionally substituted with one or more R<A> or R<1> substituents, or

19) -aryl optionally substituted with one or more R<A> or R<1> substituents,

and wherein each substituent is optionally attached to the L group if it is not already present, and wherein, when (R<1>) and R<1> are attached to a nitrogen atom, optionally they join together with the nitrogen atom to form a 3 to 7-membered ring which optionally includes one or more other heteroatom selected from N, 0 and S, optionally the is substituted with one or more R<1> or R<A>;

W is

1) -H,

2) -halogen,

3) -OR<1>,

4) -L-OH,

5) -L-OR<1>,

6) -SR<1>,

7) -CN,

8) -P(0)(OR<1>)(OR<1>),

9) -NHR<1>,

10) -N(R<1>)R<1>,

1 1) -L-NH2,

12) -L-NHR<1>,

13) -L-N(R<1>)R<1>,

14) -L-SR<1>,

15) -L-S(0)R<1>,

16) -L-S(0)2R<1>,

17) -L-P(0)(OR<1>)(OR<1>

18) -C(0)OR<1>,

19) -C(0)NH2,

20) -C(0)NHR<1>,

21) -C(0)N(R<1>)R<1>,

22) -NHC(0)R<1>,

23) -NR1C(0)R<1>, -NHC(0)0R<1>,

-NR1C(0)0R<1>,

-0C(0)NH2,

-0C(0)NHR<1>,

-0C(0)N(R)R<1>,

-0C(0)R<1>,

-C(0)R<1>,

-NHC(0)NH2,

-NHC(0)NHR<1>,

-NHC(0)N(R)R<1>,

-NR C(0)NH2,

-NR C(0)NHR<1>,

-NR C(0)N(R)R<1>,

-NHS(0)2R<1>,

-NR S(0)2R<1>,

-S(0)2NH2,

-S(0)2NHR<1>,

-S(0)2N(R)R<1>,

-S(0)R<1>,

-S(0)2R<1>,

-0S(0)2R1 ,

-S(0)20R<1>,

-benzyl optionally substituted with 1 , 2 or 3 R<A> or R<1> substituents,

-L-heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and the heteroaryl groups,

-L-heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and the heterocyclyl goups,

-L-aryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and aryl groups,

-L-NR<1>(R<1>),

-L-)2 NR<1>,

-L-(N(R1)-L)n - N(R1)R1 , -L-(N(R<1>)-L)n - heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heteroaryl groups,

-L-(N(R<1>)-L)n - heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heterocyclyl groups,

-L-(N(R<1>)-L)n - aryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and aryl groups,

-0-L-N(R)R<1> ,

-0-L- heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heteroaryl groups,

-0-L- heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heterocyclyl groups,

-0-L- aryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and aryl groups,

-0-L)2-NR<1>,

-0-L-(N(R)-L)n - N(R)R<1> ,

-0-L-(N(R<1>)-L)n - heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heteroaryl groups,

-0-L-(N(R<1>)-L)n - heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heterocyclyl groups,

-0-L-(N(R<1>)-L)n- aryl optionally substituted with one or more R<A> or R<1> substituents,

-S-L- heteroaryl optionally substituted with one or more R<A> or R<1> substituents,

-S-L- heterocyclyl optionally substituted with one or more R<A> or R<1> substituents,

-S-L- aryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and aryl groups,

-S-L)2 NR1 ,

-S-L-(N(R1)-L)"- N(R1)R1,

-S-L-(N(R<1>)-L)n - heteroaryl optionally substituted with one or more R<A> substituents, -S-L-(N(R<1>)-L)n - heterocyclyl optionally substituted with one or more R<A> substituents, -S-L-(N(R<1>)-L)n - aryl optionally substituted with one or more R<A> substituents,

-NR<1>(R<1>),

-(N(R1)-L)n - N(R1)R1 ,

-N(R1)L)2 -NR1 , 76) -(N(R1)-L)"- N(R1)RA,

77) -(N(R<1>)-L)n - heteroaryl optionally substituted with one or more R<A> or R<1> substituents,

78) -(N(R<1>)-L)n - heterocyclyl optionally substituted with one or more R<A> or R<1> substituents,

79) -(N(R<1>)-L)n - aryl optionally substituted with one or more R<A> or R<1> substituents,

80) -heteroaryl optionally substituted with one or more R<A> substituents, or

81) -aryl optionally substituted with one or more R<A> substituents,

and wherein each substituent is optionally attached to the L group if it is not already present, and wherein when two R<1> substituents are present on the same nitrogen atom, then each R<1> substituent is independently selected from the list of R<1> values described thereafter,

and wherein n is an integer equal to either 0, 1 , 2, 3, 4, or 5,

and wherein, when (R<1>) and R<1> are attached to a nitrogen atom, optionally they join together with the nitrogen atom to form a 3 to 7-membered ring which optionally includes one or more other heteroatom selected from N, 0 and S, optionally the ring is substituted with one or more R<1> or R<A>;

L is

1) -Ci-6 alkyl,

2) -C2-6 alkenyl,

3) -C2-6 alkynyl,

4) -C3-7 cycloalkyi,

5) -C3-7 cycloalkenyl,

6) heterocyclyl,

7) -Ci-6 alkyl-C3-7 cycloalkyi,

8) -Ci-6 alkyl-heterocyclyl,

9) aryl, or

10) heteroaryl,

and wherein the alkyl, the alkenyl, the alkynyl, the cycloalkyi, the cycloalkenyl, the heterocyclyl, the aryl and the heteroaryl groups are each independently optionally substituted with one or two R<A> substituent;

Ri is

1) -H,

2) -C1-6 alkyl,

3) -C2-6 alkenyl,

4) -C2-6 alkynyl, 5) -C3-7 cycloalkyl,

6) -C3-7 cycloalkenyl,

7) -C1-5 perfluorinated,

8) -heterocydyl,

9) -aryl,

10) -heteroaryl,

1 1) -benzyl, or

12) 5-[(3aS,4S,6aR)-2-oxohexahydro-1 H-thieno[3,4-d]imidazol-4-yl]pentanoyl,

and wherein the alkyi, the alkenyl, the alkynyl, the cycloalkenyl, the perfluorinated alkyi, the heterocydyl, the aryl, the heteroaryl and the benzyl groups are each independently optionally substituted with 1 , 2 or 3 R<A> or R<1> substituents;

R2 is

1) -H,

2) -C1-6 alkyi,

3) -SR ,

4) -C(0)R1,

5) -S(0)R1,

6) -S(0)2R<1>,

7) -benzyl optionally substituted with 1 , 2 or 3 R<A> or R<1> substituents,

8) -L-heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either one or both the L and the heteroaryl groups,

9) -L-heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either one or both the L and the heterocydyl groups,

10) -L-aryl optionally substituted with one or more R<A> or R<1> substituents attached on either one or both the L and the aryl groups,

11) -heteroaryl optionally substituted with one or more R<A> or R<1> substituents, or

12) -aryl optionally substituted with one or more R<A> or R<1> substituents,

and wherein each substituent is optionally attached to the L group if it is not already present;

R<A> is

1) -halogen,

2) -CFs, 3) -OH,

4) -OR<1>,

5) -L-OH,

6) -L-OR<1>,

7) -OCFs,

8) -SH,

9) -SR1 ,

10) -CN,

11) -NO2,

12) -NH2,

13) -NHR<1>,

14) -NR<1>R<1>,

15) -L-NH2,

16) -L-NHR<1>,

17) -L-NR<4>R<1>,

18) -L-SR<1>,

19) -L-S(0)R<1>,

20) -L-S(0)2R<1>,

21) -C(0)OH,

22) -C(0)OR<1>,

23) -C(0)NH2,

24) -C(0)NHR<1>,

25) -C(0)N(R<1>)R<1>,

26) -NHC(0)R<1>,

27) -NR1C(0)R<1>,

28) -NHC(0)OR<1>,

29) -NR1C(0)0R<1>,

30) -OC(0)NH2,

31) -OC(0)NHR<1>,

32) -OC(0)N(R)R<1>,

33) -OC(0)R<1>,

34) -C(0)R1, 35) -NHC(0)NH2,

36) -NHC(0)NHR1,

37) -NHC(0)N(R)R<1>,

38) -NR C(0)NH2,

39) -NR C(0)NHR<1>,

40) -NR1C(0)N(R1)R1,

41) -NHS(0)2R<1>,

42) -NR S(0)2R<1>,

43) -S(0)2NH2,

44) -S(0)2NHR<1>,

45) -S(0)2N(R)R<1>,

46) -S(0)R<1>,

47) -S(0)2R<1>,

48) -0S(0)2R<1>,

49) -S(0)20R<1>,

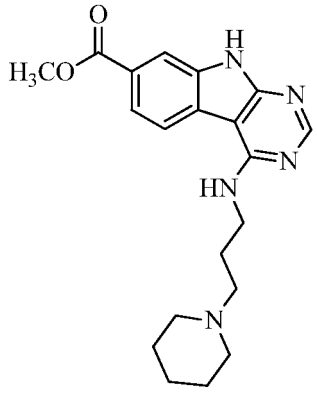
50) -benzyl,

51) -N3, or

52) -C(-N=N-)(CF3),

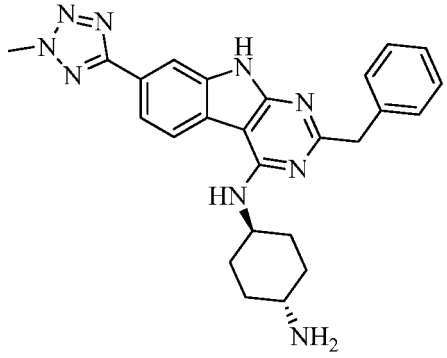
and wherein the benzyl group is optionally substituted with 1 , 2 or 3 R<A> or R<1> substituents.

[0029] In certain aspects, said pyrimido(4,5-b)indole derivative has the chemical structure



(UM729).

[0030] In cetain aspects, said pyrimido(4,5-b)indole derivative has the chemical structure



(UM171).

[0031] In certain aspects, said third medium used in the three-stage method comprises IL-2 and IL-15, and lacks a stem cell mobilizing agent and LMWH. In certain aspects, the third medium used in the three-stage method comprises, in addition to IL-2 and IL-15, one or more of SCF, IL-6, IL-7, G-CSF, or GM-CSF. In certain aspects, the third medium used in the three-stage method comprises, in addition to IL-2 and IL-15, each of SCF, IL-6, IL-7, G-CSF, and GM-CSF. In certain aspects, said IL-2 is present in said third medium at a concentration of from 10 U/mL to 10,000 U/mL and said IL-15 is present in said third medium at a concentration of from 1 ng/mL to 50 ng/mL. In certain aspects, said IL-2 is present in said third medium at a concentration of from 100 U/mL to 10,000 U/mL and said IL-15 is present in said third medium at a concentration of from 1 ng/mL to 50 ng/mL. In certain aspects, said IL-2 is present in said third medium at a concentration of from 300 U/mL to 3,000 U/mL and said IL-15 is present in said

third medium at a concentration of from 10 ng/mL to 30 ng/mL. In certain aspects, said IL-2 is present in said third medium at a concentration of about 1,000 U/mL and said IL-15 is present in said third medium at a concentration of about 20 ng/mL. In certain aspects, in said third medium, the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL. In certain aspects, in said third medium, the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in said third medium, the SCF is present at a concentration of about 22 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 20 ng/mL; the G-CSF is present at a concentration of about 0.25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL. In certain embodiments, said third medium is not GBGM®.

[0032] In certain aspects, the third medium comprises 100 ng/mL IL-7, 1000 ng/mL IL-2, 20 ng/mL IL-15, and 10 μM SR1 and lacks SCF. In certain aspects, the third medium comprises 20 ng/mL IL-7, 1000 ng/mL IL-2, 20 ng/mL IL-15, and 10 μM SR1 and lacks SCF. In certain aspects, the third medium comprises 20 ng/mL IL-7, 20 ng/mL IL-15, and 10 μM SR1 and lacks SCF. In certain aspects, the third medium comprises 100 ng/mL IL-7, 22 ng/mL SCF, 1000 ng/mL IL-2, and 20 ng/mL IL-15 and lacks SR1. In certain aspects, the third medium comprises 22 ng/mL SCF, 1000 ng/mL IL-2, and 20 ng/mL IL-15 and lacks SR1. In certain aspects, the third medium comprises 20 ng/mL IL-7, 22 ng/mL SCF, 1000 ng/mL IL-2, and 20 ng/mL IL-15 and lacks SR1. In certain aspects, the third medium comprises 20 ng/mL IL-7, 22 ng/mL SCF, and 1000 ng/mL IL-2 and lacks SR1.

[0033] In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α,

and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[0034] Generally, the particularly recited medium components do not refer to possible constituents in an undefined component of said medium, e.g., serum. For example, said Tpo, IL-2, and IL-15 are not comprised within an undefined component of the first medium, second medium or third medium, *e.g.*, said Tpo, IL-2, and IL-15 are not comprised within serum. Further, said LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and/or GM-CSF are not comprised within an undefined component of the first medium, second medium or third medium, *e.g.*, said LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and/or GM-CSF are not comprised within serum.

[0035] In certain aspects, said first medium, second medium or third medium comprises human serum-AB. In certain aspects, any of said first medium, second medium or third medium comprises 1% to 20% human serum-AB, 5% to 15% human serum-AB, or about 2, 5, or 10% human serum-AB.

[0036] In certain aspects, any of said first medium, second medium or third medium comprises 2-mercaptoethanol. In certain aspects, any of said first medium, second medium or third medium comprises gentamycin.

[0037] In certain embodiments, in the three-stage methods described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days before said culturing in said second medium. In certain embodiments, cells are cultured in said second medium for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days before said culturing in said third medium. In certain embodiments, cells are cultured in said third medium for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days, or for more than 30 days.

[0038] In one embodiment, in the three-stage methods described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for 7-13 days to produce a first population of cells; said first population of cells are cultured in said second medium for 2-6 days to produce a second population of cells; and said second population of cells are cultured in said third medium for 10-30 days, *i.e.*, the cells are cultured a total of 19-49 days.

[0039] In one embodiment, in the three-stage methods described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for 8-12 days to produce a first population of cells; said first population of cells are cultured in said second medium for 3-

5 days to produce a second population of cells; and said second population of cells are cultured in said third medium for 15-25 days, *i.e.*, the cells are cultured a total of 26-42 days.

[0040] In a specific embodiment, in the three-stage methods described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for about 10 days to produce a first population of cells; said first population of cells are cultured in said second medium for about 4 days to produce a second population of cells; and said second population of cells are cultured in said third medium for about 21 days, *i.e.*, the cells are cultured a total of about 35 days.

[0041] In certain aspects, said culturing in said first medium, second medium and third medium are all performed under static culture conditions, e.g., in a culture dish or culture flask. In certain aspects, said culturing in at least one of said first medium, second medium or third medium are performed in a spinner flask. In certain aspects, said culturing in said first medium and said second medium is performed under static culture conditions, and said culturing in said third medium is performed in a spinner flask.

[0042] In certain aspects, said culturing is performed in a spinner flask. In other aspects, said culturing is performed in a G-Rex device. In yet other aspects, said culturing is performed in a WAVE bioreactor.

[0043] In certain aspects, said hematopoietic stem or progenitor cells are initially inoculated into said first medium from 1×10^4 to 1×10^5 cells/mL. In a specific aspect, said hematopoietic stem or progenitor cells are initially inoculated into said first medium at about 3×10^4 cells/mL.

[0044] In certain aspects, said first population of cells are initially inoculated into said second medium from 5×10^4 to 5×10^5 cells/mL. In a specific aspect, said first population of cells is initially inoculated into said second medium at about 1×10^5 cells/mL.

[0045] In certain aspects said second population of cells is initially inoculated into said third medium from 1×10^5 to 5×10^6 cells/mL. In certain aspects, said second population of cells is initially inoculated into said third medium from 1×10^5 to 1×10^6 cells/mL. In a specific aspect, said second population of cells is initially inoculated into said third medium at about 5×10^5 cells/mL. In a more specific aspect, said second population of cells is initially inoculated into said third medium at about 5×10^5 cells/mL in a spinner flask. In a specific aspect, said second population of cells is initially inoculated into said third medium at about 3×10^5 cells/mL. In a

more specific aspect, said second population of cells is initially inoculated into said third medium at about 3 x 10^5 cells/mL in a static culture.

[0046] In certain aspects, the three-stage method disclosed herein produces at least 5000-fold more natural killer cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 10,000-fold more natural killer cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 50,000-fold more natural killer cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 75,000-fold more natural killer cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, the viability of said natural killer cells is determined by 7-aminoactinomycin D (7AAD) staining. In certain aspects, the viability of said natural killer cells is determined by annexin-V staining. In specific aspects, the viability of said natural killer cells is determined by both 7-AAD staining and annexin-V staining. In certain aspects, the viability of said natural killer cells is determined by trypan blue staining.

[0047] In certain aspects, the three-stage method disclosed herein produces at least 5000-fold more ILC3 cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 10,000-fold more ILC3 cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 50,000-fold more ILC3 cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 75,000-fold more ILC3 cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium.

[0048] In certain aspects, the three-stage method disclosed herein produces natural killer cells that comprise at least 20% CD56+CD3– natural killer cells. In certain aspects, the three-stage method produces natural killer cells that comprise at least 40% CD56+CD3– natural killer cells. In certain aspects, the three-stage method produces natural killer cells that comprise at least 60% CD56+CD3– natural killer cells. In certain aspects, the three-stage method produces natural killer cells that comprise at least 70% CD56+CD3– natural killer cells. In certain aspects, the

three-stage method produces natural killer cells that comprise at least 75% CD56+CD3– natural killer cells. In certain aspects, the three-stage method produces natural killer cells that comprise at least 80% CD56+CD3– natural killer cells.

[0049] In certain aspects, the three-stage method disclosed herein produces natural killer cells that comprise at least 20% CD56+CD3–CD11a+ natural killer cells. In certain aspects, the three-stage method disclosed herein produces natural killer cells that comprise at least 40% CD56+CD3– CD11a+ natural killer cells. In certain aspects, the three-stage method disclosed herein produces natural killer cells that comprise at least 60% CD56+CD3– CD11a+ natural killer cells. In certain aspects, the three-stage method disclosed herein produces natural killer cells that comprise at least 80% CD56+CD3– CD11a+ natural killer cells.

[0050] In certain aspects, the three-stage method disclosed herein produces ILC3 cells that comprise at least 20% CD56+CD3– CD11a– ILC3 cells. In certain aspects, the three-stage method disclosed herein produces ILC3 cells that comprise at least 40% CD56+CD3– CD11a– ILC3 cells. In certain aspects, the three-stage method disclosed herein produces ILC3 cells that comprise at least 60% CD56+CD3– CD11a– ILC3 cells. In certain aspects, the three-stage method disclosed herein produces natural killer cells that comprise at least 80% CD56+CD3– CD11a– ILC3 cells.

[0051] In certain aspects, the three-stage method disclosed herein, produces natural killer cells that exhibit at least 20% cytotoxicity against K562 cells when said natural killer cells and said K562 cells are co-cultured *in vitro* at a ratio of 10:1. In certain aspects, the three-stage method produces natural killer cells that exhibit at least 35% cytotoxicity against the K562 cells when said natural killer cells and said K562 cells are co-cultured *in vitro* at a ratio of 10:1. In certain aspects, the three-stage method produces natural killer cells that exhibit at least 45% cytotoxicity against the K562 cells when said natural killer cells and said K562 cells are co-cultured *in vitro* at a ratio of 10:1. In certain aspects, the three-stage method produces natural killer cells that exhibit at least 60% cytotoxicity against the K562 cells when said natural killer cells and said K562 cells are co-cultured *in vitro* at a ratio of 10:1. In certain aspects, the three-stage method produces natural killer cells that exhibit at least 75% cytotoxicity against the K562 cells when said natural killer cells and said K562 cells are co-cultured *in vitro* at a ratio of 10:1.

[0052] In certain aspects, the three-stage method disclosed herein, produces ILC3 cells that exhibit at least 20% cytotoxicity against K562 cells when said ILC3 cells and said K562 cells are

co-cultured *in vitro* at a ratio of 10:1. In certain aspects, the three-stage method produces ILC3 cells that exhibit at least 35% cytotoxicity against the K562 cells when said ILC3 cells and said K562 cells are co-cultured *in vitro* at a ratio of 10:1. In certain aspects, the three-stage method produces ILC3 cells that exhibit at least 45% cytotoxicity against the K562 cells when said ILC3 cells and said K562 cells are co-cultured *in vitro* at a ratio of 10:1. In certain aspects, the three-stage method produces ILC3 cells that exhibit at least 60% cytotoxicity against the K562 cells when said ILC3 cells and said K562 cells are co-cultured *in vitro* at a ratio of 10:1. In certain aspects, the three-stage method produces ILC3 cells that exhibit at least 75% cytotoxicity against the K562 cells when said ILC3 cells and said K562 cells are co-cultured *in vitro* at a ratio of 10:1.

[0053] In certain aspects, after said third culturing step, said third population of cells, *e.g.*, said population of natural killer cells, is cryopreserved. In certain aspects, after said fourth culturing step, said fourth population of cells, *e.g.*, said population of natural killer cells, is cryopreserved.

[0054] In certain aspects, provided herein are populations of cells comprising natural killer cells, *i.e.*, natural killers cells produced by a three-stage method described herein. Accordingly, provided herein is an isolated natural killer cell population produced by a three-stage method described herein. In a specific embodiment, said natural killer cell population comprises at least 20% CD56+CD3– natural killer cells. In a specific embodiment, said natural killer cell population comprises at least 40% CD56+CD3– natural killer cells. In a specific embodiment, said natural killer cell population comprises at least 60% CD56+CD3– natural killer cells. In a specific embodiment, said natural killer cell population comprises at least 80% CD56+CD3– natural killer cells. In specific embodiments, the natural killer cell population is formulated into a pharmaceutical composition suitable for use *in vivo*, for example, suitable for human use *in vivo*.

[0055] In certain aspects, provided herein are populations of cells comprising ILC3 cells, *i.e.*, natural killer cells produced by a three-stage method described herein. In specific embodiments, the population of cells comprising ILC3 cells is formulated into a pharmaceutical composition suitable for use *in vivo*, for example, suitable for human use *in vivo*.

[0056] In one embodiment, provided herein is an isolated NK progenitor cell population, wherein said NK progenitor cells are produced according to the three-stage method described herein. In specific embodiments, the NK progenitor cell population is formulated into a pharmaceutical composition suitable for use *in vivo*, for example, suitable for human use *in vivo*.

[0057] In another embodiment, provided herein is an isolated mature NK cell population, wherein said mature NK cells are produced according to the three-stage method described herein. In specific embodiments, the mature NK cell population is formulated into a pharmaceutical composition suitable for use *in vivo*, for example, suitable for human use *in vivo*.

[0058] In another embodiment, provided herein is an isolated ILC3 population, wherein said ILC3 cells are produced according to the three-stage method described herein. In specific embodiments, the isolated ILC3 population is formulated into a pharmaceutical composition suitable for use *in vivo*, for example, suitable for human use *in vivo*.

[0059] In another embodiment, provided herein is a cell population, wherein said cell population is the third population of cells produced by a method described herein. In another embodiment, provided herein is a cell population, wherein said cell population is the fourth population of cells produced by a method described herein.

[0060] In another embodiment, provided herein is an isolated NK cell population, wherein said NK cells are activated, wherein said activated NK cells are produced according to the three-stage method described herein. In specific embodiments, the isolated NK population is formulated into a pharmaceutical composition suitable for use *in vivo*, for example, suitable for human use *in vivo*.

[0061] Accordingly, in another aspect, provided herein is the use of NK cell populations produced using the three-stage methods described herein to suppress tumor cell proliferation, treat viral infection, or treat cancer, *e.g.*, blood cancers and solid tumors. In certain embodiments, the NK cell populations are contacted with, or used in combination with, an immunomodulatory compound, *e.g.*, an immunomodulatory compound described herein, or thalidomide. In certain embodiments, the NK cell populations are treated with, or used in combination with, an immunomodulatory compound, *e.g.*, an immunomodulatory compound described herein, or thalidomide.

[0062] In a specific embodiment, said cancer is a solid tumor. In another embodiment, said cancer is a blood cancer. In specific embodiments, the cancer is glioblastoma, primary ductal carcinoma, leukemia, acute T cell leukemia, chronic myeloid lymphoma (CML), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), lung carcinoma, colon adenocarcinoma, histiocytic lymphoma, colorectal carcinoma, colorectal adenocarcinoma,

prostate cancer, multiple myeloma, or retinoblastoma. In more specific embodiments, the cancer is AML. In more specific embodiments, the cancer is multiple myeloma.

[0063] In another specific embodiment, the hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells, from which the NK cell populations are produced, are obtained from placental perfusate, umbilical cord blood or peripheral blood. In one embodiment, the hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells, from which NK cell populations are produced, are obtained from placenta, *e.g.*, from placental perfusate. In one embodiment, the hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells, from which the NK cell populations are produced, are not obtained from umbilical cord blood. In one embodiment, the hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells, from which the NK cell populations are produced, are not obtained from peripheral blood. In another specific embodiment, the hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells, from which the NK cell populations are produced, are combined cells from placental perfusate and cord blood, *e.g.*, cord blood from the same placenta as the perfusate. In another specific embodiment, said umbilical cord blood is isolated from a placenta other than the placenta from which said placental perfusate is obtained. In certain embodiments, the combined cells can be obtained by pooling or combining the cord blood and placental perfusate. In certain embodiments, the cord blood and placental perfusate are combined at a ratio of 100:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45: 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 15:1, 10:1, 5:1, 1:1, 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, or the like by volume to obtain the combined cells. In a specific embodiment, the cord blood and placental perfusate are combined at a ratio of from 10:1 to 1:10, from 5:1 to 1:5, or from 3:1 to 1:3. In another specific embodiment, the cord blood and placental perfusate are combined at a ratio of 10:1, 5:1, 3:1, 1:1, 1:3, 1:5 or 1:10. In a more specific embodiment, the cord blood and placental perfusate are combined at a ratio of 8.5:1.5 (85%:15%).

[0064] In certain embodiments, the cord blood and placental perfusate are combined at a ratio of 100:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45: 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 15:1, 10:1, 5:1, 1:1, 1:5, 1:10, 1:15, 1:20,

1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, or the like, as determined by total nucleated cells (TNC) content to obtain the combined cells. In a specific embodiment, the cord blood and placental perfusate are combined at a ratio of from 10:1 to 10:1, from 5:1 to 1:5, or from 3:1 to 1: 3. In another specific embodiment, the cord blood and placental perfusate are combined at a ratio of 10:1, 5:1, 3:1, 1:1, 1:3, 1:5 or 1:10.

[0065] In one embodiment, therefore, provided herein is a method of treating an individual having cancer or a viral infection, comprising administering to said individual an effective amount of cells from an isolated NK cell population produced using the three-stage methods described herein. In certain embodiments, the cancer is a solid tumor. In certain embodiments, the cancer is a hematological cancer. In a specific embodiment, the hematological cancer is leukemia. In another specific embodiment, the hematological cancer is lymphoma. In another specific embodiment, the hematological cancer is acute myeloid leukemia. In another specific embodiment, the hematological cancer is chronic lymphocytic leukemia. In another specific embodiment, the hematological cancer is chronic myelogenous leukemia. In certain aspects, said natural killer cells have been cryopreserved prior to said contacting or said administering. In other aspects, said natural killer cells have not been cryopreserved prior to said contacting or said administering.

[0066] In a specific embodiment, the NK cell populations produced using the three-stage methods described herein have been treated with an immunomodulatory compound, *e.g.* an immunomodulatory compound described herein, or thalidomide, prior to said administration. In a specific embodiment, the NK cell populations produced using the three-stage methods described herein have been treated with IL2 and IL12 and IL18, IL12 and IL15, IL12 and IL18, IL2 and IL12 and IL15 and IL18, or IL2 and IL15 and IL18 prior to said administration. In another specific embodiment, the isolated NK cell population produced using the three-stage methods described herein has been pretreated with one or more of IL2, IL12, IL18, or IL15 prior to said administration. In another specific embodiment, the method comprises administering to the individual (1) an effective amount of an isolated NK cell population produced using a three-stage method described herein; and (2) an effective amount of an immunomodulatory compound or thalidomide. An "effective amount" in this context means an amount of cells in an NK cell population, and optionally immunomodulatory compound or thalidomide, that results in a detectable improvement in one or more symptoms of said cancer or said infection, compared to

an individual having said cancer or said infection who has not been administered said NK cell population and, optionally, an immunomodulatory compound or thalidomide. In a specific embodiment, said immunomodulatory compound is lenalidomide or pomalidomide. In another embodiment, the method additionally comprises administering an anticancer compound to the individual, *e.g.*, one or more of the anticancer compounds described below.

[0067] In another embodiment, provided herein is a method of suppressing the proliferation of tumor cells comprising bringing a therapeutically effective amount of an NK cell population into proximity with the tumor cells, *e.g.*, contacting the tumor cells with the cells in an NK cell population. Hereinafter, unless noted otherwise, the term "proximity" refers to sufficient proximity to elicit the desired result; *e.g.*, in certain embodiments, the term proximity refers to contact. In certain embodiments, said contacting takes place *in vitro*. In certain embodiments, said contacting takes place *ex vivo*. In other embodiments, said contacting takes place *in vivo*. A plurality of NK cells can be used in the method of suppressing the proliferation of the tumor cells comprising bringing a therapeutically effective amount of the NK cell population into proximity with the tumor cells, *e.g.*, contacting the tumor cells with the cells in the NK cell population. In certain embodiments, said tumor cells are breast cancer cells, head and neck cancer cells, or sarcoma cells. In certain embodiments, said tumor cells are primary ductal carcinoma cells, leukemia cells, acute T cell leukemia cells, chronic myeloid lymphoma (CML) cells, chronic myelogenous leukemia (CML) cells, lung carcinoma cells, colon adenocarcinoma cells, histiocytic lymphoma cells, colorectal carcinoma cells, colorectal adenocarcinoma cells, or retinoblastoma cells.

[0068] In one embodiment, provided herein are a plurality of natural killer cells for use in a method of suppressing the proliferation of tumor cells comprising contacting the tumor cells with the plurality of natural killer cells, wherein the natural killer cells are produced by the methods described herein. In certain embodiments, said contacting takes place in a human individual. In certain embodiments, said method comprises administering said natural killer cells to said individual. In certain embodiments, said tumor cells are multiple myeloma cells. In certain embodiments, said tumor cells are acute myeloid leukemia (AML) cells. In certain embodiments, said individual has relapsed/refractory AML. In certain embodiments, said individual has AML that has failed at least one non-innate lymphoid cell (ILC) therapeutic against AML. In certain embodiments, said individual is 65 years old or greater, and is in first remission. In certain

embodiments, said individual has been conditioned with fludarabine, cytarabine, or both prior to administering said NK cells. In certain embodiments, said tumor cells are breast cancer cells, head and neck cancer cells, or sarcoma cells. In certain embodiments, said tumor cells are primary ductal carcinoma cells, leukemia cells, acute T cell leukemia cells, chronic myeloid lymphoma (CML) cells, chronic myelogenous leukemia (CML) cells, lung carcinoma cells, colon adenocarcinoma cells, histiocytic lymphoma cells, colorectal carcinoma cells, colorectal adenocarcinoma cells, or retinoblastoma cells. In certain embodiments, said tumor cells are solid tumor cells, liver tumor cells, lung tumor cells, pancreatic tumor cells, renal tumor cells or glioblastoma multiforme (GBM) cells. In certain embodiments, said natural killer cells are administered with an anti-CD33 antibody, an anti-CD20 antibody, an anti-CD138 antibody or an anti-CD32 antibody. In certain embodiments, said NK cells have or have not been cryopreserved prior to said contacting or said administering.

[0069] Administration of an isolated population of NK cells or a pharmaceutical composition thereof may be systemic or local. In specific embodiments, administration is parenteral. In specific embodiments, administration of an isolated population of NK cells or a pharmaceutical composition thereof to a subject is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In specific embodiments, administration of an isolated population of NK cells or a pharmaceutical composition thereof to a subject is performed with a device, a matrix, or a scaffold. In specific embodiments, administration an isolated population of NK cells or a pharmaceutical composition thereof to a subject is by injection. In specific embodiments, administration an isolated population of NK cells or a pharmaceutical composition thereof to a subject is via a catheter. In specific embodiments, the injection of NK cells is local injection. In more specific embodiments, the local injection is directly into a solid tumor (*e.g.*, a sarcoma). In specific embodiments, administration of an isolated population of NK cells or a pharmaceutical composition thereof to a subject is by injection by syringe. In specific embodiments, administration of an isolated population of NK cells or a pharmaceutical composition thereof to a subject is via guided delivery. In specific embodiments, administration of an isolated population of NK cells or a pharmaceutical composition thereof to a subject by injection is aided by laparoscopy, endoscopy, ultrasound, computed tomography, magnetic resonance, or radiology.

[0070] In a specific embodiment, the isolated NK cell population produced using the three-stage methods described herein has been treated with an immunomodulatory compound, *e.g.* an immunomodulatory compound described herein, below, or thalidomide, and/or IL2 and IL12 and IL18, IL12 and IL15, IL12 and IL18, IL2 and IL12 and IL15 and IL18, or IL2 and IL15 and IL18, prior to said contacting or bringing into proximity. In another specific embodiment, the isolated NK cell population produced using the three-stage methods described herein has been treated with one or more of IL2, IL12, IL18, or IL15 prior to said contacting or bringing into proximity. In another specific embodiment, an effective amount of an immunomodulatory compound, *e.g.* an immunomodulatory compound described herein, below, or thalidomide is additionally brought into proximity with the tumor cells *e.g.*, the tumor cells are contacted with the immunomodulatory compound or thalidomide. An "effective amount" in this context means an amount of cells in an NK cell population, and optionally an immunomodulatory compound or thalidomide, that results in a detectable suppression of said tumor cells compared to an equivalent number of tumor cells not contacted or brought into proximity with cells in an NK cell population, and optionally an immunomodulatory compound or thalidomide. In another specific embodiment, the method further comprises bringing an effective amount of an anticancer compound, *e.g.*, an anticancer compound described below, into proximity with the tumor cells, *e.g.*, contacting the tumor cells with the anticancer compound.

[0071] In a specific embodiment of this method, the tumor cells are blood cancer cells. In another specific embodiment, the tumor cells are solid tumor cells. In another embodiment, the tumor cells are primary ductal carcinoma cells, leukemia cells, acute T cell leukemia cells, chronic myeloid lymphoma (CML) cells, acute myelogenous leukemia cells (AML), chronic myelogenous leukemia (CML) cells, glioblastoma cells, lung carcinoma cells, colon adenocarcinoma cells, histiocytic lymphoma cells, multiple myeloma cells, retinoblastoma cell, colorectal carcinoma cells, prostate cancer cells, or colorectal adenocarcinoma cells. In more specific embodiments, the tumor cells are AML cells. In more specific embodiments, the tumor cells are multiple myeloma cells. In another specific embodiment, said contacting or bringing into proximity takes place *in vitro*. In another specific embodiment, said contacting or bringing into proximity takes place *ex vivo*. In another specific embodiment, said contacting or bringing into proximity takes place *in vivo*. In a more specific embodiment, said *in vivo* contacting or bringing into proximity takes place in a human. In a specific embodiment, said tumor cells are

solid tumor cells. In a specific embodiment, said tumor cells are liver tumor cells. In a specific embodiment, said tumor cells are lung tumor cells. In a specific embodiment, said tumor cells are pancreatic tumor cells. In a specific embodiment, said tumor cells are renal tumor cells. In a specific embodiment, said tumor cells are glioblastoma multiforme (GBM) cells. In a specific embodiment, said natural killer cells are administered with an antibody. In a specific embodiment, said natural killer cells are administered with an anti-CD33 antibody. In a specific embodiment, said natural killer cells are administered with an anti-CD20 antibody. In a specific embodiment, said natural killer cells are administered with an anti-CD138 antibody. In a specific embodiment, said natural killer cells are administered with an anti-CD32 antibody.

[0072] In another aspect, provided herein is a method of treating an individual having multiple myeloma, comprising administering to the individual (1) lenalidomide; (2) melphalan; and (3) NK cells, wherein said NK cells are effective to treat multiple myeloma in said individual. In a specific embodiment, said NK cells are cord blood NK cells, or NK cells produced from cord blood hematopoietic cells, *e.g.*, hematopoietic stem cells. In another embodiment, said NK cells have been produced by any of the methods described herein for producing NK cells, *e.g.*, for producing NK cell populations using a three-stage method. In another embodiment, said NK cells have been expanded prior to said administering. In another embodiment, said lenalidomide, melphalan, and/or NK cells are administered separately from each other. In certain specific embodiments of the method of treating an individual with multiple myeloma, said NK cell populations are produced by a three-stage method, as described herein.

[0073] In another aspect, provided herein is a method of treating an individual having acute myelogenous leukemia (AML), comprising administering to the individual NK cells (optionally activated by pretreatment with IL2 and IL12 and IL18, IL12 and IL15, IL12 and IL18, IL2 and IL12 and IL15 and IL18, or IL2 and IL15 and IL18), wherein said NK cells are effective to treat AML in said individual. In a specific embodiment, the isolated NK cell population produced using the three-stage methods described herein has been pretreated with one or more of IL2, IL12, IL18, or IL15 prior to said administering. In a specific embodiment, said NK cells are cord blood NK cells, or NK cells produced from cord blood hematopoietic cells, *e.g.*, hematopoietic stem cells. In another embodiment, said NK cells have been produced by any of the methods described herein for producing NK cells, *e.g.*, for producing NK cell populations using a three-stage method as set forth herein. In certain specific embodiments of the method of

treating an individual with AML, said NK cell populations are produced by a three-stage method, as described herein. In a particular embodiment, the AML to be treated by the foregoing methods comprises refractory AML, poor-prognosis AML, or childhood AML. In certain embodiments, said individual has AML that has failed at least one non-natural killer or non-innate lymphoid cell therapeutic against AML. In specific embodiments, said individual is 65 years old or greater, and is in first remission. In specific embodiments, said individual has been conditioned with fludarabine, cytarabine, or both prior to administering said natural killer cells. In another aspect, provided herein is a method of treating an individual having chronic lymphocytic leukemia (CLL), comprising administering to the individual a therapeutically effective dose of (1) lenalidomide; (2) melphalan; (3) fludarabine; and (4) NK cells, *e.g.*, a NK cell population produced using a three-stage method described herein, wherein said NK cells are effective to treat said CLL in said individual. In a specific embodiment, said NK cells are cord blood NK cells, or NK cells produced from cord blood hematopoietic cells, *e.g.*, hematopoietic stem cells. In another embodiment, said NK cells have been produced by any of the methods described herein for producing NK cells, *e.g.*, for producing NK cell populations using a three-stage method described herein. In a specific embodiment of any of the above methods, said lenalidomide, melphalan, fludarabine, and expanded NK cells are administered to said individual separately. In certain specific embodiments of the method of treating an individual with CLL, said NK cell populations are produced by a three-stage method, as described herein.

[0074] In another embodiment, provided herein is a method of suppressing the proliferation of tumor cells comprising bringing a therapeutically effective amount of an ILC3 cell population into proximity with the tumor cells, *e.g.*, contacting the tumor cells with the cells in an ILC3 cell population. Hereinafter, unless noted otherwise, the term "proximity" refers to sufficient proximity to elicit the desired result; *e.g.*, in certain embodiments, the term proximity refers to contact. In certain embodiments, said contacting takes place *in vitro*. In certain embodiments, said contacting takes place *ex vivo*. In other embodiments, said contacting takes place *in vivo*. A plurality of ILC3 cells can be used in the method of suppressing the proliferation of the tumor cells comprising bringing a therapeutically effective amount of the ILC3 cell population into proximity with the tumor cells, *e.g.*, contacting the tumor cells with the cells in the ILC3 cell population. In certain embodiments, said tumor cells are breast cancer cells, head and neck cancer cells, or sarcoma cells. In certain embodiments, said tumor cells are primary

ductal carcinoma cells, leukemia cells, acute T cell leukemia cells, chronic myeloid lymphoma (CML) cells, chronic myelogenous leukemia (CML) cells, lung carcinoma cells, colon adenocarcinoma cells, histiocytic lymphoma cells, colorectal carcinoma cells, colorectal adenocarcinoma cells, or retinoblastoma cells.

[0075] In one embodiment, provided herein are a plurality of ILC3 cells for use in a method of suppressing the proliferation of tumor cells comprising contacting the tumor cells with the plurality of ILC3 cells, wherein the ILC3 cells are produced by the methods described herein. In certain embodiments, said contacting takes place in a human individual. In certain embodiments, said method comprises administering said ILC3 cells to said individual. In certain embodiments, said tumor cells are multiple myeloma cells. In certain embodiments, said tumor cells are acute myeloid leukemia (AML) cells. In certain embodiments, said individual has relapsed/refractory AML. In certain embodiments, said individual has AML that has failed at least one non-innate lymphoid cell (ILC) therapeutic against AML. In certain embodiments, said individual is 65 years old or greater, and is in first remission. In certain embodiments, said individual has been conditioned with fludarabine, cytarabine, or both prior to administering said ILC3 cells. In certain embodiments, said tumor cells are breast cancer cells, head and neck cancer cells, or sarcoma cells. In certain embodiments, said tumor cells are primary ductal carcinoma cells, leukemia cells, acute T cell leukemia cells, chronic myeloid lymphoma (CML) cells, chronic myelogenous leukemia (CML) cells, lung carcinoma cells, colon adenocarcinoma cells, histiocytic lymphoma cells, colorectal carcinoma cells, colorectal adenocarcinoma cells, or retinoblastoma cells. In certain embodiments, said tumor cells are solid tumor cells, liver tumor cells, lung tumor cells, pancreatic tumor cells, renal tumor cells or glioblastoma multiforme (GBM) cells. In certain embodiments, said ILC3 cells are administered with an anti-CD33 antibody, an anti-CD20 antibody, an anti-CD138 antibody or an anti-CD32 antibody. In certain embodiments, said ILC3 cells have or have not been cryopreserved prior to said contacting or said administering.

[0076] Administration of an isolated population of ILC3 cells or a pharmaceutical composition thereof may be systemic or local. In specific embodiments, administration is parenteral. In specific embodiments, administration of an isolated population of ILC3 cells or a pharmaceutical composition thereof to a subject is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In specific

embodiments, administration of an isolated population of ILC3 cells or a pharmaceutical composition thereof to a subject is performed with a device, a matrix, or a scaffold. In specific embodiments, administration an isolated population of ILC3 cells or a pharmaceutical composition thereof to a subject is by injection. In specific embodiments, administration an isolated population of ILC3 cells or a pharmaceutical composition thereof to a subject is via a catheter. In specific embodiments, the injection of ILC3 cells is local injection. In more specific embodiments, the local injection is directly into a solid tumor (*e.g.*, a sarcoma). In specific embodiments, administration of an isolated population of ILC3 cells or a pharmaceutical composition thereof to a subject is by injection by syringe. In specific embodiments, administration of an isolated population of ILC3 cells or a pharmaceutical composition thereof to a subject is via guided delivery. In specific embodiments, administration of an isolated population of ILC3 cells or a pharmaceutical composition thereof to a subject by injection is aided by laparoscopy, endoscopy, ultrasound, computed tomography, magnetic resonance, or radiology.

[0077] In a specific embodiment, the isolated ILC3 cell population produced using the three-stage methods described herein has been treated with an immunomodulatory compound, *e.g.* an immunomodulatory compound described herein, below, or thalidomide, and/or IL2 and IL12 and IL18, IL12 and IL15, IL12 and IL18, IL2 and IL12 and IL15 and IL18, or IL2 and IL15 and IL18, prior to said contacting or bringing into proximity. In a specific embodiment, the isolated NK cell population produced using the three-stage methods described herein has been treated with one or more of IL2, IL12, IL18, or IL15 prior to said contacting or bringing into proximity. In another specific embodiment, an effective amount of an immunomodulatory compound, *e.g.* an immunomodulatory compound described herein, below, or thalidomide is additionally brought into proximity with the tumor cells *e.g.*, the tumor cells are contacted with the immunomodulatory compound or thalidomide. An "effective amount" in this context means an amount of cells in an ILC3 cell population, and optionally an immunomodulatory compound or thalidomide, that results in a detectable suppression of said tumor cells compared to an equivalent number of tumor cells not contacted or brought into proximity with cells in an ILC3 cell population, and optionally an immunomodulatory compound or thalidomide. In another specific embodiment, the method further comprises bringing an effective amount of an

anticancer compound, *e.g.*, an anticancer compound described below, into proximity with the tumor cells, *e.g.*, contacting the tumor cells with the anticancer compound.

[0078] In a specific embodiment of this method, the tumor cells are blood cancer cells. In another specific embodiment, the tumor cells are solid tumor cells. In another embodiment, the tumor cells are primary ductal carcinoma cells, leukemia cells, acute T cell leukemia cells, chronic myeloid lymphoma (CML) cells, acute myelogenous leukemia cells (AML), chronic myelogenous leukemia (CML) cells, glioblastoma cells, lung carcinoma cells, colon adenocarcinoma cells, histiocytic lymphoma cells, multiple myeloma cells, retinoblastoma cell, colorectal carcinoma cells, prostate cancer cells, or colorectal adenocarcinoma cells. In another specific embodiment, said contacting or bringing into proximity takes place *in vitro*. In another specific embodiment, said contacting or bringing into proximity takes place *ex vivo*. In another specific embodiment, said contacting or bringing into proximity takes place *in vivo*. In a more specific embodiment, said *in vivo* contacting or bringing into proximity takes place in a human. In a specific embodiment, said tumor cells are solid tumor cells. In a specific embodiment, said tumor cells are liver tumor cells. In a specific embodiment, said tumor cells are lung tumor cells. In a specific embodiment, said tumor cells are pancreatic tumor cells. In a specific embodiment, said tumor cells are renal tumor cells. In a specific embodiment, said tumor cells are glioblastoma multiforme (GBM) cells. In a specific embodiment, said ILC3 cells are administered with an antibody. In a specific embodiment, said ILC3 cells are administered with an anti-CD33 antibody. In a specific embodiment, said ILC3 cells are administered with an anti-CD20 antibody. In a specific embodiment, said ILC3 cells are administered with an anti-CD138 antibody. In a specific embodiment, said ILC3 cells are administered with an anti-CD32 antibody.

[0079] In another aspect, provided herein is a method of treating an individual having multiple myeloma, comprising administering to the individual (1) lenalidomide; (2) melphalan; and (3) ILC3 cells, wherein said ILC3 cells are effective to treat multiple myeloma in said individual. In a specific embodiment, said ILC3 cells are cord blood ILC3 cells, or ILC3 cells produced from cord blood hematopoietic cells, *e.g.*, hematopoietic stem cells. In another embodiment, said ILC3 cells have been produced by any of the methods described herein for producing ILC3 cells, *e.g.*, for producing ILC3 cell populations using a three-stage method. In another embodiment, said ILC3 cells have been expanded prior to said administering. In another

embodiment, said lenalidomide, melphalan, and/or ILC3 cells are administered separately from each other. In certain specific embodiments of the method of treating an individual with multiple myeloma, said ILC3 cell populations are produced by a three-stage method, as described herein.

[0080] In another aspect, provided herein is a method of treating an individual having acute myelogenous leukemia (AML), comprising administering to the individual ILC3 cells (optionally activated by pretreatment with IL2 and IL12 and IL18, IL12 and IL15, IL12 and IL18, IL2 and IL12 and IL15 and IL18, or IL2 and IL15 and IL18), wherein said ILC3 cells are effective to treat AML in said individual. In a specific embodiment, the isolated NK cell population produced using the three-stage methods described herein has been pretreated with one or more of IL2, IL12, IL18, or IL15 prior to said administering. In a specific embodiment, said ILC3 cells are cord blood ILC3 cells, or ILC3 cells produced from cord blood hematopoietic cells, *e.g.*, hematopoietic stem cells. In another embodiment, said ILC3 cells have been produced by any of the methods described herein for producing ILC3 cells, *e.g.*, for producing ILC3 cell populations using a three-stage method as set forth herein. In certain specific embodiments of the method of treating an individual with AML, said ILC3 cell populations are produced by a three-stage method, as described herein. In a particular embodiment, the AML to be treated by the foregoing methods comprises refractory AML, poor-prognosis AML, or childhood AML. In certain embodiments, said individual has AML that has failed at least one non-ILC3 or non-innate lymphoid cell therapeutic against AML. In specific embodiments, said individual is 65 years old or greater, and is in first remission. In specific embodiments, said individual has been conditioned with fludarabine, cytarabine, or both prior to administering said ILC3 cells. In another aspect, provided herein is a method of treating an individual having chronic lymphocytic leukemia (CLL), comprising administering to the individual a therapeutically effective dose of (1) lenalidomide; (2) melphalan; (3) fludarabine; and (4) ILC3 cells, *e.g.*, a ILC3 cell population produced using a three-stage method described herein, wherein said ILC3 cells are effective to treat said CLL in said individual. In a specific embodiment, said ILC3 cells are cord blood ILC3 cells, or ILC3 cells produced from cord blood hematopoietic cells, *e.g.*, hematopoietic stem cells. In another embodiment, said ILC3 cells have been produced by any of the methods described herein for producing ILC3 cells, *e.g.*, for producing ILC3 cell populations using a three-stage method described herein. In a specific embodiment of any of the above methods, said lenalidomide, melphalan, fludarabine, and expanded ILC3 cells

are administered to said individual separately. In certain specific embodiments of the method of treating an individual with CLL, said ILC3 cell populations are produced by a three-stage method, as described herein.

[0081] In certain embodiments, the NK cell populations produced using a three-stage method described herein are cryopreserved, *e.g.*, cryopreserved using a method described herein. In a certain embodiments, the NK cell populations produced using a three-stage method described herein are cryopreserved in a cryopreservation medium, *e.g.*, a cryopreservation medium described herein. In a specific embodiment, cryopreservation of the NK progenitor cell populations and/or NK cell populations produced using a three-stage method described herein comprises (1) preparing a cell suspension solution comprising an NK progenitor cell population and/or an NK cell population produced using a three-stage method described herein; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain a cryopreserved cell suspension; (3) cooling the cryopreserved cell suspension from step (3) to obtain a cryopreserved sample; and (4) storing the cryopreserved sample below -80 ℃.

[0082] In certain embodiments of the methods of treatment or tumor suppression above, NK cell populations produced by a three-stage method described herein are combined with other natural killer cells, *e.g.*, natural killer cells isolated from placental perfusate, umbilical cord blood or peripheral blood, or produced from hematopoietic cells by a different method. In specific embodiments, the natural killer cell populations are combined with natural killer cells from another source, or made by a different method, in a ratio of about 100:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45: 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 15:1, 10:1, 5:1, 1:1, 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, or the like.

[0083] In certain embodiments, the ILC3 cell populations produced using a three-stage method described herein are cryopreserved, *e.g.*, cryopreserved using a method described herein. In a certain embodiments, the ILC3 cell populations produced using a three-stage method described herein are cryopreserved in a cryopreservation medium, *e.g.*, a cryopreservation medium described herein. In a specific embodiment, cryopreservation of the ILC3 progenitor cell populations and/or ILC3 cell populations produced using a three-stage method described herein comprises (1) preparing a cell suspension solution comprising an ILC3 progenitor cell

population and/or an ILC3 cell population produced using a three-stage method described herein; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain a cryopreserved cell suspension; (3) cooling the cryopreserved cell suspension from step (3) to obtain a cryopreserved sample; and (4) storing the cryopreserved sample below -80 °C.

[0084] In certain embodiments of the methods of treatment or tumor suppression above, ILC3 cell populations produced by a three-stage method described herein are combined with other ILC3 cells, *e.g.*, ILC3 cells isolated from placental perfusate, umbilical cord blood or peripheral blood, or produced from hematopoietic cells by a different method. In specific embodiments, the ILC3 cell populations are combined with ILC3 cells from another source, or made by a different method, in a ratio of about 100:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45: 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 15:1, 10:1, 5:1, 1:1, 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, or the like.

[0085] In another aspect, provided herein is a method of repairing the gastrointestinal tract after chemotherapy comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells are produced by a three-stage method described herein. A plurality of ILC3 cells can be used in the method of repairing the gastrointestinal tract after chemotherapy comprising administering to an individual a plurality of the ILC3 cells, wherein the ILC3 cells are produced by a three-stage method described herein.

[0086] In another aspect, provided herein is a method of protecting an individual against radiation comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells are produced by a three-stage method described herein. A plurality of ILC3 cells can be used in the method of protecting an individual against radiation comprising administering to an individual a plurality of the ILC3 cells, wherein the ILC3 cells are produced by a three-stage method described herein. In certain aspects of the method, said ILC3 cells are used as an adjunct to bone marrow transplantation.

[0087] In another aspect, provided herein is a method of reconstituting the thymus of an individual comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells are produced by a three-stage method described herein. A plurality of ILC3 cells can be used in the method of reconstituting the thymus of an individual comprising administering to an

individual a plurality of the ILC3 cells, wherein the ILC3 cells are produced by a three-stage method described herein.

[0088] In another aspect, provided herein is a composition comprising isolated NK cells produced by a three-stage method described herein. In a specific embodiment, said NK cells are produced from hematopoietic cells, *e.g.*, hematopoietic stem or progenitor cells isolated from placental perfusate, umbilical cord blood, and/or peripheral blood. In another specific embodiment, said NK cells comprise at least 70% of cells in the composition. In another specific embodiment, said NK cells comprise at least 80%, 85%, 90%, 95%, 98% or 99% of cells in the composition. In certain embodiments, at least 80%, 82%, 84%, 86%, 88% or 90% of NK cells in said composition are $CD3^-$ and $CD56^+$. In certain embodiments, at least 65%, 70%, 75%, 80%, 82%, 84%, 86%, 88% or 90% of NK cells in said composition are CD16-. In certain embodiments, at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55% or 60% of NK cells in said composition are CD94+.

[0089] In certain aspects, provided herein is a population of natural killer cells that is CD56+CD3– CD117+CD11a+, wherein said natural killer cells express perforin and/or EOMES, and do not express one or more of RORγt, aryl hydrocarbon receptor, and IL1R1. In certain aspects, said natural killer cells express perforin and EOMES, and do not express any of RORγt, aryl hydrocarbon receptor, or IL1R1. In certain aspects, said natural killer cells additionally express T-bet, GZMB, NKp46, NKp30, and NKG2D. In certain aspects, said natural killer cells express CD94. In certain aspects, said natural killer cells do not express CD94.

[0090] In certain aspects, provided herein is a population of ILC3 cells that is CD56+CD3– CD117+CD11a-, wherein said ILC3 cells express one or more of RORγt, aryl hydrocarbon receptor, and IL1R1, and do not express one or more of CD94, perforin, and EOMES. In certain aspects, said ILC3 cells express RORγt, aryl hydrocarbon receptor, and IL1R1, and do not express any of CD94, perforin, or EOMES. In certain aspects, said ILC3 cells additionally express CD226 and/or 2B4. In certain aspects, said ILC3 cells additionally express one or more of IL-22, TNFα, and DNAM-1. In certain aspects, said ILC3 cells express CD226, 2B4, IL-22, TNFα, and DNAM-1.

[0091] In certain aspects, provided herein is a method of producing a cell population comprising natural killer cells and ILC3 cells, comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin

(Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of a stem cell mobilizing agent and LMWH, to produce a third population of cells; and (d) separating CD11a+ cells and CD11a– cells from the third population of cells; and (e) combining the CD11a+ cells with the CD11a– cells in a ratio of 50:1, 40:1, 30:1, 20:1, 10:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:10, 1:20, 1:30, 1:40, or 1:50 to produce a fourth population of cells. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 50:1. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 20:1. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 10:1. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 5:1. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 1:1. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 1:5. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 1:10. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 1:20. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 1:50.

[0092] In certain aspects, a plurality of the NK cells in said population expresses one or more of the microRNAS dme-miR-7, hsa-let-7a, hsa-let-7c, hsa-let-7e, hsa-let-7g, hsa-miR-103, hsa-miR-106a, hsa-miR-10b, hsa-miR-1183, hsa-miR-124, hsa-miR-1247, hsa-miR-1248, hsa-miR-1255A, hsa-miR-126, hsa-miR-140-3p, hsa-miR-144, hsa-miR-151-3p, hsa-miR-155, hsa-miR-15a, hsa-miR-16, hsa-miR-17, hsa-miR-181a, hsa-miR-182, hsa-miR-192, hsa-miR-199a-

3p, hsa-miR-200a, hsa-miR-20a, hsa-miR-214, hsa-miR-221, hsa-miR-29a, hsa-miR-29b, hsa-miR-30b, hsa-miR-30c, hsa-miR-31, hsa-miR-335, hsa-miR-374b, hsa-miR-454, hsa-miR-484, hsa-miR-513C, hsa-miR-516-3p, hsa-miR-520h, hsa-miR-548K, hsa-miR-548P, hsa-miR-600, hsa-miR-641, hsa-miR-643, hsa-miR-874, hsa-miR-875-5p, and hsa-miR-92a-2 at a detectably higher level as peripheral blood natural killer cells. In certain aspects, a plurality of the NK cells in said population expresses one or more of the microRNAS miR188-5p, miR-339-5p, miR-19a, miR-34c, miR-18a, miR-500, miR-22, miR-222, miR-7a, miR-532-3p, miR-223, miR-26b, miR-26a, miR-191, miR-181d, miR-322, and miR342-3p at a detectably lower level than peripheral blood natural killer cells. In certain aspects, a plurality of the NK cells in said population expresses one or more of the microRNAS miR-181a, miR-30b, and miR30c at an equivalent level to peripheral blood natural killer cells.

[0093] In a specific embodiment, said NK cells are from a single individual, that is, said hemtopoietic stem and progenitor cells are from a single individual. In a more specific embodiment, said NK cells comprise natural killer cells from at least two different individuals, that is, said hemtopoietic stem and progenitor cells are from at least two different individuals. In another specific embodiment, said NK cells are from a different individual than the individual for whom treatment with the NK cells is intended, that is, said hemtopoietic stem and progenitor cells are from a different individual than the individual for whom treatment with the NK cells is intended. In another specific embodiment, said NK cells have been contacted or brought into proximity with an immunomodulatory compound or thalidomide in an amount and for a time sufficient for said NK cells to express detectably more granzyme B or perforin than an equivalent number of natural killer cells, *i.e.* NK cells, not contacted or brought into proximity with said immunomodulatory compound or thalidomide. In another specific embodiment, a composition comprising said NK cells additionally comprises an immunomodulatory compound or thalidomide. In certain embodiments, the immunomodulatory compound is a compound described below, *e.g.*, an amino-substituted isoindoline compound. In certain embodiments, the immunomodulatory compound is lenalidomide. In certain embodiments, the immunomodulatory compound is pomalidomide.

[0094] In another specific embodiment, a composition comprising said NK cells additionally comprises one or more anticancer compounds, *e.g.*, one or more of the anticancer compounds described below.

[0095] In a more specific embodiment, the composition comprises NK cells produced by a three-stage method described herein and natural killer cells from another source, or made by another method. In a specific embodiment, said other source is placental blood and/or umbilical cord blood. In another specific embodiment, said other source is peripheral blood. In more specific embodiments, the NK cells are combined with natural killer cells from another source, or made by another method in a ratio of about 100:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45: 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 15:1, 10:1, 5:1, 1:1, 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, or the like.

[0096] In another specific embodiment, the composition comprises NK cells produced using a three-stage method described herein and either isolated placental perfusate or isolated placental perfusate cells. In a more specific embodiment, said placental perfusate is from the same individual as said NK cells. In another more specific embodiment, said placental perfusate comprises placental perfusate from a different individual than said NK cells. In another specific embodiment, all, or substantially all (*e.g.*, greater than 90%, 95%, 98% or 99%) of cells in said placental perfusate are fetal cells. In another specific embodiment, the placental perfusate or placental perfusate cells, comprise fetal and maternal cells. In a more specific embodiment, the fetal cells in said placental perfusate comprise less than about 90%, 80%, 70%, 60% or 50% of the cells in said perfusate. In another specific embodiment, said perfusate is obtained by passage of a 0.9% NaCl solution through the placental vasculature. In another specific embodiment, said perfusate comprises a culture medium. In another specific embodiment, said perfusate has been treated to remove erythrocytes. In another specific embodiment, said composition comprises an immunomodulatory compound, *e.g.*, an immunomodulatory compound described below, *e.g.*, an amino-substituted isoindoline compound. In another specific embodiment, the composition additionally comprises one or more anticancer compounds, *e.g.*, one or more of the anticancer compounds described below.

[0097] In another specific embodiment, the composition comprises NK cells produced using a three-stage method described herein and placental perfusate cells. In a more specific embodiment, said placental perfusate cells are from the same individual as said NK cells. In another more specific embodiment, said placental perfusate cells are from a different individual

than said NK cells. In another specific embodiment, the composition comprises isolated placental perfusate and isolated placental perfusate cells, wherein said isolated perfusate and said isolated placental perfusate cells are from different individuals. In another more specific embodiment of any of the above embodiments comprising placental perfusate, said placental perfusate comprises placental perfusate from at least two individuals. In another more specific embodiment of any of the above embodiments comprising placental perfusate cells, said isolated placental perfusate cells are from at least two individuals. In another specific embodiment, said composition comprises an immunomodulatory compound. In another specific embodiment, the composition additionally comprises one or more anticancer compounds, *e.g.*, one or more of the anticancer compounds described below.

[0098] In another aspect, provided herein is a composition, *e.g.*, a pharmaceutical composition, comprising an isolated NK cell population, *e.g.*, produced by any embodiment of the three-stage method described herein. In a specific embodiment, said isolated NK cell population is produced from hematopoietic cells, *e.g.*, hematopoietic stem or progenitor cells isolated from placenta, *e.g.*, from placental perfusate, umbilical cord blood, and/or peripheral blood. In another specific embodiment, said isolated NK cell population comprises at least 70% of cells in the composition. In another specific embodiment, said isolated NK cell population comprises at least 80%, 85%, 90%, 95%, 98% or 99% of cells in the composition. In another specific embodiment, said NK cells comprise at least 70% of cells in the composition. In certain embodiments, at least 80%, 82%, 84%, 86%, 88% or 90% of NK cells in said composition are $CD3^-$ and $CD56^+$. In certain embodiments, at least 65%, 70%, 75%, 80%, 82%, 84%, 86%, 88% or 90% of NK cells in said composition are CD16-. In certain embodiments, at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55% or 60% of NK cells in said composition are CD94+.

[0099] In another specific embodiment, said isolated NK cells in said composition are from a single individual, that is, said hemtopoietic stem and progenitor cells are from a single individual. In a more specific embodiment, said isolated NK cells comprise NK cells from at least two different individuals, that is, said hemtopoietic stem and progenitor cells are from at least two different individuals. In another specific embodiment, said isolated NK cells in said composition are from a different individual than the individual for whom treatment with the NK cells is intended, that is, said hemtopoietic stem and progenitor cells are from a different

individual than the individual for whom treatment with the NK cells is intended. In another specific embodiment, said NK cells have been contacted or brought into proximity with an immunomodulatory compound or thalidomide in an amount and for a time sufficient for said NK cells to express detectably more granzyme B or perforin than an equivalent number of natural killer cells, *i.e.* NK cells not contacted or brought into proximity with said immunomodulatory compound or thalidomide. In another specific embodiment, said composition additionally comprises an immunomodulatory compound or thalidomide. In certain embodiments, the immunomodulatory compound is a compound described below.

[00100] In another specific embodiment, the composition additionally comprises one or more anticancer compounds, *e.g.*, one or more of the anticancer compounds described below.

[00101] In a more specific embodiment, the composition comprises NK cells from another source, or made by another method. In a specific embodiment, said other source is placental blood and/or umbilical cord blood. In another specific embodiment, said other source is peripheral blood. In more specific embodiments, the NK cell population in said composition is combined with NK cells from another source, or made by another method in a ratio of about 100:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45: 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 15:1, 10:1, 5:1, 1:1, 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, or the like.

[00102] In another specific embodiment, the composition comprises an NK cell population and either isolated placental perfusate or isolated placental perfusate cells. In a more specific embodiment, said placental perfusate is from the same individual as said NK cell population. In another more specific embodiment, said placental perfusate comprises placental perfusate from a different individual than said NK cell population. In another specific embodiment, all, or substantially all (*e.g.*, greater than 90%, 95%, 98% or 99%), of cells in said placental perfusate are fetal cells. In another specific embodiment, the placental perfusate or placental perfusate cells, comprise fetal and maternal cells. In a more specific embodiment, the fetal cells comprise less than about 90%, 80%, 70%, 60% or 50% of the cells in said placental perfusate. In another specific embodiment, said perfusate is obtained by passage of a 0.9% NaCl solution through the placental vasculature. In another specific embodiment, said perfusate comprises a culture medium. In another specific embodiment, said perfusate has been treated to

remove erythrocytes. In another specific embodiment, said composition comprises an immunomodulatory compound, *e.g.*, an immunomodulatory compound described below, *e.g.*, an amino-substituted isoindoline compound. In another specific embodiment, the composition additionally comprises one or more anticancer compounds, *e.g.*, one or more of the anticancer compounds described below.

[00103] In another specific embodiment, the composition comprises an NK cell population and placental perfusate cells. In a more specific embodiment, said placental perfusate cells are from the same individual as said NK cell population. In another more specific embodiment, said placental perfusate cells are from a different individual than said NK cell population. In another specific embodiment, the composition comprises isolated placental perfusate and isolated placental perfusate cells, wherein said isolated perfusate and said isolated placental perfusate cells are from different individuals. In another more specific embodiment of any of the above embodiments comprising placental perfusate, said placental perfusate comprises placental perfusate from at least two individuals. In another more specific embodiment of any of the above embodiments comprising placental perfusate cells, said isolated placental perfusate cells are from at least two individuals. In another specific embodiment, said composition comprises an immunomodulatory compound. In another specific embodiment, the composition additionally comprises one or more anticancer compounds, *e.g.*, one or more of the anticancer compounds described below.

3.1. Terminology

[00104] As used herein, the terms "immunomodulatory compound" and "IMiD™" do not encompass thalidomide.

[00105] As used herein, "lenalidomide" means 3-(4'aminoisoindoline-1'-one)-1-piperidine-2,6-dione (Chemical Abstracts Service name) or 2,6-Piperidinedione,3-(4-amino-1,3-dihydro-1-oxo-2H-isoindol-2-yl)- (International Union of Pure and Applied Chemistry (IUPAC) name). As used herein, "pomalidomide" means 4-amino-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione.

[00106] As used herein, "multipotent," when referring to a cell, means that the cell has the capacity to differentiate into a cell of another cell type. In certain embodiments, "a multipotent cell" is a cell that has the capacity to grow into a subset of the mammalian body's approximately

260 cell types. Unlike a pluripotent cell, a multipotent cell does not have the capacity to form all of the cell types.

[00107] As used herein, "feeder cells" refers to cells of one type that are co-cultured with cells of a second type, to provide an environment in which the cells of the second type can be maintained, and perhaps proliferate. Without being bound by any theory, feeder cells can provide, for example, peptides, polypeptides, electrical signals, organic molecules (*e.g.*, steroids), nucleic acid molecules, growth factors (*e.g.*, bFGF), other factors (*e.g.*, cytokines), and metabolic nutrients to target cells. In certain embodiments, feeder cells grow in a mono-layer.

[00108] As used herein, the "natural killer cells" or "NK cells" produced using the methods described herein, without further modification, include natural killer cells from any tissue source.

[00109] As used herein, the "ILC3 cells" produced using the methods described herein, without further modification, include ILC3 cells from any tissue source.

[00110] As used herein, "placental perfusate" means perfusion solution that has been passed through at least part of a placenta, *e.g.*, a human placenta, *e.g.*, through the placental vasculature, and includes a plurality of cells collected by the perfusion solution during passage through the placenta.

[00111] As used herein, "placental perfusate cells" means nucleated cells, *e.g.*, total nucleated cells, isolated from, or isolatable from, placental perfusate.

[00112] As used herein, "tumor cell suppression," "suppression of tumor cell proliferation," and the like, includes slowing the growth of a population of tumor cells, *e.g.*, by killing one or more of the tumor cells in said population of tumor cells, for example, by contacting or bringing, *e.g.*, NK cells or an NK cell population produced using a three-stage method described herein into proximity with the population of tumor cells, *e.g.*, contacting the population of tumor cells with NK cells or an NK cell population produced using a three-stage method described herein. In certain embodiments, said contacting takes place *in vitro* or *ex vivo*. In other embodiments, said contacting takes place *in vivo*.

[00113] As used herein, the term "hematopoietic cells" includes hematopoietic stem cells and hematopoietic progenitor cells.

[00114] As used herein, the "undefined component" is a term of art in the culture medium field that refers to components whose constituents are not generally provided or quantified.

Examples of an "undefined component" include, without limitation, serum, for example, human serum (*e.g.*, human serum AB) and fetal serum (*e.g.*, fetal bovine serum or fetal calf serum).

[00115] As used herein, "+", when used to indicate the presence of a particular cellular marker, means that the cellular marker is detectably present in fluorescence activated cell sorting over an isotype control; or is detectable above background in quantitative or semi-quantitative RT-PCR.

[00116] As used herein, "–", when used to indicate the presence of a particular cellular marker, means that the cellular marker is not detectably present in fluorescence activated cell sorting over an isotype control; or is not detectable above background in quantitative or semi-quantitative RT-PCR.

4. BRIEF DESCRIPTION OF THE FIGURES

[00117] FIG. 1A-C: Effects on (A) fold expansion, (B) cell purity (CD56+CD3-), and (C) cytotoxicity of NK cells against K562 cells at a 10:1 (E:T) ratio for the three-stage method using StemRegenin-1 (SR-1) or CH223191 at 1 μM, 10 μM, and 30 μM, as compared to previous NK cell expansion media ("NK cell exp") or dimethyl sulfoxide (DMSO).

[00118] FIG. 2: Multi-color flow cytometry of CD3-CD56+ gated cells produced by the three-stage method, showing the expression of CD11a and the natural cytotoxicity receptor NKp30, the c-lectin receptor NKG2D, DNAM-1, 2B4, the cytolytic mediators perforin and granzyme B, and EOMES, the regulator of NK cell maturation and cytolytic function.

[00119] FIG. 3: Cytotoxicity of 35-day three-stage NK cells (n=10) against tumor cell lines K562 (CML), HL-60 (AML), and RPMI8226 (multiple myeloma). Lysis was measured at a 10:1 effector-to-target ratio.

[00120] FIG. 4: Multi-color flow cytometry comparing FITC isotype control cells to three-stage NK cells in expression of perforin (top), a cytolytic mediator, and CD107 (bottom), a marker of degranulation.

[00121] FIG. 5: Production of cytokines by three-stage NK cells (n=11) when co-cultured with K562 (CML) cells at a 1:1 ratio for 24 hours.

[00122] FIG. 6A-B: The formation of an F-actin immunological synapse with polarization of perforin captured by confocal imaging of three-stage NK cells and K562 (CML) (A) and RPMI8226 (multiple myeloma) (B) cells at an effector-to-target of 1:1, 15 minutes post-incubation at 63x magnification. Cells were fixed with formaldehyde and F-Actin was stained

with Alexa-488 conjugated phalloidin, and co-staining was performed with perforin antibodies followed by Alexa Fluor 555 dye conjugated goat anti-rabbit secondary antibodies. Tumor target cells were also stained with cell tracker violet dye. Arrows indicate the NK cells, perforin, and target cells.

[00123] FIG. 7: Cytotoxicity of three-stage NK cells (n=3) against tumor cell lines K562 (CML), HL-60 (AML), and RPMI8226 (multiple myeloma). Lysis was measured at various effector-to-target ratios.

[00124] FIG. 8: CD107a expression in three-stage NK cells (n=4) upon stimulation with tumor cells (K562 or HL-60) or phorbol 12-myristate 13-acetate plus ionomycin (PMA). CD107a expression is a marker for degranulation. The results from four donors are shown.

[00125] FIG. 9: IFNγ secretion in three-stage NK cells (n=3) upon stimulation with tumor cells (K562, HL-60, or RPMI8226) or phorbol 12-myristate 13-acetate plus ionomycin (PMA). The results from three donors are shown.

[00126] FIG. 10: Cytolytic activity of three-stage NK cells (n = 2) against primary AML target cells (A1, A2, and KG1a) at an effector-to-target ratio of 3:1. Results are shown for 24 hours of incubation. The results from two donors are shown.

[00127] FIG. 11: IFNγ secretion in three-stage NK cells (n=5) upon stimulation with primary AML target cells (AML1-4, and KG1a), compared with stimulation with HL-60 (AML) tumor cell line. The results from five donors are shown. The boxes are added for ease of comparison.

[00128] FIG. 12: Multi-color flow cytometry of CD3-CD56+ gated cells produced by the three-stage method, showing the expression of CD11a and CD117. Two subsets of cells, CD11a+ and CD11a-, are shown in boxes.

[00129] FIG. 13: Multi-color flow cytometry of the CD11a+ (right) and CD11a- (left) cells from Figure 12, showing the expression of CD56 and CD94.

[00130] FIG. 14: Multi-color flow cytometry of the CD11a+ (top) and CD11a- (bottom) cells from Figure 12, showing the levels of expression of perforin, EOMES, RORγt, and IL1R1 for each subset.

[00131] FIG. 15: Quantification of the percentage of CD11a+ and CD11a- three-stage cells expressing perforin, granzyme B, EOMES, T-bet, IL1R1, RORγt, and AHR, as determined by flow cytometry. The results are shown across four donors.

[00132] FIG. 16: Quantification of the percentage of CD11a+ and CD11a- three-stage cells expressing IFNγ, GM-CSF, IL-22, IL-8, and TNFα, upon stimulation with cytokines, as determined by flow cytometry. The results are shown across four donors. The bars on the graph indicate expression as follows, from left to right: i) no stimulation, ii) stimulation with PMA and ionomycin (PMAi), (iii) stimulation with PMAi and IL23, (iv) stimulation with IL12 and IL18, and (v) stimulation with IL1b and IL23.

[00133] FIG. 17: Multi-color flow cytometry of CD3-CD56+ gated cells produced by the three-stage method, showing the expression of CD11a compared with NKp46, NKp30, and NKG2D.

[00134] FIG. 18: Multi-color flow cytometry of CD3-CD56+ gated cells produced by the three-stage method, showing the expression of CD11a compared with CD226 (DNAM-1) and CD244 (2B4).

[00135] FIG. 19: Multi-color flow cytometry of CD3-CD56+ gated cells produced by the three-stage method, showing the expression of CD11a compared with CD16, NKG2A, and KIRs.

[00136] FIG. 20A-D: Percentage of three-stage cells expressing (A) CD56 and (B) CD14/15, and percentage of CD56+ three-stage cells expressing (C) RORγt and (D) perforin, using the indicated third medium conditions from Table 6, as measured by flow cytometry. Results from two donors are shown as adjacent bars for each condition.

[00137] FIG. 21A-C: CD107a expression using FACS in (A) CD11a+ and (B) CD11a- cell subpopulations in the presence of K562 cells. The graph in (C) shows the results for four populations of CD56+CD3- three-stage NK cells.

[00138] FIG. 22: Cytotoxicity of CD11a+ (positive) and CD11a- (negative) subpopulations of a CD56+CD3- population of three-stage NK cells against K562 cells. Effector to target cell ratio is indicated on the x-axis.

[00139] FIG. 23: Cytokine secretion analysis performed in the presence and absence of co-culture of K562 target cells and CD11a positive and negative effector cells (1:1 ratio of effector:target). The left hand bar for positive and negative cell subpopulations indicates the secretion in the absence of K562 cells, whereas the right hand bar for positive and negative cell subpopulations indicates secretion in the presence of K562 cells.

5. DETAILED DESCRIPTION

[00140] Provided herein are novel methods of producing and expanding NK cells and/or ILC3 cells from hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells. Also provided herein are methods, *e.g.*, three-stage methods, of producing NK cell populations and/or ILC3 cell populations from hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells. The hematopoietic cells (*e.g.*, CD34+ hematopoietic stem cells) used to produce the NK cells and/or ILC3 cells, and NK cell populations and/or ILC3 cell populations, may be obtained from any source, for example, without limitation, placenta, umbilical cord blood, placental blood, peripheral blood, spleen or liver. In certain embodiments, the NK cells and/or ILC3 cells or NK cell populations and/or ILC3 cell populations are produced from expanded hematopoietic cells, *e.g.*, hematopoietic stem cells and/or hematopoietic progenitor cells. In one embodiment, hematopoietic cells are collected from a source of such cells, *e.g.*, placenta, for example from placental perfusate, umbilical cord blood, placental blood, peripheral blood, spleen, liver (*e.g.*, fetal liver) and/or bone marrow.

[00141] The hematopoietic cells used to produce the NK cells and/or ILC3 cells, and NK cell populations and/or ILC3 cell populations, may be obtained from any animal species. In certain embodiments, the hematopoietic stem or progenitor cells are mammalian cells. In specific embodiments, said hematopoietic stem or progenitor cells are human cells. In specific embodiments, said hematopoietic stem or progenitor cells are primate cells. In specific embodiments, said hematopoietic stem or progenitor cells are canine cells. In specific embodiments, said hematopoietic stem or progenitor cells are rodent cells.

5.1. Hematopoietic Cells

[00142] Hematopoietic cells useful in the methods disclosed herein can be any hematopoietic cells able to differentiate into NK cells and/or ILC3 cells, *e.g.*, precursor cells, hematopoietic progenitor cells, hematopoietic stem cells, or the like. Hematopoietic cells can be obtained from tissue sources such as, *e.g.*, bone marrow, cord blood, placental blood, peripheral blood, liver or the like, or combinations thereof. Hematopoietic cells can be obtained from placenta. In a specific embodiment, the hematopoietic cells are obtained from placental perfusate. In one embodiment, the hematopoietic cells are not obtained from umbilical cord blood. In one embodiment, the hematopoietic cells are not obtained from peripheral blood. Hematopoietic cells from placental perfusate can comprise a mixture of fetal and maternal

hematopoietic cells, *e.g.*, a mixture in which maternal cells comprise greater than 5% of the total number of hematopoietic cells. In certain embodiments, hematopoietic cells from placental perfusate comprise at least about 90%, 95%, 98%, 99% or 99.5% fetal cells.

[00143] In another specific embodiment, the hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells, from which the NK cell populations and/or ILC3 cell populations produced using a three-stage method described herein are produced, are obtained from placental perfusate, umbilical cord blood, fetal liver, mobilized peripheral blood, or bone marrow. In another specific embodiment, the hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells, from which the NK cell populations and/or ILC3 cell populations produced using a three-stage method described herein are produced, are combined cells from placental perfusate and cord blood, *e.g.*, cord blood from the same placenta as the perfusate. In another specific embodiment, said umbilical cord blood is isolated from a placenta other than the placenta from which said placental perfusate is obtained. In certain embodiments, the combined cells can be obtained by pooling or combining the cord blood and placental perfusate. In certain embodiments, the cord blood and placental perfusate are combined at a ratio of 100:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45: 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 15:1, 10:1, 5:1, 1:1, 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, or the like by volume to obtain the combined cells. In a specific embodiment, the cord blood and placental perfusate are combined at a ratio of from 10:1 to 1:10, from 5:1 to 1:5, or from 3:1 to 1:3. In another specific embodiment, the cord blood and placental perfusate are combined at a ratio of 10:1, 5:1, 3:1, 1:1, 1:3, 1:5 or 1:10. In a more specific embodiment, the cord blood and placental perfusate are combined at a ratio of 8.5:1.5 (85%:15%).

[00144] In certain embodiments, the cord blood and placental perfusate are combined at a ratio of 100:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45: 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 15:1, 10:1, 5:1, 1:1, 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, or the like by total nucleated cells (TNC) content to obtain the combined cells. In a specific embodiment, the cord blood and placental perfusate are combined at a ratio of from 10:1

to 10:1, from 5:1 to 1:5, or from 3:1 to 1: 3. In another specific embodiment, the cord blood and placental perfusate are combined at a ratio of 10:1, 5:1, 3:1, 1:1, 1:3, 1:5 or 1:10.

[00145] In another specific embodiment, the hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells from which said NK cell populations and/or ILC3 cell populations produced using a three-stage method described herein are produced, are from both umbilical cord blood and placental perfusate, but wherein said umbilical cord blood is isolated from a placenta other than the placenta from which said placental perfusate is obtained.

[00146] In certain embodiments, the hematopoietic cells are $CD34^+$ cells. In specific embodiments, the hematopoietic cells useful in the methods disclosed herein are $CD34^+CD38^+$ or $CD34^+CD38^-$. In a more specific embodiment, the hematopoietic cells are $CD34^+CD38^-Lin^-$. In another specific embodiment, the hematopoietic cells are one or more of $CD2^-$, $CD3^-$, $CD11b^-$, $CD11c^-$, $CD14^-$, $CD16^-$, $CD19^-$, $CD24^-$, $CD56^-$, $CD66b^-$ and/or glycophorin A^-. In another specific embodiment, the hematopoietic cells are $CD2^-$, $CD3^-$, $CD11b^-$, $CD11c^-$, $CD14^-$, $CD16^-$, $CD19^-$, $CD24^-$, $CD56^-$, $CD66b^-$ and glycophorin A^-. In another more specific embodiment, the hematopoietic cells are $CD34^+CD38^-CD33^-CD117^-$. In another more specific embodiment, the hematopoietic cells are $CD34^+CD38^-CD33^-CD117^-$ $CD235^-CD36^-$.

[00147] In another embodiment, the hematopoietic cells are $CD45^+$. In another specific embodiment, the hematopoietic cells are $CD34^+CD45^+$. In another embodiment, the hematopoietic cell is Thy-1^+. In a specific embodiment, the hematopoietic cell is $CD34^+$Thy-1^+. In another embodiment, the hematopoietic cells are $CD133^+$. In specific embodiments, the hematopoietic cells are $CD34^+CD133^+$ or $CD133^+$Thy-1^+. In another specific embodiment, the $CD34^+$ hematopoietic cells are $CXCR4^+$. In another specific embodiment, the $CD34^+$ hematopoietic cells are $CXCR4^-$. In another embodiment, the hematopoietic cells are positive for KDR (vascular growth factor receptor 2). In specific embodiments, the hematopoietic cells are $CD34^+KDR^+$, $CD133^+KDR^+$ or Thy-1^+KDR^+. In certain other embodiments, the hematopoietic cells are positive for aldehyde dehydrogenase ($ALDH^+$), *e.g.*, the cells are $CD34^+ALDH^+$.

[00148] In certain other embodiments, the $CD34^+$ cells are $CD45^-$. In specific embodiments, the $CD34^+$ cells, *e.g.*, $CD34^+$, $CD45^-$ cells express one or more, or all, of the

miRNAs hsa-miR-380, hsa-miR-512, hsa-miR-517, hsa-miR-518c, hsa-miR-519b, hsa-miR-520a, hsa-miR-337, hsa-miR-422a, hsa-miR-549, and/or hsa-miR-618.

[00149] In certain embodiments, the hematopoietic cells are $CD34^-$.

[00150] The hematopoietic cells can also lack certain markers that indicate lineage commitment, or a lack of developmental naiveté. For example, in another embodiment, the hematopoietic cells are $HLA\text{-}DR^-$. In specific embodiments, the hematopoietic cells are $CD34^+HLA\text{-}DR^-$, $CD133^+HLA\text{-}DR^-$, $Thy\text{-}1^+HLA\text{-}DR^-$ or $ALDH^+HLA\text{-}DR^-$ In another embodiment, the hematopoietic cells are negative for one or more, or all, of lineage markers CD2, CD3, CD11b, CD11c, CD14, CD16, CD19, CD24, CD56, CD66b and glycophorin A.

[00151] Thus, hematopoietic cells can be selected for use in the methods disclosed herein on the basis of the presence of markers that indicate an undifferentiated state, or on the basis of the absence of lineage markers indicating that at least some lineage differentiation has taken place. Methods of isolating cells, including hematopoietic cells, on the basis of the presence or absence of specific markers is discussed in detail below.

[00152] Hematopoietic cells used in the methods provided herein can be a substantially homogeneous population, *e.g.*, a population comprising at least about 95%, at least about 98% or at least about 99% hematopoietic cells from a single tissue source, or a population comprising hematopoietic cells exhibiting the same hematopoietic cell-associated cellular markers. For example, in various embodiments, the hematopoietic cells can comprise at least about 95%, 98% or 99% hematopoietic cells from bone marrow, cord blood, placental blood, peripheral blood, or placenta, *e.g.*, placenta perfusate.

[00153] Hematopoietic cells used in the methods provided herein can be obtained from a single individual, *e.g.*, from a single placenta, or from a plurality of individuals, *e.g.*, can be pooled. Where the hematopoietic cells are obtained from a plurality of individuals and pooled, the hematopoietic cells may be obtained from the same tissue source. Thus, in various embodiments, the pooled hematopoietic cells are all from placenta, *e.g.*, placental perfusate, all from placental blood, all from umbilical cord blood, all from peripheral blood, and the like.

[00154] Hematopoietic cells used in the methods disclosed herein can, in certain embodiments, comprise hematopoietic cells from two or more tissue sources. For example, in certain embodiments, when hematopoietic cells from two or more sources are combined for use in the methods herein, a plurality of the hematopoietic cells used to produce natural killer cells

using a three-stage method described herein comprise hematopoietic cells from placenta, *e.g.*, placenta perfusate. In various embodiments, the hematopoietic cells used to produce NK cell populations and/or ILC3 cell populations produced using a three-stage method described herein, comprise hematopoietic cells from placenta and from cord blood; from placenta and peripheral blood; from placenta and placental blood, or placenta and bone marrow. In one embodiment, the hematopoietic cells comprise hematopoietic cells from placental perfusate in combination with hematopoietic cells from cord blood, wherein the cord blood and placenta are from the same individual, *i.e.*, wherein the perfusate and cord blood are matched. In embodiments in which the hematopoietic cells comprise hematopoietic cells from two tissue sources, the hematopoietic cells from the sources can be combined in a ratio of, for example, 1:10, 2:9, 3:8, 4:7:, 5:6, 6:5, 7:4, 8:3, 9:2, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1 or 9:1.

5.1.1. Placental Hematopoietic Stem Cells

[00155] In certain embodiments, the hematopoietic cells used in the methods provided herein are placental hematopoietic cells. In one embodiment, placental hematopoietic cells are $CD34^+$. In a specific embodiment, the placental hematopoietic cells are predominantly (*e.g.*, at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98%) $CD34^+CD38^-$ cells. In another specific embodiment, the placental hematopoietic cells are predominantly (*e.g.*, at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98%) $CD34^+CD38^+$ cells. Placental hematopoietic cells can be obtained from a post-partum mammalian (*e.g.*, human) placenta by any means known to those of skill in the art, *e.g.*, by perfusion.

[00156] In another embodiment, the placental hematopoietic cell is $CD45^-$. In a specific embodiment, the hematopoietic cell is $CD34^+CD45^-$. In another specific embodiment, the placental hematopoietic cells are $CD34^+CD45^+$.

5.2. Production of Natural Killer and/or ILC3 Cells and Natural Killer Cell and/or ILC3 Cell Populations

[00157] Production of NK cells and/or ILC3 cells and NK cell and/or ILC3 cell populations by the present methods comprises expanding a population of hematopoietic cells. During cell expansion, a plurality of hematopoietic cells within the hematopoietic cell population differentiate into NK cells and/or ILC3 cells. In one aspect, provided herein is a method of producing NK cells comprising culturing hematopoietic stem cells or progenitor cells, *e.g.*, $CD34^+$ stem cells or progenitor cells, in a first medium comprising a stem cell mobilizing agent

and thrombopoietin (Tpo) to produce a first population of cells, subsequently culturing said first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells, and subsequently culturing said second population of cells in a third medium comprising IL-2 and IL-15, and lacking a stem cell mobilizing agent and LMWH, to produce a third population of cells, wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and wherein at least 70%, for example at least 80%, of the natural killer cells are viable. In certain embodiments, such natural killer cells comprise natural killer cells that are CD16-. In certain embodiments, such natural killer cells comprise natural killer cells that are CD94+. In certain embodiments, such natural killer cells comprise natural killer cells that are CD94+ or CD16+. In certain embodiments, such natural killer cells comprise natural killer cells that are CD94- or CD16-. In certain embodiments, such natural killer cells comprise natural killer cells that are CD94+ and CD16+. In certain embodiments, such natural killer cells comprise natural killer cells that are CD94- and CD16-. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00158] In one aspect, provided herein is a method of producing NK cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments,

said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00159] In one aspect, provided herein is a method of producing NK cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of stem cell factor (SCF) and LMWH, to produce a third population of cells; wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00160] In one aspect, provided herein is a method of producing NK cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of SCF, a stem cell mobilizing agent, and LMWH, to produce a third population of cells; wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments,

none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00161] In one aspect, provided herein is a method of producing NK cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of a stem cell mobilizing agent and LMWH, to produce a third population of cells; and (d) isolating CD11a+ cells from the third population of cells to produce a fourth population of cells; wherein the fourth population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00162] In certain embodiments, of any of the above embodiments, said natural killer cells express perforin and EOMES. In certain embodiments, said natural killer cells do not express either RORγt or IL1R1.

[00163] In one aspect, provided herein is a method of producing ILC3 cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first

medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00164] In one aspect, provided herein is a method of producing ILC3 cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising a stem cell mobilizing agent, IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00165] In one aspect, provided herein is a method of producing ILC3 cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising SCF, IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00166] In one aspect, provided herein is a method of producing ILC3 cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising a stem cell mobilizing agent, SCF, IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00167] In one aspect, provided herein is a method of producing ILC3 cells comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of a stem cell mobilizing agent and LMWH, to produce a third population of cells; and (d) isolating CD11a- cells, or removing CD11a+ cells, from the third population of cells to produce a fourth population of cells; wherein the fourth population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00168] In certain embodiments, said ILC3 cells express RORγt and IL1R1. In certain embodiments, said ILC3 cells do not express either perforin or EOMES.

5.2.1. Production of NK Cell and/or ILC3 Cell Populations Using a Three-Stage Method

[00169] In one embodiment, provided herein is a three-stage method of producing NK cell and/or ILC3 cell populations. In certain embodiments, the method of expansion and differentiation of the hematopoietic cells, as described herein, to produce NK cell and/or ILC3 cell populations according to a three-stage method described herein comprises maintaining the cell population comprising said hematopoietic cells at between about 2×10^4 and about 6×10^6 cells per milliliter. In certain aspects, said hematopoietic stem or progenitor cells are initially inoculated into said first medium from 1×10^4 to 1×10^5 cells/mL. In a specific aspect, said hematopoietic stem or progenitor cells are initially inoculated into said first medium at about 3×10^4 cells/mL.

[00170] In certain aspects, said first population of cells are initially inoculated into said second medium from 5×10^4 to 5×10^5 cells/mL. In a specific aspect, said first population of cells is initially inoculated into said second medium at about 1×10^5 cells/mL.

[00171] In certain aspects said second population of cells is initially inoculated into said third medium from 1×10^5 to 5×10^6 cells/mL. In certain aspects, said second population of cells is initially inoculated into said third medium from 1×10^5 to 1×10^6 cells/mL. In a specific aspect, said second population of cells is initially inoculated into said third medium at about 5×10^5 cells/mL. In a more specific aspect, said second population of cells is initially inoculated into said third medium at about 5×10^5 cells/mL in a spinner flask. In a specific aspect, said second population of cells is initially inoculated into said third medium at about 3×10^5 cells/mL. In a more specific aspect, said second population of cells is initially inoculated into said third medium at about 3×10^5 cells/mL in a static culture.

[00172] In a certain embodiment, the three-stage method comprises a first stage ("stage 1") comprising culturing hematopoietic stem cells or progenitor cells, *e.g.*, $CD34^+$ stem cells or progenitor cells, in a first medium for a specified time period, *e.g.*, as described herein, to produce a first population of cells. In certain embodiments, the first medium comprises a stem cell mobilizing agent and thrombopoietin (Tpo). In certain embodiments, the first medium

comprises in addition to a stem cell mobilizing agent and Tpo, one or more of LMWH, Flt-3L, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In a specific embodiment, the first medium comprises each of the first medium comprises in addition to a stem cell mobilizing agent and Tpo, each of LMWH, Flt-3L, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In a specific embodiment, the first medium lacks added LMWH. In a specific embodiment, the first medium lacks added desulphated glycosaminoglycans. In a specific embodiment, the first medium lacks LMWH. In a specific embodiment, the first medium lacks desulphated glycosaminoglycans. In a specific embodiment, the first medium comprises each of the first medium comprises in addition to a stem cell mobilizing agent and Tpo, each of Flt-3L, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In specific embodiments, the first medium lacks leukemia inhibiting factor (LIF), macrophage inhibitory protein-1alpha (MIP-1α) or both.

[00173] In certain embodiments, subsequently, in "stage 2" said cells are cultured in a second medium for a specified time period, *e.g.*, as described herein, to produce a second population of cells. In certain embodiments, the second medium comprises a stem cell mobilizing agent and interleukin-15 (IL-15), and lacks Tpo. In certain embodiments, the second medium comprises, in addition to a stem cell mobilizing agent and IL-15, one or more of LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In certain embodiments, the second medium comprises, in addition to a stem cell mobilizing agent and IL-15, each of LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In a specific embodiment, the second medium lacks added LMWH. In a specific embodiment, the second medium lacks added desulphated glycosaminoglycans. In a specific embodiment, the second medium lacks heparin, *e.*g., LMWH. In a specific embodiment, the second medium lacks desulphated glycosaminoglycans. In certain embodiments, the second medium comprises, in addition to a stem cell mobilizing agent and IL-15, each of Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In specific embodiments, the second medium lacks leukemia inhibiting factor (LIF), macrophage inhibitory protein-1alpha (MIP-1α) or both.

[00174] In certain embodiments, subsequently, in "stage 3" said cells are cultured in a third medium for a specified time period, *e.g.*, as described herein, to produce a third population of cell, *e.g.*, natural killer cells. In certain embodiments, the third medium comprises IL-2 and IL-15, and lacks a stem cell mobilizing agent and LMWH. In certain embodiments, the third medium comprises in addition to IL-2 and IL-15, one or more of SCF, IL-6, IL-7, G-CSF, and

GM-CSF. In certain embodiments, the third medium comprises, in addition to IL-2 and IL-15, each of SCF, IL-6, IL-7, G-CSF, and GM-CSF. In specific embodiments, the first medium lacks one, two, or all three of LIF, MIP-1α, and Flt3L. In specific embodiments, the third medium lacks added desulphated glycosaminoglycans. In specific embodiments, the third medium lacks desulphated glycosaminoglycans. In specific embodiments, the third medium lacks heparin, *e.g.*, LMWH.

[00175] In a specific embodiment, the three-stage method is used to produce NK cell and/or ILC3 cell populations. In certain embodiments, the three-stage method is conducted in the absence of stromal feeder cell support. In certain embodiments, the three-stage method is conducted in the absence of exogenously added steroids (*e.g.*, cortisone, hydrocortisone, or derivatives thereof).

[00176] In certain aspects, said first medium used in the three-stage method comprises a stem cell mobilizing agent and thrombopoietin (Tpo). In certain aspects, the first medium used in the three-stage method comprises, in addition to a stem cell mobilizing agent and Tpo, one or more of Low Molecular Weight Heparin (LMWH), Flt-3 Ligand (Flt-3L), stem cell factor (SCF), IL-6, IL-7, granulocyte colony-stimulating factor (G-CSF), or granulocyte-macrophage-stimulating factor (GM-CSF). In certain aspects, the first medium used in the three-stage method comprises, in addition to a stem cell mobilizing agent and Tpo, each of LMWH, Flt-3L, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In certain aspects, the first medium used in the three-stage method comprises, in addition to a stem cell mobilizing agent and Tpo, each of Flt-3L, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In a specific aspect, the first medium lacks added LMWH. In a specific aspect, the first medium lacks added desulphated glycosaminoglycans. In a specific aspect, the first medium lacks LMWH. In a specific aspect, the first medium lacks desulphated glycosaminoglycans. In certain aspects, said Tpo is present in the first medium at a concentration of from 1 ng/mL to 100 ng/mL, from 1 ng/mL to 50 ng/mL, from 20 ng/mL to 30 ng/mL, or about 25 ng/mL. In certain aspects, in the first medium, the LMWH is present at a concentration of from 1U/mL to 10U/mL; the Flt-3L is present at a concentration of from 1 ng/mL to 50 ng/mL; the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL

to 0.1 ng/mL. In certain aspects, in the first medium, the Flt-3L is present at a concentration of from 1 ng/mL to 50 ng/mL; the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL. In certain aspects, in the first medium, the LMWH is present at a concentration of from 4U/mL to 5U/mL; the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the first medium, the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the first medium, the LMWH is present at a concentration of about 4.5U/mL; the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about .25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL. In certain aspects, in the first medium, the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about .25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL. In certain embodiments, said first medium additionally comprises one or more of the following: antibiotics such as gentamycin; antioxidants such as transferrin, insulin, and/or beta-mercaptoethanol; sodium selenite; ascorbic acid; ethanolamine; and glutathione. In certain embodiments, the medium that provides the base for the first medium is a cell/tissue culture medium known to those of skill in the art, *e.g.*, a commercially available cell/tissue culture medium such as SCGM™, STEMMACS™, GBGM®, AIM-V®, X-VIVO™ 10, X-VIVO™ 15,

OPTMIZER, STEMSPAN® H3000, CELLGRO COMPLETE™, DMEM:Ham's F12 ("F12") (*e.g.*, 2:1 ratio, or high glucose or low glucose DMEM), Advanced DMEM (Gibco), EL08-1D2, Myelocult™ H5100, IMDM, and/or RPMI-1640; or is a medium that comprises components generally included in known cell/tissue culture media, such as the components included in GBGM®, AIM-V®, X-VIVO™ 10, X-VIVO™ 15, OPTMIZER, STEMSPAN® H3000, CELLGRO COMPLETE™, DMEM:Ham's F12 ("F12") (*e.g.*, 2:1 ratio, or high glucose or low glucose DMEM), Advanced DMEM (Gibco), EL08-1D2, Myelocult™ H5100, IMDM, and/or RPMI-1640. In certain embodiments, said first medium is not GBGM®. In specific embodiments of any of the above embodiments, the first medium lacks LIF, MIP-1α, or both.

[00177] In certain aspects, said second medium used in the three-stage method comprises a stem cell mobilizing agent and interleukin-15 (IL-15), and lacks Tpo. In certain aspects, the second medium used in the three-stage method comprises, in addition to a stem cell mobilizing agent and IL-15, one or more of LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In certain aspects, the second medium used in the three-stage method comprises, in addition to a stem cell mobilizing agent and IL-15, each of LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In certain aspects, the second medium used in the three-stage method comprises, in addition to a stem cell mobilizing agent and IL-15, each of Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF. In a specific aspect, the second medium lacks added LMWH. In a specific aspect, the second medium lacks added desulphated glycosaminoglycans. In a specific aspect, the second medium lacks LMWH. In a specific aspect, the second medium lacks desulphated glycosaminoglycans. In certain aspects, said IL-15 is present in said second medium at a concentration of from 1 ng/mL to 50 ng/mL, from 10 ng/mL to 30 ng/mL, or about 20 ng/mL. In certain aspects, in said second medium, the LMWH is present at a concentration of from 1U/mL to 10U/mL; the Flt-3L is present at a concentration of from 1 ng/mL to 50 ng/mL; the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL. In certain aspects, in said second medium, the Flt-3L is present at a concentration of from 1 ng/mL to 50 ng/mL; the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50

ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL. In certain aspects, in the second medium, the LMWH is present in the second medium at a concentration of from 4U/mL to 5U/mL; the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the second medium, the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the second medium, the LMWH is present in the second medium at a concentration of from 4U/mL to 5U/mL; the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the second medium, the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in the second medium, the LMWH is present in the second medium at a concentration of about 4.5U/mL; the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about 0.25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL. In certain aspects, in the second medium, the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL; the IL-6 is present at a concentration of

about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about 0.25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL. In certain embodiments, said second medium additionally comprises one or more of the following: antibiotics such as gentamycin; antioxidants such as transferrin, insulin, and/or beta-mercaptoethanol; sodium selenite; ascorbic acid; ethanolamine; and glutathione. In certain embodiments, the medium that provides the base for the second medium is a cell/tissue culture medium known to those of skill in the art, *e.g.*, a commercially available cell/tissue culture medium such as SCGM™, STEMMACS™, GBGM®, AIM-V®, X-VIVO™ 10, X-VIVO™ 15, OPTMIZER, STEMSPAN® H3000, CELLGRO COMPLETE™, DMEM:Ham's F12 ("F12") (*e.g.*, 2:1 ratio, or high glucose or low glucose DMEM), Advanced DMEM (Gibco), EL08-1D2, Myelocult™ H5100, IMDM, and/or RPMI-1640; or is a medium that comprises components generally included in known cell/tissue culture media, such as the components included in GBGM®, AIM-V®, X-VIVO™ 10, X-VIVO™ 15, OPTMIZER, STEMSPAN® H3000, CELLGRO COMPLETE™, DMEM:Ham's F12 ("F12") (*e.g.*, 2:1 ratio, or high glucose or low glucose DMEM), Advanced DMEM (Gibco), EL08-1D2, Myelocult™ H5100, IMDM, and/or RPMI-1640. In certain embodiments, said second medium is not GBGM®. In specific embodiments of any of the above embodiments, the first medium lacks LIF, MIP-1α, or both.

[00178] In certain aspects, said third medium used in the three-stage method comprises IL-2 and IL-15, and lacks a stem cell mobilizing agent and LMWH. In certain aspects, said third medium used in the three-stage method comprises IL-2 and IL-15, and lacks LMWH. In certain aspects, said third medium used in the three-stage method comprises IL-2 and IL-15, and lacks SCF and LMWH. In certain aspects, said third medium used in the three-stage method comprises IL-2 and IL-15, and lacks SCF, a stem cell mobilizing agent and LMWH. In certain aspects, said third medium used in the three-stage method comprises a stem cell mobilizing agent, IL-2 and IL-15, and lacks LMWH. In certain aspects, said third medium used in the three-stage method comprises SCF, IL-2 and IL-15, and lacks LMWH. In certain aspects, said third medium used in the three-stage method comprises a stem cell mobilizing agent, SCF, IL-2 and IL-15, and lacks LMWH. In certain aspects, said third medium used in the three-stage method comprises IL-2 and IL-15, and lacks a stem cell mobilizing agent and LMWH. In certain aspects, the third medium used in the three-stage method comprises, in addition to IL-2 and IL-15, one or more of SCF, IL-6, IL-7, G-CSF, or GM-CSF. In certain aspects, the third medium used in the three-

stage method comprises, in addition to IL-2 and IL-15, each of SCF, IL-6, IL-7, G-CSF, and GM-CSF. In certain aspects, said IL-2 is present in said third medium at a concentration of from 10 U/mL to 10,000 U/mL and said IL-15 is present in said third medium at a concentration of from 1 ng/mL to 50 ng/mL. In certain aspects, said IL-2 is present in said third medium at a concentration of from 100 U/mL to 10,000 U/mL and said IL-15 is present in said third medium at a concentration of from 1 ng/mL to 50 ng/mL. In certain aspects, said IL-2 is present in said third medium at a concentration of from 300 U/mL to 3,000 U/mL and said IL-15 is present in said third medium at a concentration of from 10 ng/mL to 30 ng/mL. In certain aspects, said IL-2 is present in said third medium at a concentration of about 1,000 U/mL and said IL-15 is present in said third medium at a concentration of about 20 ng/mL. In certain aspects, in said third medium, the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL. In certain aspects, in said third medium, the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL. In certain aspects, in said third medium, the SCF is present at a concentration of about 22 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 20 ng/mL; the G-CSF is present at a concentration of about 0.25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL. In certain aspects, the third medium comprises 100 ng/mL IL-7, 1000 ng/mL IL-2, 20 ng/mL IL-15, and 10 μM SR1 and lacks SCF. In certain aspects, the third medium comprises 20 ng/mL IL-7, 1000 ng/mL IL-2, 20 ng/mL IL-15, and 10 μM SR1 and lacks SCF. In certain aspects, the third medium comprises 20 ng/mL IL-7, 20 ng/mL IL-15, and 10 μM SR1 and lacks SCF. In certain aspects, the third medium comprises 100 ng/mL IL-7, 22 ng/mL SCF, 1000 ng/mL IL-2, and 20 ng/mL IL-15 and lacks SR1. In certain aspects, the third medium comprises 22 ng/mL SCF, 1000 ng/mL IL-2, and 20 ng/mL IL-15 and lacks SR1. In certain aspects, the third medium comprises 20 ng/mL IL-7, 22 ng/mL SCF, 1000 ng/mL IL-2, and 20 ng/mL IL-15 and lacks SR1. In certain aspects, the third medium comprises 20 ng/mL IL-7, 22 ng/mL SCF,

and 1000 ng/mL IL-2 and lacks SR1. In specific embodiments of any of the above embodiments, the first medium lacks one, two, or all three of LIF, MIP-1α, Flt-3L.

[00179] In certain embodiments, said third medium additionally comprises one or more of the following: antibiotics such as gentamycin; antioxidants such as transferrin, insulin, and/or beta-mercaptoethanol; sodium selenite; ascorbic acid; ethanolamine; and glutathione. In certain embodiments, the medium that provides the base for the third medium is a cell/tissue culture medium known to those of skill in the art, *e.g.*, a commercially available cell/tissue culture medium such as SCGM™, STEMMACS™, GBGM®, AIM-V®, X-VIVO™ 10, X-VIVO™ 15, OPTMIZER, STEMSPAN® H3000, CELLGRO COMPLETE™, DMEM:Ham's F12 ("F12") (*e.g.*, 2:1 ratio, or high glucose or low glucose DMEM), Advanced DMEM (Gibco), EL08-1D2, Myelocult™ H5100, IMDM, and/or RPMI-1640; or is a medium that comprises components generally included in known cell/tissue culture media, such as the components included in GBGM®, AIM-V®, X-VIVO™ 10, X-VIVO™ 15, OPTMIZER, STEMSPAN® H3000, CELLGRO COMPLETE™, DMEM:Ham's F12 ("F12") (*e.g.*, 2:1 ratio, or high glucose or low glucose DMEM), Advanced DMEM (Gibco), EL08-1D2, Myelocult™ H5100, IMDM, and/or RPMI-1640. In certain embodiments, said third medium is not GBGM®.

[00180] Generally, the particularly recited medium components do not refer to possible constituents in an undefined component of said medium. For example, said Tpo, IL-2, and IL-15 are not comprised within an undefined component of the first medium, second medium or third medium, *e.g.*, said Tpo, IL-2, and IL-15 are not comprised within serum. Further, said LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and/or GM-CSF are not comprised within an undefined component of the first medium, second medium or third medium, *e.g.*, said LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and/or GM-CSF are not comprised within serum.

[00181] In certain aspects, said first medium, second medium or third medium comprises human serum-AB. In certain aspects, any of said first medium, second medium or third medium comprises 1% to 20% human serum-AB, 5% to 15% human serum-AB, or about 2, 5, or 10% human serum-AB.

[00182] In certain embodiments, in the three-stage methods described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days. In certain embodiments, in the three-stage methods described herein, cells are cultured in said second medium for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days. In certain embodiments, in the three-stage methods described herein, cells are cultured in said third medium for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days, or for more than 30 days.

[00183] In a specific embodiment, in the three-stage methods described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for 7-13 days to produce a first population of cells, before said culturing in said second medium; said first population of cells are cultured in said second medium for 2-6 days to produce a second population of cells before said culturing in said third medium; and said second population of cells are cultured in said third medium for 10-30 days, *i.e.*, the cells are cultured a total of 19-49 days.

[00184] In a specific embodiment, in the three-stage methods described herein, in the three-stage methods described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for 8-12 days to produce a first population of cells, before said culturing in said second medium; said first population of cells are cultured in said second medium for 3-5 days to produce a second population of cells before said culturing in said third medium; and said second population of cells are cultured in said third medium for 15-25 days, *i.e.*, the cells are cultured a total of 26-42 days.

[00185] In a specific embodiment, in the three-stage methods described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for about 10 days to produce a first population of cells, before said culturing in said second medium; said first population of cells are cultured in said second medium for about 4 days to produce a second population of cells before said culturing in said third medium; and said second population of cells are cultured in said third medium for about 21 days, *i.e.*, the cells are cultured a total of about 35 days.

[00186] In certain aspects, the three-stage method disclosed herein produces at least 5000-fold more natural killer cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 10,000-fold more natural killer cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 50,000-fold more natural killer cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 75,000-fold more natural killer cells as compared to the number of

hematopoietic stem cells initially inoculated into said first medium. In certain aspects, the viability of said natural killer cells is determined by 7-aminoactinomycin D (7AAD) staining. In certain aspects, the viability of said natural killer cells is determined by annexin-V staining. In specific aspects, the viability of said natural killer cells is determined by both 7-AAD staining and annexin-V staining. In certain aspects, the viability of said natural killer cells is determined by trypan blue staining.

[00187] In certain aspects, the three-stage method disclosed herein produces at least 5000-fold more ILC3 cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 10,000-fold more ILC3 cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 50,000-fold more ILC3 cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium. In certain aspects, said three-stage method produces at least 75,000-fold more ILC3 cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium.

[00188] In certain aspects, the three-stage method produces natural killer cells that comprise at least 20% CD56+CD3– natural killer cells. In certain aspects, the three-stage method produces natural killer cells that comprise at least 40% CD56+CD3– natural killer cells. In certain aspects, the three-stage method produces natural killer cells that comprise at least 60% CD56+CD3– natural killer cells. In certain aspects, the three-stage method produces natural killer cells that comprise at least 70% CD56+CD3– natural killer cells. In certain aspects, the three-stage method produces natural killer cells that comprise at least 80% CD56+CD3– natural killer cells.

[00189] In certain aspects, the three-stage method disclosed herein produces natural killer cells that comprise at least 20% CD56+CD3–CD11a+ natural killer cells. In certain aspects, the three-stage method disclosed herein produces natural killer cells that comprise at least 40% CD56+CD3– CD11a+ natural killer cells. In certain aspects, the three-stage method disclosed herein produces natural killer cells that comprise at least 60% CD56+CD3– CD11a+ natural killer cells. In certain aspects, the three-stage method disclosed herein produces natural killer cells that comprise at least 80% CD56+CD3– CD11a+ natural killer cells.

[00190] In certain aspects, the three-stage method disclosed herein produces ILC3 cells that comprise at least 20% CD56+CD3– CD11a– ILC3 cells. In certain aspects, the three-stage method disclosed herein produces ILC3 cells that comprise at least 40% CD56+CD3– CD11a– ILC3 cells. In certain aspects, the three-stage method disclosed herein produces ILC3 cells that comprise at least 60% CD56+CD3– CD11a– ILC3 cells. In certain aspects, the three-stage method disclosed herein produces natural killer cells that comprise at least 80% CD56+CD3– CD11a– ILC3 cells.

[00191] In certain aspects, the three-stage method produces natural killer cells that exhibit at least 20% cytotoxicity against K562 cells when said natural killer cells and said K562 cells are co-cultured *in vitro* or *ex vivo* at a ratio of 10:1. In certain aspects, the three-stage method produces natural killer cells that exhibit at least 35% cytotoxicity against the K562 cells when said natural killer cells and said K562 cells are co-cultured *in vitro* or *ex vivo* at a ratio of 10:1. In certain aspects, the three-stage method produces natural killer cells that exhibit at least 45% cytotoxicity against the K562 cells when said natural killer cells and said K562 cells are co-cultured *in vitro* or *ex vivo* at a ratio of 10:1. In certain aspects, the three-stage method produces natural killer cells that exhibit at least 60% cytotoxicity against the K562 cells when said natural killer cells and said K562 cells are co-cultured *in vitro* or *ex vivo* at a ratio of 10:1. In certain aspects, the three-stage method produces natural killer cells that exhibit at least 75% cytotoxicity against the K562 cells when said natural killer cells and said K562 cells are co-cultured *in vitro* or *ex vivo* at a ratio of 10:1.

[00192] In certain aspects, the three-stage method produces ILC3 cells that exhibit at least 20% cytotoxicity against K562 cells when said ILC3 cells and said K562 cells are co-cultured *in vitro* or *ex vivo* at a ratio of 10:1. In certain aspects, the three-stage method produces ILC3 cells that exhibit at least 35% cytotoxicity against the K562 cells when said ILC3 cells and said K562 cells are co-cultured *in vitro* or *ex vivo* at a ratio of 10:1. In certain aspects, the three-stage method produces ILC3 cells that exhibit at least 45% cytotoxicity against the K562 cells when said ILC3 cells and said K562 cells are co-cultured *in vitro* or *ex vivo* at a ratio of 10:1. In certain aspects, the three-stage method produces ILC3 cells that exhibit at least 60% cytotoxicity against the K562 cells when said ILC3 cells and said K562 cells are co-cultured *in vitro* or *ex vivo* at a ratio of 10:1. In certain aspects, the three-stage method produces ILC3 cells that exhibit

at least 75% cytotoxicity against the K562 cells when said ILC3 cells and said K562 cells are co-cultured *in vitro* or *ex vivo* at a ratio of 10:1.

[00193] In certain aspects, after said third culturing step, said third population of cells, *e.g.*, said population of natural killer cells and/or ILC3 cells, is cryopreserved. In certain aspects, after said fourth step, said fourth population of cells, *e.g.*, said population of natural killer cells and/or ILC3 cells, is cryopreserved.

[00194] In certain aspects, provided herein are populations of cells comprising natural killer cells, *i.e.*, natural killers cells produced by a three-stage method described herein. Accordingly, provided herein is an isolated natural killer cell population produced by a three-stage method described herein. In a specific embodiment, said natural killer cell population comprises at least 20% CD56+CD3– natural killer cells. In a specific embodiment, said natural killer cell population comprises at least 40% CD56+CD3– natural killer cells. In a specific embodiment, said natural killer cell population comprises at least 60% CD56+CD3– natural killer cells. In a specific embodiment, said natural killer cell population comprises at least 80% CD56+CD3– natural killer cells. In a specific embodiment, said natural killer cell population comprises at least 60% CD16- cells. In a specific embodiment, said natural killer cell population comprises at least 80% CD16- cells. In a specific embodiment, said natural killer cell population comprises at least 20% CD94+ cells. In a specific embodiment, said natural killer cell population comprises at least 40% CD94+ cells.

[00195] In certain aspects, provided herein is a population of natural killer cells that is CD56+CD3– CD117+CD11a+, wherein said natural killer cells express perforin and/or EOMES, and do not express one or more of RORγt, aryl hydrocarbon receptor (AHR), and IL1R1. In certain aspects, said natural killer cells express perforin and EOMES, and do not express any of RORγt, aryl hydrocarbon receptor, or IL1R1. In certain aspects, said natural killer cells additionally express T-bet, GZMB, NKp46, NKp30, and NKG2D. In certain aspects, said natural killer cells express CD94. In certain aspects, said natural killer cells do not express CD94.

[00196] In certain aspects, provided herein is a population of ILC3 cells that is CD56+CD3– CD117+CD11a-, wherein said ILC3 cells express one or more of RORγt, aryl hydrocarbon receptor, and IL1R1, and do not express one or more of CD94, perforin, and EOMES. In certain aspects, said ILC3 cells express RORγt, aryl hydrocarbon receptor, and

IL1R1, and do not express any of CD94, perforin, or EOMES. In certain aspects, said ILC3 cells additionally express CD226 and/or 2B4. In certain aspects, said ILC3 cells additionally express one or more of IL-22, TNFα, and DNAM-1. In certain aspects, said ILC3 cells express CD226, 2B4, IL-22, TNFα, and DNAM-1.

[00197] In certain aspects, provided herein is a method of producing a cell population comprising natural killer cells and ILC3 cells, comprising (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of a stem cell mobilizing agent and LMWH, to produce a third population of cells; and (d) separating CD11a+ cells and CD11a– cells from the third population of cells; and (e) combining the CD11a+ cells with the CD11a– cells in a ratio of 50:1, 40:1, 30:1, 20:1, 10:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:10, 1:20, 1:30, 1:40, or 1:50 to produce a fourth population of cells. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 50:1. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 20:1. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 10:1. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 5:1. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 1:1. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 1:5. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 1:10. In certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 1:20. In

certain aspects, in the fourth population of cells, the CD11a+ cells and CD11a– cells are combined in a ratio of 1:50.

5.3. Stem Cell Mobilizing Factors

5.3.1. Chemistry definitions

[00198] To facilitate understanding of the disclosure of stem cell mobilizing factors set forth herein, a number of terms are defined below.

[00199] Generally, the nomenclature used herein and the laboratory procedures in biology, cellular biology, biochemistry, organic chemistry, medicinal chemistry, and pharmacology described herein are those well known and commonly employed in the art. Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[00200] The term "about" or "approximately" means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined. In certain embodiments, the term "about" or "approximately" means within 1, 2, 3, or 4 standard deviations. In certain embodiments, the term "about" or "approximately" means within 50%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.05% of a given value or range.

[00201] The term "aryl hydrocarbon receptor" or "AHR" refers to a protein encoded by the AHR gene in humans, or a variant thereof (for example, *see* GenBank Accession Nos. P35869.2 and AAH70080.1) .

[00202] The term "aryl hydrocarbon receptor antagonist," "AHR antagonist," "aryl hydrocarbon receptor inhibitor," or "AHR inhibitor" refers to a compound that downregulates or reduces the activity of an aryl hydrocarbon receptor.

[00203] The term "alkyl" refers to a linear or branched saturated monovalent hydrocarbon radical, wherein the alkyl is optionally substituted with one or more substituents Q as described herein. The term "alkyl" also encompasses both linear and branched alkyl, unless otherwise specified. In certain embodiments, the alkyl is a linear saturated monovalent hydrocarbon radical that has 1 to 20 (C_{1-20}), 1 to 15 (C_{1-15}), 1 to 10 (C_{1-10}), or 1 to 6 (C_{1-6}) carbon atoms, or branched saturated monovalent hydrocarbon radical of 3 to 20 (C_{3-20}), 3 to 15 (C_{3-15}), 3 to 10 (C_{3-10}), or 3 to 6 (C_{3-6}) carbon atoms. As used herein, linear C_{1-6} and branched C_{3-6} alkyl groups are also referred as "lower alkyl." Examples of alkyl groups include, but are not limited to, methyl,

ethyl, propyl (including all isomeric forms), *n*-propyl, isopropyl, butyl (including all isomeric forms), *n*-butyl, isobutyl, *sec*-butyl, *t*-butyl, pentyl (including all isomeric forms), and hexyl (including all isomeric forms). For example, C_{1-6} alkyl refers to a linear saturated monovalent hydrocarbon radical of 1 to 6 carbon atoms or a branched saturated monovalent hydrocarbon radical of 3 to 6 carbon atoms.

[00204] The term "alkylene" refers to a linear or branched saturated divalent hydrocarbon radical, wherein the alkylene is optionally substituted with one or more substituents Q as described herein. For example, C_{1-6} alkylene refers to a linear saturated divalent hydrocarbon radical of 1 to 6 carbon atoms or a branched saturated divalent hydrocarbon radical of 3 to 6 carbon atoms. In certain embodiments, the alkylene is a linear saturated divalent hydrocarbon radical that has 1 to 20 (C_{1-20}), 1 to 15 (C_{1-15}), 1 to 10 (C_{1-10}), or 1 to 6 (C_{1-6}) carbon atoms, or branched saturated divalent hydrocarbon radical of 3 to 20 (C_{3-20}), 3 to 15 (C_{3-15}), 3 to 10 (C_{3-10}), or 3 to 6 (C_{3-6}) carbon atoms. As used herein, linear C_{1-6} and branched C_{3-6} alkylene groups are also referred as "lower alkylene." Examples of alkylene groups include, but are not limited to, methylene, ethylene, propylene (including all isomeric forms), *n*-propylene, isopropylene, butylene (including all isomeric forms), *n*-butylene, isobutylene, *t*-butylene, pentylene (including all isomeric forms), and hexylene (including all isomeric forms).

[00205] The term "alkenyl" refers to a linear or branched monovalent hydrocarbon radical, which contains one or more, in one embodiment, one, two, three, four, or five, in another embodiment, one, carbon-carbon double bond(s). The alkenyl is optionally substituted with one or more substituents Q as described herein. The term "alkenyl" also embraces radicals having "*cis*" and "*trans*" configurations, or alternatively, "Z" and "E" configurations, as appreciated by those of ordinary skill in the art. As used herein, the term "alkenyl" encompasses both linear and branched alkenyl, unless otherwise specified. For example, C_{2-6} alkenyl refers to a linear unsaturated monovalent hydrocarbon radical of 2 to 6 carbon atoms or a branched unsaturated monovalent hydrocarbon radical of 3 to 6 carbon atoms. In certain embodiments, the alkenyl is a linear monovalent hydrocarbon radical of 2 to 20 (C_{2-20}), 2 to 15 (C_{2-15}), 2 to 10 (C_{2-10}), or 2 to 6 (C_{2-6}) carbon atoms, or a branched monovalent hydrocarbon radical of 3 to 20 (C_{3-20}), 3 to 15 (C_{3-15}), 3 to 10 (C_{3-10}), or 3 to 6 (C_{3-6}) carbon atoms. Examples of alkenyl groups include, but are not limited to, ethenyl, propen-1-yl, propen-2-yl, allyl, butenyl, and 4-methylbutenyl.

[00206] The term "alkenylene" refers to a linear or branched divalent hydrocarbon radical, which contains one or more, in one embodiment, one to five, in another embodiment, one, carbon-carbon double bond(s). The alkenylene is optionally substituted with one or more substituents Q as described herein. The term "alkenylene" embraces radicals having a "*cis*" or "*trans*" configuration or a mixture thereof, or alternatively, a "Z" or "E" configuration or a mixture thereof, as appreciated by those of ordinary skill in the art. For example, C_{2-6} alkenylene refers to a linear unsaturated divalent hydrocarbon radical of 2 to 6 carbon atoms or a branched unsaturated divalent hydrocarbon radical of 3 to 6 carbon atoms. In certain embodiments, the alkenylene is a linear divalent hydrocarbon radical of 2 to 20 (C_{2-20}), 2 to 15 (C_{2-15}), 2 to 10 (C_{2-10}), or 2 to 6 (C_{2-6}) carbon atoms, or a branched divalent hydrocarbon radical of 3 to 20 (C_{3-20}), 3 to 15 (C_{3-15}), 3 to 10 (C_{3-10}), or 3 to 6 (C_{3-6}) carbon atoms. Examples of alkenylene groups include, but are not limited to, ethenylene, allylene, propenylene, butenylene, and 4-methylbutenylene.

[00207] The term "alkynyl" refers to a linear or branched monovalent hydrocarbon radical, which contains one or more, in one embodiment, one, two, three, four, or five, in another embodiment, one, carbon-carbon triple bond(s). The alkynyl is optionally substituted with one or more substituents Q as described herein. The term "alkynyl" also encompasses both linear and branched alkynyl, unless otherwise specified. In certain embodiments, the alkynyl is a linear monovalent hydrocarbon radical of 2 to 20 (C_{2-20}), 2 to 15 (C_{2-15}), 2 to 10 (C_{2-10}), or 2 to 6 (C_{2-6}) carbon atoms, or a branched monovalent hydrocarbon radical of 3 to 20 (C_{3-20}), 3 to 15 (C_{3-15}), 3 to 10 (C_{3-10}), or 3 to 6 (C_{3-6}) carbon atoms. Examples of alkynyl groups include, but are not limited to, ethynyl (–C≡CH) and propargyl (–CH_2C≡CH). For example, C_{2-6} alkynyl refers to a linear unsaturated monovalent hydrocarbon radical of 2 to 6 carbon atoms or a branched unsaturated monovalent hydrocarbon radical of 3 to 6 carbon atoms.

[00208] The term "alkynylene" refers to a linear or branched divalent hydrocarbon radical, which contains one or more, in one embodiment, one to five, in another embodiment, one, carbon-carbon triple bond(s). The alkynylene is optionally substituted with one or more substituents Q as described herein. For example, C_{2-6} alkynylene refers to a linear unsaturated divalent hydrocarbon radical of 2 to 6 carbon atoms or a branched unsaturated divalent hydrocarbon radical of 3 to 6 carbon atoms. In certain embodiments, the alkynylene is a linear divalent hydrocarbon radical of 2 to 20 (C_{2-20}), 2 to 15 (C_{2-15}), 2 to 10 (C_{2-10}), or 2 to 6 (C_{2-6})

carbon atoms, or a branched divalent hydrocarbon radical of 3 to 20 (C_{3-20}), 3 to 15 (C_{3-15}), 3 to 10 (C_{3-10}), or 3 to 6 (C_{3-6}) carbon atoms. Examples of alkynylene groups include, but are not limited to, ethynylene, propynylene (including all isomeric forms, *e.g.*, 1-propynylene and propargylene), butynylene (including all isomeric forms, *e.g.*, 1-butyn-1-ylene and 2-butyn-1-ylene), pentynylene (including all isomeric forms, *e.g.*, 1-pentyn-1-ylene and 1-methyl-2-butyn-1-ylene), and hexynylene (including all isomeric forms, *e.g.*, 1-hexyn-1-ylene).

[00209] The term "cycloalkyl" refers to a cyclic saturated or non-aromatic unsaturated, bridged or non-bridged monovalent hydrocarbon radical, which is optionally substituted with one or more substituents Q as described herein. In certain embodiments, the cycloalkyl is a cyclic saturated bridged or non-bridged monovalent hydrocarbon radical. In certain embodiments, the cycloalkyl has from 3 to 20 (C_{3-20}), from 3 to 15 (C_{3-15}), from 3 to 10 (C_{3-10}), or from 3 to 7 (C_{3-7}) carbon atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, bicyclo[2.1.1]hexyl, bicyclo[2.2.1]heptyl, decalinyl, and adamantyl.

[00210] The term "cycloalkylene" refers to a cyclic divalent hydrocarbon radical, which is optionally substituted with one or more substituents Q as described herein. In one embodiment, cycloalkyl groups is saturated or unsaturated but non-aromatic, and/or bridged, and/or non-bridged, and/or fused bicyclic groups. In certain embodiments, the cycloalkylene has from 3 to 20 (C_{3-20}), from 3 to 15 (C_{3-15}), from 3 to 10 (C_{3-10}), or from 3 to 7 (C_{3-7}) carbon atoms. Examples of cycloalkylene groups include, but are not limited to, cyclopropylene (*e.g.*, 1,1-cyclopropylene and 1,2-cyclopropylene), cyclobutylene (*e.g.*, 1,1-cyclobutylene, 1,2-cyclobutylene, or 1,3-cyclobutylene), cyclopentylene (*e.g.*, 1,1-cyclopentylene, 1,2-cyclopentylene, or 1,3-cyclopentylene), cyclohexylene (*e.g.*, 1,1-cyclohexylene, 1,2-cyclohexylene, 1,3-cyclohexylene, or 1,4-cyclohexylene), cycloheptylene (*e.g.*, 1,1-cycloheptylene, 1,2-cycloheptylene, 1,3-cycloheptylene, or 1,4-cycloheptylene), decalinylene, and adamantylene.

[00211] The term "aryl" refers to a monocyclic aromatic carbocyclic group and/or multicyclic monovalent aromatic carbocyclic group that contain at least one aromatic hydrocarbon ring. In certain embodiments, the aryl has from 6 to 20 (C_{6-20}), from 6 to 15 (C_{6-15}), or from 6 to 10 (C_{6-10}) ring atoms. Examples of aryl groups include, but are not limited to, phenyl, naphthyl, fluorenyl, azulenyl, anthryl, phenanthryl, pyrenyl, biphenyl, and terphenyl. In

certain embodiments, the term "aryl" refers to a bicyclic or tricyclic carbon ring, where one of the rings is aromatic and the others of which can be saturated, partially unsaturated, or aromatic, for example, dihydronaphthyl, indenyl, indanyl, or tetrahydronaphthyl (tetralinyl). The aryl is optionally substituted with one or more substituents Q as described herein.

[00212] The term "arylene" refers to a divalent monocyclic aromatic group and/or divalent polycyclic aromatic group that contain at least one aromatic carbon ring. In certain embodiments, the arylene has from 6 to 20 (C_{6-20}), from 6 to 15 (C_{6-15}), or from 6 to 10 (C_{6-10}) ring atoms. Examples of arylene groups include, but are not limited to, phenylene, naphthylene, fluorenylene, azulenylene, anthrylene, phenanthrylene, pyrenylene, biphenylene, and terphenylene. Arylene also refers to bicyclic or tricyclic carbon rings, where one of the rings is aromatic and the others of which can be saturated, partially unsaturated, or aromatic, for example, dihydronaphthylene, indenylene, indanylene, or tetrahydronaphthylene (tetralinylene). The arylene is optionally substituted with one or more substituents Q as described herein.

[00213] The term "aralkyl" or "arylalkyl" refers to a monovalent alkyl group substituted with one or more aryl groups. In certain embodiments, the aralkyl has from 7 to 30 (C_{7-30}), from 7 to 20 (C_{7-20}), or from 7 to 16 (C_{7-16}) carbon atoms. Examples of aralkyl groups include, but are not limited to, benzyl, 1-phenylethyl, 2-phenylethyl, and 3-phenylpropyl. The aralkyl is optionally substituted with one or more substituents Q as described herein.

[00214] The term "heteroaryl" refers to a monovalent monocyclic aromatic group or monovalent polycyclic aromatic group that contain at least one aromatic ring, wherein at least one aromatic ring contains one or more heteroatoms, each of which is independently selected from O, S, N, and P, in the ring. For clarity, the terms "aryl" and "heteroaryl" as used herein are mutually exclusive, *i.e.*, "aryl" groups do not include "heteroaryl" groups, and vice versa. A heteroaryl group is bonded to the rest of a molecule through its aromatic ring. Each ring of a heteroaryl group can contain one or two O atoms, one or two S atoms, one to four N atoms, and/or one or two P atoms, provided that the total number of heteroatoms in each ring is four or less and each ring contains at least one carbon atom. In certain embodiments, the heteroaryl has from 5 to 20, from 5 to 15, or from 5 to 10 ring atoms. Examples of monocyclic heteroaryl groups include, but are not limited to, furanyl, imidazolyl, isothiazolyl, isoxazolyl, oxadiazolyl, oxadiazolyl, oxazolyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridyl, pyrimidinyl, pyrrolyl, thiadiazolyl, thiazolyl, thienyl, tetrazolyl, triazinyl, and triazolyl. Examples of bicyclic

heteroaryl groups include, but are not limited to, benzofuranyl, benzimidazolyl, benzoisoxazolyl, benzopyranyl, benzothiadiazolyl, benzothiazolyl, benzothienyl, benzotriazolyl, benzoxazolyl, furopyridyl, imidazopyridinyl, imidazothiazolyl, indolizinyl, indolyl, indazolyl, isobenzofuranyl, isobenzothienyl, isoindolyl, isoquinolinyl, isothiazolyl, naphthyridinyl, oxazolopyridinyl, phthalazinyl, pteridinyl, purinyl, pyridopyridyl, pyrrolopyridyl, quinolinyl, quinoxalinyl, quinazolinyl, thiadiazolopyrimidyl, and thienopyridyl. Examples of tricyclic heteroaryl groups include, but are not limited to, acridinyl, benzindolyl, carbazolyl, dibenzofuranyl, perimidinyl, phenanthrolinyl, phenanthridinyl, phenarsazinyl, phenazinyl, phenothiazinyl, phenoxazinyl, and xanthenyl. The heteroaryl is optionally substituted with one or more substituents Q as described herein.

[00215] The term "heteroarylene" refers to a divalent monocyclic aromatic group or divalent polycyclic aromatic group that contain at least one aromatic ring, wherein at least one aromatic ring contains one or more heteroatoms independently selected from O, S, and N in the ring. For clarity, the terms "arylene" and "heteroarylene" as used herein are mutually exclusive, *i.e.*, "arylene" groups do not include "heteroarylene" groups, and vice versa. A heteroarylene group is bonded to the rest of a molecule through its aromatic ring. Each ring of a heteroarylene group can contain one or two O atoms, one or two S atoms, and/or one to four N atoms, provided that the total number of heteroatoms in each ring is four or less and each ring contains at least one carbon atom. In certain embodiments, the heteroarylene has from 5 to 20, from 5 to 15, or from 5 to 10 ring atoms. Examples of monocyclic heteroarylene groups include, but are not limited to, furanylene, imidazolylene, isothiazolylene, isoxazolylene, oxadiazolylene, oxadiazolylene, oxazolylene, pyrazinylene, pyrazolylene, pyridazinylene, pyridylene, pyrimidinylene, pyrrolylene, thiadiazolylene, thiazolylene, thienylene, tetrazolylene, triazinylene, and triazolylene. Examples of bicyclic heteroarylene groups include, but are not limited to, benzofuranylene, benzimidazolylene, benzoisoxazolylene, benzopyranylene, benzothiadiazolylene, benzothiazolylene, benzothienylene, benzotriazolylene, benzoxazolylene, furopyridylene, imidazopyridinylene, imidazothiazolylene, indolizinylene, indolylene, indazolylene, isobenzofuranylene, isobenzothienylene, isoindolylene, isoquinolinylene, isothiazolylene, naphthyridinylene, oxazolopyridinylene, phthalazinylene, pteridinylene, purinylene, pyridopyridylene, pyrrolopyridylene, quinolinylene, quinoxalinylene, quinazolinylene, thiadiazolopyrimidylene, and thienopyridylene. Examples of tricyclic

heteroarylene groups include, but are not limited to, acridinylene, benzindolylene, carbazolylene, dibenzofuranylene, perimidinylene, phenanthrolinylene, phenanthridinylene, phenarsazinylene, phenazinylene, phenothiazinylene, phenoxazinylene, and xanthenylene. The heteroarylene is optionally substituted with one or more substituents Q as described herein.

[00216] The term "heterocyclyl" or "heterocyclic" refers to a monovalent monocyclic non-aromatic ring system or monovalent polycyclic ring system that contains at least one non-aromatic ring, wherein one or more of the non-aromatic ring atoms are heteroatoms, each of which is independently selected from O, S, N, and P; and the remaining ring atoms are carbon atoms. In certain embodiments, the heterocyclyl or heterocyclic group has from 3 to 20, from 3 to 15, from 3 to 10, from 3 to 8, from 4 to 7, or from 5 to 6 ring atoms. A heterocyclyl group is bonded to the rest of a molecule through its non-aromatic ring. In certain embodiments, the heterocyclyl is a monocyclic, bicyclic, tricyclic, or tetracyclic ring system, which can be spiro, fused, or bridged, and in which nitrogen or sulfur atoms can be optionally oxidized, nitrogen atoms can be optionally quaternized, and some rings can be partially or fully saturated, or aromatic. The heterocyclyl can be attached to the main structure at any heteroatom or carbon atom which results in the creation of a stable compound. Examples of heterocyclic groups include, but are not limited to, azepinyl, benzodioxanyl, benzodioxolyl, benzofuranonyl, benzopyranonyl, benzopyranyl, benzotetrahydrofuranyl, benzotetrahydrothienyl, benzothiopyranyl, benzoxazinyl, β-carbolinyl, chromanyl, chromonyl, cinnolinyl, coumarinyl, decahydroisoquinolinyl, dihydrobenzisothiazinyl, dihydrobenzisoxazinyl, dihydrofuryl, dihydroisoindolyl, dihydropyranyl, dihydropyrazolyl, dihydropyrazinyl, dihydropyridinyl, dihydropyrimidinyl, dihydropyrrolyl, dioxolanyl, 1,4-dithianyl, furanonyl, imidazolidinyl, imidazolinyl, indolinyl, isobenzotetrahydrofuranyl, isobenzotetrahydrothienyl, isochromanyl, isocoumarinyl, isoindolinyl, isothiazolidinyl, isoxazolidinyl, morpholinyl, octahydroindolyl, octahydroisoindolyl, oxazolidinonyl, oxazolidinyl, oxiranyl, piperazinyl, piperidinyl, 4-piperidonyl, pyrazolidinyl, pyrazolinyl, pyrrolidinyl, pyrrolinyl, quinuclidinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydropyranyl, tetrahydrothienyl, thiamorpholinyl, thiazolidinyl, tetrahydroquinolinyl, and 1,3,5-trithianyl. The heterocyclyl is optionally substituted with one or more substituents Q as described herein.

[00217] The term "heterocyclylene" refers to a divalent monocyclic non-aromatic ring system or divalent polycyclic ring system that contains at least one non-aromatic ring, wherein

one or more of the non-aromatic ring atoms are heteroatoms independently selected from O, S, and N; and the remaining ring atoms are carbon atoms. In certain embodiments, the heterocyclylene group has from 3 to 20, from 3 to 15, from 3 to 10, from 3 to 8, from 4 to 7, or from 5 to 6 ring atoms. In certain embodiments, the heterocyclylene is a monocyclic, bicyclic, tricyclic, or tetracyclic ring system, which can be fused or bridged, and in which nitrogen or sulfur atoms can be optionally oxidized, nitrogen atoms can be optionally quaternized, and some rings can be partially or fully saturated, or aromatic. The heterocyclylene can be attached to the main structure at any heteroatom or carbon atom which results in the creation of a stable compound. Examples of such heterocyclylene groups include, but are not limited to, azepinylene, benzodioxanylene, benzodioxolylene, benzofuranonylene, benzopyranonylene, benzopyranylene, benzotetrahydrofuranylene, benzotetrahydrothienylene, benzothiopyranylene, benzoxazinylene, β-carbolinylene, chromanylene, chromonylene, cinnolinylene, coumarinylene, decahydroisoquinolinylene, dihydrobenzisothiazinylene, dihydrobenzisoxazinylene, dihydrofurylene, dihydroisoindolylene, dihydropyranylene, dihydropyrazolylene, dihydropyrazinylene, dihydropyridinylene, dihydropyrimidinylene, dihydropyrrolylene, dioxolanylene, 1,4-dithianylene, furanonylene, imidazolidinylene, imidazolinylene, indolinylene, isobenzotetrahydrofuranylene, isobenzotetrahydrothienylene, isochromanylene, isocoumarinylene, isoindolinylene, isothiazolidinylene, isoxazolidinylene, morpholinylene, octahydroindolylene, octahydroisoindolylene, oxazolidinonylene, oxazolidinylene, oxiranylene, piperazinylene, piperidinylene, 4-piperidonylene, pyrazolidinylene, pyrazolinylene, pyrrolidinylene, pyrrolinylene, quinuclidinylene, tetrahydrofurylene, tetrahydroisoquinolinylene, tetrahydropyranylene, tetrahydrothienylene, thiamorpholinylene, thiazolidinylene, tetrahydroquinolinylene, and 1,3,5-trithianylene. The heterocyclylene is optionally substituted with one or more substituents Q as described herein.

[00218] The term "halogen", "halide" or "halo" refers to fluorine, chlorine, bromine, and/or iodine.

[00219] The term "haloalkyl" refers to an alkyl group substituted with one or more, in one embodiment, one, two, or three, halo groups, where the alkyl is as defined herein. The haloalkyl is optionally substituted with one or more substituents Q as described herein.

[00220] The term "alkoxy" refers to –O-alkyl, where the alkyl is as defined herein.

[00221] The term "haloalkoxy" refers to –O-haloalkyl, where the haloalkyl is as defined herein.

[00222] The term "optionally substituted" is intended to mean that a group or substituent, such as an alkyl, alkylene, alkenyl, alkenylene, alkynyl, alkynylene, cycloalkyl, cycloalkylene, aryl, arylene, aralkyl (*e.g.*, benzyl), heteroaryl, heteroarylene, heterocyclyl, and heterocyclylene group, may be substituted with one or more substituents Q, each of which is independently selected from, *e.g.*, (a) oxo (=O), cyano (–CN), halo, and nitro ($–NO_2$); (b) C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-14} aryl, C_{7-15} aralkyl, heteroaryl, and heterocyclyl, each of which is further optionally substituted with one or more, in one embodiment, one, two, three, four, or five, substituents Q^a; and (c) $–C(O)R^a$, $–C(O)OR^a$, $–C(O)NR^bR^c$, $–C(NR^a)NR^bR^c$, $–OR^a$, $–OC(O)R^a$, $–OC(O)OR^a$, $–OC(O)NR^bR^c$, $–OC(=NR^a)NR^bR^c$, $–OS(O)R^a$, $–OS(O)_2R^a$, $–OS(O)NR^bR^c$, $–OS(O)_2NR^bR^c$, $–NR^bR^c$, $–NR^aC(O)R^d$, $–NR^aC(O)OR^d$, $–NR^aC(O)NR^bR^c$, $–NR^aC(=NR^d)NR^bR^c$, $–NR^aS(O)R^d$, $–NR^aS(O)_2R^d$, $–NR^aS(O)NR^bR^c$, $–NR^aS(O)_2NR^bR^c$, $–P(O)R^aR^d$, $–P(O)(OR^a)R^d$, $–P(O)(OR^a)(OR^d)$, $–SR^a$, $–S(O)R^a$, $–S(O)_2R^a$, $–S(O)NR^bR^c$, and $–S(O)_2NR^bR^c$, wherein each R^a, R^b, R^c, and R^d is independently (i) hydrogen; (ii) C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-14} aryl, C_{7-15} aralkyl, heteroaryl, or heterocyclyl, each of which is optionally substituted with one or more, in one embodiment, one, two, three, or four, substituents Q^a; or (iii) R^b and R^c together with the N atom to which they are attached form heteroaryl or heterocyclyl, each of which is optionally substituted with one or more, in one embodiment, one, two, three, or four, substituents Q^a. As used herein, all groups described herein that can be substituted are "optionally substituted," unless otherwise specified.

[00223] In one embodiment, each substituent Q^a is independently selected from the group consisting of (a) oxo, cyano, halo, and nitro; and (b) C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-14} aryl, C_{7-15} aralkyl, heteroaryl, and heterocyclyl; and (c) $–C(O)R^e$, $–C(O)OR^e$, $–C(O)NR^fR^g$, $–C(NR^e)NR^fR^g$, $–OR^e$, $–OC(O)R^e$, $–OC(O)OR^e$, $–OC(O)NR^fR^g$, $–OC(=NR^e)NR^fR^g$, $–OS(O)R^e$, $–OS(O)_2R^e$, $–OS(O)NR^fR^g$, $–OS(O)_2NR^fR^g$, $–NR^fR^g$, $–NR^eC(O)R^h$, $–NR^eC(O)OR^h$, $–NR^eC(O)NR^fR^g$, $–NR^eC(=NR^h)NR^fR^g$, $–NR^eS(O)R^h$, $–NR^eS(O)_2R^h$, $–NR^eS(O)NR^fR^g$, $–NR^eS(O)_2NR^fR^g$, $–P(O)R^eR^h$, $–P(O)(OR^e)R^h$, $–P(O)(OR^e)(OR^h)$, $–SR^e$, $–S(O)R^e$, $–S(O)_2R^e$, $–S(O)NR^fR^g$, and $–S(O)_2NR^fR^g$; wherein each R^e, R^f, R^g, and R^h is independently (i) hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10}

cycloalkyl, C_{6-14} aryl, C_{7-15} aralkyl, heteroaryl, or heterocyclyl; or (ii) R^f and R^g together with the N atom to which they are attached form heteroaryl or heterocyclyl.

[00224] In certain embodiments, "optically active" and "enantiomerically active" refer to a collection of molecules, which has an enantiomeric excess of no less than about 50%, no less than about 70%, no less than about 80%, no less than about 90%, no less than about 91%, no less than about 92%, no less than about 93%, no less than about 94%, no less than about 95%, no less than about 96%, no less than about 97%, no less than about 98%, no less than about 99%, no less than about 99.5%, or no less than about 99.8%. In certain embodiments, the compound comprises about 95% or more of the desired enantiomer and about 5% or less of the less preferred enantiomer based on the total weight of the two enantiomers in question.

[00225] In describing an optically active compound, the prefixes R and S are used to denote the absolute configuration of the optically active compound about its chiral center(s). The (+) and (-) are used to denote the optical rotation of an optically active compound, that is, the direction in which a plane of polarized light is rotated by the optically active compound. The (-) prefix indicates that an optically active compound is levorotatory, that is, the compound rotates the plane of polarized light to the left or counterclockwise. The (+) prefix indicates that an optically active compound is dextrorotatory, that is, the compound rotates the plane of polarized light to the right or clockwise. However, the sign of optical rotation, (+) and (-), is not related to the absolute configuration of a compound, R and S.

[00226] The term "isotopic variant" refers to a compound that contains an unnatural proportion of an isotope at one or more of the atoms that constitute such a compound. In certain embodiments, an "isotopic variant" of a compound contains unnatural proportions of one or more isotopes, including, but not limited to, hydrogen (^{1}H), deuterium (^{2}H), tritium (^{3}H), carbon-11 (^{11}C), carbon-12 (^{12}C), carbon-13 (^{13}C), carbon-14 (^{14}C), nitrogen-13 (^{13}N), nitrogen-14 (^{14}N), nitrogen-15 (^{15}N), oxygen-14 (^{14}O), oxygen-15 (^{15}O), oxygen-16 (^{16}O), oxygen-17 (^{17}O), oxygen-18 (^{18}O), fluorine-17 (^{17}F), fluorine-18 (^{18}F), phosphorus-31 (^{31}P), phosphorus-32 (^{32}P), phosphorus-33 (^{33}P), sulfur-32 (^{32}S), sulfur-33 (^{33}S), sulfur-34 (^{34}S), sulfur-35 (^{35}S), sulfur-36 (^{36}S), chlorine-35 (^{35}Cl), chlorine-36 (^{36}Cl), chlorine-37 (^{37}Cl), bromine-79 (^{79}Br), bromine-81 (^{81}Br), iodine-123 (^{123}I), iodine-125 (^{125}I), iodine-127 (^{127}I), iodine-129 (^{129}I), and iodine-131 (^{131}I). In certain embodiments, an "isotopic variant" of a compound is in a stable form, that is, non-radioactive. In certain embodiments, an "isotopic variant" of a compound contains

unnatural proportions of one or more isotopes, including, but not limited to, hydrogen (^{1}H), deuterium (^{2}H), carbon-12 (^{12}C), carbon-13 (^{13}C), nitrogen-14 (^{14}N), nitrogen-15 (^{15}N), oxygen-16 (^{16}O), oxygen-17 (^{17}O), oxygen-18 (^{18}O), fluorine-17 (^{17}F), phosphorus-31 (^{31}P), sulfur-32 (^{32}S), sulfur-33 (^{33}S), sulfur-34 (^{34}S), sulfur-36 (^{36}S), chlorine-35 (^{35}Cl), chlorine-37 (^{37}Cl), bromine-79 (^{79}Br), bromine-81 (^{81}Br), and iodine-127 (^{127}I). In certain embodiments, an "isotopic variant" of a compound is in an unstable form, that is, radioactive. In certain embodiments, an "isotopic variant" of a compound contains unnatural proportions of one or more isotopes, including, but not limited to, tritium (^{3}H), carbon-11 (^{11}C), carbon-14 (^{14}C), nitrogen-13 (^{13}N), oxygen-14 (^{14}O), oxygen-15 (^{15}O), fluorine-18 (^{18}F), phosphorus-32 (^{32}P), phosphorus-33 (^{33}P), sulfur-35 (^{35}S), chlorine-36 (^{36}Cl), iodine-123 (^{123}I), iodine-125 (^{125}I), iodine-129 (^{129}I), and iodine-131 (^{131}I). It will be understood that, in a compound as provided herein, any hydrogen can be ^{2}H, for example, or any carbon can be ^{13}C, for example, or any nitrogen can be ^{15}N, for example, or any oxygen can be ^{18}O, for example, where feasible according to the judgment of one of skill. In certain embodiments, an "isotopic variant" of a compound contains unnatural proportions of deuterium (D).

[00227] The term "solvate" refers to a complex or aggregate formed by one or more molecules of a solute, *e.g.*, a compound provided herein, and one or more molecules of a solvent, which present in a stoichiometric or non-stoichiometric amount. Suitable solvents include, but are not limited to, water, methanol, ethanol, *n*-propanol, isopropanol, and acetic acid. In certain embodiments, the solvent is pharmaceutically acceptable. In one embodiment, the complex or aggregate is in a crystalline form. In another embodiment, the complex or aggregate is in a noncrystalline form. Where the solvent is water, the solvate is a hydrate. Examples of hydrates include, but are not limited to, a hemihydrate, monohydrate, dihydrate, trihydrate, tetrahydrate, and pentahydrate.

[00228] The phrase "an enantiomer, a mixture of enantiomers, a mixture of two or more diastereomers, or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof" has the same meaning as the phrase "(i) an enantiomer, a mixture of enantiomers, a mixture of two or more diastereomers, or an isotopic variant of the compound referenced therein; (ii) a pharmaceutically acceptable salt, solvate, hydrate, or prodrug of the compound referenced therein; or (iii) a pharmaceutically acceptable salt, solvate, hydrate, or

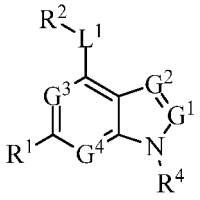
prodrug of an enantiomer, a mixture of enantiomers, a mixture of two or more diastereomers, or an isotopic variant of the compound referenced therein."

5.3.2. Stem cell mobilizing compounds

[00229] In one embodiment, the stem cell mobilizing compound is an aryl hydrocarbon receptor inhibitor, *e.g.*, an aryl hydrocarbon receptor antagonist.

[00230] In another embodiment, the stem cell mobilizing compound is a 5,6-fused heteroaryl compound, including, but not limited to, those described in U.S. Pat. App. Pub. Nos. 2010/0183564, 2014/0023626, and 2014/0114070, the disclosure of each of which is incorporated herein by reference in its entirety.

[00231] In yet another embodiment, the stem cell mobilizing compound is a compound of Formula I:



(I)

[00232] or an enantiomer, a mixture of enantiomers, a mixture of two or more diastereomers, or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof; wherein:

[00233] G^1 is N and CR^3;

[00234] G^2, G^3, and G^4 are each independently CH and N; with the proviso that at least one of G^3 and G^4 is N, and at least one of G^1 and G^2 is not N;

[00235] L^1 is $–NR^{1a}–$, $–NR^{1a}(CH_2)_{1\text{-}3}–$, $–NR^{1a}CH(C(O)OCH_3)CH_2–$, $–NR^{1a}(CH_2)_2NR^{1c}–$, $–NR^{1a}(CH_2)_2S–$, $–NR^{1a}CH_2CH(CH_3)CH_2–$, $–NR^{1a}CH_2CH(OH)–$, or $–NR^{1a}CH(CH_3)CH_2–$;

[00236] R^1 is (i) hydrogen; or (ii) phenyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, thienyl, thiazolyl, pyridinyl, pyrimidinyl, pyrrolidinyl, pyrazinyl, pyridazinyl, benzoimidazolyl, isoquinolinyl, imidazopyridinyl, or benzothienyl, each of which is optionally substituted by one, two, or three substituents, where each substituent is independently cyano, halo, $C_{1\text{-}4}$ alkyl, $C_{1\text{-}4}$ alkoxy, $C_{1\text{-}4}$ haloalkyl, $C_{1\text{-}4}$ haloalkoxy, hydroxyl, amino, $–C(O)R^{1a}$, $–C(O)OR^{1a}$, $–C(O)NR^{1a}R^{1b}$, $–SR^{1a}$, $–S(O)R^{1a}$, or $–S(O)_2R^{1a}$;

[00237] R^2 is (i) $-NR^{1a}C(O)R^{1c}$, $-NR^{1c}C(O)NR^{1a}R^{1b}$, or $-S(O)_2NR^{1a}R^{1b}$; or (ii) phenyl, pyrrolopyridin-3-yl, indolyl, thienyl, pyridinyl, 1,2,4-triazolyl, 2-oxoimidazolidinyl, pyrazolyl, 2-oxo-2,3-dihydro-1*H*-benzoimidazolyl, or indazolyl, each of which is optionally substituted with one, two, or three substituents, where each substituent is independently hydroxyl, halo, methyl, methoxy, amino, $-O(CH_2)_{1-3}NR^{1a}R^{1b}$, $-OS(O)_2NR^{1a}R^{1b}$, $-NR^{1a}S(O)_2R^{1b}$, or $-S(O)_2NR^{1a}R^{1b}$;

[00238] R^3 is hydrogen, C_{1-4} alkyl, or biphenyl; with the proviso that at least one of R^1 and R^3 is not hydrogen;

[00239] R^4 is C_{1-10} alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-3-yl, benzhydryl, tetrahydro-2*H*-pyran-3-yl, tetrahydro-2*H*-pyran-4-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1*H*-1,2,3-triazol-4-yl)ethyl, each of which is optionally substituted with one, two, or three substituents, where each substituent is independently hydroxyl, C_{1-4} alkyl, or C_{1-4} haloalkyl; and

[00240] each R^{1a}, R^{1b}, and R^{1c} is independently hydrogen or C_{1-4} alkyl; or R^{1a} and R^{1b} together with the N atom to which they are attached form heterocyclyl.

[00241] In one embodiment, in Formula I, G^1 is CR^3, in one embodiment, CH; G^2, G^3, and G^4 are each N; and R^1, R^2, R^3, R^4, and L^1 are each as defined herein.

[00242] In another embodiment, in Formula I, G^1, G^3, and G^4 are each N; G^2 is CH; and R^1, R^2, R^4, and L^1 are each as defined herein.

[00243] In yet another embodiment, in Formula I, G^1 is CR^3, in one embodiment, CH; G^2 and G^3 are each N; G^4 is CH; and R^1, R^2, R^3, R^4, and L^1 are each as defined herein.

[00244] In yet another embodiment, in Formula I, G^1 is CR^3, in one embodiment, CH; G^2 and G^4 are each N; G^3 is CH; and R^1, R^2, R^3, R^4, and L^1 are each as defined herein.

[00245] In yet another embodiment, in Formula I, G^1 is CR^3, in one embodiment, CH; G^2 is CH; G^3 and G^4 are each N; and R^1, R^2, R^3, R^4, and L^1 are each as defined herein.

[00246] In still embodiment, in Formula I,

[00247] G^1 is CH;

[00248] G^2, G^3, and G^4 are each N;

[00249] R^1 is benzothienyl, optionally substituted by one, two, or three substituents, each of which is independently cyano, halo, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, C_{1-4} haloalkoxy, hydroxyl, amino, $-C(O)R^{1a}$, $-C(O)OR^{1a}$, $-C(O)NR^{1a}R^{1b}$, $-SR^{1a}$, $-S(O)R^{1a}$, or $-S(O)_2R^{1a}$;

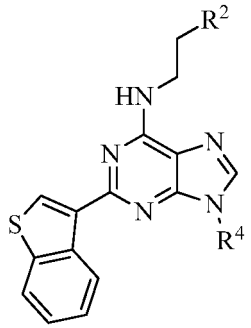
[00250] R^2 is phenyl, optionally substituted with one, two, or three substituents, each of which is independently hydroxyl, halo, methyl, methoxy, amino, $-O(CH_2)_{1-3}NR^{1a}R^{1b}$, $-OS(O)_2NR^{1a}R^{1b}$, $-NR^{1a}S(O)_2R^{1b}$, or $-S(O)_2NR^{1a}R^{1b}$;

[00251] R^4 is C_{1-10} alkyl, optionally substituted with one, two, or three substituents, each of which is independently hydroxyl, C_{1-4} alkyl, or C_{1-4} haloalkyl;

[00252] L^1 is $-NR^{1a}(CH_2)_2-$; and

[00253] R^{1a} and R^{1b} are each as defined herein.

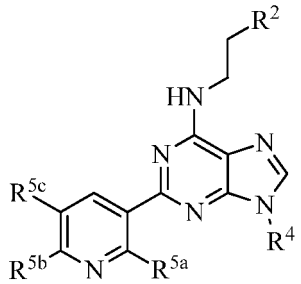
[00254] In yet another embodiment, the stem cell mobilizing compound is a compound of Formula II:



(II)

[00255] or an enantiomer, a mixture of enantiomers, a mixture of two or more diastereomers, or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof; wherein R^2 and R^4 are each as defined herein.

[00256] In yet another embodiment, the stem cell mobilizing compound is a compound of Formula III:

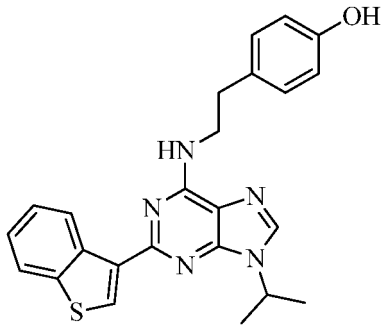


(III)

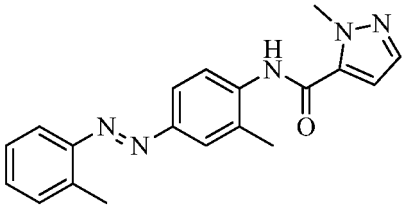
[00257] or an enantiomer, a mixture of enantiomers, a mixture of two or more diastereomers, or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate,

hydrate, or prodrug thereof; wherein R^2 and R^4 are each as defined herein; and R^{5a}, R^{5b}, and R^{5c} are each independently hydrogen, cyano, methyl, halo, trifluoromethyl, or $-SO_2CH_3$.

[00258] In yet another embodiment, the stem cell mobilizing compound is 4-(2-(2-(benzo[*b*]thien-3-yl)-9-isopropyl-9*H*-purin-6-ylamino)ethyl)phenol. In certain embodiments, the stem cell mobilizing compound is StemRegenin-1 (SR-1), having the structure of:

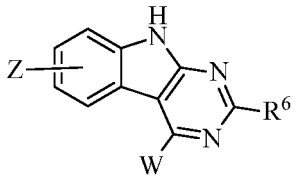


[00259] In yet another embodiment, the stem cell mobilizing compound is 1-methyl-*N*-(2-methyl-4-(2-(2-methylphenyl)diazenyl)phenyl)-1*H*-pyrazole-5-carboxamide. In certain embodiments, the stem cell mobilizing compound is CH223191, which has the structure of:



[00260] In yet another embodiment, the stem cell mobilizing compound is a pyrimido(4,5-*b*)indole.

[00261] In yet another embodiment, the stem cell mobilizing compound is a compound of Formula IV:



(IV)

[00262] or an enantiomer, a mixture of enantiomers, a mixture of two or more diastereomers, or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof; wherein:

[00263] Z is cyano, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-14} aryl, C_{7-15} aralkyl, benzyl, heteroaryl, heterocyclyl, –L– C_{6-14} aryl, –L–heteroaryl, –L–heterocyclyl, $-C(O)R^{1a}$, $-C(O)OR^{1a}$, $-C(O)NHR^{1a}$, $-C(O)N(R^{1a})R^{1b}$, $-P(O)(OR^{1a})(OR^{1c})$, $-SR^{1a}$, $-S(O)R^{1a}$, $-S(O)_2R^{1a}$, $-S(O)_2NH_2$, $-S(O)_2NHR^{1a}$, or $-S(O)_2N(R^{1a})R^{1b}$;

[00264] W is hydrogen, halo, cyano, C_{6-14} aryl, benzyl, heteroaryl, heterocyclyl, –L–C_{6-14} aryl, –L–heteroaryl, –L–heterocyclyl, –L–OH, $-L-OR^{1a}$, $-L-NH_2$, $-L-NHR^{1a}$, $-L-N(R^{1a})R^{1b}$, $-L-SR^{1a}$, $-L-S(O)R^{1a}$, $-L-S(O)_2R^{1a}$, $-L-P(O)(OR^{1a})(OR^{1c})$, $-L-(N(R^{1c})-L)_n-N(R^{1a})R^{1b}$, $-L-(N(R^{1c})-L)_n-C_{6-14}$ aryl, $-L-(N(R^{1c})-L)_n-$heteroaryl, $-L-(N(R^{1c})-L)_n-$heterocyclyl, $-O-L-N(R^{1a})R^{1b}$, –O–L–C_{6-14} aryl, –O–L–heteroaryl, –O–L–heterocyclyl, $-O-L-(N(R^{1c})-L)_n-N(R^{1a})R^{1b}$, $-O-L-(N(R^{1c})-L)_n-C_{6-14}$ aryl, $-O-L-(N(R^{1c})-L)_n-$heteroaryl, $-O-L-(N(R^{1c})-L)_n-$heterocyclyl, $-S-L-N(R^{1a})R^{1b}$, –S–L–C_{6-14} aryl, –S–L–heteroaryl, –S–L–heterocyclyl, $-S-L-(N(R^{1c})-L)_n-N(R^{1a})R^{1b}$, $-S-L-(N(R^{1c})-L)_n-C_{6-14}$ aryl, $-S-L-(N(R^{1c})-L)_n-$heteroaryl, $-S-L-(N(R^{1c})-L)_n-$heterocyclyl, $-(N(R^{1c})-L)_n-N(R^{1a})R^{1b}$, $-(N(R^{1c})-L)_n-C_{6-14}$ aryl, $-(N(R^{1c})-L)_n-$heteroaryl, $-(N(R^{1c})-L)_n-$heterocyclyl, $-C(O)R^{1a}$, $-C(O)OR^{1a}$, $-C(O)NH_2$, $-C(O)NHR^{1a}$, $-C(O)N(R^{1a})R^{1b}$, $-NHR^{1a}$, $-N(R^{1a})R^{1b}$, $-NHC(O)R^{1a}$, $-NR^{1a}C(O)R^{1c}$, $-NHC(O)OR^{1a}$, $-NR^{1a}C(O)OR^{1c}$, $-NHC(O)NH_2$, $-NHC(O)NHR^{1a}$, $-NHC(O)N(R^{1a})R^{1b}$, $-NR^{1a}C(O)NH_2$, $-NR^{1c}C(O)NHR^{1a}$, $-NR^{1c}C(O)N(R^{1a})R^{1b}$, $-NHS(O)_2R^{1a}$, $-NR^{1c}S(O)_2R^{1a}$, $-OR^{1a}$, $-OC(O)R^{1a}$, $-OC(O)OR^{1a}$, $-OC(O)NH_2$, $-OC(O)NHR^{1a}$, $-OC(O)N(R^{1a})R^{1b}$, $-OS(O)_2R^{1a}$, $-P(O)(OR^{1a})(OR^{1c})$, $-SR^{1a}$, $-S(O)R^{1a}$, $-S(O)_2R^{1a}$, $-S(O)_2NH_2$, $-S(O)_2NHR^{1a}$, $-S(O)_2N(R^{1a})R^{1b}$, or $-S(O)_2OR^{1a}$;

[00265] each L is independently C_{1-6} alkylene, C_{2-6} alkenylene, C_{2-6} alkynylene, C_{3-7} cycloalkylene, C_{6-14} arylene, heteroarylene, heterocyclylene, C_{1-6} alkylene-C_{3-7} cycloalkylene, or C_{1-6} alkylene-heterocyclylene;

[00266] R^6 is hydrogen, C_{1-6} alkyl, C_{6-14} aryl, benzyl, heteroaryl, $-C(O)R^{1a}$, $-SR^{1a}$, $-S(O)R^{1a}$, $-S(O)_2R^{1a}$, –L–C_{6-14} aryl, –L–heteroaryl, or –L–heterocyclyl;

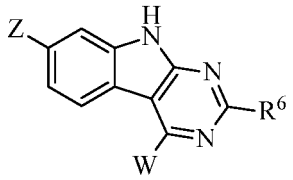
[00267] each n is independently an integer of 1, 2, 3, 4, or 5; and

[00268] each R^{1a}, R^{1b}, and R^{1c} is independently (i) hydrogen; (ii) C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-14} aryl, C_{7-15} aralkyl, heteroaryl, or heterocyclyl; or (iii) R^{1a} and R^{1b} together with the N atom to which they are attached form heterocyclyl;

[00269] wherein each alkyl, alkylene, alkenyl, alkenylene, alkynyl, alkynylene, cycloalkyl, cycloalkylene, aryl, benzyl, arylene, heteroaryl, heteroarylene, heterocyclyl, and heterocyclylene is optionally substituted with one or more, in one embodiment, one, two, three, or four, substituents Q, wherein each substituent Q is independently selected from (a) oxo, cyano, halo, and nitro; (b) C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-14} aryl, C_{7-15} aralkyl, heteroaryl, and heterocyclyl, each of which is further optionally substituted with one or more, in one embodiment, one, two, three, or four, substituents Q^a; and (c) $-C(O)R^a$, $-C(O)OR^a$, $-C(O)NR^bR^c$, $-C(NR^a)NR^bR^c$, $-OR^a$, $-OC(O)R^a$, $-OC(O)OR^a$, $-OC(O)NR^bR^c$, $-OC(=NR^a)NR^bR^c$, $-OS(O)R^a$, $-OS(O)_2R^a$, $-OS(O)NR^bR^c$, $-OS(O)_2NR^bR^c$, $-NR^bR^c$, $-NR^aC(O)R^d$, $-NR^aC(O)OR^d$, $-NR^aC(O)NR^bR^c$, $-NR^aC(=NR^d)NR^bR^c$, $-NR^aS(O)R^d$, $-NR^aS(O)_2R^d$, $-NR^aS(O)NR^bR^c$, $-NR^aS(O)_2NR^bR^c$, $-SR^a$, $-S(O)R^a$, $-S(O)_2R^a$, $-S(O)NR^bR^c$, and $-S(O)_2NR^bR^c$, wherein each R^a, R^b, R^c, and R^d is independently (i) hydrogen; (ii) C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-14} aryl, C_{7-15} aralkyl, heteroaryl, or heterocyclyl, each of which is further optionally substituted with one or more, in one embodiment, one, two, three, or four, substituents Q^a; or (iii) R^b and R^c together with the N atom to which they are attached form heterocyclyl, which is further optionally substituted with one or more, in one embodiment, one, two, three, or four, substituents Q^a;

[00270] wherein each Q^a is independently selected from the group consisting of (a) oxo, cyano, halo, and nitro; (b) C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-14} aryl, C_{7-15} aralkyl, heteroaryl, and heterocyclyl; and (c) $-C(O)R^e$, $-C(O)OR^e$, $-C(O)NR^fR^g$, $-C(NR^e)NR^fR^g$, $-OR^e$, $-OC(O)R^e$, $-OC(O)OR^e$, $-OC(O)NR^fR^g$, $-OC(=NR^e)NR^fR^g$, $-OS(O)R^e$, $-OS(O)_2R^e$, $-OS(O)NR^fR^g$, $-OS(O)_2NR^fR^g$, $-NR^fR^g$, $-NR^eC(O)R^h$, $-NR^eC(O)OR^h$, $-NR^eC(O)NR^fR^g$, $-NR^eC(=NR^h)NR^fR^g$, $-NR^eS(O)R^h$, $-NR^eS(O)_2R^h$, $-NR^eS(O)NR^fR^g$, $-NR^eS(O)_2NR^fR^g$, $-SR^e$, $-S(O)R^e$, $-S(O)_2R^e$, $-S(O)NR^fR^g$, and $-S(O)_2NR^fR^g$; wherein each R^e, R^f, R^g, and R^h is independently (i) hydrogen; (ii) C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-14} aryl, C_{7-15} aralkyl, heteroaryl, or heterocyclyl; or (iii) R^f and R^g together with the N atom to which they are attached form heterocyclyl.

[00271] In yet another embodiment, the stem cell mobilizing compound is a compound of Formula V:



(V)

[00272] or an enantiomer, a mixture of enantiomers, a mixture of two or more diastereomers, or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof; wherein R^6, W, and Z are each as defined herein.

[00273] In one embodiment, in Formula IV or V,

[00274] Z is cyano, heteroaryl, or $-C(O)OR^{1a}$;

[00275] W is heterocyclyl, –L–heterocyclyl, –O–L–heterocyclyl, $-(N(R^{1c})-L)_n-N(R^{1a})R^{1b}$, $-(N(R^{1c})-L)_n-$heterocyclyl, $-NHR^{1a}$, or $-N(R^{1a})R^{1b}$;

[00276] each L is independently C_{1-6} alkylene or C_{3-7} cycloalkylene;

[00277] R^6 is hydrogen, C_{1-6} alkyl, benzyl, $-C(O)R^{1a}$, $-L-C_{6-14}$ aryl, or –L–heteroaryl;

[00278] each n is independently an integer of 1; and

[00279] R^{1a}, R^{1b}, and R^{1c} are each as defined herein;

[00280] wherein each alkyl, alkylene, cycloalkylene, aryl, benzyl, heteroaryl, and heterocyclyl is optionally substituted with one or more substituents Q as defined herein.

[00281] In another embodiment, in Formula IV or V,

[00282] Z is cyano, 5-membered heteroaryl, or $-C(O)O-C_{1-6}$ alkyl;

[00283] W is heterocyclyl, –L–heterocyclyl, –O–L–heterocyclyl, $-(N(R^{1c})-L)_n-N(R^{1a})R^{1b}$, $-(N(R^{1c})-L)_n-$heterocyclyl, $-NHR^{1a}$, or $-N(R^{1a})R^{1b}$;

[00284] each L is independently C_{1-6} alkylene or C_{3-7} cycloalkylene;

[00285] R^6 is hydrogen, methyl, benzyl, $-L-C_{6-14}$ aryl, or –L–heteroaryl;

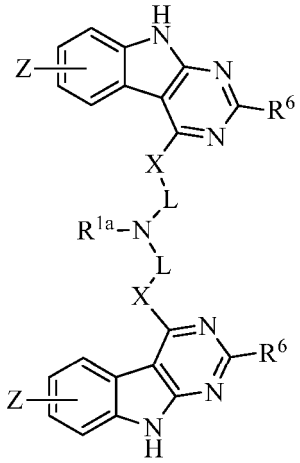
[00286] each n is independently an integer of 1; and

[00287] R^{1a}, R^{1b}, and R^{1c} are each as defined herein;

[00288] wherein each alkylene, cycloalkylene, aryl, benzyl, heteroaryl, and heterocyclyl is optionally substituted with one or more substituents Q as defined herein.

[00289] In one embodiment, in Formula IV or V, W is $-L-N(R^{1a})R^{1b}$, $-L-(N(R^{1c})-L)_n-N(R^{1a})R^{1b}$, $-O-L-N(R^{1a})R^{1b}$, $-O-L-(N(R^{1c})-L)_n-N(R^{1a})R^{1b}$, $-S-L-N(R^{1a})R^{1b}$, $-S-L-(N(R^{1c})-L)_n-N(R^{1a})R^{1b}$, or $-(N(R^{1c})-L)_n-N(R^{1a})R^{1b}$; and R^6, R^{1a}, R^{1b}, R^{1c}, L, and Z are each as defined herein.

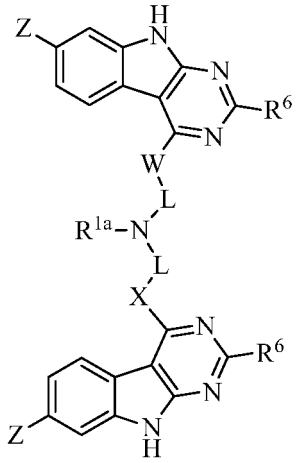
[00290] In yet another embodiment, the stem cell mobilizing compound is a compound of Formula VI:



(VI)

[00291] or an enantiomer, a mixture of enantiomers, a mixture of two or more diastereomers, or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof; wherein X is a bond, O, S, or NR^{1c}; and R^{1a}, R^{1c}, R^6, L, and Z are each as defined herein.

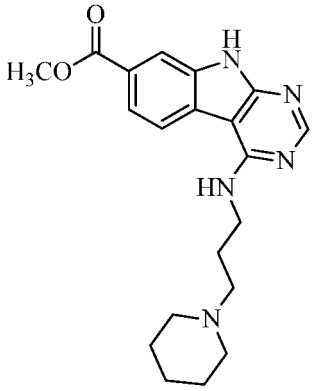
[00292] In still another embodiment, the stem cell mobilizing compound is a compound of Formula VII:



(VII)

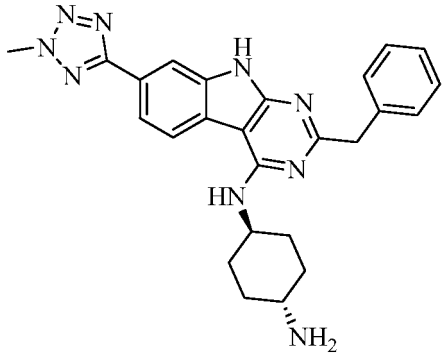
[00293] or an enantiomer, a mixture of enantiomers, a mixture of two or more diastereomers, or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof; wherein R^{1a}, R^6, L, X, and Z are each as defined herein.

[00294] In yet another embodiment, the stem cell mobilizing compound is a compound having the structure of:



(UM729).

[00295] In yet another embodiment, the stem cell mobilizing compound is a compound having the structure of:



(UM171).

[00296] In yet another embodiment, the stem cell mobilizing compound is resveratrol, tetraethylenepentamine (TEPA), alpha naphthoflavone, 3'-methoxy-4'-nitroflavone, 3,4-dimethoxyflavone, 4',5,7-trihydroxyflavone (apigenin), 6-methyl-1,3,8-trichlorodibenzofuran, epigallocatechin, or epigallocatechingallate.

[00297] In yet another embodiment, the stem cell mobilizing compound is resveratrol. In certain embodiments, the stem cell mobilizing compound is (*Z*)-resveratrol. In certain embodiments, the stem cell mobilizing compound is (*E*)-resveratrol.

[00298] In still another embodiment, the stem cell mobilizing compound is tetraethylenepentamine (TEPA).

[00299] All of the compounds described herein are either commercially available or can be prepared according to the methods described in the patents or patent publications disclosed herein. Further, optically pure compounds can be asymmetrically synthesized or resolved using known resolving agents or chiral columns as well as other standard synthetic organic chemistry

techniques. Additional information on stem cell mobilizing compounds, their preparation, and use can be found, for example, in U.S. Pat. App. Pub. Nos. 2010/0183564, 2014/0023626, and 2014/0114070; and Kim *et al.*, *Mol. Pharmacol.*, **2006,** *69*, 1871-1878; the disclosures of each of which is incorporated by reference herein in its entirety.

[00300] The groups or variables, G^1, G^2, G^3, G^4, R^1, R^2, R^3, R^4, R^{5a}, R^{5b}, R^{5c}, R^6, X, L, L^1, X, W, Z, and n, in Formulae provided herein, *e.g.,* Formulae I to VII, are further defined in the embodiments described herein. All combinations of the embodiments provided herein for such groups and/or variables are within the scope of this disclosure.

[00301] In certain embodiments, G^1 is N. In certain embodiments, G^1 is CR^3, wherein R^3 is as defined herein. In certain embodiments, G^1 is CH.

[00302] In certain embodiments, G^2 is N. In certain embodiments, G^2 is CH.

[00303] In certain embodiments, G^3 is N. In certain embodiments, G^3 is CH.

[00304] In certain embodiments, G^4 is N. In certain embodiments, G^4 is CH.

[00305] In certain embodiments, R^1 is hydrogen. In certain embodiments, R^1 is phenyl optionally substituted as described herein. In certain embodiments, R^1 is furanyl optionally substituted as described herein. In certain embodiments, R^1 is pyrrolyl optionally substituted as described herein. In certain embodiments, R^1 is imidazolyl optionally substituted as described herein. In certain embodiments, R^1 is pyrazolyl optionally substituted as described herein. In certain embodiments, R^1 is thienyl optionally substituted as described herein. In certain embodiments, R^1 is thiazolyl optionally substituted as described herein. In certain embodiments, R^1 is pyridinyl optionally substituted as described herein. In certain embodiments, R^1 is pyrimidinyl optionally substituted as described herein. In certain embodiments, R^1 is pyrrolidinyl optionally substituted as described herein. In certain embodiments, R^1 is pyrazinyl optionally substituted as described herein. In certain embodiments, R^1 is pyridazinyl optionally substituted as described herein. In certain embodiments, R^1 is benzoimidazolyl optionally substituted as described herein. In certain embodiments, R^1 is isoquinolinyl optionally substituted as described herein. In certain embodiments, R^1 is imidazopyridinyl optionally substituted as described herein. In certain embodiments, R^1 is benzothienyl optionally substituted as described herein.

[00306] In certain embodiments, R^2 is $-NR^{1a}C(O)R^{1c}$, wherein R^{1a} and R^{1c} are each as defined herein. In certain embodiments, R^2 is $-NR^{1c}C(O)NR^{1a}R^{1b}$, wherein R^{1a}, R^{1b}, and R^{1c} are

each as defined herein. In certain embodiments, R^2 is $-S(O)_2NR^{1a}R^{1b}$, wherein R^{1a} and R^{1b} are each as defined herein. In certain embodiments, R^2 is phenyl optionally substituted as described herein. In certain embodiments, R^2 is pyrrolopyridin-3-yl optionally substituted as described herein. In certain embodiments, R^2 is indolyl optionally substituted as described herein. In certain embodiments, R^2 is thienyl optionally substituted as described herein. In certain embodiments, R^2 is pyridinyl optionally substituted as described herein. In certain embodiments, R^2 is 1,2,4-triazolyl optionally substituted as described herein. In certain embodiments, R^2 is 2-oxoimidazolidinyl optionally substituted as described herein. In certain embodiments, R^2 is pyrazolyl optionally substituted as described herein. In certain embodiments, R^2 is 2-oxo-2,3-dihydro-1*H*-benzoimidazolyl optionally substituted as described herein. In certain embodiments, R^2 is indazolyl optionally substituted as described herein.

[00307] In certain embodiments, R^3 is hydrogen. In certain embodiments, R^3 is C_{1-4} alkyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, R^3 is biphenyl, optionally substituted with one or more substituents Q as described herein.

[00308] In certain embodiments, R^4 is C_{1-10} alkyl optionally substituted as described herein. In certain embodiments, R^4 is prop-1-en-2-yl optionally substituted as described herein. In certain embodiments, R^4 is cyclohexyl optionally substituted as described herein. In certain embodiments, R^4 is cyclopropyl optionally substituted as described herein. In certain embodiments, R^4 is 2-(2-oxopyrrolidin-1-yl)ethyl optionally substituted as described herein. In certain embodiments, R^4 is oxetan-3-yl optionally substituted as described herein. In certain embodiments, R^4 is benzhydryl optionally substituted as described herein. In certain embodiments, R^4 is tetrahydro-2*H*-pyran-3-yl optionally substituted as described herein. In certain embodiments, R^4 is tetrahydro-2*H*-pyran-4-yl optionally substituted as described herein. In certain embodiments, R^4 is phenyl optionally substituted as described herein. In certain embodiments, R^4 is tetrahydrofuran-3-yl optionally substituted as described herein. In certain embodiments, R^4 is benzyl optionally substituted as described herein. In certain embodiments, R^4 is (4-pentylphenyl)(phenyl)methyl optionally substituted as described herein. In certain embodiments, R^4 is 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1*H*-1,2,3-triazol-4-yl)ethyl optionally substituted as described herein.

[00309] In certain embodiments, L^1 is $-NR^{1a}-$, wherein R^{1a} is as defined herein. In certain embodiments, L^1 is $-NR^{1a}(CH_2)_{1-3}-$, wherein R^{1a} is as defined herein. In certain embodiments, L^1 is $-NR^{1a}CH(C(O)OCH_3)CH_2-$, wherein R^{1a} is as defined herein. In certain embodiments, L^1 is $-NR^{1a}(CH_2)_2NR^{1c}-$, wherein R^{1a} and R^{1c} are each as defined herein. In certain embodiments, L^1 is $-NR^{1a}(CH_2)_2S-$, wherein R^{1a} is as defined herein. In certain embodiments, L^1 is $-NR^{1a}CH_2CH(CH_3)CH_2-$, wherein R^{1a} is as defined herein. In certain embodiments, L^1 is $-NR^{1a}CH_2CH(OH)-$, wherein R^{1a} is as defined herein. In certain embodiments, L^1 is $-NR^{1a}CH(CH_3)CH_2-$, wherein R^{1a} is as defined herein.

[00310] In certain embodiments, R^{5a} is hydrogen. In certain embodiments, R^{5a} is cyano. In certain embodiments, R^{5a} is methyl. In certain embodiments, R^{5a} is halo. In certain embodiments, R^{5a} is fluoro, chloro, or bromo. In certain embodiments, R^{5a} is trifluoromethyl. In certain embodiments, R^{5a} is $-SO_2CH_3$.

[00311] In certain embodiments, R^{5b} is hydrogen. In certain embodiments, R^{5b} is cyano. In certain embodiments, R^{5b} is methyl. In certain embodiments, R^{5b} is halo. In certain embodiments, R^{5b} is fluoro, chloro, or bromo. In certain embodiments, R^{5b} is trifluoromethyl. In certain embodiments, R^{5b} is $-SO_2CH_3$.

[00312] In certain embodiments, R^{5c} is hydrogen. In certain embodiments, R^{5c} is cyano. In certain embodiments, R^{5c} is methyl. In certain embodiments, R^{5c} is halo. In certain embodiments, R^{5c} is fluoro, chloro, or bromo. In certain embodiments, R^{5c} is trifluoromethyl. In certain embodiments, R^{5c} is $-SO_2CH_3$.

[00313] In certain embodiments, L is C_{1-6} alkylene, optionally substituted with one or more substituents Q as described herein. In certain embodiments, L is ethylene, propylene, or butylenes, each optionally substituted with one or more substituents Q as described herein. In certain embodiments, L is C_{2-6} alkenylene, optionally substituted with one or more substituents Q as described herein. In certain embodiments, L is C_{2-6} alkynylene, optionally substituted with one or more substituents Q as described herein. In certain embodiments, L is C_{3-7} cycloalkylene, optionally substituted with one or more substituents Q as described herein. In certain embodiments, L is cyclohexylene, optionally substituted with one or more substituents Q as described herein. In certain embodiments, L is C_{6-14} arylene, optionally substituted with one or more substituents Q as described herein. In certain embodiments, L is heteroarylene, optionally substituted with one or more substituents Q as described herein. In certain embodiments, L is

heterocyclylene, optionally substituted with one or more substituents Q as described herein. In certain embodiments, L is C_{1-6} alkylene-C_{3-7} cycloalkylene, optionally substituted with one or more substituents Q as described herein. In certain embodiments, L is C_{1-6} alkylene-heterocyclylene, optionally substituted with one or more substituents Q as described herein.

[00314] In certain embodiments, R^6 is hydrogen. In certain embodiments, R^6 is C_{1-6} alkyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, R^6 is methyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, R^6 is C_{6-14} aryl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, R^6 is benzyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, R^6 is heteroaryl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, R^6 is $-C(O)R^{1a}$, where R^{1a} is as defined herein. In certain embodiments, R^6 is $-SR^{1a}$, where R^{1a} is as defined herein. In certain embodiments, R^6 is $-S(O)R^{1a}$, where R^{1a} is as defined herein. In certain embodiments, R^6 is $-S(O)_2R^{1a}$, where R^{1a} is as defined herein. In certain embodiments, R^6 is –L–C_{6-14} aryl, where L is as defined herein. In certain embodiments, R^6 is –L–heteroaryl, where L is as defined herein. In certain embodiments, R^6 is or –L–heterocyclyl, where L is as defined herein.

[00315] In certain embodiments, W is hydrogen. In certain embodiments, W is halo. In certain embodiments, W is cyano. In certain embodiments, W is C_{6-14} aryl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, W is benzyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, W is heteroaryl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, W is heterocyclyl, optionally substituted with one or more substituents Q as described herein.

[00316] In certain embodiments, W is –L–C_{6-14} aryl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein. In certain embodiments, W is

–L–heteroaryl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein. In certain embodiments, W is –L–heterocyclyl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein. In certain embodiments, W is –L–OH, where L is as defined herein. In certain embodiments, W is –L–

OR^{1a}, where R^{1a} and L are each as defined herein. In certain embodiments, W is $–L–NH_2$, where L is as defined herein. In certain embodiments, W is $–L–NHR^{1a}$, where R^{1a} and L are each as defined herein. In certain embodiments, W is $–L–N(R^{1a})R^{1b}$, where R^{1a}, R^{1b}, and L are each as defined herein. In certain embodiments, W is $–L–SR^{1a}$, where R^{1a} and L are each as defined herein. In certain embodiments, W is $–L–S(O)R^{1a}$, where R^{1a} and L are each as defined herein. In certain embodiments, W is $–L–S(O)_2R^{1a}$, where R^{1a} and L are each as defined herein. In certain embodiments, W is $–L–P(O)(OR^{1a})(OR^{1c})$, where R^{1a}, R^{1c}, and L are each as defined herein.

[00317] In certain embodiments, W is $–L–(N(R^{1c})–L)_n–N(R^{1a})R^{1b}$, where R^{1a}, R^{1b}, R^{1c}, L and n are each as defined herein. In certain embodiments, W is $–L–(N(R^{1c})–L)_n–C_{6\text{-}14}$ aryl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein. In certain embodiments, W is $–L–(N(R^{1c})–L)_n–$heteroaryl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein. In certain embodiments, W is $–L–(N(R^{1c})–L)_n–$heterocyclyl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein.

[00318] In certain embodiments, W is $–O–L–N(R^{1a})R^{1b}$, where R^{1a}, R^{1b}, and L are each as defined herein. In certain embodiments, W is $–O–L–C_{6\text{-}14}$ aryl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein. In certain embodiments, W is –O–L–heteroaryl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein. In certain embodiments, W is –O–L–heterocyclyl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein.

[00319] In certain embodiments, W is $–O–L–(N(R^{1c})–L)_n–N(R^{1a})R^{1b}$, where R^{1a}, R^{1b}, R^{1c}, L, and n are each as defined herein. In certain embodiments, W is $–O–L–(N(R^{1c})–L)_n–C_{6\text{-}14}$ aryl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein. In certain embodiments, W is $–O–L–(N(R^{1c})–L)_n–$ heteroaryl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein. In certain embodiments, W is $–O–L–(N(R^{1c})–L)_n–$heterocyclyl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein.

[00320] In certain embodiments, W is –S–L–$N(R^{1a})R^{1b}$, where R^{1a}, R^{1b}, and L are each as defined herein. In certain embodiments, W is –S–L–C_{6-14} aryl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein. In certain embodiments, W is –S–L–heteroaryl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein. In certain embodiments, W is –S–L–heterocyclyl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein.

[00321] In certain embodiments, W is –S–L–$(N(R^{1c})–L)_n–N(R^{1a})R^{1b}$, where R^{1a}, R^{1b}, R^{1c}, L, and n are each as defined herein. In certain embodiments, W is –S–L–$(N(R^{1c})–L)_n–C_{6-14}$ aryl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein. In certain embodiments, W is –S–L–$(N(R^{1c})–L)_n$–heteroaryl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein. In certain embodiments, W is –S–L–$(N(R^{1c})–L)_n$–heterocyclyl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein.

[00322] In certain embodiments, W is –$(N(R^{1c})–L)_n–N(R^{1a})R^{1b}$, where R^{1a}, R^{1b}, R^{1c}, L, and n are each as defined herein. In certain embodiments, W is –$(N(R^{1c})–L)_n–C_{6-14}$ aryl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein. In certain embodiments, W is –$(N(R^{1c})–L)_n$–heteroaryl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein. In certain embodiments, W is –$(N(R^{1c})–L)_n$–heterocyclyl, optionally substituted with one or more substituents Q as described herein, where R^{1c}, L, and n are each as defined herein.

[00323] In certain embodiments, W is –$C(O)R^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is –$C(O)OR^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is –$C(O)NH_2$. In certain embodiments, W is –$C(O)NHR^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is –$C(O)N(R^{1a})R^{1b}$, where R^{1a} and R^{1b} are each as defined herein. In certain embodiments, W is –NHR^{1a}, where R^{1a} is as defined herein. In certain embodiments, W is –$N(R^{1a})R^{1b}$, where R^{1a} and R^{1b} are each as defined herein. In certain embodiments, W is –$NHC(O)R^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is –$NR^{1a}C(O)R^{1c}$, where R^{1a} and R^{1c} are each as defined herein. In certain embodiments, W is –$NHC(O)OR^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is –$NR^{1a}C(O)OR^{1c}$, where R^{1a} and

R^{1c} are each as defined herein. In certain embodiments, W is $-NHC(O)NH_2$. In certain embodiments, W is $-NHC(O)NHR^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is $-NHC(O)N(R^{1a})R^{1b}$, where R^{1a} and R^{1b} are each as defined herein. In certain embodiments, W is $-NR^{1a}C(O)NH_2$, where R^{1a} is as defined herein. In certain embodiments, W is $-NR^{1c}C(O)NHR^{1a}$, where R^{1a} and R^{1c} are each as defined herein. In certain embodiments, W is $-NR^{1c}C(O)N(R^{1a})R^{1b}$, where R^{1a}, R^{1b}, and R^{1c} are each as defined herein. In certain embodiments, W is $-NHS(O)_2R^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is $-NR^{1c}S(O)_2R^{1a}$, where R^{1a} and R^{1c} are each as defined herein. In certain embodiments, W is $-OR^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is $-OC(O)R^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is $-OC(O)OR^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is $-OC(O)NH_2$. In certain embodiments, W is $-OC(O)NHR^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is $-OC(O)N(R^{1a})R^{1b}$, where R^{1a} and R^{1b} are each as defined herein. In certain embodiments, W is $-OS(O)_2R^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is $-P(O)(OR^{1a})(OR^{1c})$, where R^{1a} and R^{1c} are each as defined herein. In certain embodiments, W is $-SR^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is $-S(O)R^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is $-S(O)_2R^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is $-S(O)_2NH_2$. In certain embodiments, W is $-S(O)_2NHR^{1a}$, where R^{1a} is as defined herein. In certain embodiments, W is $-S(O)_2N(R^{1a})R^{1b}$, where R^{1a} and R^{1b} are each as defined herein. In certain embodiments, W is $-S(O)_2OR^{1a}$, where R^{1a} is as defined herein.

[00324] In certain embodiments, Z is cyano. In certain embodiments, Z is C_{1-6} alkyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, Z is C_{2-6} alkenyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, Z is C_{2-6} alkynyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, Z is C_{3-10} cycloalkyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, Z is C_{6-14} aryl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, Z is C_{7-15} aralkyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, Z is benzyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, Z is heteroaryl, optionally substituted with one or more substituents Q as described herein. In certain

embodiments, Z is 5-membered heteroaryl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, Z is tetrazolyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, Z is 1,2,4-oxadiazolyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, Z is heterocyclyl, optionally substituted with one or more substituents Q as described herein. In certain embodiments, Z is –L– C_{6-14} aryl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein. In certain embodiments, Z is –L–heteroaryl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein. In certain embodiments, Z is –L–heterocyclyl, optionally substituted with one or more substituents Q as described herein, where L is as defined herein.

[00325] In certain embodiments, Z is $–C(O)R^{1a}$, wherein R^{1a} is as defined herein. In certain embodiments, Z is $–C(O)OR^{1a}$, wherein R^{1a} is as defined herein. In certain embodiments, Z is
$–C(O)OC_{1-6}$ alkyl, wherein the alkyl is optionally substituted with one or more substituents Q as defined herein. In certain embodiments, Z is $–C(O)OCH_3$. In certain embodiments, Z is $–C(O)NHR^{1a}$, wherein R^{1a} is as defined herein. In certain embodiments, Z is $–C(O)N(R^{1a})R^{1b}$, wherein R^{1a} and R^{1b} are each as defined herein. In certain embodiments, Z is $–P(O)(OR^{1a})(OR^{1c})$, wherein R^{1a} and R^{1c} are each as defined herein. In certain embodiments, Z is $–SR^{1a}$, wherein R^{1a} is as defined herein. In certain embodiments, Z is $–S(O)R^{1a}$, wherein R^{1a} is as defined herein. In certain embodiments, Z is $–S(O)_2R^{1a}$, wherein R^{1a} is as defined herein. In certain embodiments, Z is $–S(O)_2NH_2$. In certain embodiments, Z is $–S(O)_2NHR^{1a}$, wherein R^{1a} is as defined herein. In certain embodiments, Z is $–S(O)_2N(R^{1a})R^{1b}$, wherein R^{1a} and R^{1b} are each as defined herein.

[00326] In certain embodiments, X is a bond. In certain embodiments, X is O. In certain embodiments, X is S. In certain embodiments, X is NR^{1c}, where R^{1c} is as defined herein.

[00327] In certain embodiments, n is 1. In certain embodiments, n is 2. In certain embodiments, n is 3. In certain embodiments, n is 4. In certain embodiments, n is 5.

[00328] In certain embodiments, the compounds provided herein show activity as antagonists of an AHR.

[00329] The compounds provided herein may be enantiomerically pure, such as a single enantiomer or a single diastereomer, or be stereoisomeric mixtures, such as a mixture of

enantiomers, *e.g.*, a racemic mixture of two enantiomers; or a mixture of two or more diastereomers. As such, one of skill in the art will recognize that administration of a compound in its (*R*) form is equivalent, for compounds that undergo epimerization *in vivo*, to administration of the compound in its (*S*) form. Conventional techniques for the preparation/isolation of individual enantiomers include synthesis from a suitable optically pure precursor, asymmetric synthesis from achiral starting materials, or resolution of an enantiomeric mixture, for example, chiral chromatography, recrystallization, resolution, diastereomeric salt formation, or derivatization into diastereomeric adducts followed by separation.

5.4. Isolation of NK Cells

[00330] Methods of isolating natural killer cells are known in the art and can be used to isolate the natural killer cells, *e.g.*, NK cells produced using the three-stage method, described herein. For example, NK cells can be isolated or enriched, for example, by staining cells, in one embodiment, with antibodies to CD56 and CD3, and selecting for $CD56^{+}CD3^{-}$ cells. In certain embodiments, the NK cells are enriched for $CD56^{+}CD3^{-}$ cells in comparison with total cells produced using the three-stage method, described herein. NK cells, *e.g.*, cells produced using the three-stage method, described herein, can be isolated using a commercially available kit, for example, the NK Cell Isolation Kit (Miltenyi Biotec). NK cells, *e.g.*, cells produced using the three-stage method, described herein, can also be isolated or enriched by removal of cells other than NK cells in a population of cells that comprise the NK cells, *e.g.*, cells produced using the three-stage method, described herein. For example, NK cells, *e.g.*, cells produced using the three-stage method, described herein, may be isolated or enriched by depletion of cells displaying non-NK cell markers using, *e.g.*, antibodies to one or more of CD3, CD4, CD14, CD19, CD20, CD36, CD66b, CD123, HLA DR and/or CD235a (glycophorin A). Negative isolation can be carried out using a commercially available kit, *e.g.*, the NK Cell Negative Isolation Kit (Dynal Biotech). Cells isolated by these methods may be additionally sorted, *e.g.*, to separate CD11a+ and CD11a- cells, and/or CD117+ and CD117- cells, and/or $CD16^{+}$ and $CD16^{-}$ cells, and/or $CD94^{+}$ and $CD94^{-}$. In certain embodiments, cells, *e.g.*, cells produced by the three-step methods described herein, are sorted to separate CD11a+ and CD11a- cells. In specific embodiments, CD11a+ cells are isolated. In certain embodiments, the cells are enriched for $CD11a^{+}$ cells in comparison with total cells produced using the three-stage method, described

herein. In specific embodiments, CD11a- cells are isolated. In certain embodiments, the cells are enriched for CD11a- cells in comparison with total cells produced using the three-stage method, described herein. In certain embodiments, cells are sorted to separate CD117+ and CD117- cells. In specific embodiments, CD117+ cells are isolated. In certain embodiments, the cells are enriched for $CD117^+$ cells in comparison with total cells produced using the three-stage method, described herein. In specific embodiments, CD117- cells are isolated. In certain embodiments, the cells are enriched for CD117- cells in comparison with total cells produced using the three-stage method, described herein. In certain embodiments, cells are sorted to separate $CD16^+$ and $CD16^-$ cells. In specific embodiments, $CD16^+$ cells are isolated. In certain embodiments, the cells are enriched for $CD16^+$ cells in comparison with total cells produced using the three-stage method, described herein. In specific embodiments, $CD16^-$ cells are isolated. In certain embodiments, the cells are enriched for CD16- cells in comparison with total cells produced using the three-stage method, described herein. In certain embodiments, cells are sorted to separate $CD94^+$ and $CD94^-$ cells. In specific embodiments, $CD94^+$ cells are isolated. In certain embodiments, the cells are enriched for $CD94^+$ cells in comparison with total cells produced using the three-stage method, described herein. In specific embodiments, $CD94^-$ cells are isolated. In certain embodiments, the cells are enriched for CD94- cells in comparison with total cells produced using the three-stage method, described herein. In certain embodiments, isolation is performed using magnetic separation. In certain embodiments, isolation is performed using flow cytometry.

[00331] Methods of isolating ILC3 cells are known in the art and can be used to isolate the ILC3 cells, *e.g.*, ILC3 cells produced using the three-stage method, described herein. For example, ILC3 cells can be isolated or enriched, for example, by staining cells, in one embodiment, with antibodies to CD56, CD3, and CD11a, and selecting for $CD56^+CD3^-CD11a^-$ cells. ILC3 cells, *e.g.*, cells produced using the three-stage method, described herein, can also be isolated or enriched by removal of cells other than ILC3 cells in a population of cells that comprise the ILC3 cells, *e.g.*, cells produced using the three-stage method, described herein. For example, ILC3 cells, *e.g.*, cells produced using the three-stage method, described herein, may be isolated or enriched by depletion of cells displaying non-ILC3 cell markers using, *e.g.*, antibodies to one or more of CD3, CD4, CD11a, CD14, CD19, CD20, CD36, CD66b, CD94, CD123, HLA DR and/or CD235a (glycophorin A). Cells isolated by these methods may be

additionally sorted, *e.g.*, to separate $CD117^+$ and $CD117^-$ cells. NK cells can be isolated or enriched, for example, by staining cells, in one embodiment, with antibodies to CD56, CD3, CD94, and CD11a, and selecting for $CD56^+CD3^-CD94^+CD11a^+$ cells. NK cells, *e.g.*, cells produced using the three-stage method, described herein, can also be isolated or enriched by removal of cells other than NK cells in a population of cells that comprise the NK cells, *e.g.*, cells produced using the three-stage method, described herein. In certain embodiments, the NK cells are enriched for $CD56^+CD3^-CD94^+CD11a^+$ cells in comparison with total cells produced using the three-stage method, described herein.

[00332] In one embodiment, ILC3 cells are isolated or enriched by selecting for $CD56^+CD3^-CD11a^-$ cells. In certain embodiments, the ILC3 cells are enriched for $CD56^+CD3^-CD11a^-$ cells in comparison with total cells produced using the three-stage method, described herein. In one embodiment, ILC3 cells are isolated or enriched by selecting for $CD56^+CD3^-CD11a^-CD117+$ cells. In certain embodiments, the ILC3 cells are enriched for $CD56^+CD3^-CD11a^-CD117+$ cells in comparison with total cells produced using the three-stage method, described herein. In one embodiment, ILC3 cells are isolated or enriched by selecting for $CD56^+CD3^-CD11a^-CD117^+CDIL1R1^+$ cells. In certain embodiments, the ILC3 cells are enriched for $CD56^+CD3^-CD11a^-CD117^+CDIL1R1^+$ cells in comparison with total cells produced using the three-stage method, described herein.

[00333] In one embodiment, NK cells are isolated or enriched by selecting for $CD56^+CD3^-CD94^+CD11a^+$ cells. In certain embodiments, the NK cells are enriched for $CD56^+CD3^-CD94^+CD11a^+$ cells in comparison with total cells produced using the three-stage method, described herein. In one embodiment, NK cells are isolated or enriched by selecting for $CD56^+CD3^-CD94^+CD11a^+CD117^-$ cells. In certain embodiments, the NK cells are enriched for $CD56^+CD3^-CD94^+CD11a^+CD117^-$ cells in comparison with total cells produced using the three-stage method, described herein.

[00334] Cell separation can be accomplished by, *e.g.*, flow cytometry, fluorescence-activated cell sorting (FACS), or, in one embodiment, magnetic cell sorting using microbeads conjugated with specific antibodies. The cells may be isolated, *e.g.*, using a magnetic activated cell sorting (MACS) technique, a method for separating particles based on their ability to bind magnetic beads (*e.g.*, about 0.5-100 μm diameter) that comprise one or more specific antibodies, *e.g.*, anti-CD56 antibodies. Magnetic cell separation can be performed and automated using, *e.g.*,

an AUTOMACS™ Separator (Miltenyi). A variety of useful modifications can be performed on the magnetic microspheres, including covalent addition of antibody that specifically recognizes a particular cell surface molecule or hapten. The beads are then mixed with the cells to allow binding. Cells are then passed through a magnetic field to separate out cells having the specific cell surface marker. In one embodiment, these cells can then isolated and re-mixed with magnetic beads coupled to an antibody against additional cell surface markers. The cells are again passed through a magnetic field, isolating cells that bound both the antibodies. Such cells can then be diluted into separate dishes, such as microtiter dishes for clonal isolation.

5.5. Placental Perfusate

[00335] NK cells and/or ILC3 cells, *e.g.*, NK cell and/or ILC3 cell populations produced according to the three-stage method described herein may be produced from hematopoietic cells, *e.g.*, hematopoietic stem or progenitors from any source, *e.g.*, placental tissue, placental perfusate, umbilical cord blood, placental blood, peripheral blood, spleen, liver, or the like. In certain embodiments, the hematopoietic stem cells are combined hematopoietic stem cells from placental perfusate and from cord blood from the same placenta used to generate the placental perfusate. Placental perfusate comprising placental perfusate cells that can be obtained, for example, by the methods disclosed in U.S. Patent Nos. 7,045,148 and 7,468,276 and U.S. Patent Application Publication No. 2009/0104164, the disclosures of which are hereby incorporated in their entireties.

5.5.1. Cell Collection Composition

[00336] The placental perfusate and perfusate cells, from which hematopoietic stem or progenitors may be isolated, or useful in tumor suppression or the treatment of an individual having tumor cells, cancer or a viral infection, *e.g.*, in combination with the NK cells and/or ILC3 cells, *e.g.*, NK cell and/or ILC3 cell populations produced according to the three-stage method provided herein, can be collected by perfusion of a mammalian, *e.g.*, human post-partum placenta using a placental cell collection composition. Perfusate can be collected from the placenta by perfusion of the placenta with any physiologically-acceptable solution, *e.g.*, a saline solution, culture medium, or a more complex cell collection composition. A cell collection composition suitable for perfusing a placenta, and for the collection and preservation of perfusate

cells is described in detail in related U.S. Application Publication No. 2007/0190042, which is incorporated herein by reference in its entirety.

[00337] The cell collection composition can comprise any physiologically-acceptable solution suitable for the collection and/or culture of stem cells, for example, a saline solution (*e.g.*, phosphate-buffered saline, Kreb's solution, modified Kreb's solution, Eagle's solution, 0.9% NaCl. *etc.*), a culture medium (*e.g.*, DMEM, H.DMEM, *etc.*), and the like.

[00338] The cell collection composition can comprise one or more components that tend to preserve placental cells, that is, prevent the placental cells from dying, or delay the death of the placental cells, reduce the number of placental cells in a population of cells that die, or the like, from the time of collection to the time of culturing. Such components can be, *e.g.*, an apoptosis inhibitor (*e.g.*, a caspase inhibitor or JNK inhibitor); a vasodilator (*e.g.*, magnesium sulfate, an antihypertensive drug, atrial natriuretic peptide (ANP), adrenocorticotropin, corticotropin-releasing hormone, sodium nitroprusside, hydralazine, adenosine triphosphate, adenosine, indomethacin or magnesium sulfate, a phosphodiesterase inhibitor, *etc.*); a necrosis inhibitor (*e.g.*, 2-(1H-Indol-3-yl)-3-pentylamino-maleimide, pyrrolidine dithiocarbamate, or clonazepam); a TNF-α inhibitor; and/or an oxygen-carrying perfluorocarbon (*e.g.*, perfluorooctyl bromide, perfluorodecyl bromide, *etc.*).

[00339] The cell collection composition can comprise one or more tissue-degrading enzymes, *e.g.*, a metalloprotease, a serine protease, a neutral protease, a hyaluronidase, an RNase, or a DNase, or the like. Such enzymes include, but are not limited to, collagenases (*e.g.*, collagenase I, II, III or IV, a collagenase from *Clostridium histolyticum*, *etc.*); dispase, thermolysin, elastase, trypsin, LIBERASE, hyaluronidase, and the like.

[00340] The cell collection composition can comprise a bacteriocidally or bacteriostatically effective amount of an antibiotic. In certain non-limiting embodiments, the antibiotic is a macrolide (*e.g.*, tobramycin), a cephalosporin (*e.g.*, cephalexin, cephradine, cefuroxime, cefprozil, cefaclor, cefixime or cefadroxil), a clarithromycin, an erythromycin, a penicillin (*e.g.*, penicillin V) or a quinolone (*e.g.*, ofloxacin, ciprofloxacin or norfloxacin), a tetracycline, a streptomycin, *etc.* In a particular embodiment, the antibiotic is active against Gram(+) and/or Gram(–) bacteria, *e.g.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and the like.

[00341] The cell collection composition can also comprise one or more of the following compounds: adenosine (about 1 mM to about 50 mM); D-glucose (about 20 mM to about 100 mM); magnesium ions (about 1 mM to about 50 mM); a macromolecule of molecular weight greater than 20,000 daltons, in one embodiment, present in an amount sufficient to maintain endothelial integrity and cellular viability (*e.g.*, a synthetic or naturally occurring colloid, a polysaccharide such as dextran or a polyethylene glycol present at about 25 g/l to about 100 g/l, or about 40 g/l to about 60 g/l); an antioxidant (*e.g.*, butylated hydroxyanisole, butylated hydroxytoluene, glutathione, vitamin C or vitamin E present at about 25 μM to about 100 μM); a reducing agent (*e.g.*, N-acetylcysteine present at about 0.1 mM to about 5 mM); an agent that prevents calcium entry into cells (*e.g.*, verapamil present at about 2 μM to about 25 μM); nitroglycerin (*e.g.*, about 0.05 g/L to about 0.2 g/L); an anticoagulant, in one embodiment, present in an amount sufficient to help prevent clotting of residual blood (*e.g.*, heparin or hirudin present at a concentration of about 1000 units/l to about 100,000 units/l); or an amiloride containing compound (*e.g.*, amiloride, ethyl isopropyl amiloride, hexamethylene amiloride, dimethyl amiloride or isobutyl amiloride present at about 1.0 μM to about 5 μM).

5.5.2. Collection and Handling of Placenta

[00342] Generally, a human placenta is recovered shortly after its expulsion after birth. In one embodiment, the placenta is recovered from a patient after informed consent and after a complete medical history of the patient is taken and is associated with the placenta. In one embodiment, the medical history continues after delivery.

[00343] Prior to recovery of perfusate, the umbilical cord blood and placental blood are removed. In certain embodiments, after delivery, the cord blood in the placenta is recovered. The placenta can be subjected to a conventional cord blood recovery process. Typically a needle or cannula is used, with the aid of gravity, to exsanguinate the placenta (*see*, *e.g.*, Anderson, U.S. Patent No. 5,372,581; Hessel *et al.*, U.S. Patent No. 5,415,665). The needle or cannula is usually placed in the umbilical vein and the placenta can be gently massaged to aid in draining cord blood from the placenta. Such cord blood recovery may be performed commercially, *e.g.*, LifeBank Inc., Cedar Knolls, N.J., ViaCord, Cord Blood Registry and CryoCell. In one embodiment, the placenta is gravity drained without further manipulation so as to minimize tissue disruption during cord blood recovery.

[00344] Typically, a placenta is transported from the delivery or birthing room to another location, *e.g.*, a laboratory, for recovery of cord blood and collection of perfusate. The placenta can be transported in a sterile, thermally insulated transport device (maintaining the temperature of the placenta between 20-28 °C), for example, by placing the placenta, with clamped proximal umbilical cord, in a sterile zip-lock plastic bag, which is then placed in an insulated container. In another embodiment, the placenta is transported in a cord blood collection kit substantially as described in U.S. Patent No. 7,147,626. In one embodiment, the placenta is delivered to the laboratory four to twenty-four hours following delivery. In certain embodiments, the proximal umbilical cord is clamped, for example within 4-5 cm (centimeter) of the insertion into the placental disc prior to cord blood recovery. In other embodiments, the proximal umbilical cord is clamped after cord blood recovery but prior to further processing of the placenta.

[00345] The placenta, prior to collection of the perfusate, can be stored under sterile conditions and at either room temperature or at a temperature of 5 to 25 °C (centigrade). The placenta may be stored for a period of longer than forty eight hours, or for a period of four to twenty-four hours prior to perfusing the placenta to remove any residual cord blood. The placenta can be stored in an anticoagulant solution at a temperature of 5 °C to 25 °C (centigrade). Suitable anticoagulant solutions are well known in the art. For example, a solution of heparin or warfarin sodium can be used. In one embodiment, the anticoagulant solution comprises a solution of heparin (*e.g.*, 1% w/w in 1:1000 solution). In some embodiments, the exsanguinated placenta is stored for no more than 36 hours before placental perfusate is collected.

5.5.3. Placental Perfusion

[00346] Methods of perfusing mammalian placentae and obtaining placental perfusate are disclosed, *e.g.*, in Hariri, U.S. Patent Nos. 7,045,148 and 7,255,879, and in U.S. Application Publication Nos. 2009/0104164, 2007/0190042 and 20070275362, issued as U.S. Pat No. 8,057,788, the disclosures of which are hereby incorporated by reference herein in their entireties.

[00347] Perfusate can be obtained by passage of perfusion solution, *e.g.*, saline solution, culture medium or cell collection compositions described above, through the placental vasculature. In one embodiment, a mammalian placenta is perfused by passage of perfusion solution through either or both of the umbilical artery and umbilical vein. The flow of perfusion solution through the placenta may be accomplished using, *e.g.*, gravity flow into the placenta. For example, the perfusion solution is forced through the placenta using a pump, *e.g.*, a

peristaltic pump. The umbilical vein can be, *e.g.*, cannulated with a cannula, *e.g.*, a TEFLON® or plastic cannula, that is connected to a sterile connection apparatus, such as sterile tubing. The sterile connection apparatus is connected to a perfusion manifold.

[00348] In preparation for perfusion, the placenta can be oriented in such a manner that the umbilical artery and umbilical vein are located at the highest point of the placenta. The placenta can be perfused by passage of a perfusion solution through the placental vasculature, or through the placental vasculature and surrounding tissue. In one embodiment, the umbilical artery and the umbilical vein are connected simultaneously to a pipette that is connected via a flexible connector to a reservoir of the perfusion solution. The perfusion solution is passed into the umbilical vein and artery. The perfusion solution exudes from and/or passes through the walls of the blood vessels into the surrounding tissues of the placenta, and is collected in a suitable open vessel from the surface of the placenta that was attached to the uterus of the mother during gestation. The perfusion solution may also be introduced through the umbilical cord opening and allowed to flow or percolate out of openings in the wall of the placenta which interfaced with the maternal uterine wall. In another embodiment, the perfusion solution is passed through the umbilical veins and collected from the umbilical artery, or is passed through the umbilical artery and collected from the umbilical veins, that is, is passed through only the placental vasculature (fetal tissue).

[00349] In one embodiment, for example, the umbilical artery and the umbilical vein are connected simultaneously, *e.g.*, to a pipette that is connected via a flexible connector to a reservoir of the perfusion solution. The perfusion solution is passed into the umbilical vein and artery. The perfusion solution exudes from and/or passes through the walls of the blood vessels into the surrounding tissues of the placenta, and is collected in a suitable open vessel from the surface of the placenta that was attached to the uterus of the mother during gestation. The perfusion solution may also be introduced through the umbilical cord opening and allowed to flow or percolate out of openings in the wall of the placenta which interfaced with the maternal uterine wall. Placental cells that are collected by this method, which can be referred to as a "pan" method, are typically a mixture of fetal and maternal cells.

[00350] In another embodiment, the perfusion solution is passed through the umbilical veins and collected from the umbilical artery, or is passed through the umbilical artery and

collected from the umbilical veins. Placental cells collected by this method, which can be referred to as a "closed circuit" method, are typically almost exclusively fetal.

[00351] The closed circuit perfusion method can, in one embodiment, be performed as follows. A post-partum placenta is obtained within about 48 hours after birth. The umbilical cord is clamped and cut above the clamp. The umbilical cord can be discarded, or can processed to recover, *e.g.*, umbilical cord stem cells, and/or to process the umbilical cord membrane for the production of a biomaterial. The amniotic membrane can be retained during perfusion, or can be separated from the chorion, *e.g.*, using blunt dissection with the fingers. If the amniotic membrane is separated from the chorion prior to perfusion, it can be, *e.g.*, discarded, or processed, *e.g.*, to obtain stem cells by enzymatic digestion, or to produce, *e.g.*, an amniotic membrane biomaterial, *e.g.*, the biomaterial described in U.S. Application Publication No. 2004/0048796. After cleaning the placenta of all visible blood clots and residual blood, *e.g.*, using sterile gauze, the umbilical cord vessels are exposed, *e.g.*, by partially cutting the umbilical cord membrane to expose a cross-section of the cord. The vessels are identified, and opened, *e.g.*, by advancing a closed alligator clamp through the cut end of each vessel. The apparatus, *e.g.*, plastic tubing connected to a perfusion device or peristaltic pump, is then inserted into each of the placental arteries. The pump can be any pump suitable for the purpose, *e.g.*, a peristaltic pump. Plastic tubing, connected to a sterile collection reservoir, *e.g.*, a blood bag such as a 250 mL collection bag, is then inserted into the placental vein. Alternatively, the tubing connected to the pump is inserted into the placental vein, and tubes to a collection reservoir(s) are inserted into one or both of the placental arteries. The placenta is then perfused with a volume of perfusion solution, *e.g.*, about 750 ml of perfusion solution. Cells in the perfusate are then collected, *e.g.*, by centrifugation.

[00352] In one embodiment, the proximal umbilical cord is clamped during perfusion, and, more specifically, can be clamped within 4-5 cm (centimeter) of the cord's insertion into the placental disc.

[00353] The first collection of perfusion fluid from a mammalian placenta during the exsanguination process is generally colored with residual red blood cells of the cord blood and/or placental blood. The perfusion fluid becomes more colorless as perfusion proceeds and the residual cord blood cells are washed out of the placenta. Generally from 30 to 100 mL of

perfusion fluid is adequate to initially flush blood from the placenta, but more or less perfusion fluid may be used depending on the observed results.

[00354] In certain embodiments, cord blood is removed from the placenta prior to perfusion (*e.g.*, by gravity drainage), but the placenta is not flushed (*e.g.*, perfused) with solution to remove residual blood. In certain embodiments, cord blood is removed from the placenta prior to perfusion (*e.g.*, by gravity drainage), and the placenta is flushed (*e.g.*, perfused) with solution to remove residual blood.

[00355] The volume of perfusion liquid used to perfuse the placenta may vary depending upon the number of placental cells to be collected, the size of the placenta, the number of collections to be made from a single placenta, *etc*. In various embodiments, the volume of perfusion liquid may be from 50 mL to 5000 mL, 50 mL to 4000 mL, 50 mL to 3000 mL, 100 mL to 2000 mL, 250 mL to 2000 mL, 500 mL to 2000 mL, or 750 mL to 2000 mL. Typically, the placenta is perfused with 700-800 mL of perfusion liquid following exsanguination.

[00356] The placenta can be perfused a plurality of times over the course of several hours or several days. Where the placenta is to be perfused a plurality of times, it may be maintained or cultured under aseptic conditions in a container or other suitable vessel, and perfused with a cell collection composition, or a standard perfusion solution (*e.g.*, a normal saline solution such as phosphate buffered saline ("PBS") with or without an anticoagulant (*e.g.*, heparin, warfarin sodium, coumarin, bishydroxycoumarin), and/or with or without an antimicrobial agent (*e.g.*, β-mercaptoethanol (0.1 mM); antibiotics such as streptomycin (*e.g.*, at 40-100 μg/ml), penicillin (*e.g.*, at 40 U/ml), amphotericin B (*e.g.*, at 0.5 μg/ml). In one embodiment, an isolated placenta is maintained or cultured for a period of time without collecting the perfusate, such that the placenta is maintained or cultured for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours, or 2 or 3 or more days before perfusion and collection of perfusate. The perfused placenta can be maintained for one or more additional time(s), *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours, and perfused a second time with, *e.g.*, 700-800 mL perfusion fluid. The placenta can be perfused 1, 2, 3, 4, 5 or more times, for example, once every 1, 2, 3, 4, 5 or 6 hours. In one embodiment, perfusion of the placenta and collection of perfusion solution, *e.g.*, placental cell collection composition, is repeated until the number of recovered nucleated cells falls below 100 cells/ml. The perfusates at different time points can be further processed individually to recover time-dependent

populations of cells, *e.g.*, total nucleated cells. Perfusates from different time points can also be pooled.

5.5.4. Placental Perfusate and Placental Perfusate Cells

[00357] Typically, placental perfusate from a single placental perfusion comprises about 100 million to about 500 million nucleated cells, including hematopoietic cells from which NK cells and/or ILC3 cells, *e.g.*, NK cells and/or ILC3 cells produced according to the three-stage method described herein, may be produced by the method disclosed herein. In certain embodiments, the placental perfusate or perfusate cells comprise $CD34^+$ cells, *e.g.*, hematopoietic stem or progenitor cells. Such cells can, in a more specific embodiment, comprise $CD34^+CD45^-$ stem or progenitor cells, $CD34^+CD45^+$ stem or progenitor cells, or the like. In certain embodiments, the perfusate or perfusate cells are cryopreserved prior to isolation of hematopoietic cells therefrom. In certain other embodiments, the placental perfusate comprises, or the perfusate cells comprise, only fetal cells, or a combination of fetal cells and maternal cells.

5.6. NK Cells

5.6.1. NK Cells Produced by Three-Stage Method

[00358] In another embodiment, provided herein is an isolated NK cell population, wherein said NK cells are produced according to the three-stage method described above.

[00359] In one embodiment, provided herein is an isolated NK cell population produced by a three-stage method described herein, wherein said NK cell population comprises a greater percentage of CD3–CD56+ cells than an NK progenitor cell population produced by a three-stage method described herein, *e.g.*, an NK progenitor cell population produced by the same three-stage method with the exception that the third culture step used to produce the NK progenitor cell population was of shorter duration than the third culture step used to produce the NK cell population. In a specific embodiment, said NK cell population comprises about 70% or more, in some embodiments, 75%, 80%, 85%, 90%, 95%, 98%, or 99% CD3–CD56+ cells. In another specific embodiment, said NK cell population comprises no less than 80%, 85%, 90%, 95%, 98%, or 99% CD3–CD56+ cells. In another specific embodiment, said NK cell population comprises between 70%-75%, 75%-80%, 80%-85%, 85%-90%, 90%-95%, or 95%-99% CD3–CD56+ cells.

[00360] In certain embodiments, said $CD3^-CD56^+$ cells in said NK cell population comprises $CD3^-CD56^+$ cells that are additionally $NKp46^+$. In certain embodiments, said $CD3^-CD56^+$ cells in said NK cell population comprises $CD3^-CD56^+$ cells that are additionally CD16-. In certain embodiments, said $CD3^-CD56^+$ cells in said NK cell population comprises $CD3^-CD56^+$ cells that are additionally CD16+. In certain embodiments, said $CD3^-CD56^+$ cells in said NK cell population comprises $CD3^-CD56^+$ cells that are additionally CD94-. In certain embodiments, said $CD3^-CD56^+$ cells in said NK cell population comprises $CD3^-CD56^+$ cells that are additionally CD94+. In certain embodiments, said $CD3^-CD56^+$ cells in said NK cell population comprises $CD3^-CD56^+$ cells that are additionally $CD11a^+$. In certain embodiments, said $CD3^-CD56^+$ cells in said NK cell population comprises $CD3^-CD56^+$ cells that are additionally $NKp30^+$. In certain embodiments, said $CD3^-CD56^+$ cells in said NK cell population comprises $CD3^-CD56^+$ cells that are additionally $CD161^+$. In certain embodiments, said $CD3^-CD56^+$ cells in said NK cell population comprises $CD3^-CD56^+$ cells that are additionally DNAM-1^+. In certain embodiments, said $CD3^-CD56^+$ cells in said NK cell population comprises $CD3^-CD56^+$ cells that are additionally T-bet^+.

[00361] In one embodiment, an NK cell population produced by a three-stage method described herein comprises cells which are CD117+. In one embodiment, an NK cell population produced by a three-stage method described herein comprises cells which are NKG2D+. In one embodiment, an NK cell population produced by a three-stage method described herein comprises cells which are NKp44+. In one embodiment, an NK cell population produced by a three-stage method described herein comprises cells which are CD244+. In one embodiment, an NK cell population produced by a three-stage method described herein comprises cells which express perforin. In one embodiment, an NK cell population produced by a three-stage method described herein comprises cells which express EOMES. In one embodiment, an NK cell population produced by a three-stage method described herein comprises cells which express granzyme B. In one embodiment, an NK cell population produced by a three-stage method described herein comprises cells which secrete IFNγ, GM-CSF and/or TNFα.

5.7. ILC3 Cells

5.7.1. ILC3 Cells Produced by Three-Stage Method

[00362] In another embodiment, provided herein is an isolated ILC3 cell population, wherein said ILC3 cells are produced according to the three-stage method described above.

[00363] In one embodiment, provided herein is an isolated ILC3 cell population produced by a three-stage method described herein, wherein said ILC3 cell population comprises a greater percentage of CD3–CD56+ cells than an ILC3 progenitor cell population produced by a three-stage method described herein, *e.g.*, an ILC3 progenitor cell population produced by the same three-stage method with the exception that the third culture step used to produce the ILC3 progenitor cell population was of shorter duration than the third culture step used to produce the ILC3 cell population. In a specific embodiment, said ILC3 cell population comprises about 70% or more, in some embodiments, 75%, 80%, 85%, 90%, 95%, 98%, or 99% CD3–CD56+ cells. In another specific embodiment, said ILC3 cell population comprises no less than 80%, 85%, 90%, 95%, 98%, or 99% CD3–CD56+ cells. In another specific embodiment, said ILC3 cell population comprises between 70%-75%, 75%-80%, 80%-85%, 85%-90%, 90%-95%, or 95%-99% CD3–CD56+ cells.

[00364] In certain embodiments, said $CD3^-CD56^+$ cells in said ILC3 cell population comprises $CD3^-CD56^+$ cells that are additionally $NKp46^-$. In certain embodiments, said $CD3^-CD56^+$ cells in said ILC3 cell population comprises $CD3^-CD56^+$ cells that are additionally CD16-. In certain embodiments, said $CD3^-CD56^+$ cells in said ILC3 cell population comprises $CD3^-CD56^+$ cells that are additionally IL1R1+. In certain embodiments, said $CD3^-CD56^+$ cells in said ILC3 cell population comprises $CD3^-CD56^+$ cells that are additionally CD94-. In certain embodiments, said $CD3^-CD56^+$ cells in said ILC3 cell population comprises $CD3^-CD56^+$ cells that are additionally RORγt+. In certain embodiments, said $CD3^-CD56^+$ cells in said ILC3 cell population comprises $CD3^-CD56^+$ cells that are additionally $CD11a^-$. In certain embodiments, said $CD3^-CD56^+$ cells in said ILC3 cell population comprises $CD3^-CD56^+$ cells that are additionally T-bet+.

[00365] In one embodiment, an ILC3 cell population produced by a three-stage method described herein comprises cells which are CD117+. In one embodiment, an ILC3 cell population produced by a three-stage method described herein comprises cells which are $NKG2D^-$. In one embodiment, an ILC3 cell population produced by a three-stage method described herein comprises cells which are $NKp30^-$. In one embodiment, an ILC3 cell population produced by a three-stage method described herein comprises cells which are CD244+. In one embodiment, an ILC3 cell population produced by a three-stage method described herein comprises cells which are DNAM-1+. In one embodiment, an ILC3 cell

population produced by a three-stage method described herein comprises cells which express AHR. In one embodiment, an ILC3 cell population produced by a three-stage method described herein comprises cells which do not express perforin. In one embodiment, an ILC3 cell population produced by a three-stage method described herein comprises cells which do not express EOMES. In one embodiment, an ILC3 cell population produced by a three-stage method described herein comprises cells which do not express granzyme B. In one embodiment, an ILC3 cell population produced by a three-stage method described herein comprises cells which secrete IL-22 and/or IL-8.

[00366] In certain aspects, cell populations produced by the three-stage method described herein comprise CD11a+ cells and CD11a– cells in a ratio of 50:1, 40:1, 30:1, 20:1, 10:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:10, 1:20, 1:30, 1:40, or 1:50. In certain aspects, a population of cells described herein comprises CD11a+ cells and CD11a– cells in a ratio of 50:1. In certain aspects, a population of cells described herein comprises CD11a+ cells and CD11a– cells in a ratio of 20:1. In certain aspects, a population of cells described herein comprises CD11a+ cells and CD11a– cells in a ratio of 10:1. In certain aspects, a population of cells described herein comprises CD11a+ cells and CD11a– cells in a ratio of 5:1. In certain aspects, a population of cells described herein comprises CD11a+ cells and CD11a– cells in a ratio of 1:1. In certain aspects, a population of cells described herein comprises CD11a+ cells and CD11a– cells in a ratio of 1:5. In certain aspects, a population of cells described herein comprises CD11a+ cells and CD11a– cells in a ratio of 1:10. In certain aspects, a population of cells described herein comprises CD11a+ cells and CD11a– cells in a ratio of 1:20. In certain aspects, a population of cells described herein comprises CD11a+ cells and CD11a– cells in a ratio of 1:50.

[00367] In certain aspects, cell populations described herein are produced by combining the CD11a+ cells with the CD11a– cells in a ratio of 50:1, 40:1, 30:1, 20:1, 10:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:10, 1:20, 1:30, 1:40, or 1:50 to produce a combined population of cells. In certain aspects, a combined population of cells described herein comprises CD11a+ cells and CD11a– cells combined in a ratio of 50:1. In certain aspects, a combined population of cells described herein comprises CD11a+ cells and CD11a– cells combined in a ratio of 20:1. In certain aspects, a combined population of cells described herein comprises CD11a+ cells and CD11a– cells combined in a ratio of 10:1. In certain aspects, a combined population of cells

described herein comprises CD11a+ cells and CD11a– cells combined in a ratio of 5:1. In certain aspects, a combined population of cells described herein comprises CD11a+ cells and CD11a– cells combined in a ratio of 1:1. In certain aspects, a combined population of cells described herein comprises CD11a+ cells and CD11a– cells combined in a ratio of 1:5. In certain aspects, a combined population of cells described herein comprises CD11a+ cells and CD11a– cells combined in a ratio of 1:10. In certain aspects, a combined population of cells described herein comprises CD11a+ cells and CD11a– cells combined in a ratio of 1:20. In certain aspects, a combined population of cells described herein comprises CD11a+ cells and CD11a– cells combined in a ratio of 1:50.

[00368] In certain aspects, cell populations produced by the three-stage method described herein comprise NK cells and ILC3 cells in a ratio of 50:1, 40:1, 30:1, 20:1, 10:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:10, 1:20, 1:30, 1:40, or 1:50. In certain aspects, a population of cells described herein comprises NK cells and ILC3 cells in a ratio of 50:1. In certain aspects, a population of cells described herein comprises NK cells and ILC3 cells in a ratio of 20:1. In certain aspects, a population of cells described herein comprises NK cells and ILC3 cells in a ratio of 10:1. In certain aspects, a population of cells described herein comprises NK cells and ILC3 cells in a ratio of 5:1. In certain aspects, a population of cells described herein comprises NK cells and ILC3 cells in a ratio of 1:1. In certain aspects, a population of cells described herein comprises NK cells and ILC3 cells in a ratio of 1:5. In certain aspects, a population of cells described herein comprises NK cells and ILC3 cells in a ratio of 1:10. In certain aspects, a population of cells described herein comprises NK cells and ILC3 cells in a ratio of 1:20. In certain aspects, a population of cells described herein comprises NK cells and ILC3 cells in a ratio of 1:50.

[00369] In certain aspects, cell populations described herein are produced by combining the NK cells with the ILC3 cells in a ratio of 50:1, 40:1, 30:1, 20:1, 10:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:10, 1:20, 1:30, 1:40, or 1:50 to produce a combined population of cells. In certain aspects, a combined population of cells described herein comprises NK cells and ILC3 cells combined in a ratio of 50:1. In certain aspects, a combined population of cells described herein comprises NK cells and ILC3 cells combined in a ratio of 20:1. In certain aspects, a combined population of cells described herein comprises NK cells and ILC3 cells combined in a ratio of 10:1. In certain aspects, a combined population of cells described herein comprises NK

cells and ILC3 cells combined in a ratio of 5:1. In certain aspects, a combined population of cells described herein comprises NK cells and ILC3 cells combined in a ratio of 1:1. In certain aspects, a combined population of cells described herein comprises NK cells and ILC3 cells combined in a ratio of 1:5. In certain aspects, a combined population of cells described herein comprises NK cells and ILC3 cells combined in a ratio of 1:10. In certain aspects, a combined population of cells described herein comprises NK cells and ILC3 cells combined in a ratio of 1:20. In certain aspects, a combined population of cells described herein comprises NK cells and ILC3 cells combined in a ratio of 1:50.

5.8. NK Cells and/or ILC3 Cells In Combination With Placental Perfusate

[00370] Further provided herein are compositions comprising NK cells and/or ILC3 cells according to the three-stage method described herein, in combination with placental perfusate, placental perfusate cells and/or adherent placental cells, *e.g.*, for use in suppressing the proliferation of a tumor cell or plurality of tumor cells.

5.8.1. Combinations of NK Cells and/or ILC3 Cells and Perfusate or Perfusate Cells

[00371] Further provided herein are compositions comprising combinations of NK cell and/or ILC3 cell populations produced according to the three-stage method described herein, and placental perfusate and/or placental perfusate cells. In one embodiment, for example, provided herein is a volume of placental perfusate supplemented with NK cells and/or ILC3 cells produced using the methods described herein. In specific embodiments of a volume of placental perfusate supplemented with NK cells and ILC3 cells, the NK cells and ILC3 cells are present in ratios as described herein. In specific embodiments, for example, each milliliter of placental perfusate is supplemented with about 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8 or more NK cells and/or ILC3 cells produced using the methods described herein. In another embodiment, placental perfusate cells are supplemented with NK cells and/or ILC3 cells produced using the methods described herein. In certain other embodiments, when placental perfusate cells are combined with NK cells and/or ILC3 cells produced using the methods described herein, the placental perfusate cells generally comprise about, greater than about, or fewer than about, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 8%, 6%, 4%, 2% or 1% of the total number of cells. In certain other embodiments, when NK cells and/or ILC3

cells produced using the methods described herein are combined with a plurality of placental perfusate cells and/or combined natural killer cells, the NK cells and/or ILC3 cells or NK cell populations generally comprise about, greater than about, or fewer than about, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 8%, 6%, 4%, 2% or 1% of the total number of cells. In certain other embodiments, when NK cells and/or ILC3 cells produced using the methods described herein are used to supplement placental perfusate, the volume of solution (*e.g.*, saline solution, culture medium or the like) in which the cells are suspended comprises about, greater than about, or less than about, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 8%, 6%, 4%, 2% or 1% of the total volume of perfusate plus cells, where the NK cells and/or ILC3 cells are suspended to about 1 x 10^4, 5 x 10^4, 1 x 10^5, 5 x 10^5, 1 x 10^6, 5 x 10^6, 1 x 10^7, 5 x 10^7, 1 x 10^8, 5 x 10^8 or more cells per milliliter prior to supplementation.

[00372] In other embodiments, any of the above combinations of cells is, in turn, combined with umbilical cord blood or nucleated cells from umbilical cord blood.

[00373] Further provided herein is pooled placental perfusate that is obtained from two or more sources, *e.g.*, two or more placentas, and combined, *e.g.*, pooled. Such pooled perfusate can comprise approximately equal volumes of perfusate from each source, or can comprise different volumes from each source. The relative volumes from each source can be randomly selected, or can be based upon, *e.g.*, a concentration or amount of one or more cellular factors, *e.g.*, cytokines, growth factors, hormones, or the like; the number of placental cells in perfusate from each source; or other characteristics of the perfusate from each source. Perfusate from multiple perfusions of the same placenta can similarly be pooled.

[00374] Similarly, provided herein are placental perfusate cells, and placenta-derived intermediate natural killer cells, that are obtained from two or more sources, *e.g.*, two or more placentas, and pooled. Such pooled cells can comprise approximately equal numbers of cells from the two or more sources, or different numbers of cells from one or more of the pooled sources. The relative numbers of cells from each source can be selected based on, *e.g.*, the number of one or more specific cell types in the cells to be pooled, *e.g.*, the number of $CD34^+$ cells, *etc.*

[00375] Further provided herein are NK cells and/or ILC3 cells produced using the methods described herein, and combinations of such cells with placental perfusate and/or placental perfusate cells, that have been assayed to determine the degree or amount of tumor

suppression (that is, the potency) to be expected from, *e.g.*, a given number of NK cells and/or ILC3 cells or NK cell and/or ILC3 cell populations or a given volume of perfusate. For example, an aliquot or sample number of cells is contacted or brought into proximity with a known number of tumor cells under conditions in which the tumor cells would otherwise proliferate, and the rate of proliferation of the tumor cells in the presence of placental perfusate, perfusate cells, placental natural killer cells, or combinations thereof, over time (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks, or longer) is compared to the proliferation of an equivalent number of the tumor cells in the absence of perfusate, perfusate cells, placental natural killer cells, or combinations thereof. The potency of the cells can be expressed, *e.g.*, as the number of cells or volume of solution required to suppress tumor cell growth, *e.g.*, by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or the like.

[00376] In certain embodiments, NK cells and/or ILC3 cells produced using the methods described herein, are provided as pharmaceutical grade administrable units. Such units can be provided in discrete volumes, *e.g.*, 15 mL, 20 mL, 25 mL, 30 nL. 35 mL, 40 mL, 45 mL, 50 mL, 55 mL, 60 mL, 65 mL, 70 mL, 75 mL, 80 mL, 85 mL, 90 mL, 95 mL, 100 mL, 150 mL, 200 mL, 250 mL, 300 mL, 350 mL, 400 mL, 450 mL, 500 mL, or the like. Such units can be provided so as to contain a specified number of cells, *e.g.*, NK cells and/or ILC3 cells or NK cell and/or ILC3 cell populations in combination with other NK cells and/or ILC3 cells or perfusate cells, *e.g.*, 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8 or more cells per milliliter, or 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 5×10^{10}, 1×10^{11} or more cells per unit. In specific embodiments, the units can comprise about, at least about, or at most about 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6 or more NK cells per milliliter, or 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 5×10^{10}, 1×10^{11} or more cells per unit. Such units can be provided to contain specified numbers of NK cells and/or ILC3 cells or NK cell and/or ILC3 cell populations and/or any of the other cells.

[00377] In the above embodiments, the NK cells and/or ILC3 cells or NK cell and/or ILC3 cell populations or combinations of NK cells and/or ILC3 cells or NK cell and/or ILC3 cell populations with other NK cells and/or ILC3 cells, perfusate cells or perfusate can be autologous to a recipient (that is, obtained from the recipient), or allogeneic to a recipient (that is, obtained from at last one other individual from said recipient).

[00378] In certain embodiments, each unit of cells is labeled to specify one or more of volume, number of cells, type of cells, whether the unit has been enriched for a particular type of cell, and/or potency of a given number of cells in the unit, or a given number of milliliters of the unit, that is, whether the cells in the unit cause a measurable suppression of proliferation of a particular type or types of tumor cell.

5.8.2. Combinations of NK Cells and/or ILC3 Cells With Adherent Placental Stem Cells

[00379] In other embodiments, the NK cells and/or ILC3 cells produced using the methods described herein, *e.g.*, NK cell and/or ILC3 cell populations produced using the three-stage method described herein, either alone or in combination with placental perfusate or placental perfusate cells, are supplemented with isolated adherent placental cells, *e.g.*, placental stem cells and placental multipotent cells as described, *e.g.*, in Hariri U.S. Patent Nos. 7,045,148 and 7,255,879, and in U.S. Patent Application Publication No. 2007/0275362, the disclosures of which are incorporated herein by reference in their entireties. In specific embodiments, NK cells and ILC3 cells, the NK cells and ILC3 cells are present in ratios as described herein. "Adherent placental cells" means that the cells are adherent to a tissue culture surface, *e.g.*, tissue culture plastic. The adherent placental cells useful in the compositions and methods disclosed herein are generally not trophoblasts, embryonic germ cells or embryonic stem cells.

[00380] The NK cells and/or ILC3 cells produced using the methods described herein, *e.g.*, NK cell and/or ILC3 cell populations, either alone or in combination with placental perfusate or placental perfusate cells can be supplemented with, *e.g.*, 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8 or more adherent placental cells per milliliter, or 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 5×10^{10}, 1×10^{11} or more adherent placental cells. The adherent placental cells in the combinations can be, *e.g.*, adherent placental cells that have been cultured for, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, or 40 population doublings, or more.

[00381] Isolated adherent placental cells, when cultured in primary cultures or expanded in cell culture, adhere to the tissue culture substrate, *e.g.*, tissue culture container surface (*e.g.*, tissue culture plastic). Adherent placental cells in culture assume a generally fibroblastoid, stellate appearance, with a number of cytoplasmic processes extending from the central cell body.

Adherent placental cells are, however, morphologically distinguishable from fibroblasts cultured under the same conditions, as the adherent placental cells exhibit a greater number of such processes than do fibroblasts. Morphologically, adherent placental cells are also distinguishable from hematopoietic stem cells, which generally assume a more rounded, or cobblestone, morphology in culture.

[00382] The isolated adherent placental cells, and populations of adherent placental cells, useful in the compositions and methods provided herein, express a plurality of markers that can be used to identify and/or isolate the cells, or populations of cells that comprise the adherent placental cells. The adherent placental cells, and adherent placental cell populations useful in the compositions and methods provided herein include adherent placental cells and adherent placental cell-containing cell populations obtained directly from the placenta, or any part thereof (*e.g.*, amnion, chorion, amnion-chorion plate, placental cotyledons, umbilical cord, and the like). The adherent placental stem cell population, in one embodiment, is a population (that is, two or more) of adherent placental stem cells in culture, *e.g.*, a population in a container, *e.g.*, a bag.

[00383] The adherent placental cells generally express the markers CD73, CD105, and CD200, and/or OCT-4, and do not express CD34, CD38, or CD45. Adherent placental stem cells can also express HLA-ABC (MHC-1) and HLA-DR. These markers can be used to identify adherent placental cells, and to distinguish the adherent placental cells from other cell types. Because the adherent placental cells can express CD73 and CD105, they can have mesenchymal stem cell-like characteristics. Lack of expression of CD34, CD38 and/or CD45 identifies the adherent placental stem cells as non-hematopoietic stem cells.

[00384] In certain embodiments, the isolated adherent placental cells described herein detectably suppress cancer cell proliferation or tumor growth.

[00385] In certain embodiments, the isolated adherent placental cells are isolated placental stem cells. In certain other embodiments, the isolated adherent placental cells are isolated placental multipotent cells. In a specific embodiment, the isolated adherent placental cells are $CD34^-$, $CD10^+$ and $CD105^+$ as detected by flow cytometry. In a more specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ adherent placental cells are placental stem cells. In another more specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ placental cells are multipotent adherent placental cells. In another specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ placental cells have the potential to differentiate into cells of a neural phenotype, cells of an

osteogenic phenotype, or cells of a chondrogenic phenotype. In a more specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ adherent placental cells are additionally $CD200^+$. In another more specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ adherent placental cells are additionally $CD90^+$ or $CD45^-$, as detected by flow cytometry. In another more specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ adherent placental cells are additionally $CD90^+$ or $CD45^-$, as detected by flow cytometry. In a more specific embodiment, the $CD34^-$, $CD10^+$, $CD105^+$, $CD200^+$ adherent placental cells are additionally $CD90^+$ or $CD45^-$, as detected by flow cytometry. In another more specific embodiment, the $CD34^-$, $CD10^+$, $CD105^+$, $CD200^+$ adherent placental cells are additionally $CD90^+$ and $CD45^-$, as detected by flow cytometry. In another more specific embodiment, the $CD34^-$, $CD10^+$, $CD105^+$, $CD200^+$, $CD90^+$, $CD45^-$ adherent placental cells are additionally $CD80^-$ and $CD86^-$, as detected by flow cytometry.

[00386] In one embodiment, the isolated adherent placental cells are $CD200^+$, HLA-G^+. In a specific embodiment, said isolated adherent placental cells are also $CD73^+$ and $CD105^+$. In another specific embodiment, said isolated adherent placental cells are also $CD34^-$, $CD38^-$ or $CD45^-$. In a more specific embodiment, said isolated adherent placental cells are also $CD34^-$, $CD38^-$, $CD45^-$, $CD73^+$ and $CD105^+$. In another embodiment, said isolated adherent placental cells produce one or more embryoid-like bodies when cultured under conditions that allow the formation of embryoid-like bodies.

[00387] In another embodiment, the isolated adherent placental cells are $CD73^+$, $CD105^+$, $CD200^+$. In a specific embodiment of said populations, said isolated adherent placental cells are also HLA-G^+. In another specific embodiment, said isolated adherent placental cells are also $CD34^-$, $CD38^-$ or $CD45^-$. In another specific embodiment, said isolated adherent placental cells are also $CD34^-$, $CD38^-$ and $CD45^-$. In a more specific embodiment, said isolated adherent placental cells are also $CD34^-$, $CD38^-$, $CD45^-$, and HLA-G^+. In another specific embodiment, said isolated adherent placental cells produce one or more embryoid-like bodies when cultured under conditions that allow the formation of embryoid-like bodies.

[00388] In another embodiment, the isolated adherent placental cells are $CD200^+$, OCT-4^+. In a specific embodiment, said isolated adherent placental cells are also $CD73^+$ and $CD105^+$. In another specific embodiment, said isolated adherent placental cells are also HLA-G^+. In another specific embodiment, said isolated adherent placental cells are also $CD34^-$, $CD38^-$ and $CD45^-$. In a more specific embodiment, said isolated adherent placental cells are also $CD34^-$, $CD38^-$,

$CD45^-$, $CD73^+$, $CD105^+$ and $HLA\text{-}G^+$. In another specific embodiment, the isolated adherent placental cells also produce one or more embryoid-like bodies when cultured under conditions that allow the formation of embryoid-like bodies.

[00389] In another embodiment, the isolated adherent placental cells are $CD73^+$, $CD105^+$ and $HLA\text{-}G^+$. In a specific embodiment, said isolated adherent placental cells are also $CD34^-$, $CD38^-$ or $CD45^-$. In another specific embodiment, said isolated adherent placental cells also $CD34^-$, $CD38^-$ and $CD45^-$. In another specific embodiment, said adherent stem cells are also $OCT\text{-}4^+$. In another specific embodiment, said adherent stem cells are also $CD200^+$. In a more specific embodiment, said adherent stem cells are also $CD34^-$, $CD38^-$, $CD45^-$, $OCT\text{-}4^+$ and $CD200^+$.

[00390] In another embodiment, the isolated adherent placental cells are $CD73^+$, $CD105^+$ stem cells, wherein said cells produce one or more embryoid-like bodies under conditions that allow formation of embryoid-like bodies. In a specific embodiment, said isolated adherent placental cells are also $CD34^-$, $CD38^-$ or $CD45^-$. In another specific embodiment, isolated adherent placental cells are also $CD34^-$, $CD38^-$ and $CD45^-$. In another specific embodiment, isolated adherent placental cells are also $OCT\text{-}4^+$. In a more specific embodiment, said isolated adherent placental cells are also $OCT\text{-}4^+$, $CD34^-$, $CD38^-$ and $CD45^-$.

[00391] In another embodiment, the adherent placental stem cells are $OCT\text{-}4^+$ stem cells, wherein said adherent placental stem cells produce one or more embryoid-like bodies when cultured under conditions that allow the formation of embryoid-like bodies, and wherein said stem cells have been identified as detectably suppressing cancer cell proliferation or tumor growth.

[00392] In various embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50% at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of said isolated adherent placental cells are $OCT\text{-}4^+$. In a specific embodiment of the above populations, said isolated adherent placental cells are also $CD73^+$ and $CD105^+$. In another specific embodiment, said isolated adherent placental cells are also $CD34^-$, $CD38^-$, or $CD45^-$. In another specific embodiment, said stem cells are $CD200^+$. In a more specific embodiment, said isolated adherent placental cells are also $CD73^+$, $CD105^+$, $CD200^+$, $CD34^-$, $CD38^-$, and $CD45^-$. In another specific embodiment, said isolated adherent placental cells have been expanded, for example,

passaged at least once, at least three times, at least five times, at least 10 times, at least 15 times, or at least 20 times.

[00393] In a more specific embodiment of any of the above embodiments, the isolated adherent placental cells express ABC-p (a placenta-specific ABC transporter protein; *see*, *e.g.*, Allikmets *et al.*, *Cancer Res.* 58(23):5337-9 (1998)).

[00394] In another embodiment, the isolated adherent placental cells $CD29^+$, $CD44^+$, $CD73^+$, $CD90^+$, $CD105^+$, $CD200^+$, $CD34^-$ and $CD133^-$. In another embodiment, the isolated adherent placental cells constitutively secrete IL-6, IL-8 and monocyte chemoattractant protein (MCP-1).

[00395] Each of the above-referenced isolated adherent placental cells can comprise cells obtained and isolated directly from a mammalian placenta, or cells that have been cultured and passaged at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30 or more times, or a combination thereof. Tumor cell suppressive pluralities of the isolated adherent placental cells described above can comprise about, at least, or no more than, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 5×10^{10}, 1×10^{11} or more isolated adherent placental cells.

5.8.3. Compositions Comprising Adherent Placental Cell Conditioned Media

[00396] Also provided herein is the use of a composition comprising NK cells and/or ILC3 cells produced using the methods described herein, *e.g.*, NK cell and/or ILC3 cell populations produced using the three-stage method described herein, and additionally conditioned medium, wherein said composition is tumor suppressive, or is effective in the treatment of cancer or viral infection. In specific embodiments, the NK cells and ILC3 cells are present in ratios as described herein. Adherent placental cells as described herein can be used to produce conditioned medium that is tumor cell suppressive, anti-cancer or anti-viral that is, medium comprising one or more biomolecules secreted or excreted by the cells that have a detectable tumor cell suppressive effect, anti-cancer effect or antiviral effect. In various embodiments, the conditioned medium comprises medium in which the cells have proliferated (that is, have been cultured) for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more days. In other embodiments, the conditioned medium comprises medium in which such cells have grown to at least 30%, 40%, 50%, 60%, 70%, 80%, 90% confluence, or up to 100% confluence. Such

conditioned medium can be used to support the culture of a separate population of cells, *e.g.*, placental cells, or cells of another kind. In another embodiment, the conditioned medium provided herein comprises medium in which isolated adherent placental cells, *e.g.*, isolated adherent placental stem cells or isolated adherent placental multipotent cells, and cells other than isolated adherent placental cells, *e.g.*, non-placental stem cells or multipotent cells, have been cultured.

[00397] Such conditioned medium can be combined with any of, or any combination of NK cells and/or ILC3 cells produced using the methods described herein, placental perfusate, or placental perfusate cells to form a composition that is tumor cell suppressive, anticancer or antiviral. In certain embodiments, the composition comprises less than half conditioned medium by volume, *e.g.*, about, or less than about, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% by volume.

[00398] Thus, in one embodiment, provided herein is a composition comprising NK cells and/or ILC3 cells produced using the methods described herein and culture medium from a culture of isolated adherent placental cells, wherein said isolated adherent placental cells (a) adhere to a substrate; and (b) are $CD34^-$, $CD10^+$ and $CD105^+$; wherein said composition detectably suppresses the growth or proliferation of tumor cells, or is anti-cancer or antiviral. In a specific embodiment, the isolated adherent placental cells are $CD34^-$, $CD10^+$ and $CD105^+$ as detected by flow cytometry. In a more specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ adherent placental cells are placental stem cells. In another more specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ placental cells are multipotent adherent placental cells. In another specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ placental cells have the potential to differentiate into cells of a neural phenotype, cells of an osteogenic phenotype, or cells of a chondrogenic phenotype. In a more specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ adherent placental cells are additionally $CD200^+$. In another more specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ adherent placental cells are additionally $CD90^+$ or $CD45^-$, as detected by flow cytometry. In another more specific embodiment, the isolated $CD34^-$, $CD10^+$, $CD105^+$ adherent placental cells are additionally $CD90^+$ or $CD45^-$, as detected by flow cytometry. In a more specific embodiment, the $CD34^-$, $CD10^+$, $CD105^+$, $CD200^+$ adherent placental cells are additionally $CD90^+$ or $CD45^-$, as detected by flow cytometry. In another more specific embodiment, the $CD34^-$, $CD10^+$, $CD105^+$, $CD200^+$ adherent placental cells are

additionally $CD90^+$ and $CD45^-$, as detected by flow cytometry. In another more specific embodiment, the $CD34^-$, $CD10^+$, $CD105^+$, $CD200^+$, $CD90^+$, $CD45^-$ adherent placental cells are additionally $CD80^-$ and $CD86^-$, as detected by flow cytometry.

[00399] In another embodiment, provided herein is a composition comprising NK cells and/or ILC3 cells produced using the methods described herein, and culture medium from a culture of isolated adherent placental cells, wherein said isolated adherent placental cells (a) adhere to a substrate; and (b) express CD200 and HLA-G, or express CD73, CD105, and CD200, or express CD200 and OCT-4, or express CD73, CD105, and HLA-G, or express CD73 and CD105 and facilitate the formation of one or more embryoid-like bodies in a population of placental cells that comprise the placental stem cells when said population is cultured under conditions that allow formation of embryoid-like bodies, or express OCT-4 and facilitate the formation of one or more embryoid-like bodies in a population of placental cells that comprise the placental stem cells when said population is cultured under conditions that allow formation of embryoid-like bodies; wherein said composition detectably suppresses the growth or proliferation of tumor cells, or is anti-cancer or antiviral. In a specific embodiment, the composition further comprises a plurality of said isolated placental adherent cells. In another specific embodiment, the composition comprises a plurality of non-placental cells. In a more specific embodiment, said non-placental cells comprise $CD34^+$ cells, *e.g.*, hematopoietic progenitor cells, such as peripheral blood hematopoietic progenitor cells, cord blood hematopoietic progenitor cells, or placental blood hematopoietic progenitor cells. The non-placental cells can also comprise stem cells, such as mesenchymal stem cells, *e.g.*, bone marrow-derived mesenchymal stem cells. The non-placental cells can also be one or more types of adult cells or cell lines. In another specific embodiment, the composition comprises an anti-proliferative agent, *e.g.*, an anti-MIP-1α or anti-MIP-1β antibody.

[00400] In a specific embodiment, culture medium conditioned by one of the cells or cell combinations described above is obtained from a plurality of isolated adherent placental cells co-cultured with a plurality of tumor cells at a ratio of about 1:1, about 2:1, about 3:1, about 4:1, or about 5:1 isolated adherent placental cells to tumor cells. For example, the conditioned culture medium or supernatant can be obtained from a culture comprising about 1×10^5 isolated adherent placental cells, about 1×10^6 isolated adherent placental cells, about 1×10^7 isolated adherent placental cells, or about 1×10^8 isolated adherent placental cells, or more. In another

specific embodiment, the conditioned culture medium or supernatant is obtained from a co-culture comprising about 1 x 10^5 to about 5 x 10^5 isolated adherent placental cells and about 1 x 10^5 tumor cells; about 1 x 10^6 to about 5 x 10^6 isolated adherent placental cells and about 1 x 10^6 tumor cells; about 1 x 10^7 to about 5 x 10^7 isolated adherent placental cells and about 1 x 10^7 tumor cells; or about 1 x 10^8 to about 5 x 10^8 isolated adherent placental cells and about 1 x 10^8 tumor cells.

5.9. Preservation of Cells

[00401] Cells, *e.g.*, NK cells and/or ILC3 cells produced using the methods described herein, *e.g.*, NK cell and/or ILC3 cell populations produced using the three-stage method described herein, or placental perfusate cells comprising hematopoietic stem cells or progenitor cells, can be preserved, that is, placed under conditions that allow for long-term storage, or under conditions that inhibit cell death by, *e.g.*, apoptosis or necrosis.

[00402] Placental perfusate can be produced by passage of a cell collection composition through at least a part of the placenta, *e.g.*, through the placental vasculature. The cell collection composition comprises one or more compounds that act to preserve cells contained within the perfusate. Such a placental cell collection composition can comprise an apoptosis inhibitor, necrosis inhibitor and/or an oxygen-carrying perfluorocarbon, as described in related U.S. Application Publication No. 20070190042, the disclosure of which is hereby incorporated by reference in its entirety.

[00403] In one embodiment, perfusate or a population of placental cells are collected from a mammalian, *e.g.*, human, post-partum placenta by bringing the perfusate or population of cells into proximity with a cell collection composition comprising an inhibitor of apoptosis and an oxygen-carrying perfluorocarbon, wherein said inhibitor of apoptosis is present in an amount and for a time sufficient to reduce or prevent apoptosis in the population of placental cells, *e.g.*, adherent placental cells, for example, placental stem cells or placental multipotent cells, as compared to a population of cells not contacted or brought into proximity with the inhibitor of apoptosis. For example, the placenta can be perfused with the cell collection composition, and placental cells, *e.g.*, total nucleated placental cells, are isolated therefrom. In a specific embodiment, the inhibitor of apoptosis is a caspase inhibitor. In another specific embodiment, said inhibitor of apoptosis is a JNK inhibitor. In a more specific embodiment, said JNK inhibitor does not modulate differentiation or proliferation of adherent placental cells, *e.g.*, adherent

placental stem cells or adherent placental multipotent cells. In another embodiment, the cell collection composition comprises said inhibitor of apoptosis and said oxygen-carrying perfluorocarbon in separate phases. In another embodiment, the cell collection composition comprises said inhibitor of apoptosis and said oxygen-carrying perfluorocarbon in an emulsion. In another embodiment, the cell collection composition additionally comprises an emulsifier, *e.g.*, lecithin. In another embodiment, said apoptosis inhibitor and said perfluorocarbon are between about 0 ºC and about 25 ºC at the time of bringing the placental cells into proximity with the cell collection composition. In another more specific embodiment, said apoptosis inhibitor and said perfluorocarbon are between about 2 ºC and 10 ºC, or between about 2 ºC and about 5 ºC, at the time of bringing the placental cells into proximity with the cell collection composition. In another more specific embodiment, said bringing into proximity is performed during transport of said population of cells. In another more specific embodiment, said bringing into proximity is performed during freezing and thawing of said population of cells.

[00404] In another embodiment, placental perfusate and/or placental cells can be collected and preserved by bringing the perfusate and/or cells into proximity with an inhibitor of apoptosis and an organ-preserving compound, wherein said inhibitor of apoptosis is present in an amount and for a time sufficient to reduce or prevent apoptosis of the cells, as compared to perfusate or placental cells not contacted or brought into proximity with the inhibitor of apoptosis. In a specific embodiment, the organ-preserving compound is UW solution (described in U.S. Patent No. 4,798,824; also known as VIASPAN™; *see also* Southard *et al.*, *Transplantation* 49(2):251-257 (1990) or a solution described in Stern *et al.*, U.S. Patent No. 5,552,267, the disclosures of which are hereby incorporated by reference in their entireties. In another embodiment, said organ-preserving composition is hydroxyethyl starch, lactobionic acid, raffinose, or a combination thereof. In another embodiment, the placental cell collection composition additionally comprises an oxygen-carrying perfluorocarbon, either in two phases or as an emulsion.

[00405] In another embodiment of the method, placental cells are brought into proximity with a cell collection composition comprising an apoptosis inhibitor and oxygen-carrying perfluorocarbon, organ-preserving compound, or combination thereof, during perfusion. In another embodiment, placental cells are brought into proximity with said cell collection compound after collection by perfusion.

[00406] Typically, during placental cell collection, enrichment and isolation, it is preferable to minimize or eliminate cell stress due to hypoxia and mechanical stress. In another embodiment of the method, therefore, placental perfusate or a population of placental cells is exposed to a hypoxic condition during collection, enrichment or isolation for less than six hours during said preservation, wherein a hypoxic condition is a concentration of oxygen that is less than normal blood oxygen concentration. In a more specific embodiment, said perfusate or population of placental cells is exposed to said hypoxic condition for less than two hours during said preservation. In another more specific embodiment, said population of placental cells is exposed to said hypoxic condition for less than one hour, or less than thirty minutes, or is not exposed to a hypoxic condition, during collection, enrichment or isolation. In another specific embodiment, said population of placental cells is not exposed to shear stress during collection, enrichment or isolation.

[00407] Cells, *e.g.*, placental perfusate cells, hematopoietic cells, *e.g.*, $CD34^+$ hematopoietic stem cells; NK cells and/or ILC3 cells produced using the methods described herein; isolated adherent placental cells provided herein can be cryopreserved, *e.g.*, in cryopreservation medium in small containers, *e.g.*, ampoules or septum vials. In certain embodiments, cells provided herein are cryopreserved at a concentration of about $1 \times 10^4 - 5 \times 10^8$ cells per mL. In specific embodiments, cells provided herein are cryopreserved at a concentration of about $1 \times 10^6 - 1.5 \times 10^7$ cells per mL. In more specific embodiments, cells provided herein are cryopreserved at a concentration of about 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 1.5×10^7 cells per mL.

[00408] Suitable cryopreservation medium includes, but is not limited to, normal saline, culture medium including, *e.g.*, growth medium, or cell freezing medium, for example commercially available cell freezing medium, *e.g.*, C2695, C2639 or C6039 (Sigma); CryoStor® CS2, CryoStor® CS5 or CryoStor®CS10 (BioLife Solutions). In one embodiment, cryopreservation medium comprises DMSO (dimethylsulfoxide), at a concentration of, *e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10% (v/v). Cryopreservation medium may comprise additional agents, for example, methylcellulose, dextran, albumin (*e.g.*, human serum albumin), trehalose, and/or glycerol. In certain embodiments, the cryopreservation medium comprises about 1%-10% DMSO, about 25%-75% dextran and/or about 20-60% human serum albumin (HSA). In certain embodiments, the cryopreservation medium comprises about 1%-10% DMSO, about 25%-75%

trehalose and/or about 20-60% human HSA. In a specific embodiment, the cryopreservation medium comprises 5% DMSO, 55% dextran and 40% HSA. In a more specific embodiment, the cryopreservation medium comprises 5% DMSO, 55% dextran (10% w/v in normal saline) and 40% HSA. In another specific embodiment, the cryopreservation medium comprises 5% DMSO, 55% trehalose and 40% HSA. In a more specific embodiment, the cryopreservation medium comprises 5% DMSO, 55% trehalose (10% w/v in normal saline) and 40% HSA. In another specific embodiment, the cryopreservation medium comprises CryoStor® CS5. In another specific embodiment, the cryopreservation medium comprises CryoStor®CS10.

[00409] Cells provided herein can be cryopreserved by any of a variety of methods, and at any stage of cell culturing, expansion or differentiation. For example, cells provided herein can be cryopreserved right after isolation from the origin tissues or organs, *e.g.*, placental perfusate or umbilical cord blood, or during, or after either the first, second, or third step of the methods outlined above. In certain embodiments, the hematopoietic cells, *e.g.*, hematopoietic stem or progenitor cells are cryopreserved within about 1, 5, 10, 15, 20, 30, 45 minutes or within about 1, 2, 4, 6, 10, 12, 18, 20 or 24 hours after isolation from the origin tissues or organs. In certain embodiments, said cells are cryopreserved within 1, 2 or 3 days after isolation from the origin tissues or organs. In certain embodiments, said cells are cryopreserved after being cultured in a first medium as described above, for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 days. In some embodiments, said cells are cryopreserved after being cultured in a first medium as described above, for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 days, and in a second medium for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 days as described above. In some embodiments, when NK cells are made using a three-stage method described herein, said cells are cryopreserved after being cultured in a first medium about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 days; and/or after being cultured in a second medium about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 days; and/or after being cultured in a third medium about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 days. In a specific embodiment, NK cells and/or ILC3 cells are made using a three-stage method described herein, and said cells are cryopreserved after being cultured in a

first medium for 10 days; after being cultured in a second medium for 4 days; and after being cultured in a third medium for 21 days.

[00410] In one aspect, provided herein is a method of cryopreserving a population of NK cells and/or ILC3 cells, *e.g.*, NK cells and/or ILC3 cells produced by a three-stage method described herein. In one embodiment, said method comprises: culturing hematopoietic stem cells or progenitor cells, *e.g.*, $CD34^+$ stem cells or progenitor cells, in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells, subsequently culturing said first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells, and subsequently culturing said second population of cells in a third medium comprising IL-2 and IL-15, and lacking a stem cell mobilizing agent and LMWH, to produce a third population of cells, wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, CD16– or CD16+, and CD94+ or CD94-, and wherein at least 70%, or at least 80%, of the natural killer cells are viable, and next, cryopreserving the NK cells in a cryopreservation medium. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In a specific embodiment, said cryopreservation step further comprises (1) preparing a cell suspension solution; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain cryopreserved cell suspension; (3) cooling the cryopreserved cell suspension from step (3) to obtain a cryopreserved sample; and (4) storing the cryopreserved sample below -80 °C. In certain embodiments, the method includes no intermediary steps.

[00411] In one embodiment, said method comprises: (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third

medium comprising IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+ and next, cryopreserving the NK cells in a cryopreservation medium. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In a specific embodiment, said cryopreservation step further comprises (1) preparing a cell suspension solution; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain cryopreserved cell suspension; (3) cooling the cryopreserved cell suspension from step (3) to obtain a cryopreserved sample; and (4) storing the cryopreserved sample below -80 ℃. In certain embodiments, the method includes no intermediary steps.

[00412] In one embodiment, said method comprises: (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of stem cell factor (SCF) and LMWH, to produce a third population of cells; wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+ and next, cryopreserving the NK cells in a cryopreservation medium. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In a specific embodiment, said cryopreservation step further comprises (1) preparing a cell suspension solution; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain cryopreserved cell suspension; (3) cooling the

cryopreserved cell suspension from step (3) to obtain a cryopreserved sample; and (4) storing the cryopreserved sample below -80 °C. In certain embodiments, the method includes no intermediary steps.

[00413] In one embodiment, said method comprises: (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of SCF, a stem cell mobilizing agent, and LMWH, to produce a third population of cells; wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+ and next, cryopreserving the NK cells in a cryopreservation medium. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In a specific embodiment, said cryopreservation step further comprises (1) preparing a cell suspension solution; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain cryopreserved cell suspension; (3) cooling the cryopreserved cell suspension from step (3) to obtain a cryopreserved sample; and (4) storing the cryopreserved sample below -80 °C. In certain embodiments, the method includes no intermediary steps.

[00414] In one embodiment, said method comprises: (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of a stem cell mobilizing agent and LMWH, to produce a third population of cells; and (d) isolating CD11a+ cells from the third population of cells to produce a fourth population of cells; wherein the fourth population of cells

comprises natural killer cells that are CD56+, CD3-, and CD11a+ and next, cryopreserving the NK cells in a cryopreservation medium. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In a specific embodiment, said cryopreservation step further comprises (1) preparing a cell suspension solution; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain cryopreserved cell suspension; (3) cooling the cryopreserved cell suspension from step (3) to obtain a cryopreserved sample; and (4) storing the cryopreserved sample below -80 °C. In certain embodiments, the method includes no intermediary steps.

[00415] In one embodiment, said method comprises: (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a- and next, cryopreserving the ILC3 cells in a cryopreservation medium. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In a specific embodiment, said cryopreservation step further comprises (1) preparing a cell suspension solution; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain cryopreserved cell suspension; (3) cooling the cryopreserved cell suspension from step (3) to obtain a

cryopreserved sample; and (4) storing the cryopreserved sample below -80 °C. In certain embodiments, the method includes no intermediary steps.

[00416] In one embodiment, said method comprises: (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising a stem cell mobilizing agent, IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a- and next, cryopreserving the ILC3 cells in a cryopreservation medium. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In a specific embodiment, said cryopreservation step further comprises (1) preparing a cell suspension solution; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain cryopreserved cell suspension; (3) cooling the cryopreserved cell suspension from step (3) to obtain a cryopreserved sample; and (4) storing the cryopreserved sample below -80 °C. In certain embodiments, the method includes no intermediary steps.

[00417] In one embodiment, said method comprises: (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising SCF, IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a- and next, cryopreserving the ILC3 cells in a cryopreservation medium. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF)

and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In a specific embodiment, said cryopreservation step further comprises (1) preparing a cell suspension solution; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain cryopreserved cell suspension; (3) cooling the cryopreserved cell suspension from step (3) to obtain a cryopreserved sample; and (4) storing the cryopreserved sample below -80 ℃. In certain embodiments, the method includes no intermediary steps.

[00418] In one embodiment, said method comprises: (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and (c) culturing the second population of cells in a third medium comprising a stem cell mobilizing agent, SCF, IL-2 and IL-15, and lacking LMWH, to produce a third population of cells; wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a- and next, cryopreserving the ILC3 cells in a cryopreservation medium. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In a specific embodiment, said cryopreservation step further comprises (1) preparing a cell suspension solution; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain cryopreserved cell suspension; (3) cooling the cryopreserved cell suspension from step (3) to obtain a cryopreserved sample; and (4) storing the cryopreserved sample below -80 ℃. In certain embodiments, the method includes no intermediary steps.

[00419] In one embodiment, said method comprises: (a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells; (b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; (c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of a stem cell mobilizing agent and LMWH, to produce a third population of cells; and (d) isolating CD11a- cells from the third population of cells to produce a fourth population of cells; wherein the fourth population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a- and next, cryopreserving the ILC3 cells in a cryopreservation medium. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin. In a specific embodiment, said cryopreservation step further comprises (1) preparing a cell suspension solution; (2) adding cryopreservation medium to the cell suspension solution from step (1) to obtain cryopreserved cell suspension; (3) cooling the cryopreserved cell suspension from step (3) to obtain a cryopreserved sample; and (4) storing the cryopreserved sample below -80 °C. In certain embodiments, the method includes no intermediary steps.

[00420] Cells provided herein can be cooled in a controlled-rate freezer, *e.g.*, at about 0.1, 0.3, 0.5, 1, or 2 °C/min during cryopreservation. In one embodiment, the cryopreservation temperature is about -80 °C to about -180 °C, or about -125 °C to about -140 °C. Cryopreserved cells can be transferred to liquid nitrogen prior to thawing for use. In some embodiments, for example, once the ampoules have reached about -90 °C, they are transferred to a liquid nitrogen storage area. Cryopreserved cells can be thawed at a temperature of about 25 °C to about 40 °C, more specifically can be thawed to a temperature of about 37 °C. In certain embodiments, the cryopreserved cells are thawed after being cryopreserved for about 1, 2, 4, 6, 10, 12, 18, 20 or 24 hours, or for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 days. In certain embodiments, the cryopreserved cells are thawed after being

cryopreserved for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 months. In certain embodiments, the cryopreserved cells are thawed after being cryopreserved for about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 years.

[00421] Suitable thawing medium includes, but is not limited to, normal saline, plasmalyte culture medium including, for example, growth medium, *e.g.*, RPMI medium. In certain embodiments, the thawing medium comprises one or more of medium supplements (*e.g.*, nutrients, cytokines and/or factors). Medium supplements suitable for thawing cells provided herein include, for example without limitation, serum such as human serum AB, fetal bovine serum (FBS) or fetal calf serum (FCS), vitamins, human serum albumin (HSA), bovine serum albumin (BSA), amino acids (*e.g.*, L-glutamine), fatty acids (*e.g.*, oleic acid, linoleic acid or palmitic acid), insulin (*e.g.*, recombinant human insulin), transferrin (iron saturated human transferrin), β-mercaptoethanol, stem cell factor (SCF), Fms-like-tyrosine kinase 3 ligand (Flt3-L), cytokines such as interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-15 (IL-15), thrombopoietin (Tpo) or heparin. In a specific embodiment, the thawing medium useful in the methods provided herein comprises RPMI. In another specific embodiment, said thawing medium comprises plasmalyte. In another specific embodiment, said thawing medium comprises about 0.5-20% FBS. In another specific embodiment, said thawing medium comprises about 1, 2, 5, 10, 15 or 20% FBS. In another specific embodiment, said thawing medium comprises about 0.5%-20% HSA. In another specific embodiment, said thawing medium comprises about 1, 2.5, 5, 10, 15, or 20% HSA. In a more specific embodiment, said thawing medium comprises RPMI and about 10% FBS. In another more specific embodiment, said thawing medium comprises plasmalyte and about 5% HSA.

[00422] The cryopreservation methods provided herein can be optimized to allow for long-term storage, or under conditions that inhibit cell death by, *e.g.*, apoptosis or necrosis. In one embodiments, the post-thaw cells comprise greater than 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98% of viable cells, as determined by, *e.g.*, automatic cell counter or trypan blue method. In another embodiment, the post-thaw cells comprise about 0.5, 1, 5, 10, 15, 20 or 25% of dead cells. In another embodiment, the post-thaw cells comprise about 0.5, 1, 5, 10, 15, 20 or 25% of early apoptotic cells. In another embodiment, about 0.5, 1, 5, 10, 15 or 20% of post-thaw cells undergo apoptosis after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 days after being thawed, *e.g.*, as determined by an apoptosis assay

(*e.g.*, TO-PRO3 or AnnV/PI Apoptosis assay kit). In certain embodiments, the post-thaw cells are re-cryopreserved after being cultured, expanded or differentiated using methods provided herein.

5.10. Compositions Comprising NK Cells and/or ILC3 Cells

5.10.1. NK Cells and/or ILC3 Cells Produced Using The Three-Stage Method

[00423] In some embodiments, provided herein is a composition, *e.g.*, a pharmaceutical composition, comprising an isolated NK cell and/or ILC3 cell population produced using the three-stage method described herein. In a specific embodiment, said isolated NK cell and/or ILC3 cell population is produced from hematopoietic cells, *e.g.*, hematopoietic stem or progenitor cells isolated from placental perfusate, umbilical cord blood, and/or peripheral blood. In another specific embodiment, said isolated NK cell and/or ILC3 cell population comprises at least 50% of cells in the composition. In another specific embodiment, said isolated NK cell and/or ILC3 cell population, *e.g.*, $CD3^-CD56^+$ cells, comprises at least 80%, 85%, 90%. 95%, 98% or 99% of cells in the composition. In certain embodiments, no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 40% of the cells in said isolated NK cell and/or ILC3 cell population are $CD3^-CD56^+$ cells. In certain embodiments, said $CD3^-CD56^+$ cells are $CD16^-$.

[00424] NK cell and/or ILC3 cell populations produced using the three-stage method described herein, can be formulated into pharmaceutical compositions for use *in vivo*. Such pharmaceutical compositions comprise a population of NK cells and/or ILC3 cells in a pharmaceutically-acceptable carrier, *e.g.*, a saline solution or other accepted physiologically-acceptable solution for *in vivo* administration. Pharmaceutical compositions of the invention can comprise any of the NK cell and/or ILC3 cell populations described elsewhere herein.

[00425] The pharmaceutical compositions of the invention comprise populations of cells that comprise 50% viable cells or more (that is, at least 50% of the cells *in* the population are functional or living). Preferably, at least 60% of the cells in the population are viable. More preferably, at least 70%, 80%, 90%, 95%, or 99% of the cells in the population in the pharmaceutical composition are viable.

[00426] The pharmaceutical compositions of the invention can comprise one or more compounds that, *e.g.*, facilitate engraftment; stabilizers such as albumin, dextran 40, gelatin, hydroxyethyl starch, and the like.

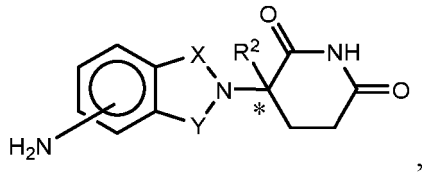
[00427] When formulated as an injectable solution, in one embodiment, the pharmaceutical composition of the invention comprises about 1.25% HSA and about 2.5% dextran. Other injectable formulations, suitable for the administration of cellular products, may be used.

[00428] In one embodiment, the compositions, *e.g.*, pharmaceutical compositions, provided herein are suitable for systemic or local administration. In specific embodiments, the compositions, *e.g.*, pharmaceutical compositions, provided herein are suitable for parenteral administration. In specific embodiments, the compositions, *e.g.*, pharmaceutical compositions, provided herein are suitable for injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In specific embodiments, the compositions, *e.g.*, pharmaceutical compositions, provided herein are suitable for administration via a device, a matrix, or a scaffold. In specific embodiments, the compositions, *e.g.*, pharmaceutical compositions provided herein are suitable for injection. In specific embodiments, the compositions, *e.g.*, pharmaceutical compositions, provided herein are suitable for administration via a catheter. In specific embodiments, the compositions, *e.g.*, pharmaceutical compositions, provided herein are suitable for local injection. In more specific embodiments, the compositions, *e.g.*, pharmaceutical compositions, provided herein are suitable for local injection directly into a solid tumor (*e.g.*, a sarcoma). In specific embodiments, the compositions, *e.g.*, pharmaceutical compositions, provided herein are suitable for injection by syringe. In specific embodiments, the compositions, *e.g.*, pharmaceutical compositions, provided herein are suitable for administration via guided delivery. In specific embodiments, the compositions, *e.g.*, pharmaceutical compositions, provided herein are suitable for injection aided by laparoscopy, endoscopy, ultrasound, computed tomography, magnetic resonance, or radiology.

[00429] In certain embodiments, the compositions, *e.g.*, pharmaceutical compositions provided herein, comprising NK cells and/or ILC3 cells produced using the methods described herein, are provided as pharmaceutical grade administrable units. Such units can be provided in discrete volumes, *e.g.*, 15 mL, 20 mL, 25 mL, 30 nL. 35 mL, 40 mL, 45 mL, 50 mL, 55 mL, 60 mL, 65 mL, 70 mL, 75 mL, 80 mL, 85 mL, 90 mL, 95 mL, 100 mL, 150 mL, 200 mL, 250 mL, 300 mL, 350 mL, 400 mL, 450 mL, 500 mL, or the like. Such units can be provided so as to contain a specified number of cells, *e.g.*, NK cells and/or ILC3 cells, *e.g.*, 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8 or more cells per milliliter, or 1 x

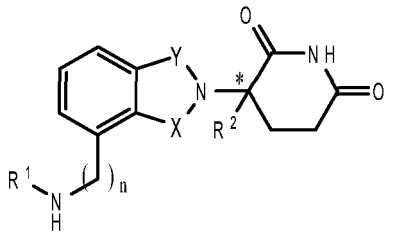
10^4, 5 x 10^4, 1 x 10^5, 5 x 10^5, 1 x 10^6, 5 x 10^6, 1 x 10^7, 5 x 10^7, 1 x 10^8, 5 x 10^8, 1 x 10^9, 5 x 10^9, 1 x 10^{10}, 5 x 10^{10}, 1 x 10^{11} or more cells per unit. In specific embodiments, the units can comprise about, at least about, or at most about 1 x 10^4, 5 x 10^4, 1 x 10^5, 5 x 10^5, 1 x 10^6, 5 x 10^6 or more NK cells and/or ILC3 cells per milliliter, or 1 x 10^4, 5 x 10^4, 1 x 10^5, 5 x 10^5, 1 x 10^6, 5 x 10^6, 1 x 10^7, 5 x 10^7, 1 x 10^8, 5 x 10^8, 1 x 10^9, 5 x 10^9, 1 x 10^{10}, 5 x 10^{10}, 1 x 10^{11} or more cells per unit. Such units can be provided to contain specified numbers of NK cells and/or ILC3 cells or NK cell and/or ILC3 cell populations and/or any of the other cells. In specific embodiments, the NK cells and ILC3 cells are present in ratios provided herein.

[00430] In another specific embodiment, said isolated NK cells and/or ILC3 cells in said composition are from a single individual. In a more specific embodiment, said isolated NK cells and/or ILC3 cells comprise NK cells and/or ILC3 cells from at least two different individuals. In another specific embodiment, said isolated NK cells and/or ILC3 cells in said composition are from a different individual than the individual for whom treatment with the NK cells and/or ILC3 cells is intended. In another specific embodiment, said NK cells have been contacted or brought into proximity with an immunomodulatory compound or thalidomide in an amount and for a time sufficient for said NK cells to express detectably more granzyme B or perforin than an equivalent number of natural killer cells, *i.e.* NK cells not contacted or brought into proximity with said immunomodulatory compound or thalidomide. In another specific embodiment, said composition additionally comprises an immunomodulatory compound or thalidomide. In certain embodiments, the immunomodulatory compound is a compound described below. *See*, *e.g.*, U.S. Patent No. 7,498,171, the disclosure of which is hereby incorporated by reference in its entirety. In certain embodiments, the immunomodulatory compound is an amino-substituted isoindoline. In one embodiment, the immunomodulatory compound is 3-(4-amino-1-oxo-1,3-dihydroisoindol-2-yl)-piperidine-2,6-dione; 3-(4'aminoisolindoline-1'-one)-1-piperidine-2,6-dione; 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione; or 4-Amino-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione. In another embodiment, the immunomodulatory compound is pomalidomide, or lenalidomide. In another embodiment, said immunomodulatory compound is a compound having the structure



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wherein one of X and Y is C=O, the other of X and Y is C=O or CH_2, and R^2 is hydrogen or lower alkyl, or a pharmaceutically acceptable salt, hydrate, solvate, clathrate, enantiomer, diastereomer, racemate, or mixture of stereoisomers thereof. In another embodiment, said immunomodulatory compound is a compound having the structure



,

wherein one of X and Y is C=O and the other is CH_2 or C=O;

R^1 is H, (C_1–C_8)alkyl, (C_3–C_7)cycloalkyl, (C_2–C_8)alkenyl, (C_2-C_8)alkynyl, benzyl, aryl, (C_0-C_4)alkyl–(C_1-C_6)heterocycloalkyl, (C_0-C_4)alkyl–(C_2-C_5)heteroaryl, $C(O)R^3$, $C(S)R^3$, $C(O)OR^4$, (C_1-C_8)alkyl–$N(R^6)_2$, (C_1-C_8)alkyl–OR^5, (C_1-C_8)alkyl–$C(O)OR^5$, $C(O)NHR^3$, $C(S)NHR^3$, $C(O)NR^3R^{3'}$, $C(S)NR^3R^{3'}$ or (C_1-C_8)alkyl–$O(CO)R^5$;

R^2 is H, F, benzyl, (C_1-C_8)alkyl, (C_2-C_8)alkenyl, or (C_2-C_8)alkynyl;

R^3 and $R^{3'}$ are independently (C_1-C_8)alkyl, (C_3-C_7)cycloalkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, benzyl, aryl, (C_0-C_4)alkyl–(C_1-C_6)heterocycloalkyl, (C_0-C_4)alkyl–(C_2-C_5)heteroaryl, (C_0-C_8)alkyl–$N(R^6)_2$, (C_1-C_8)alkyl–OR^5, (C_1-C_8)alkyl–$C(O)OR^5$, (C_1-C_8)alkyl–$O(CO)R^5$, or $C(O)OR^5$;

R^4 is (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, (C_1-C_4)alkyl–OR^5, benzyl, aryl, (C_0-C_4)alkyl–(C_1-C_6)heterocycloalkyl, or (C_0-C_4)alkyl–(C_2-C_5)heteroaryl;

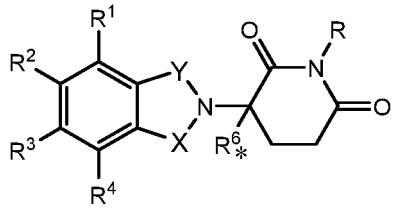
R^5 is (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, benzyl, aryl, or (C_2-C_5)heteroaryl;

each occurrence of R^6 is independently H, (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, benzyl, aryl, (C_2-C_5)heteroaryl, or (C_0-C_8)alkyl–C(O)O–R^5 or the R^6 groups can join to form a heterocycloalkyl group;

n is 0 or 1; and

* represents a chiral-carbon center;

or a pharmaceutically acceptable salt, hydrate, solvate, clathrate, enantiomer, diastereomer, racemate, or mixture of stereoisomers thereof. In another embodiment, said immunomodulatory compound is a compound having the structure



wherein:

one of X and Y is C=O and the other is CH_2 or C=O;

R is H or CH_2OCOR';

(i) each of R^1, R^2, R^3, or R^4, independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms or (ii) one of R^1, R^2, R^3, or R^4 is nitro or $-NHR^5$ and the remaining of R^1, R^2, R^3, or R^4 are hydrogen;

R^5 is hydrogen or alkyl of 1 to 8 carbons

R^6 hydrogen, alkyl of 1 to 8 carbon atoms, benzo, chloro, or fluoro;

R' is R^7-CHR^{10}-$N(R^8R^9)$;

R^7 is m-phenylene or p-phenylene or $-(C_nH_{2n})-$ in which n has a value of 0 to 4;

each of R^8 and R^9 taken independently of the other is hydrogen or alkyl of 1 to 8 carbon atoms, or R^8 and R^9 taken together are tetramethylene, pentamethylene, hexamethylene, or $-CH_2CH_2X_1CH_2CH_2-$ in which X_1 is -O-, -S-, or -NH-;

R^{10} is hydrogen, alkyl of to 8 carbon atoms, or phenyl; and

* represents a chiral-carbon center;

or a pharmaceutically acceptable salt, hydrate, solvate, clathrate, enantiomer, diastereomer, racemate, or mixture of stereoisomers thereof.

[00431] In another specific embodiment, the composition additionally comprises one or more anticancer compounds, *e.g.*, one or more of the anticancer compounds described below.

[00432] In a more specific embodiment, the composition comprises NK cells and/or ILC3 cells from another source, or made by another method. In a specific embodiment, said other source is placental blood and/or umbilical cord blood. In another specific embodiment, said other source is peripheral blood. In more specific embodiments, the NK cell and/or ILC3 cell

population in said composition is combined with NK cells and/or ILC3 cells from another source, or made by another method in a ratio of about 100:1, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45: 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 15:1, 10:1, 5:1, 1:1, 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, or the like.

[00433] In another specific embodiment, the composition comprises an NK cell and/or ILC3 cell population produced using the three-stage method described herein and either isolated placental perfusate or isolated placental perfusate cells. In a more specific embodiment, said placental perfusate is from the same individual as said NK cell and/or ILC3 cell population. In another more specific embodiment, said placental perfusate comprises placental perfusate from a different individual than said NK cell and/or ILC3 cell population. In another specific embodiment, all, or substantially all (*e.g.*, greater than 90%, 95%, 98% or 99%) of cells in said placental perfusate are fetal cells. In another specific embodiment, the placental perfusate or placental perfusate cells, comprise fetal and maternal cells. In a more specific embodiment, the fetal cells in said placental perfusate comprise less than about 90%, 80%, 70%, 60% or 50% of the cells in said perfusate. In another specific embodiment, said perfusate is obtained by passage of a 0.9% NaCl solution through the placental vasculature. In another specific embodiment, said perfusate comprises a culture medium. In another specific embodiment, said perfusate has been treated to remove erythrocytes. In another specific embodiment, said composition comprises an immunomodulatory compound, *e.g.*, an immunomodulatory compound described below, *e.g.*, an amino-substituted isoindoline compound. In another specific embodiment, the composition additionally comprises one or more anticancer compounds, *e.g.*, one or more of the anticancer compounds described below.

[00434] In another specific embodiment, the composition comprises an NK cell and/or ILC3 cell population and placental perfusate cells. In a more specific embodiment, said placental perfusate cells are from the same individual as said NK cell and/or ILC3 cell population. In another more specific embodiment, said placental perfusate cells are from a different individual than said NK cell and/or ILC3 cell population. In another specific embodiment, the composition comprises isolated placental perfusate and isolated placental perfusate cells, wherein said isolated perfusate and said isolated placental perfusate cells are

from different individuals. In another more specific embodiment of any of the above embodiments comprising placental perfusate, said placental perfusate comprises placental perfusate from at least two individuals. In another more specific embodiment of any of the above embodiments comprising placental perfusate cells, said isolated placental perfusate cells are from at least two individuals. In another specific embodiment, said composition comprises an immunomodulatory compound. In another specific embodiment, the composition additionally comprises one or more anticancer compounds, *e.g.*, one or more of the anticancer compounds described below.

5.11. Uses of NK Cells and/or ILC3 Cells Produced Using the Three-Stage Method

[00435] The NK cells and/or ILC3 cells produced using the methods described herein, *e.g.*, NK cell and/or ILC3 cell produced according to the three-stage method described herein, provided herein can be used in methods of treating individuals having cancer, *e.g.*, individuals having solid tumor cells and/or blood cancer cells, or persons having a viral infection. In some such embodiments, an effective dosage of NK cells and/or ILC3 cells produced using the methods described herein ranges from 1×10^4 to 5×10^4, 5×10^4 to 1×10^5, 1×10^5 to 5×10^5, 5×10^5 to 1×10^6, 1×10^6 to 5×10^6, 5×10^6 to 1×10^7, or more cells/kilogram body weight. The NK cells and/or ILC3 cells produced using the methods described herein, can also be used in methods of suppressing proliferation of tumor cells.

5.11.1. Treatment of Individuals Having Cancer

[00436] In one embodiment, provided herein is a method of treating an individual having a cancer, for example, a blood cancer or a solid tumor, comprising administering to said individual a therapeutically effective amount of NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein. In one embodiment, provided herein is a method of treating an individual having a cancer, for example, a blood cancer or a solid tumor, comprising administering to said individual a therapeutically effective amount of ILC3 cells produced using the methods described herein, *e.g.*, ILC3 cell populations produced using the three-stage method described herein. In certain embodiments, the individual has a deficiency of natural killer cells, *e.g.*, a deficiency of NK cells active against the individual's cancer. In a specific embodiment, the method additionally comprises administering to said individual isolated placental perfusate or isolated placental perfusate cells,

e.g., a therapeutically effective amount of placental perfusate or isolated placental perfusate cells. In another specific embodiment, the method comprises additionally administering to said individual an effective amount of an immunomodulatory compound, *e.g.*, an immunomodulatory compound described above, or thalidomide. As used herein, an "effective amount" is an amount that, *e.g.*, results in a detectable improvement of, lessening of the progression of, or elimination of, one or more symptoms of a cancer from which the individual suffers.

[00437] Administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof may be systemic or local. In specific embodiments, administration is parenteral. In specific embodiments, administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In specific embodiments, administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is performed with a device, a matrix, or a scaffold. In specific embodiments, administration an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is by injection. In specific embodiments, administration an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is via a catheter. In specific embodiments, the injection of NK cells and/or ILC3 cells is local injection. In more specific embodiments, the local injection is directly into a solid tumor (*e.g.*, a sarcoma). In specific embodiments, administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is by injection by syringe. In specific embodiments, administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is via guided delivery. In specific embodiments, administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject by injection is aided by laparoscopy, endoscopy, ultrasound, computed tomography, magnetic resonance, or radiology.

[00438] In a specific embodiment, the cancer is a blood cancer, *e.g.*, a leukemia or a lymphoma. In more specific embodiments, the cancer is an acute leukemia, *e.g.*, acute T cell leukemia, acute myelogenous leukemia (AML), acute promyelocytic leukemia, acute myeloblastic leukemia, acute megakaryoblastic leukemia, precursor B acute lymphoblastic leukemia, precursor T acute lymphoblastic leukemia, Burkitt's leukemia (Burkitt's lymphoma),

or acute biphenotypic leukemia; a chronic leukemia, *e.g.*, chronic myeloid lymphoma, chronic myelogenous leukemia (CML), chronic monocytic leukemia, chronic lymphocytic leukemia (CLL)/Small lymphocytic lymphoma, or B-cell prolymphocytic leukemia; hairy cell lymphoma; T-cell prolymphocytic leukemia; or a lymphoma, *e.g.*, histiocytic lymphoma, lymphoplasmacytic lymphoma (*e.g.*, Waldenström macroglobulinemia), splenic marginal zone lymphoma, plasma cell neoplasm (*e.g.*, plasma cell myeloma, plasmacytoma, a monoclonal immunoglobulin deposition disease, or a heavy chain disease), extranodal marginal zone B cell lymphoma (MALT lymphoma), nodal marginal zone B cell lymphoma (NMZL), follicular lymphoma, mantle cell lymphoma, diffuse large B cell lymphoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, T cell large granular lymphocytic leukemia, aggressive NK cell leukemia, adult T cell leukemia/lymphoma, extranodal NK/T cell lymphoma, nasal type, enteropathy-type T cell lymphoma, hepatosplenic T cell lymphoma, blastic NK cell lymphoma, mycosis fungoides (Sezary syndrome), a primary cutaneous CD30-positive T cell lymphoproliferative disorder (*e.g.*, primary cutaneous anaplastic large cell lymphoma or lymphomatoid papulosis), angioimmunoblastic T cell lymphoma, peripheral T cell lymphoma, unspecified, anaplastic large cell lymphoma, a Hodgkin's lymphoma or a nodular lymphocyte-predominant Hodgkin's lymphoma. In another specific embodiment, the cancer is multiple myeloma or myelodysplastic syndrome.

[00439] In certain other specific embodiments, the cancer is a solid tumor, *e.g.*, a carcinoma, such as an adenocarcinoma, an adrenocortical carcinoma, a colon adenocarcinoma, a colorectal adenocarcinoma, a colorectal carcinoma, a ductal cell carcinoma, a lung carcinoma, a thyroid carcinoma, a nasopharyngeal carcinoma, a melanoma (*e.g.*, a malignant melanoma), a non-melanoma skin carcinoma, or an unspecified carcinoma; a desmoid tumor; a desmoplastic small round cell tumor; an endocrine tumor; an Ewing sarcoma; a germ cell tumor (*e.g.*, testicular cancer, ovarian cancer, choriocarcinoma, endodermal sinus tumor, germinoma, *etc.*); a hepatosblastoma; a hepatocellular carcinoma; a neuroblastoma; a non-rhabdomyosarcoma soft tissue sarcoma; an osteosarcoma; a retinoblastoma; a rhabdomyosarcoma; or a Wilms tumor. In another embodiment, the solid tumor is pancreatic cancer or breast cancer. In other embodiments, the solid tumor is an acoustic neuroma; an astrocytoma (*e.g.*, a grade I pilocytic astrocytoma, a grade II low-grade astrocytoma; a grade III anaplastic astrocytoma; or a grade IV glioblastoma multiforme); a chordoma; a craniopharyngioma; a glioma (*e.g.*, a brain stem glioma;

an ependymoma; a mixed glioma; an optic nerve glioma; or a subependymoma); a glioblastoma; a medulloblastoma; a meningioma; a metastatic brain tumor; an oligodendroglioma; a pineoblastoma; a pituitary tumor; a primitive neuroectodermal tumor; or a schwannoma. In another embodiment, the cancer is prostate cancer. In another embodiment, the cancer is liver cancer. In another embodiment, the cancer is lung cancer. In another embodiment, the cancer is renal cancer.

[00440] In certain embodiments, the individual having a cancer, for example, a blood cancer or a solid tumor, *e.g.*, an individual having a deficiency of natural killer cells, is an individual that has received a bone marrow transplant before said administering. In certain embodiments, the bone marrow transplant was in treatment of said cancer. In certain other embodiments, the bone marrow transplant was in treatment of a condition other than said cancer. In certain embodiments, the individual received an immunosuppressant in addition to said bone marrow transplant. In certain embodiments, the individual who has had a bone marrow transplant exhibits one or more symptoms of graft-versus-host disease (GVHD) at the time of said administration. In certain other embodiments, the individual who has had a bone marrow transplant is administered said cells before a symptom of GVHD has manifested.

[00441] In certain specific embodiments, the individual having a cancer, for example, a blood cancer, has received at least one dose of a TNFα inhibitor, *e.g.*, ETANERCEPT® (Enbrel), prior to said administering. In specific embodiments, said individual received said dose of a TNFα inhibitor within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 months of diagnosis of said cancer. In a specific embodiment, the individual who has received a dose of a TNFα inhibitor exhibits acute myeloid leukemia. In a more specific embodiment, the individual who has received a dose of a TNFα inhibitor and exhibits acute myeloid leukemia further exhibits deletion of the long arm of chromosome 5 in blood cells. In another embodiment, the individual having a cancer, for example, a blood cancer, exhibits a Philadelphia chromosome.

[00442] In certain other embodiments, the cancer, for example, a blood cancer or a solid tumor, in said individual is refractory to one or more anticancer drugs. In a specific embodiment, the cancer is refractory to GLEEVEC® (imatinib mesylate).

[00443] In certain embodiments, the cancer, for example, a blood cancer, in said individual responds to at least one anticancer drug; in this embodiment, placental perfusate, isolated placental perfusate cells, isolated natural killer cells, *e.g.*, placental natural killer cells,

e.g., placenta-derived intermediate natural killer cells, isolated combined natural killer cells, or NK cells described herein, and/or combinations thereof, and optionally an immunomodulatory compound, are added as adjunct treatments or as a combination therapy with said anticancer drug. In certain other embodiments, the individual having a cancer, for example, a blood cancer, has been treated with at least one anticancer drug, and has relapsed, prior to said administering. In certain embodiments, the individual to be treated has a refractory cancer. In one embodiment, the cancer treatment method with the cells described herein protects against (*e.g.*, prevents or delays) relapse of cancer. In one embodiment, the cancer treatment method described herein results in remission of the cancer for 1 month or more, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or more, 1 year or more, 2 years or more, 3 years or more, or 4 years or more.

[00444] In one embodiment, provided herein is a method of treating an individual having multiple myeloma, comprising administering to the individual (1) lenalidomide; (2) melphalan; and (3) NK cells, wherein said NK cells are effective to treat multiple myeloma in said individual. In a specific embodiment, said NK cells are cord blood NK cells, or NK cells produced from cord blood hematopoietic cells, *e.g.*, hematopoietic stem cells. In another embodiment, said NK cells have been produced by a three-stage method described herein for producing NK cells. In another embodiment, said lenalidomide, melphalan, and/or NK cells are administered separately from each other. In certain specific embodiments of the method of treating an individual with multiple myeloma, said NK cells are produced by a method comprising: culturing hematopoietic stem cells or progenitor cells, *e.g.*, $CD34^+$ stem cells or progenitor cells, in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells, subsequently culturing said first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells, and subsequently culturing said second population of cells in a third medium comprising IL-2 and IL-15, and lacking a stem cell mobilizing agent and LMWH, to produce a third population of cells, wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, CD16– or CD16+, and CD94+ or CD94-, and wherein at least 70%, or at least 80%, of the natural killer cells are viable. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific

embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00445] In another embodiment, provided herein is a method of treating an individual having acute myelogenous leukemia (AML), comprising administering to the individual NK cells (optionally activated by pretreatment with IL2 alone, or IL-15 alone, IL2 and IL12 and IL18, IL12 and IL15, IL12 and IL18, IL2 and IL12 and IL15 and IL18, or IL2 and IL15 and IL18), wherein said NK cells are effective to treat AML in said individual. In a specific embodiment, the isolated NK cell population produced using the three-stage methods described herein has been pretreated with one or more of IL2, IL12, IL18, or IL15 prior to said administering. In a specific embodiment, said NK cells are cord blood NK cells, or NK cells produced from cord blood hematopoietic cells, *e.g.*, hematopoietic stem cells. In another embodiment, said NK cells have been produced by a three-stage method described herein for producing NK cells. In certain specific embodiments of the method of treating an individual with AML, said NK cells are produced by a three-stage method, as described herein. In a particular embodiment, the AML to be treated by the foregoing methods comprises refractory AML, poor-prognosis AML, or childhood AML. Methods known in the art for administering NK cells for the treatment of refractory AML, poor-prognosis AML, or childhood AML may be adapted for this purpose; see, *e.g.*, Miller et al., 2005, Blood 105:3051-3057; Rubnitz et al., 2010, J Clin Oncol. 28:955-959, each of which is incorporated herein by reference in its entirety. In certain embodiments, said individual has AML that has failed at least one non-natural killer cell therapeutic against AML. In specific embodiments, said individual is 65 years old or greater, and is in first remission. In specific embodiments, said individual has been conditioned with fludarabine, cytarabine, or both prior to administering said natural killer cells.

[00446] In one embodiment, provided herein is a method of treating an individual having multiple myeloma, comprising administering to the individual (1) lenalidomide; (2) melphalan; and (3) ILC3 cells, wherein said ILC3 cells are effective to treat multiple myeloma in said individual. In a specific embodiment, said ILC3 cells are cord blood ILC3 cells, or ILC3 cells produced from cord blood hematopoietic cells, *e.g.*, hematopoietic stem cells. In another embodiment, said ILC3 cells have been produced by a three-stage method described herein for producing ILC3 cells. In another embodiment, said lenalidomide, melphalan, and/or ILC3 cells

are administered separately from each other. In certain specific embodiments of the method of treating an individual with multiple myeloma, said ILC3 cells are produced by a method comprising: culturing hematopoietic stem cells or progenitor cells, *e.g.*, $CD34^+$ stem cells or progenitor cells, in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells, subsequently culturing said first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells, and subsequently culturing said second population of cells in a third medium comprising IL-2 and IL-15, and lacking a stem cell mobilizing agent and LMWH, to produce a third population of cells, wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, CD16– or CD16+, and CD94+ or CD94-, and wherein at least 70%, or at least 80%, of the natural killer cells are viable. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00447] In another embodiment, provided herein is a method of treating an individual having acute myelogenous leukemia (AML), comprising administering to the individual ILC3 cells (optionally activated by pretreatment with IL2 and IL12 and IL18, IL12 and IL15, IL12 and IL18, IL2 and IL12 and IL15 and IL18, or IL2 and IL15 and IL18), wherein said ILC3 cells are effective to treat AML in said individual. In a specific embodiment, the ILC3 cell population produced using the three-stage methods described herein has been pretreated with one or more of IL2, IL12, IL18, or IL15 prior to said administering. In a specific embodiment, said ILC3 cells are cord blood ILC3 cells, or ILC3 cells produced from cord blood hematopoietic cells, *e.g.*, hematopoietic stem cells. In another embodiment, said ILC3 cells have been produced by a three-stage method described herein for producing ILC3 cells. In certain specific embodiments of the method of treating an individual with AML, said ILC3 cells are produced by a three-stage method, as described herein. In a particular embodiment, the AML to be treated by the foregoing methods comprises refractory AML, poor-prognosis AML, or childhood AML. Methods known in the art for administering ILC3 cells for the treatment of refractory AML,

poor-prognosis AML, or childhood AML may be adapted for this purpose; see, *e.g.*, Miller et al., 2005, Blood 105:3051-3057; Rubnitz et al., 2010, J Clin Oncol. 28:955-959, each of which is incorporated herein by reference in its entirety. In certain embodiments, said individual has AML that has failed at least one non-natural killer cell therapeutic against AML. In specific embodiments, said individual is 65 years old or greater, and is in first remission. In specific embodiments, said individual has been conditioned with fludarabine, cytarabine, or both prior to administering said natural killer cells.

[00448] In other specific embodiments of the method of treating an individual with AML, said NK cells are produced by a method comprising: culturing hematopoietic stem cells or progenitor cells, *e.g.*, $CD34^+$ stem cells or progenitor cells, in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells, subsequently culturing said first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells, and subsequently culturing said second population of cells in a third medium comprising IL-2 and IL-15, and lacking a stem cell mobilizing agent and LMWH, to produce a third population of cells, wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, CD16– or CD16+, and CD94+ or CD94-, and wherein at least 70%, or at least 80%, of the natural killer cells are viable. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

[00449] In another embodiment, provided herein is a method of treating an individual having chronic lymphocytic leukemia (CLL), comprising administering to the individual a therapeutically effective dose of (1) lenalidomide; (2) melphalan; (3) fludarabine; and (4) NK cells, *e.g.*, NK cells produced by a three-stage method described herein, wherein said NK cells are effective to treat said CLL in said individual. In a specific embodiment, said NK cells are cord blood NK cells, or NK cells produced from cord blood hematopoietic stem cells. In another embodiment, said NK cells have been produced by a three-stage method described herein for

producing NK cells. In a specific embodiment of any of the above methods, said lenalidomide, melphalan, fludarabine, and expanded NK cells are administered to said individual separately. In certain specific embodiments of the method of treating an individual with CLL, said NK cells are produced by a method comprising: culturing hematopoietic stem cells or progenitor cells, *e.g.*, $CD34^+$ stem cells or progenitor cells, in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells, subsequently culturing said first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells, and subsequently culturing said second population of cells in a third medium comprising IL-2 and IL-15, and lacking a stem cell mobilizing agent and LMWH, to produce a third population of cells, wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, CD16– or CD16+, and CD94+ or CD94-, and wherein at least 70%, or at least 80%, of the natural killer cells are viable. In certain embodiments, said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α). In certain embodiments, said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L). In specific embodiments, said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L. In certain embodiments, none of the first medium, second medium or third medium comprises heparin, *e.g.*, low-molecular weight heparin.

5.11.2. Suppression of Tumor Cell Proliferation

[00450] Further provided herein is a method of suppressing the proliferation of tumor cells, comprising bringing NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, into proximity with the tumor cells, *e.g.*, contacting the tumor cells with NK cells produced using the methods described herein. A plurality of the NK cells can thus be used in the method of suppressing the proliferation of the tumor cells comprising bringing a therapeutically effective amount of the NK cell population into proximity with the tumor cells, *e.g.*, contacting the tumor cells with the cells in the NK cell population. Optionally, isolated placental perfusate or isolated placental perfusate cells is brought into proximity with the tumor cells and/or NK cells produced using the methods described herein. In another specific embodiment, an immunomodulatory compound, *e.g.*, an immunomodulatory compound described above, or thalidomide is additionally brought into proximity with the tumor cells and/or NK cells produced using the methods described herein,

such that proliferation of the tumor cells is detectably reduced compared to tumor cells of the same type not brought into proximity with NK cells produced using the methods described herein. Optionally, isolated placental perfusate or isolated placental perfusate cells are brought into proximity with the tumor cells and/or NK cells produced using the methods described herein that have been contacted or brought into proximity with an immunomodulatory compound.

[00451] Also provided herein is a method of suppressing the proliferation of tumor cells, comprising bringing ILC3 cells produced using the methods described herein, *e.g.*, ILC3 cell populations produced using the three-stage method described herein, into proximity with the tumor cells, *e.g.*, contacting the tumor cells with ILC3 cells produced using the methods described herein. A plurality of the ILC3 cells can thus be used in the method of suppressing the proliferation of the tumor cells comprising bringing a therapeutically effective amount of the ILC3 cell population into proximity with the tumor cells, *e.g.*, contacting the tumor cells with the cells in the ILC3 cell population. Optionally, isolated placental perfusate or isolated placental perfusate cells is brought into proximity with the tumor cells and/or ILC3 cells produced using the methods described herein. In another specific embodiment, an immunomodulatory compound, *e.g.*, an immunomodulatory compound described above, or thalidomide is additionally brought into proximity with the tumor cells and/or ILC3 cells produced using the methods described herein, such that proliferation of the tumor cells is detectably reduced compared to tumor cells of the same type not brought into proximity with ILC3 cells produced using the methods described herein. Optionally, isolated placental perfusate or isolated placental perfusate cells are brought into proximity with the tumor cells and/or ILC3 cells produced using the methods described herein that have been contacted or brought into proximity with an immunomodulatory compound.

[00452] As used herein, in certain embodiments, "contacting," or "bringing into proximity," with respect to cells, in one embodiment encompasses direct physical, *e.g.*, cell-cell, contact between placental perfusate, placental perfusate cells, natural killer cells, *e.g.*, NK cell populations produced according to the three-stage method described herein, ILC3 cells, *e.g.*, ILC3 cell populations produced according to the three-stage method described herein, and/or isolated combined natural killer cells and the tumor cells. In another embodiment, "contacting" encompasses presence in the same physical space, *e.g.*, placental perfusate, placental perfusate cells, natural killer cells, *e.g.*, placental intermediate natural killer cells, natural killer cells

described herein, *e.g.*, NK cell populations produced according to the three-stage method described herein, ILC3 cells described herein, *e.g.*, ILC3 cell populations produced according to the three-stage method described herein, and/or isolated combined natural killer cells are placed in the same container (*e.g.*, culture dish, multiwell plate) as tumor cells. In another embodiment, "contacting" placental perfusate, placental perfusate cells, combined natural killer cells, placental intermediate natural killer cells, or natural killer cells described herein, *e.g.*, NK cell populations produced according to the three-stage method described herein or ILC3 cells described herein, *e.g.*, ILC3 cell populations produced according to the three-stage method described herein, and tumor cells is accomplished, *e.g.*, by injecting or infusing the placental perfusate or cells, *e.g.*, placental perfusate cells, combined natural killer cells, natural killer cells, *e.g.*, placental intermediate natural killer cells, or ILC3 cells, into an individual, *e.g.*, a human comprising tumor cells, *e.g.*, a cancer patient. "Contacting," in the context of immunomodulatory compounds and/or thalidomide, means, *e.g.*, that the cells and the immunomodulatory compound and/or thalidomide are directly physically contacted with each other, or are placed within the same physical volume (*e.g.*, a cell culture container or an individual).

[00453] In a specific embodiment, the tumor cells are blood cancer cells, *e.g.*, leukemia cells or lymphoma cells. In more specific embodiments, the cancer is an acute leukemia, *e.g.*, acute T cell leukemia cells, acute myelogenous leukemia (AML) cells, acute promyelocytic leukemia cells, acute myeloblastic leukemia cells, acute megakaryoblastic leukemia cells, precursor B acute lymphoblastic leukemia cells, precursor T acute lymphoblastic leukemia cells, Burkitt's leukemia (Burkitt's lymphoma) cells, or acute biphenotypic leukemia cells; chronic leukemia cells, *e.g.*, chronic myeloid lymphoma cells, chronic myelogenous leukemia (CML) cells, chronic monocytic leukemia cells, chronic lymphocytic leukemia (CLL)/Small lymphocytic lymphoma cells, or B-cell prolymphocytic leukemia cells; hairy cell lymphoma cells; T-cell prolymphocytic leukemia cells; or lymphoma cells, *e.g.*, histiocytic lymphoma cells, lymphoplasmacytic lymphoma cells (*e.g.*, Waldenström macroglobulinemia cells), splenic marginal zone lymphoma cells, plasma cell neoplasm cells (*e.g.*, plasma cell myeloma cells, plasmacytoma cells, monoclonal immunoglobulin deposition disease, or a heavy chain disease), extranodal marginal zone B cell lymphoma (MALT lymphoma) cells, nodal marginal zone B cell lymphoma (NMZL) cells, follicular lymphoma cells, mantle cell lymphoma cells, diffuse large B cell lymphoma cells, mediastinal (thymic) large B cell lymphoma cells, intravascular large B cell

lymphoma cells, primary effusion lymphoma cells, T cell large granular lymphocytic leukemia cells, aggressive NK cell leukemia cells, adult T cell leukemia/lymphoma cells, extranodal NK/T cell lymphoma - nasal type cells, enteropathy-type T cell lymphoma cells, hepatosplenic T cell lymphoma cells, blastic NK cell lymphoma cells, mycosis fungoides (Sezary syndrome), primary cutaneous CD30-positive T cell lymphoproliferative disorder (*e.g.*, primary cutaneous anaplastic large cell lymphoma or lymphomatoid papulosis) cells, angioimmunoblastic T cell lymphoma cells, peripheral T cell lymphoma - unspecified cells, anaplastic large cell lymphoma cells, Hodgkin lymphoma cells or nodular lymphocyte-predominant Hodgkin lymphoma cells. In another specific embodiment, the tumor cells are multiple myeloma cells or myelodysplastic syndrome cells.

[00454] In specific embodiments, the tumor cells are solid tumor cells, *e.g.*, carcinoma cells, for example, adenocarcinoma cells, adrenocortical carcinoma cells, colon adenocarcinoma cells, colorectal adenocarcinoma cells, colorectal carcinoma cells, ductal cell carcinoma cells, lung carcinoma cells, thyroid carcinoma cells, nasopharyngeal carcinoma cells, melanoma cells (*e.g.*, malignant melanoma cells), non-melanoma skin carcinoma cells, or unspecified carcinoma cells; desmoid tumor cells; desmoplastic small round cell tumor cells; endocrine tumor cells; Ewing sarcoma cells; germ cell tumor cells (*e.g.*, testicular cancer cells, ovarian cancer cells, choriocarcinoma cells, endodermal sinus tumor cells, germinoma cells, *etc.*); hepatosblastoma cells; hepatocellular carcinoma cells; neuroblastoma cells; non-rhabdomyosarcoma soft tissue sarcoma cells; osteosarcoma cells; retinoblastoma cells; rhabdomyosarcoma cells; or Wilms tumor cells. In another embodiment, the tumor cells are pancreatic cancer cells or breast cancer cells. In other embodiments, the solid tumor cells are acoustic neuroma cells; astrocytoma cells (*e.g.*, grade I pilocytic astrocytoma cells, grade II low-grade astrocytoma cells; grade III anaplastic astrocytoma cells; or grade IV glioblastoma multiforme cells); chordoma cells; craniopharyngioma cells; glioma cells (*e.g.*, brain stem glioma cells; ependymoma cells; mixed glioma cells; optic nerve glioma cells; or subependymoma cells); glioblastoma cells; medulloblastoma cells; meningioma cells; metastatic brain tumor cells; oligodendroglioma cells; pineoblastoma cells; pituitary tumor cells; primitive neuroectodermal tumor cells; or schwannoma cells. In another embodiment, the tumor cells are prostate cancer cells.

[00455] As used herein, "therapeutically beneficial" and "therapeutic benefits" include, but are not limited to, *e.g.*, reduction in the size of a tumor; lessening or cessation of expansion

of a tumor; reducing or preventing metastatic disease; reduction in the number of cancer cells in a tissue sample, *e.g.*, a blood sample, per unit volume; the clinical improvement in any symptom of the particular cancer or tumor said individual has, the lessening or cessation of worsening of any symptom of the particular cancer the individual has, *etc.*

5.11.3. Treatment of cancers using NK cells and/or ILC3 cells and other anticancer agents

[00456] Treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, can be part of an anticancer therapy regimen that includes one or more other anticancer agents. Likewise, treatment of an individual having cancer using the ILC3 cells produced using the methods described herein, *e.g.*, ILC3 cell populations produced using the three-stage method described herein, can be part of an anticancer therapy regimen that includes one or more other anticancer agents. In addition or alternatively, treatment of an individual having cancer using the NK cells and/or ILC3 cells produced using the methods described herein can be used to supplement an anticancer therapy that includes one or more other anticancer agents. Such anticancer agents are well-known in the art and include anti-inflammatory agents, immumodulatory agents, cytotoxic agents, cancer vaccines, chemotherapeutics, HDAC inhibitors (*e.g.*, HDAC6i (ACY-241)), and siRNAs. Specific anticancer agents that may be administered to an individual having cancer, *e.g.*, an individual having tumor cells, in addition to the NK cells produced using the methods described herein and optionally perfusate, perfusate cells, natural killer cells other than NK cells produced using the methods described herein include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; adriamycin; adrucil; aldesleukin; altretamine; ambomycin; ametantrone acetate; amsacrine; anastrozole; anthramycin; asparaginase (*e.g.*, from Erwinia chrysan; Erwinaze); asperlin; avastin (bevacizumab); azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; celecoxib (COX-2 inhibitor); Cerubidine; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel;

doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflomithine hydrochloride; elsamitrucin; Elspar; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; Etopophos; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; Idamycin; idarubicin hydrochloride; ifosfamide; ilmofosine; iproplatin; irinotecan; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; Proleukin; Purinethol; puromycin; puromycin hydrochloride; pyrazofurin; Rheumatrex; riboprine; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; Tabloid; talisomycin; tecogalan sodium; taxotere; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; Toposar; toremifene citrate; trestolone acetate; Trexall; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; and zorubicin hydrochloride.

[00457] Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-azacytidine; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic

protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptosar (also called Campto; irinotecan) camptothecin derivatives; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; CC-122; CC-220; CC-486; cecropin B; cetrorelix; chlorlns; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidenmin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; doxorubicin; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine (*e.g.*, Fludara); fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; homoharringtonine (HHT, omacetaxine mepesuccinate); hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imatinib (*e.g.*, GLEEVEC®), imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin;

lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide + estrogen + progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; anti-EGFR antibody (*e.g.*, Erbitux (cetuximab)); anti-CD19 antibody; anti-CD20 antibody (*e.g.*, rituximab); anti-CS-1 antibody (*e.g.*, elotuzumab (BMS/AbbVie)); anti-CD38 antiobdy (*e.g.*, daratumumab (Genmab/Janssen Biotech); anti-CD138 antibody (*e.g.*, indatuximab (Biotest AG Dreieich)); anti-PD-1 antibody; anti- PD-L1 antibody (*e.g.*, durvalumab (AstraZeneca)); anti-NKG2A antibody (*e.g.*, monalizumab (IPH2201; Innate Pharma)); anti-DLL4 antibody (e.g., demcizumab (Oncomed/Celgene)); anti-DLL4 and anti-VEGF bispecific antibody; anti-RSPO3 antibody; anti-TIGIT antibody; ICOS agonist antibody; anti-disialoganglioside (GD2) antibody (*e.g.*, monoclonal antibody 3F8 or ch14.18); anti-ErbB2 antibody (*e.g.*, herceptin); human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; oblimersen (GENASENSE®); O^6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin (*e.g.*, Floxatin); oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based

immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; Vectibix (panitumumab)velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; Welcovorin (leucovorin); Xeloda (capecitabine); zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

[00458] Treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, can be part of an anticancer therapy regimen that includes one or more immune checkpoint modulator. In certain embodiments, the immune checkpoint modulator modulates an immune checkpoint molecule such as CD28, OX40, Glucocorticoid-Induced Tumour-necrosis factor Receptor-related protein (GITR), CD137 (4-1BB), CD27, Herpes Virus Entry Mediator (HVEM), T cell Immunoglobulin and Mucin-domain containing-3 (TIM-3), Lymphocyte-Activation Gene 3 (LAG-3), Cytotoxic T-Lymphocyte-associated Antigen-4 (CTLA-4), V-domain Immunoglobulin Suppressor of T cell Activation (VISTA), B and T Lymphocyte

Attenuator (BTLA), PD-1, and/or PD-L1. In certain embodiments, the immune checkpoint molecule is an antibody or antigen-binding fragment thereof.

[00459] In certain embodiments, the immune checkpoint modulator is an agonist of an immune checkpoint molecule. In certain embodiments, the immune checkpoint molecule is CD28, OX40, Glucocorticoid-Induced Tumour-necrosis factor Receptor-related protein (GITR), CD137 (4-1BB), CD27, ICOS (CD278); Inducible T-cell Costimulator) and/or Herpes Virus Entry Mediator (HVEM). In certain embodiments, the immune checkpoint modulator is an antibody or antigen-binding fragment thereof.

[00460] In certain embodiments, the immune checkpoint modulator is an antagonist of an immune checkpoint molecule. In certain embodiments, the immune checkpoint molecule is T cell Immunoglobulin and Mucin-domain containing-3 (TIM-3), Lymphocyte-Activation Gene 3 (LAG-3), Cytotoxic T-Lymphocyte-associated Antigen-4 (CTLA-4), V-domain Immunoglobulin Suppressor of T cell Activation (VISTA), B and T Lymphocyte Attenuator (BTLA), PD-1, and/or PD-L1. In certain embodiments, the immune checkpoint modulator is an antibody or antigen-binding fragment thereof.

[00461] In certain embodiments, the immune checkpoint modulator is an antibody or antigen-binding fragment thereof. In certain embodiments, the antibody or antibody-binding fragment thereof binds PD-1. In certain embodiments, the antibody or antibody-binding fragment thereof that binds PD-1 is nivolumab (OPDIVO®, BMS-936558, MDX-1106, ONO-4538; Bristol-Myers Squibb, Ono Pharmaceuticals, Inc.), pembrolizumab (KEYTRUDA®, lambrolizumab, MK-3475; Merck), pidilizumab (CT-011; Curetech, Medivation); MEDI0680 (AMP-514; MedImmune, AstraZeneca); PDR-001 (Novartis), SHR1210, or INCSHR1210; Incyte, Jiangsu Hengrui). In certain embodiments, the antibody or antigen-binding fragment thereof binds PD-L1. In certain embodiments, the antibody or antigen-binding fragment thereof that binds PD-L1 is durvalumab (MEDI4736; MedImmune, AstraZeneca), BMS-936559 (MDX-1105; Bristol-Myers Squibb), avelumab (MSB0010718C; Merck Serono, Pfizer), or atezolizumab (MPDL-3280A; Genentech, Roche). In certain embodiments, the antibody or antibody-binding fragment thereof binds LAG-3. In certain embodiments, the antibody or antibody-binding fragment thereof that binds LAG-3 is BMS-986016 (Bristol-Myers Squibb), GSK2831781 (GlaxoSmithKline), or LAG525 (Novartis). In certain embodiments, the antibody or antibody-binding fragment thereof binds CTLA-4. In certain embodiments, the antibody or

antibody-binding fragment thereof that binds CTLA-4 is ipilimumab (YERVOY™, BMS-734016, MDX010, MDX-101; Bristol-Myers Squibb), or tremelimumab (CP-675,206; MedImmune, AstraZeneca). In certain embodiments, the antibody or antibody-binding fragment thereof binds OX40. In certain embodiments, the antibody or antibody-binding fragment thereof that binds OX40 is MEDI6469 (MedImmune, AstraZeneca), MEDI0562 (MedImmune, AstraZeneca), or KHK4083 (Kyowa Hakko Kirin). In certain embodiments, the antibody or antibody-binding fragment thereof binds GITR. In certain embodiments, the antibody or antibody-binding fragment thereof that binds GITR is TRX518 (Leap Therapeutics) or MEDI1873 (MedImmune, AstraZeneca). In certain embodiments, the antibody or antibody-binding fragment thereof binds CD137 (4-1BB). In certain embodiments, the antibody or antibody-binding fragment thereof that binds CD137 (4-1BB) is PF-2566 (PF-05082566; Pfizer), or urelumab (BMS-663513; Bristol-Myers Squibb). In certain embodiments, the antibody or antibody-binding fragment thereof binds CD27. In certain embodiments, the antibody or antibody-binding fragment thereof that binds CD27 is varilumab (CDX-1127; Celldex Therapies).

[00462] In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes lenalidomide or pomalidomide. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an HDAC inhibitor. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an anti-CS-1 antibody. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an anti-CD38 antibody. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an anti-CD138 antibody.

In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an anti-PD-1 antibody. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an anti-PD-L1 antibody. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an anti-NKG2A antibody. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an anti-CD20 antibody (*e.g.*, rituximab; RITUXAN®). In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes CC-122. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes CC-220. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an anti-DLL4 antibody (*e.g.*, demcizumab). In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an anti-DLL4 and anti-VEGF bispecific antibody. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an anti-RSPO3 antibody. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described

herein, is part of an anticancer therapy regimen that includes an anti-TIGIT antibody. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes an ICOS agonist antibody. In certain embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein, is part of an anticancer therapy regimen that includes homoharringtonine (*e.g.*, omacetaxine mepesuccinate).

[00463] In some embodiments, treatment of an individual having cancer using the NK cells produced using the methods described herein is part of an anticancer therapy regimen for antibody-dependent cell-mediated cytotoxicity (ADCC). In some embodiments, treatment of an individual having cancer using the ILC3 cells produced using the methods described herein is part of an anticancer therapy regimen for antibody-dependent cell-mediated cytotoxicity (ADCC). In one embodiment, the ADCC regimen comprises administration of one or more antibodies (*e.g.*, an antibody described in the foregoing paragraph) in combination with NK cells and/or ILC3 cells produced using the methods described herein. Several types of cancer can be treated using such ADCC methods, including but not limited to acute lymphoblastic leukemia (ALL) or other B-cell malignancies (lymphomas and leukemias), neuroblastoma, melanoma, breast cancers, and head and neck cancers. In specific embodiments, the ADCC therapy comprises administration of one or more of the following antibodies anti-EGFR antibody (*e.g.*, Erbitux (cetuximab)), anti-CD19 antibody, anti-CD20 antibody (*e.g.*, rituximab), anti-disialoganglioside (GD2) antibody (*e.g.*, monoclonal antibody 3F8 or ch14.18), or anti-ErbB2 antibody (*e.g.*, herceptin), in combination with NK cells and/or ILC3 cells produced using the methods described herein. In one embodiment, the ADCC regimen comprises administration of an anti-CD33 antibody in combination with NK cells and/or ILC3 cells produced using the methods described herein. In one embodiment, the ADCC regimen comprises administration of an anti-CD20 antibody in combination with NK cells and/or ILC3 cells produced using the methods described herein. In one embodiment, the ADCC regimen comprises administration of an anti-CD138 antibody in combination with NK cells and/or ILC3 cells produced using the methods described herein. In one embodiment, the ADCC regimen comprises administration of an anti-CD32 antibody in combination with NK cells and/or ILC3 cells produced using the methods described herein.

5.11.4. Treatment of Viral Infection

[00464] In another embodiment, provided herein is a method of treating an individual having a viral infection, comprising administering to said individual a therapeutically effective amount of NK cells produced using the methods described herein, *e.g.*, NK cell populations produced using the three-stage method described herein. In another embodiment, provided herein is a method of treating an individual having a viral infection, comprising administering to said individual a therapeutically effective amount of ILC3 cells produced using the methods described herein, *e.g.*, ILC3 cell populations produced using the three-stage method described herein. In certain embodiments, the individual has a deficiency of natural killer cells, *e.g.*, a deficiency of NK cells or other innate lymphoid cells active against the individual's viral infection. In certain specific embodiments, said administering additionally comprises administering to the individual one or more of isolated placental perfusate, isolated placental perfusate cells, isolated natural killer cells, *e.g.*, placental natural killer cells, *e.g.*, placenta-derived intermediate natural killer cells, isolated combined natural killer cells, and/or combinations thereof. In certain specific embodiments, the NK cells and/or ILC3 cells produced using the methods described herein are contacted or brought into proximity with an immunomodulatory compound, *e.g.*, an immunomodulatory compound above, or thalidomide, prior to said administration. In certain other specific embodiments, said administering comprises administering an immunomodulatory compound, *e.g.*, an immunomodulatory compound described above, or thalidomide, to said individual in addition to said NK cells and/or ILC3 cells produced using the methods described herein, wherein said amount is an amount that, *e.g.*, results in a detectable improvement of, lessening of the progression of, or elimination of, one or more symptoms of said viral infection. In specific embodiments, the viral infection is an infection by a virus of the Adenoviridae, Picornaviridae, Herpesviridae, Hepadnaviridae, Flaviviridae, Retroviridae, Orthomyxoviridae, Paramyxoviridae, Papilommaviridae, Rhabdoviridae, or Togaviridae family. In more specific embodiments, said virus is human immunodeficiency virus (HIV).coxsackievirus, hepatitis A virus (HAV), poliovirus, Epstein-Barr virus (EBV), herpes simplex type 1 (HSV1), herpes simplex type 2 (HSV2), human cytomegalovirus (CMV), human herpesvirus type 8 (HHV8), herpes zoster virus (varicella zoster virus (VZV) or shingles virus), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV), influenza virus (*e.g.*, influenza A virus, influenza B virus,

influenza C virus, or thogotovirus), measles virus, mumps virus, parainfluenza virus, papillomavirus, rabies virus, or rubella virus.

[00465] In other more specific embodiments, said virus is adenovirus species A, serotype 12, 18, or 31; adenovirus species B, serotype 3, 7, 11, 14, 16, 34, 35, or 50; adenovirus species C, serotype 1, 2, 5, or 6; species D, serotype 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36, 37, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49, or 51; species E, serotype 4; or species F, serotype 40 or 41.

[00466] In certain other more specific embodiments, the virus is Apoi virus (APOIV), Aroa virus (AROAV), bagaza virus (BAGV), Banzi virus (BANV), Bouboui virus (BOUV), Cacipacore virus (CPCV), Carey Island virus (CIV), Cowbone Ridge virus (CRV), Dengue virus (DENV), Edge Hill virus (EHV), Gadgets Gully virus (GGYV), Ilheus virus (ILHV), Israel turkey meningoencephalomyelitis virus (ITV), Japanese encephalitis virus (JEV), Jugra virus (JUGV), Jutiapa virus (JUTV), kadam virus (KADV), Kedougou virus (KEDV), Kokobera virus (KOKV), Koutango virus (KOUV), Kyasanur Forest disease virus (KFDV), Langat virus (LGTV), Meaban virus (MEAV), Modoc virus (MODV), Montana myotis leukoencephalitis virus (MMLV), Murray Valley encephalitis virus (MVEV), Ntaya virus (NTAV), Omsk hemorrhagic fever virus (OHFV), Powassan virus (POWV), Rio Bravo virus (RBV), Royal Farm virus (RFV), Saboya virus (SABV), St. Louis encephalitis virus (SLEV), Sal Vieja virus (SVV), San Perlita virus (SPV), Saumarez Reef virus (SREV), Sepik virus (SEPV), Tembusu virus (TMUV), tick-borne encephalitis virus (TBEV), Tyuleniy virus (TYUV), Uganda S virus (UGSV), Usutu virus (USUV), Wesselsbron virus (WESSV), West Nile virus (WNV), Yaounde virus (YAOV), Yellow fever virus (YFV), Yokose virus (YOKV), or Zika virus (ZIKV).

[00467] In other embodiments, the NK cells produced using the methods described herein, and optionally placental perfusate and/or perfusate cells, are administered to an individual having a viral infection as part of an antiviral therapy regimen that includes one or more other antiviral agents. Specific antiviral agents that may be administered to an individual having a viral infection include, but are not limited to: imiquimod, podofilox, podophyllin, interferon alpha (IFNα), reticolos, nonoxynol-9, acyclovir, famciclovir, valaciclovir, ganciclovir, cidofovir; amantadine, rimantadine; ribavirin; zanamavir and oseltaumavir; protease inhibitors such as indinavir, nelfinavir, ritonavir, or saquinavir; nucleoside reverse transcriptase inhibitors such as

didanosine, lamivudine, stavudine, zalcitabine, or zidovudine; and non-nucleoside reverse transcriptase inhibitors such as nevirapine, or efavirenz.

5.11.5. Other Treatment Uses for ILC3 cells

[00468] Provided herein are ILC3 cells that can be used in all the methods as providedherein. Exemplary methods in which ILC3 cells can be used are disclosed in the following aspects.

[00469] In another aspect, provided herein is a method of repairing the gastrointestinal tract after chemotherapy comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells are by a three-stage method described herein.

[00470] In another aspect, provided herein is a method of protecting an individual against radiation comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells are produced by a three-stage method described herein. In certain aspects, said ILC3 cells are used as an adjunct to bone marrow transplantation.

[00471] In another aspect, provided herein is a method of reconstituting the thymus of an individual comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells are produced by a three-stage method described herein.

[00472] In another aspect, provided herein is a method of promoting protective immunity to pathogens in an individual comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells are produced by a three-stage method described herein. In certain aspects, promoting protective immunity to pathogens is performed to treat intestinal infection. In certain aspects, promoting protective immunity to pathogens is performed to prevent intestinal infection. In certain aspects, the intestinal infection is *Citrobacter rodentium*.

[00473] In another aspect, provided herein is a method of tumor rejection comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells have been produced by a three-stage method described herein.

[00474] In another aspect, provided herein is a method of maintaining tissue integrity during organogenesis comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells have been produced by a three-stage method described herein.

[00475] In another aspect, provided herein is a method of tissue repair comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells have been produced by a three-stage method described herein.

[00476] In another aspect, provided herein is a method of regulation of inflammation comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells have been produced by a three-stage method described herein.

5.11.6. Administration

[00477] Determination of the number of cells, *e.g.*, placental perfusate cells, *e.g.*, nucleated cells from placental perfusate, combined natural killer cells, ILC3 cells, and/or isolated natural killer cells, *e.g.*, NK cell populations produced using the three-stage method described herein, and determination of the amount of an immunomodulatory compound, *e.g.*, an immunomodulatory compound, or thalidomide, can be performed independently of each other.

[00478] Administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof may be systemic or local. In specific embodiments, administration is parenteral. In specific embodiments, administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In specific embodiments, administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is performed with a device, a matrix, or a scaffold. In specific embodiments, administration an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is by injection. In specific embodiments, administration an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is via a catheter. In specific embodiments, the injection of NK cells and/or ILC3 cells is local injection. In more specific embodiments, the local injection is directly into a solid tumor (*e.g.*, a sarcoma). In specific embodiments, administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is by injection by syringe. In specific embodiments, administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject is via guided delivery. In specific embodiments, administration of an isolated population of NK cells and/or ILC3 cells or a pharmaceutical composition thereof to a subject by injection is aided by laparoscopy, endoscopy, ultrasound, computed tomography, magnetic resonance, or radiology.

5.11.6.1. Administration of Cells

[00479] In certain embodiments, NK cells and/or ILC3 cells produced using the methods described herein, *e.g.*, NK cell and/or ILC3 cell populations produced using the three-stage method described herein, are used, *e.g.*, administered to an individual, in any amount or number that results in a detectable therapeutic benefit to the individual, *e.g.*, an effective amount, wherein the individual has a viral infection, cancer, or tumor cells, for example, an individual having tumor cells, a solid tumor or a blood cancer, *e.g.*, a cancer patient. Such cells can be administered to such an individual by absolute numbers of cells, *e.g.*, said individual can be administered at about, at least about, or at most about, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 5×10^{10}, or 1×10^{11} NK cells and/or ILC3 cells produced using the methods described herein. In other embodiments, NK cells and/or ILC3 cells produced using the methods described herein can be administered to such an individual by relative numbers of cells, *e.g.*, said individual can be administered at about, at least about, or at most about, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 5×10^{10}, or 1×10^{11} NK cells and/or ILC3 cells produced using the methods described herein per kilogram of the individual. In other embodiments, NK cells and/or ILC3 cells produced using the methods described herein can be administered to such an individual by relative numbers of cells, *e.g.*, said individual can be administered at about, at least about, or at most about, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, or 5×10^8 NK cells and/or ILC3 cells produced using the methods described herein per kilogram of the individual. NK cells and/or ILC3 cells produced using the methods described herein can be administered to such an individual according to an approximate ratio between a number of NK cells and/or ILC3 cells produced using the methods described herein, and optionally placental perfusate cells and/or natural killer cells other than NK cells and/or ILC3 cells produced using the methods described herein, and a number of tumor cells in said individual (*e.g.*, an estimated number). For example, NK cells and/or ILC3 cells produced using the methods described herein can be administered to said individual in a ratio of about, at least about or at most about 1:1, 1:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1 or 100:1 to the number of tumor cells in the individual. The number of tumor cells in such an individual can be estimated, *e.g.*, by counting the number of tumor cells in a sample of tissue from the individual, *e.g.*, blood sample, biopsy, or the like. In specific

embodiments, *e.g.*, for solid tumors, said counting is performed in combination with imaging of the tumor or tumors to obtain an approximate tumor volume. In a specific embodiment, an immunomodulatory compound or thalidomide, *e.g.*, an effective amount of an immunomodulatory compound or thalidomide, are administered to the individual in addition to the NK cells and/or ILC3 cells produced using the methods described herein, optionally placental perfusate cells and/or natural killer cells other than NK cells and/or ILC3 cells produced using the methods described herein.

[00480] In certain embodiments, the method of suppressing the proliferation of tumor cells, *e.g.*, in an individual; treatment of an individual having a deficiency in the individual's natural killer cells; or treatment of an individual having a viral infection; or treatment of an individual having cancer, *e.g.*, an individual having tumor cells, a blood cancer or a solid tumor, comprises bringing the tumor cells into proximity with, or administering to said individual, a combination of NK cells and/or ILC3 cells produced using the methods described herein and one or more of placental perfusate and/or placental perfusate cells. In specific embodiments, the method additionally comprises bringing the tumor cells into proximity with, or administering to the individual, an immunomodulatory compound or thalidomide.

[00481] In a specific embodiment, for example, treatment of an individual having a deficiency in the individual's natural killer cells (*e.g.*, a deficiency in the number of NK cells or in the NK cells' reactivity to a cancer, tumor or virally-infected cells); or treatment of an individual having a cancer or a viral infection, or suppression of tumor cell proliferation, comprises bringing said tumor cells into proximity with, or administering to said individual, NK cells and/or ILC3 cells produced using the methods described herein supplemented with isolated placental perfusate cells or placental perfusate. In specific embodiments, about 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8 or more NK cells produced using the methods described herein per milliliter, or 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 5×10^{10}, 1×10^{11} or more NK cells produced using the methods described herein are supplemented with about, or at least about, 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8 or more isolated placental perfusate cells per milliliter, or 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 5×10^{10}, 1×10^{11} or more isolated placental perfusate cells. In other more specific embodiments, about 1×10^4, 5×10^4, 1

x 10^5, 5 x 10^5, 1 x 10^6, 5 x 10^6, 1 x 10^7, 5 x 10^7, 1 x 10^8, 5 x 10^8 or more NK cells produced using the methods described herein or 1 x 10^4, 5 x 10^4, 1 x 10^5, 5 x 10^5, 1 x 10^6, 5 x 10^6, 1 x 10^7, 5 x 10^7, 1 x 10^8, 5 x 10^8, 1 x 10^9, 5 x 10^9, 1 x 10^{10}, 5 x 10^{10}, 1 x 10^{11} or more NK cells produced using the methods described herein are supplemented with about, or at least about, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 mL of perfusate, or about 1 unit of perfusate.

[00482] In another specific embodiment, treatment of an individual having a deficiency in the individual's natural killer cells; treatment of an individual having cancer; treatment of an individual having a viral infection; or suppression of tumor cell proliferation, comprises bringing the tumor cells into proximity with, or administering to the individual, NK cells and/or ILC3 cells produced using the methods described herein, wherein said cells are supplemented with adherent placental cells, *e.g.*, adherent placental stem cells or multipotent cells, *e.g.*, $CD34^-$, $CD10^+$, $CD105^+$, $CD200^+$ tissue culture plastic-adherent placental cells. In specific embodiments, the NK cells and/or ILC3 cells produced using the methods described herein are supplemented with about 1 x 10^4, 5 x 10^4, 1 x 10^5, 5 x 10^5, 1 x 10^6, 5 x 10^6, 1 x 10^7, 5 x 10^7, 1 x 10^8, 5 x 10^8 or more adherent placental stem cells per milliliter, or 1 x 10^4, 5 x 10^4, 1 x 10^5, 5 x 10^5, 1 x 10^6, 5 x 10^6, 1 x 10^7, 5 x 10^7, 1 x 10^8, 5 x 10^8, 1 x 10^9, 5 x 10^9, 1 x 10^{10}, 5 x 10^{10}, 1 x 10^{11} or more adherent placental cells, *e.g.*, adherent placental stem cells or multipotent cells.

[00483] In another specific embodiment, treatment of an individual having a deficiency in the individual's natural killer cells; treatment of an individual having cancer; treatment of an individual having a viral infection; or suppression of tumor cell proliferation, is performed using an immunomodulatory compound or thalidomide in combination with NK cells and/or ILC3 cells produced using the methods described herein, wherein said cells are supplemented with conditioned medium, *e.g.*, medium conditioned by $CD34^-$, $CD10^+$, $CD105^+$, $CD200^+$ tissue culture plastic-adherent placental cells, *e.g.*, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.1, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mL of stem cell-conditioned culture medium per unit of perfusate, or per 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, or 10^{11} NK cells and/or ILC3 cells produced using the methods described herein. In certain embodiments, the tissue culture plastic-adherent placental cells are the multipotent adherent placental cells described in U.S. Patent Nos. 7,468,276 and8,057,788, the disclosures of which are incorporated herein by reference in their entireties. In another specific

embodiment, the method additionally comprises bringing the tumor cells into proximity with, or administering to the individual, an immunomodulatory compound or thalidomide.

[00484] In another specific embodiment, treatment of an individual having a deficiency in the individual's natural killer cells; treatment of an individual having cancer; treatment of an individual having a viral infection; or suppression of tumor cell proliferation, in which said NK cells and/or ILC3 cells produced using the methods described herein are supplemented with placental perfusate cells, the perfusate cells are brought into proximity with interleukin-2 (IL-2) for a period of time prior to said bringing into proximity. In certain embodiments, said period of time is about, at least, or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46 or 48 hours prior to said bringing into proximity.

[00485] The NK cells and/or ILC3 cells produced using the methods described herein and optionally perfusate or perfusate cells, can be administered once to an individual having a viral infection, an individual having cancer, or an individual having tumor cells, during a course of anticancer therapy; or can be administered multiple times, *e.g.*, once every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 hours, or once every 1, 2, 3, 4, 5, 6 or 7 days, or once every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 24, 36 or more weeks during therapy. In embodiments in which cells and an immunomodulatory compound or thalidomide are used, the immunomodulatory compound or thalidomide, and cells or perfusate, can be administered to the individual together, *e.g.*, in the same formulation; separately, *e.g.*, in separate formulations, at approximately the same time; or can be administered separately, *e.g.*, on different dosing schedules or at different times of the day. Similarly, in embodiments in which cells and an antiviral compound or anticancer compound are used, the antiviral compound or anticancer compound, and cells or perfusate, can be administered to the individual together, *e.g.*, in the same formulation; separately, *e.g.*, in separate formulations, at approximately the same time; or can be administered separately, *e.g.*, on different dosing schedules or at different times of the day. The NK cells and/or ILC3 cells produced using the methods described herein and perfusate or perfusate cells, can be administered without regard to whether NK cells and/or ILC3 cells produced using the methods described herein, perfusate, or perfusate cells have been administered to the individual in the past.

6. KITS

[00486] Provided herein is a pharmaceutical pack or kit comprising one or more containers filled with one or more of the compositions described herein, *e.g.*, a composition comprising NK cells and/or ILC3 cells produced by a method described herein, *e.g.*, NK cell and/or ILC3 cell populations produced using the three-stage method described herein. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00487] The kits encompassed herein can be used in accordance with the methods described herein, *e.g.*, methods of suppressing the growth of tumor cells and/or methods of treating cancer, *e.g.*, hematologic cancer, and/or methods of treating viral infection. In one embodiment, a kit comprises NK cells and/or ILC3 cells produced by a method described herein or a composition thereof, in one or more containers. In a specific embodiment, provided herein is a kit comprising an NK cell and/or ILC3 cell population produced by a three-stage method described herein, or a composition thereof.

7. EXAMPLES

7.1. Example 1: Three-stage method of producing natural killer cells from hematopoietic stem or progenitor cells

[00488] $CD34^+$ cells are cultured in the following medium formulations for the indicated number of days, and aliquots of cells are taken for assessment of cell count, cell viability, characterization of natural killer cell differentiation and functional evaluation.

[00489] Stage 1 medium: 90% Stem Cell Growth Medium (SCGM) (CellGro®), 10% Human Serum-AB, supplemented with 4.5 U/mL low molecular weight heparin (LMWH), 25 ng/mL recombinant human thrombopoietin (TPO), 25 ng/mL recombinant human Flt3L, 27 ng/mL recombinant human stem cell factor (SCF), 25 ng/mL recombinant human IL-7, 0.05 ng/mL recombinant human IL-6 (500-fold), 0.25 ng/mL recombinant human granulocyte colony-stimulating factor (G-CSF) (50-fold), 0.01 ng/mL recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) (500-fold), 0.10% gentamicin, and 1 to 10μm StemRegenin-1 (SR-1).

[00490] Stage 2 medium: 90% SCGM, 10% Human Serum-AB, supplemented with 4.5 U/mL low molecular weight heparin (LMWH), 25 ng/mL recombinant human Flt3L, 27 ng/mL recombinant human SCF, 25 ng/mL recombinant human IL-7, 20 ng/mL recombinant human IL-15, 0.05 ng/mL recombinant human IL-6 (500-fold), 0.25 ng/mL recombinant human G-CSF (50-fold), 0.01 ng/mL recombinant human GM-CSF (500-fold), 0.10% gentamicin, and 1 to 10μm SR1.

[00491] Stage 3 medium: 90% STEMMACS™, 10% Human Serum-AB, 0.025 mM 2-mercaptoethanol (55 mM), supplemented with 22 ng/mL recombinant human SCF, 1000 U/mL recombinant human IL-2, 20 ng/mL recombinant human IL-7, 20 ng/mL recombinant human IL-15, 0.05 ng/mL recombinant human IL-6 (500-fold), 0.25 ng/mL recombinant human G-CSF (50-fold), 0.01 ng/mL recombinant human GM-CSF (500-fold), and 0.10% gentamicin.

[00492] Cells are seeded at Day 0 at 3×10^4 cells/mL in Stage 1 media, and cells are tested for purity by a CD34+ and CD45+ count and viability by 7AAD staining. At Day 5 cells are counted and seeded to a concentration of 1×10^5 cells/mL with Stage 1 medium. At Day 7 cells are counted and seeded to a concentration of 1×10^5 cells/mL with Stage 1 medium.

[00493] At Day 10, cells are counted and seeded to a concentration of 1×10^5 cells/mL in Stage 2 medium. At Day 12, cells are counted and seeded to a concentration of 3×10^5 cells/mL in Stage 2 medium.

[00494] Alternatively, the following protocol is used through Day 14: Cells seeded at Day 0 at 7.5×10^3 cells/mL in Stage 1 media, and cells are tested for purity by a CD34+ and CD45+ count and viability by 7AAD staining. At Day 7 cells are counted and seeded to a concentration of 3×10^5 cells/mL with Stage 1 medium. At Day 9 cells are counted and seeded to a concentration of 3×10^5 cells/mL with Stage 2 medium. At Day 12, cells are counted and seeded to a concentration of 3×10^5 cells/mL in Stage 2 medium.

[00495] For dynamic differentiation in spinner flasks, at Day 14, cells are centrifuged to concentrate, counted and seeded to a concentration of 5×10^5 cells/mL in Stage 3 medium. At Day 17, cells are centrifuged, counted and seeded to a concentration of 7.5×10^5 cells/mL in Stage 3 medium. At Day 21, cells are centrifuged, counted, phenotyped for CD56, CD3, CD16, and CD94, assayed for viability by 7AAD staining, and seeded to a concentration of 1×10^6 cells/mL in Stage 3 medium. At Day 24, cells are counted and seeded to a concentration of 1×10^6 cells/mL in Stage 3 medium. From Days 25 to 27, volume is added at 5 mL per day of Stage 3

medium. At Day 28, cells are counted and seeded to a concentration of 1×10^6 cells/mL in Stage 3 medium. At Day 31, cells are counted and seeded to a concentration of 1×10^6 cells/mL in Stage 3 medium. From Days 32 to 34, volume is added at 5 mL per day of Stage 3 medium. At Day 35, cells are harvested, counted, phenotyped, and assayed for cytotoxicity.

[00496] For static differentiation, at Day 14, cells are centrifuged to concentrate, counted and seeded to a concentration of 3×10^5 cells/mL in Stage 3 medium. At Day 17, cells are counted and seeded to a concentration of 3×10^5 cells/mL in Stage 3 medium. At Day 19, cells are counted and seeded to a concentration of 3×10^5 cells/mL in Stage 3 medium. At Day 21, cells are centrifuged, counted, phenotyped for CD56, CD3, CD16, and CD94, assayed for viability by 7AAD staining, and seeded to a concentration of 5×10^6 cells/mL in Stage 3 medium. At Day 24, cells are centrifuged, counted and seeded to a concentration of 7.5×10^6 cells/mL in Stage 3 medium. At Day 26, cells are counted and seeded to a concentration of 7.5×10^6 cells/mL in Stage 3 medium. At Day 28, cells are counted and seeded to a concentration of 1×10^6 cells/mL in Stage 3 medium. At Day 31, cells are centrifuged, counted and seeded to a concentration of 1×10^6 cells/mL in Stage 3 medium. At Day 33, cells are centrifuged, counted and seeded to a concentration of 1×10^6 cells/mL in Stage 3 medium. At Day 35, cells are harvested, counted, phenotyped, and assayed for cytotoxicity.

[00497] For harvest, cells are spun at $400\times g$ for seven minutes, followed by suspension of the pellet in an equal volume of Plasmalyte A. The suspension is spun at $400\times g$ for seven minutes, and the resulting pellet is suspended in 10% HSA (w/v), 60% Plasmalyte A (v/v) at the target cell concentration. The cells are then strained through a 70 μm mesh, the final container is filled, an aliquot of the cells are tested for viability, cytotoxicity, purity, and cell count, and the remainder is packaged.

7.2. Example 2: Evaluation of concentration of SR-1 and CH223191 in three-stage method

[00498] Stemregenin-1 (SR-1) was evaluated as a component of Stage 1 and Stage 2 media using the three-stage method outlined in Example 1, above, at concentrations of 1 μM, 10 μM, and 30 μM. The same concentrations of CH223191 in the three-stage method were also evaluated. SR-1 at 10 μM resulted in a higher cytotoxicity than the other two concentrations tested. Comparable effects on fold expansion, cell purity (CD56+CD3-), and cytotoxicity of K562 cells at a 10:1 (E:T) ratio were observed for SR-1 and CH223191 at both 10 μM and 1 μM

concentrations (Figures 1A-C). Both SR-1 and CH223191 also showed similar effects and trends regarding Day 7 and Day 14 expression of CD34.

7.3. Example 3: Characterization of three-stage NK cells

[00499] **METHODS**

[00500] UCB CD34+ cells were cultivated in presence of cytokines including thrombopoietin, SCF, Flt3 ligand, IL-7, IL-15 and IL-2 for 35 days to produce three-stage NK cells, as described in Example 1. Multi-color flow cytometry was used to determine the phenotypic characteristics of three-stage NK cells. Eleven 6-marker panels utilizing 35 NK subtype and other surface markers (*see* Table 1) were evaluated.

[00501] Table 1. Surface markers, including 35 NK subtype surface markers, used to evaluate phenotypic characteristics of three-stage NK cells.

SURFACE MARKERS			
CD16	NKB1 (KIR3DL1)	NKG2C	CD7
CD56	KIR2DL3	CCR5 (CD195)	NKp80
7AAD	CD11a	CXCR3 (CD183)	CD44
CD94	CD122 (IL-2Rb)	NKp30	CD85j (LIR1, ILT2)
NKp46	CD62L	NKG2A	CRACC
CD3	CD117	CD2	CD14
KIR2DL4 (CD158d)	CCL3 (MIP1a)	CD27	CD45
CD25	CD226	CD57	HLA-ABC
NKG2D	CD161	CD96	CD19
CD69	CD11b	CXCR4 (CD184)	
KIR2DL1	CD132	2B4 (CD244)	
	NKp44		

[00502] Cytotoxicity assays were performed by co-culturing three-stage NK cells with tumor cell lines for 4 hours. Furthermore, supernatants were collected to analyze secreted perforin, granzymes and cytokines.

[00503] To further investigate cytolytic activity, immune synapse formation was monitored. NK-sensitive target cells (K562, chronic myelogenous leukemia cells) were labeled

with CellTracker Violet (Life Techology). NK cell/target cell conjugates were formed by suspending equal volumes and cell numbers of NK effector cells (1×10^6/ml) and target cells in culture medium on coverslip for 15 min at 37°C. Cells were then fixed with 3% methanol-free formaldehyde and permeabilized. F-Actin was stained with Alexa-488 conjugated phalloidin (Life Techology). For perforin, CD2, or LFA-1 antibody co-staining with F-actin, slides were incubated for 1 h with primary antibodies followed by the addition of the Alexa Fluor 555 dye-conjugated goat anti-rabbit secondary antibody (Life Technology). Confocal imaging was performed using a Leica SP8 LIAchroics Compact Unit with Inverted DMI 6000 microscope outfitted with 2 HyD detectors.

[00504] **RESULTS**

[00505] Using the cultivation process described in Example 1, a highly pure population (88.3% ± 6.3%) of CD3-CD56+ NK cells was routinely achieved. Three-stage NK cells displayed a developmentally intermediate immunophenotype, evidenced by low / negative expression of CD16 and KIRs. Three-stage NK cells expressed the natural cytotoxicity receptors (NKp30, NKp46 and NKp44), the c-lectin receptors (CD94, NKG2D and CD161), DNAM-1, 2B4, CD117, and CD11a (Figure 2). Cytolytic mediators (perforin and granzymes) and EOMES, the regulator of NK cell maturation and cytolytic function, were also detected in three-stage NK cells (Figure 2).

[00506] Three-stage NK cells exhibited cytotoxicity against hematological tumor cell lines *in vitro*. At an effector-to-target ratio of 10:1, three-stage NK cells exerted lysis towards cell lines, including CML (K562, 70.3% ± 14.8%), AML (HL-60, 31.0% ± 17.8%) and multiple myeloma (RPMI8266, 32.4% ± 19.5%) (Figure 3). The three-stage NK cells also demonstrated high perforin production and a high degree of granulation (Figure 4). When co-cultured with K562 cells at a 1:1 ratio for 24 hours, three-stage NK cells produced functional cytokines including IFNγ, TNFα and GM-CSF (Figure 5 and Table 2).

[00507] Table 2. Three-stage NK cells produce functional cytokines when co-cultured with K562 at 1:1 for 24 hours.

	Average (pg/mL) @ E:T of 1:1 (n=11) against K562	Range (Min, Max) (n=11)
PERFORIN	3933.09	423, 11058
GRANZYME B	1976.35	46.80, 4281
IFN-γ	1323.20	12.55, 4251.06
GMCSF	1471.64	116.00, 4362.00
IL10	3.95	2.6, 6.33
GRANZYME-A	32065.73	820, 74697
TNF-A	482.90	16.62, 1841.00
MCP-1	1671.63	2.96, 6042
Cytotoxicity (10:1)	66.99%	52.79%, 79.76%
Cytotoxicity (2.5:1)	43.63%	18.46%, 76.94%

[00508] At an effector-to-target (E:T) ratio of 1:1, confocal imaging revealed that three-stage NK cells, when in contact with tumor cells, formed an F-actin immunological synapse with polarization of perforin (Figure 6A-B), demonstrating high cytolytic activity.

[00509] Furthermore, in the presence of anti-CD20 (rituximab, 10 μg/mL), the cytotoxicity of three-stage NK cells against Daudi cells (Burkitt's lymphoma, a lymphoblastoid cell line resistant to NK killing) increased from 7.3% ± 8.0% to 35.1% ± 5.7%, demonstrating potent antibody-dependent cell-medicated cytotoxicity (ADCC).

7.4. Example 4: Further Characterization of Three-Stage NK Cells

[00510] The cytotoxicity of three-stage NK cells against CML, AML, and multiple myeloma cells (K562, HL-60, and RPMI8226, respectively) at various effector to target ratios was examined, as shown in Figure 7. In the presence of K562, HL60, or PMA (phorbol 12-myristate 13-acetate), different levels of CD107a expression were observed, an indicator of degranulation (Figure 8). Likewise, an increase in IFNγ production by three-stage NK cells was observed when cocultured with K562 and HL60 cells lines, or upon PMA stimulation (Figure 9). Up to 40% specific lysis was observed in the presence of primary AML targets at an effector-to-target ratio of 3:1 after 24 hours of incubation, and a differential susceptibility of AML targets to NK killing was observed (Figure 10). A wide range of IFNγ production levels were observed across tumor cell lines and primary targets, as well as donor variation for both the three-stage NK cells and primary AML targets (Figure 11).

[00511] Three-stage NK cells were shown to produce various cytolytic enzymes and cytokines in the presence of various tumor cells lines or primary AML targets (AML1-4), as shown in Table 3.

[00512] Table 3. The average cytokine secretion (pg/1 × 10^6 cells) is shown at an effector-to-target ration of 1:1 against various primary and tumor cells.

pg/1E6	NK+ K562	NK+ HL-60	NK+ KG1a	NK+ RPMI	NK+ AML1	NK+ AML2	NK+ AML3	NK+ AML4	NK+ PMA
N=	5	5	2	3	2	2	3	3	5
PERFORIN	4292	3430	2787	30	419	596	1462	1662	8466
IFNG	750	71	6	2	5	148	4	70	26601
GRANZYME-A	49192	36560	23867	274	10241	12003	51316	71886	147792
GRANZYME-B	8858	6638	1276	2	1015	1699	1071	2123	22606
GMCSF	1920	434	46	4	16	646	50	1311	70340
TNF-A	5272	2110	30	17	46	306	138	554	7564
MCP-1	1739	37004	146	153	131	1173	173	466	3811

[00513] In summary, three-stage NK cells showed cytolytic activity across various tumor cell lines, exhibited a degranulation capacity when in contact with tumor cells and upon activation by PMA, and secreted IFNγ, perforin, granzyme A, and granzyme B when cocultured with tumor cells or upon activation by PMA. Furthermore, the three-stage NK cells exhibited a 24 hour cytolytic activity against primary AML targets at an effector to target ratio of 3:1 and showed the capacity to secrete IFNγ against primary AML cells.

7.5. Example 5: Two populations of three-stage NK cells

[00514] Using FACS, the CD56+CD3- population of three-stage NK cells was evaluated on a CD11a vs. CD117 plot, showing two populations of cells (Figure 12). The CD11a- fraction (ILC3) was shown to be CD94-, and the CD11a+ fraction (NK) was shown to contain both CD94- and CD94+ cells (Figure 13). Furthermore, the $CD11a^-$ and CD11a+ cells could be distinguished based on their expression of perforin, eomesodermin (EOMES), RAR-related orphan receptor gamma t (RORγt) and interleukin-1 receptor 1 (IL1R1), with CD11a+ cells expressing perforin and EOMES but not RORγt and IL1R1, and CD11a- cells expressing RORγt and IL1R1, but not perforin and EOMES (Figure 14). Consistent expression patterns of perforin, granzyme B, EOMES, T-bet, IL1R1, RORγt and aryl hydrocarbon receptor (AHR) were shown across donors, with perforin, granzyme B, EOMES, and T-bet primarily expressed by CD11a+ cells, and IL1R1, RORγt and AHR by CD11a- cells. (Figure 15). The CD11a+ population were

hypothesized to be traditional NK cells, whereas the CD11a- population were hypothesized to be ILC3 cells.

[00515] To perform a functional analysis of the two populations, an ICCS (intracellular cytokine staining) assay was used to detect cytokine expression in the three-stage NK cells. Stimulation was performed for 6 hours with 1) PMA + ionomycin, a trigger for IFNγ, 2) PMA + ionomycin and IL-23 (to detect IL-22), and 3) cytokines IL-12+IL-18 and IL1b/IL-23 to trigger CD11a+ and CD11a- cells, respectively. The following cytokines were then analyzed in the context of CD56/CD3/ CD117/CD11a/CD94 expression: IFNγ, GM-CSF, TNFα, which are NK-specific, and IL-22 an IL-8, which are ILC3-specific. It was determined that CD11a+ cells expressed IFNγ and GM-CSF, wherein CD11a- cells expressed IL-22 and IL-8 (Figure 16). Both CD11a+ cells and CD11a- cells expressed TNFα (Figure 16). Furthermore, it was shown that GM-CSF and IL-22/IL-8 expression were mutually exclusive, and that TNFα can be co-expressed with IL-22 and IL-8 (Figure 16).

[00516] Expression of activating receptors in the three-stage NK subset populations was also determined. It was found that major NK activating receptors NKp46, NKp30, and NKG2D were only expressed in CD11a+ cells (Figure 17). CD226 (DNAM-1) and 2B4 (CD244) were capable of being expressed on CD11a- cells (Figure 18). Expression of CD16 and KIR was low, and was restricted to CD94+ cells (Figure 19). The total expression of these activating receptors in the three-stage NK cells is summarized in Table 4 for several donors.

[00517] Table 4. Total expression of activating receptors in three-stage NK cells from various donors

% of Total Cells	**NKp30+**	**NKp46+**	**NKG2D+**	**DNAM-1+**	**2B4+**	**NKG2A+**	**CD16+**
Donor 1	12.5	7.5	8.8	14.1	33.8	6.9	2.6
Donor 2	24.2	19.2	23.2	36.2		12	2.2
Donor 3	13.8	10.6	12.2	30.3		9.6	2.3
Donor 4	23.6	19.0	24.0	29.2	25.5	12.0	3.4
Donor 5	35.5	27.6	33.9	47.5	33.2	16.3	8.8

[00518] The CD11a- cells present in the three-stage NK cells recapitulate expression patterns previously described for ILC3 cells, respectively, for example, with respect to RORγt and AHR, and the secretion of IL-22 upon stimulation. However, the CD11a- ("ILC3") cell

population found in the three-stage cells has characteristics not previously described for ILC3 cells, for example, the expression of T-bet, TNFα, DNAM-1, and 2B4.

7.6. Example 6: Functional Assessment of Two Populations of Three-Stage NK cells

[00519] **METHODS**

[00520] Three-stage NK cells (CD3-CD56+) were subjected to enrichment of either CD11a+ or CD94+ cells by FITC selection Kit (Stem Cell, Cat#18552) following the protocol provided by the manufacturer. After enrichment, purity of the selected population was evaluated by CD56-PerCP (BD Pharmingen, Cat#560842), CD3-APC-H7 (BD Pharmingen, Cat#560176), CD11a- FITC (BD Pharmingen, Cat#555383), or CD94-FITC (BD Pharmingen, Cat#555388). The cytotoxic activity of selected population was tested by 4h cytotoxicity assay against K562 at different E (effector):T (target) ratio as indicated. In brief, K562 cells were labeled with PKH26 (Sigma-Aldrich, Cat# PKH26-GL), placed in 96-well U-bottom tissue culture plates and incubated with NK cells at different E:T ratio as indicated in 200 μL RPMI1640 supplemented with 10% FBS. After 4h incubation at 37°C in 5% CO_2, cells were harvested and TO-PRO-3 (Invitrogen, Cat# T3605) was added to cultures at 1 μM final concentration followed by FACS analysis using BD FACSCanto II. Cytotoxicity was expressed as the percentage of dead cells (PKH26+TO-PRO-3+) within the total PKH26+ target tumor cells.

[00521] **RESULTS AND DISCUSSION**

[00522] As shown in Table 5, 98% purity of CD11a+, or 91% CD94+ cells was achieved with the enrichment procedure. The CD11a+ population showed the highest cytotoxicity (67.4%) against K562 at an E:T ratio of 2.5:1 while the unfractionated three-stage NK cells exhibited 61.3% cytotoxicity and CD11a- cells exhibited 36.0% cytotoxicity.

[00523] Table 5. Purity of CD11a/CD94 after enrichment

Cell Fractionation	CD94%	CD11a%
whole	13.1	19
CD94+ fraction	91.4	
CD11a+ fraction		97.9
CD11a- fraction		7.48

[00524] These results show that NK-enriched CD11a+CD3-CD56+ cells exhibit high cytotoxicity against K562 cells, and that ILC3-enriched CD11a-CD3-CD56+ cells exhibit

cytolytic activity as well at a lower level when compared with unfractionated cells or NK-enriched cells. The cytotoxicity exhibited by the CD11a-CD3-CD56+ cell population is another unique property of the three-stage ILC3 cells compared to the ILC3 cells described previously.

7.7. Example 7: Effect of Varying Cytokine Concentrations on the Purity and Activity of the Three-Stage NK cells

[00525] A total of 15 different conditions were evaluated to determine the effect of varying components of the third medium of Example 1 on the two (CD11a+ and CD11a-) populations found in the three-stage NK cells. Table 6 summarizes the different conditions tested.

[00526] Table 6. *Fifteen conditions with varying third medium components.*

	Condition #	IL-7 (ng/ml)	SCF (ng/ml)	IL-2 (ng/ml)	SR1	IL-15 (ng/ml)
1	1a	100	22	1000	0uM	20
2	1b	100	22	1000	10uM	20
3	2a	100	0	1000	0uM	20
4	2b	100	0	1000	10uM	20
5	3a	20	22	1000	0uM	20
6	3b	20	22	1000	10uM	20
7	4a	20	0	1000	0uM	20
8	4b	20	0	1000	10uM	20
9	5a	0	22	1000	0uM	20
10	6a	0	0	1000	0uM	20
11	7a	20	22	0	0uM	20
12	7b	20	22	0	10uM	20
13	8a	20	0	0	0uM	20
14	8b	20	0	0	10uM	20
15	9a	20	22	1000	0uM	0

[00527] A flow cytometry panel (Table 7) was utilized for evaluation on days 0, 7, 14, 21, 28, and 35, including cell surface markers CD56, CD3, CD117, CD94, IL1R1, CD11a, CD34, and CD14/CD15 and intracellular markers RORγt, AHR, and perforin.

[00528] Table 7. Panel of antibodies used for flow cytometry evaluation.

Fluorophore	**Antibody**
APCCy7	CD56
AmCyan	Viability/CD3
BV605	CD117
PerCPCy5.5	CD94
AF700	IL1R1
PECY7	CD11a
BV421	CD34
BV711	CD14+CD15
APC	RORγt
PE	AHR
FITC	Perforin

[00529] The expression data at day 28 is shown in Figures 20A-D. A summary of the results over the 15 conditions can be found in Table 8.

[00530] Table 8. Effect of varying components of the third medium on the two (CD11a+ and CD11a-) populations found in the three-stage NK cells.

Conditions						D28 results for Donor 1			D28 results for Donor 2		
#	IL-7 (ng/ml)	SCF (ng/ml)	IL-2 (ng/ml)	IL-15 (ng/ml)	SR1	%CD56+	%RORgt+ of CD56+	%Perforin+ of CD56+	%CD56+	%RORgt+ of CD56+	%Perforin+ of CD56+
1a	100	22	1000	20	0uM	67	77	4	55	75	14
1b	100	22	1000	20	10uM	27	53	26	19	21	71
2a	100	0	1000	20	0uM	37	42	27	14	19	66
2b	100	0	1000	20	10uM	22	7	79	16	4	88
3a	20	22	1000	20	0uM	54	70	8	53	65	15
3b	20	22	1000	20	10uM	27	54	31	24	38	52
4a	20	0	1000	20	0uM	40	41	37	11	17	68
4b	20	0	1000	20	10uM	27	10	77	20	3	93
5a	0	22	1000	20	0uM	50	66	10	32	63	27

6a	0	0	1000	20	0uM	22	37	41	11	14	74
7a	20	22	0	20	0uM	50	60	10	44	53	28
7b	20	22	0	20	10uM	27	53	31	18	41	49
8a	20	0	0	20	0uM	43	55	20	13	16	60
8b	20	0	0	20	10uM	26	7	75	14	3	86
9a	20	22	1000	0	0uM	67	64	3	57	78	10

[00531] In summary, low SCF in the third medium and the presence of SR1 in the third medium was found to favor NK (CD11a+) cell production, whereas the absence of SR1 in the third medium was found to favor ILC3 (CD11a-) cell production.

7.8. Example 8: Additional Characterization and Functional Assessment of Two Populations of Three-Stage NK cells

[00532] Using FACS, the CD56+CD3– population of three-stage NK cells was evaluated on a CD11a vs. CD117 plot, and the two populations of cells, the CD11a– fraction and the CD11a+ fraction, were shown to be distinguishable by CD107a expression in the presence of K562 cells (Fig. 21A-B). The CD11a+ fraction was shown to be capable of degranulation, as measured by a significantly higher CD107a+ fraction in the presence of K562 cells (Fig. 21C).

[00533] The CD11a+ cells, making up an 81% fraction of the CD56+CD3– population of three-stage NK cells, and the CD11a– cells, making up a 5% fraction of the CD56+CD3- population of three-stage NK cells were tested for cytotoxicity against K562 cells (Fig. 22). The CD11a+ enriched population exhibited a higher K562 cytotoxicity compared to the CD11a– enriched population, especially at higher effector:target cell ratios (Fig. 22).

[00534] Cytokine secretion analysis was then conducted by overnight co-culture of with K562 target cells and CD11a positive and negative cells, respectively, as effector cells at a 1:1 ratio of effector:target. Supernatant was then collected and analyzed by multiplex. In the presence and absence of K562 cells, the CD11a- and CD11a+ cells could be distinguished in their expression of certain tested cytokines, including Granzyme A, Granzyme B, IFN-γ, TNFα, perforin, GM-CSF, and IL-2 (Table 9 and Figure 23). Cytokines IL-17A, IL-22, TNFβ, and IL-10 were found at low levels or below detection (Table 9).

[00535] Table 9. Cytokine secretion analysis of CD11a positive and negative cells in the presence and absence of K562 target cells.

		GzA (pg/mL)	GzB (ng/mL)	IFN-γ (pg/mL)	IL-10 (pg/mL)	TNFα (pg/mL)	Perf (pg/mL)	GM-CSF (ng/mL)	IL-17A (pg/mL)	IL-22 (pg/mL)	IL-2 (pg/mL)	TNFβ (pg/mL)
CD11a-	NK alone	32233	282.62	1.65	below	106.45	502.87	below	below	below	141.87	24.64
CD11a+	NK alone	23322	3182	19.78	3	46.62	2021	0.21	below	below	681.98	below
CD11a-	NK+ K562 1:1	16034	236.17	1.99	below	71.63	382.37	0.19	below	below	99.25	23.47
CD11a+	NK+ K562 1:1	30648	6632	1169	2.43	711.62	2133	4.31	below	below	417.45	25.47

[00536] Table 10 provides a summary of CD11a positive and negative cells, as characterized above.

[00537] Table 10. Summary of characterization of CD11a positive and negative cells.

	CD11a+	**CD11a-**
Phenotype		
Surface markers	CD56+CD3-CD94+	CD56+CD3-CD117+ILR1+
Intracellular	Perforin+ Granzyme+ IFN-γ+	
Transcription Markers	T-bet+ EOMES+	RORγt+ AHR+
Function		
Cytotoxicity	High	Low
Degranulation	Yes	Low
Cytokine Secretion	Granzyme A Granzyme B IFN-γ TNFα (low) Perforin GM-CSF IL-2	Granzyme A Granzyme B TNFα (low) Perforin IL-2

Equivalents:

[00538] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

[00539] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication, patent or patent

application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

WHAT IS CLAIMED IS:

1. A method of producing a cell population comprising natural killer cells, comprising the steps of:

(a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells;

(b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and

(c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of a stem cell mobilizing agent and low molecular weight heparin (LMWH), to produce a third population of cells;

wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and wherein at least 80% of the natural killer cells are viable.

2. The method of claim 1, wherein said third population of cells comprises natural killer cells that are CD94+ or CD16+.

3. The method of claim 1, wherein said third population of cells comprises natural killer cells that are CD94- or CD16-.

4. The method of claim 1, wherein said third population of cells comprises natural killer cells that are CD94+ and CD16+.

5. The method of claim 1, wherein said third population of cells comprises natural killer cells that are CD94- and CD16-.

6. A method of producing a cell population comprising natural killer cells, comprising the steps of:

(a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells;

(b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and

(c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking LMWH, to produce a third population of cells;

wherein the third population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+.

7. The method of claim 6, wherein the third medium lacks stem cell factor (SCF).

8. The method of claim 7, wherein the third medium lacks LMWH.

9. The method of claim 7, comprising a further step of (d) isolating CD11a+ cells from the third population of cells to produce a fourth population of cells;

wherein the fourth population of cells comprises natural killer cells that are CD56+, CD3-, and CD11a+.

10. The method of any one of claim 6-9, wherein said natural killer cells express perforin and EOMES.

11. The method of any one of claim 6-10, wherein said natural killer cells do not express either RORγt or IL1R1.

12. A method of producing a cell population comprising ILC3 cells, comprising the steps of:

(a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells;

(b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells; and

(c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking LMWH, to produce a third population of cells;

wherein the third population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-.

13. The method of claim 12, wherein the third medium comprises a stem cell mobilizing agent.

14. The method of claim 12, wherein the third medium comprises SCF.

15. The method of claim 12, wherein the third medium comprises a stem cell mobilizing agent and SCF.

16. A method of producing a cell population comprising ILC3 cells, comprising the steps of:

(a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells;

(b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells;

(c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of a stem cell mobilizing agent and LMWH, to produce a third population of cells; and

(d) isolating CD11a- cells from the third population of cells to produce a fourth population of cells;

wherein the fourth population of cells comprises ILC3 cells that are CD56+, CD3-, and CD11a-.

17. The method of any one of claim 12-16, wherein said ILC3 cells express RORγt and IL1R1.

18. The method of any one of claim 12-17, wherein said ILC3 cells do not express either perforin or EOMES.

19. The method of any one of claims 1-18, wherein said third medium lacks desulphated glycosaminoglycans.

20. The method of any one of claim 1-19, wherein said hematopoietic stem or progenitor cells are mammalian cells.

21. The method of claim 20, wherein said hematopoietic stem or progenitor cells are human cells.

22. The method of claim 20, wherein said hematopoietic stem or progenitor cells are primate cells.

23. The method of claim 20, wherein said hematopoietic stem or progenitor cells are canine cells.

24. The method of claim 20, wherein said hematopoietic stem or progenitor cells are rodent cells.

25. The method of claim 20, wherein said hematopoietic stem or progenitor cells are cells from a mammal other than a human, primate, canine or rodent.

26. The method of any one of claims 1-25, wherein said hematopoietic stem or progenitor cells are CD34+ hematopoietic stem cells.

27. The method of any one of claims 1-26, wherein said hematopoietic stem or progenitor cells are placental cells.

28. The method of claim 27, wherein said placental cells are obtained from, or obtainable from, human placental perfusate.

29. The method of claim 27, wherein said placental cells are obtained from, or obtainable from, nucleated cells isolated from human placental perfusate.

30. The method of any one of claims 1-26, wherein said hematopoietic stem or progenitor cells are obtained from, or obtainable from, umbilical cord blood.

31. The method of any one of claims 1-26, wherein said hematopoietic stem or progenitor cells are fetal liver cells.

32. The method of any one of claims 1-26, wherein said hematopoietic stem or progenitor cells are mobilized peripheral blood cells.

33. The method of any one of claims 1-26, wherein said hematopoietic stem or progenitor cells are bone marrow cells.

34. The method of any one of claims 1-33, wherein said Tpo is present in the first medium at a concentration of from 1 ng/mL to 50 ng/mL.

35. The method of claim 34, wherein said Tpo is present in the first medium at a concentration of from 20 ng/mL to 30 ng/mL.

36. The method of claim 34, wherein said Tpo is present in the first medium at a concentration of about 25 ng/mL.

37. The method of any one of claims 1-36, wherein said IL-15 is present in said second medium at a concentration of from 1 ng/mL to 50 ng/mL.

38. The method of claim 37, wherein said IL-15 is present in said second medium at a concentration of from 10 ng/mL to 30 ng/mL.

39. The method of claim 37, wherein said IL-15 is present in said second medium at a concentration of about 20 ng/mL.

40. The method of any one of claims 1-39, wherein said IL-2 is present in said third medium at a concentration of from 10 U/mL to 10,000 U/mL and said IL-15 is present in said third medium at a concentration of from 1 ng/mL to 50 ng/mL.

41. The method of any one of claims 1-39, wherein said IL-2 is present in said third medium at a concentration of from 300 U/mL to 3,000 U/mL and said IL-15 is present in said third medium at a concentration of from 10 ng/mL to 30 ng/mL.

42. The method of any one of claims 1-39, wherein said IL-2 is present in said third medium at a concentration of about 1,000 U/mL and said IL-15 is present in said third medium at a concentration of about 20 ng/mL.

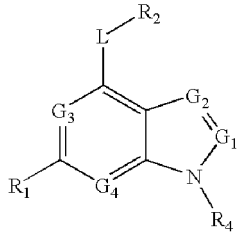
43. The method of any of claims 1-42, wherein said Tpo, IL-2, and IL-15 are not comprised within an undefined component of the first medium, second medium or third medium.

44. The method of any of claims 1-42, wherein said Tpo, IL-2, and IL-15 are not comprised within serum.

45. The method of any of claims 1-44, wherein said stem cell mobilizing agent is an aryl hydrocarbon receptor inhibitor.

46. The method of claim 45, wherein said aryl hydrocarbon receptor inhibitor is resveratrol.

47. The method of claim 45, wherein said aryl hydrocarbon receptor inhibitor is compound of the formula



in which:

G_1 is selected from N and CR_3;

G_2, G_3 and G_4 are independently selected from CH and N; with the proviso that at least 1 of G_3 and G_4 is N; with the proviso that G_1 and G_2 are not both N;

L is selected from --$NR_{5a}(CH_2)_{0-3}$--, --$NR_{5a}CH(C(O)OCH_3)CH_2$--, --$NR_{5a}(CH_2)_2NR_{5b}$--, --$NR_{5a}(CH_2)_2S$--, --$NR_{5a}CH_2CH(CH_3)CH_2$--, --$NR_{5a}CH_2CH(OH)$-- and --

$NR_{5a}CH(CH_3)CH_2$--; wherein R_{5a} and R_{5b} are independently selected from hydrogen and C_{1-4}alkyl;

R_1 is selected from hydrogen, phenyl, thiophenyl, furanyl, 1H-benzoimidazolyl, isoquinolinyl, 1H-imidazopyridinyl, benzothiophenyl, pyrimidinyl, 1H-pyrazolyl, pyridinyl, 1H-imidazolyl, pyrrolidinyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl and thiazolyl; wherein said phenyl, thiophenyl, furanyl, 1H-benzoimidazolyl, isoquinolinyl, 1H-imidazopyridinyl, benzothiophenyl, pyrimidinyl, 1H-pyrazolyl, pyridinyl, 1H-imidazolyl, pyrrolidinyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl or thiazolyl of R_1 can be optionally substituted by 1 to 3 radicals independently selected from cyano, hydroxy, C_{1-4}alkyl, C_{1-4}alkoxy, halo, halo-substituted-C_{1-4}alkyl, halo-substituted-C_{1-4}alkoxy, hydroxy, amino, --$C(O)R_{8a}$, --$S(O)_{0-2}R_{8a}$, --$C(O)OR_{8a}$ and --$C(O)NR_{8a}R_{8b}$; wherein R_{8a} and R_{8b} are independently selected from hydrogen and C_{1-4}alkyl; with the proviso that R_1 and R_3 are not both hydrogen;

R_2 is selected from --$S(O)_2NR_{6a}R_{6b}$, --$NR_{9a}C(O)R_{9b}$, --$NR_{6a}C(O)NR_{6b}R_{6c}$, phenyl, 1H-pyrrolopyridin-3-yl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl and 1H-indazolyl; wherein R_{6a}, R_{6b} and R_{6c} are independently selected from hydrogen and C_{1-4}alkyl; wherein said phenyl, 1H-pyrrolopyridin-3-yl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl or 1H-indazolyl of R_2 is optionally substituted with 1 to 3 radicals independently selected from hydroxy, halo, methyl, methoxy, amino, --$O(CH_2)_nNR_{7a}R_{7b}$, --$S(O)_2NR_{7a}R_{7b}$, --$OS(O)_2NR_{7a}R_{7b}$ and --$NR_{7a}S(O)_2R_{7b}$; wherein R_{7a} and R_{7b} are independently selected from hydrogen and C_{1-4}alkyl;

R_3 is selected from hydrogen, C_{1-4}alkyl and biphenyl; and

R_4 is selected from C_{1-10}alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-3-yl, tetrahydro-2H-pyran-4-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl; wherein said alkyl, cyclopropyl, cyclohexyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-3-yl, oxetan-2-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, tetrahydro-2H-pyran-4-yl, phenyl, tetrahydrofuran-3-yl,

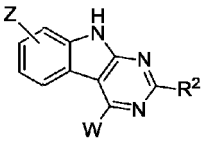
tetrahydrofuran-2-yl, benzyl, (4-pentylphenyl)(phenyl)methyl or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl can be optionally substituted with 1 to 3 radicals independently selected from hydroxy, C_{1-4}alkyl and halo-substituted-C_{1-4}alkyl; or a salt thereof.

48. The method of claim 45, wherein said aryl hydrocarbon receptor inhibitor is StemRegenin-1 (SR-1) (4-(2-(2-(benzo[b]thiophen-3-yl)-9-isopropyl-9H-purin- 6-ylamino)ethyl)phenol).

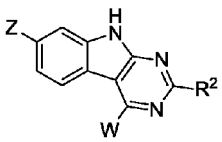
49. The method of claim 45, wherein said aryl hydrocarbon receptor inhibitor is the compound CH223191 (1-Methyl-N-[2-methyl-4-[2-(2-methylphenyl)diazenyl]phenyl-1H-pyrazole-5-carboxamide].

50. The method of any claims 1-44, wherein the stem cell mobilizing agent is a pyrimido(4,5-b)indole derivative.

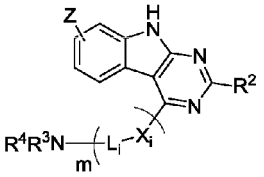
51. The method of claim 50, wherein said pyrimido(4,5-b)indole derivative is one or more of:



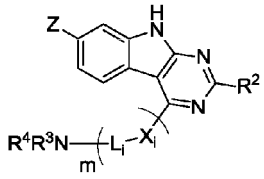
I



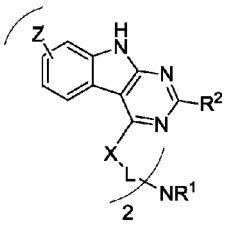
II



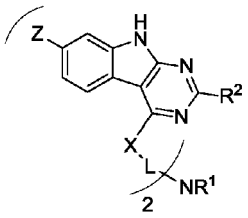
III



IV



V



VI

or a salt or a prodrug thereof, wherein:

Z is

1) -P(O) (OR<1>) (OR<1>),

2) -C(0)OR<1>,

3) -C(0)NHR<1>,

4) -C(0)N(R)R<1>,

5) -C(0)R<1>,

6) -CN,

7) -SR ,

8) -S(0)2NH2,

9) -S(0)2NHR<1>,

10) -S(0)2N(R)R<1>,

1 1) -S(0)R<1>,

12) -S(0)2R<1>,

13) -L,

14) -benzyl optionally substituted with 1 , 2 or 3 R<A> or R<1> substituents,

15) -L-heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and the heteroaryl groups,

16) -L-heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either one or both the L and the heterocyclyl groups, 17) -L-aryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and the heteroaryl groups,

18) -heteroaryl optionally substituted with one or more R<A> or R<1> substituents, or

19) -aryl optionally substituted with one or more R<A> or R<1> substituents,

and wherein each substituent is optionally attached to the L group if it is not already present, and wherein, when (R<1>) and R<1> are attached to a nitrogen atom, optionally they join together with the nitrogen atom to form a 3 to 7-membered ring which optionally includes one or more other heteroatom selected from N, 0 and S, optionally the is substituted with one or more R<1> or R<A>;

W is

1) -H,

2) -halogen,

3) -OR<1>,

4) -L-OH,

5) -L-OR<1>,

6) -SR<1>,

7) -CN,

8) -P(0)(OR<1>)(OR<1>),

9) -NHR<1>,

10) -N(R<1>)R<1>,

1 1) -L-NH2,

12) -L-NHR<1>,

13) -L-N(R<1>)R<1>,

14) -L-SR<1>,

15) -L-S(0)R<1>,

16) -L-S(0)2R<1>,

17) -L-P(0)(OR<1>)(OR<1>

18) -C(0)OR<1>,

19) -C(0)NH2,

20) -C(0)NHR<1>,

21) -C(0)N(R<1>)R<1>,

22) -NHC(0)R<1>,

23) -NR1C(0)R<1>, -NHC(0)0R<1>,

-NR1C(0)0R<1>,

-0C(0)NH2,

-0C(0)NHR<1>,

-0C(0)N(R)R<1>,

-0C(0)R<1>,

-C(0)R<1>,

-NHC(0)NH2,

-NHC(0)NHR<1>,

-NHC(0)N(R)R<1>,

-NR C(0)NH2,

-NR C(0)NHR<1>,

-NR C(0)N(R)R<1>,

-NHS(0)2R<1>,

-NR S(0)2R<1>,

-S(0)2NH2,

-S(0)2NHR<1>,

-S(0)2N(R)R<1>,

-S(0)R<1>,

-S(0)2R<1>,

-0S(0)2R1 ,

-S(0)20R<1>,

-benzyl optionally substituted with 1 , 2 or 3 R<A> or R<1> substituents,

-L-heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and the heteroaryl groups,

-L-heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and the heterocyclyl goups,

-L-aryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and aryl groups,

-L-NR<1>(R<1>),

-L-)2 NR<1>,

-L-(N(R1)-L)n - N(R1)R1 , -L-(N(R<1>)-L)n - heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heteroaryl groups,

-L-(N(R<1>)-L)n - heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heterocyclyl groups,

-L-(N(R<1>)-L)n - aryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and aryl groups,

-0-L-N(R)R<1> ,

-0-L- heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heteroaryl groups,

-0-L- heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heterocyclyl groups,

-0-L- aryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and aryl groups,

-0-L)2-NR<1>,

-0-L-(N(R)-L)n - N(R)R<1> ,

-0-L-(N(R<1>)-L)n - heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heteroaryl groups,

-0-L-(N(R<1>)-L)n - heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and heterocyclyl groups,

-0-L-(N(R<1>)-L)n- aryl optionally substituted with one or more R<A> or R<1> substituents,

-S-L- heteroaryl optionally substituted with one or more R<A> or R<1> substituents,

-S-L- heterocyclyl optionally substituted with one or more R<A> or R<1> substituents,

-S-L- aryl optionally substituted with one or more R<A> or R<1> substituents attached on either or both the L and aryl groups,

-S-L)2 NR1 ,

-S-L-(N(R1)-L)"- N(R1)R1,

-S-L-(N(R<1>)-L)n - heteroaryl optionally substituted with one or more R<A> substituents, -S-L-(N(R<1>)-L)n - heterocyclyl optionally substituted with one or more R<A> substituents, -S-L-(N(R<1>)-L)n - aryl optionally substituted with one or more R<A> substituents,

-NR<1>(R<1>),

-(N(R1)-L)n - N(R1)R1 ,

-N(R1)L)2 -NR1 , 76) -(N(R1)-L)"- N(R1)RA,

77) -(N(R<1>)-L)n - heteroaryl optionally substituted with one or more R<A> or R<1> substituents,

78) -(N(R<1>)-L)n - heterocyclyl optionally substituted with one or more R<A> or R<1> substituents,

79) -(N(R<1>)-L)n - aryl optionally substituted with one or more R<A> or R<1> substituents,

80) -heteroaryl optionally substituted with one or more R<A> substituents, or

81) -aryl optionally substituted with one or more R<A> substituents,

and wherein each substituent is optionally attached to the L group if it is not already present, and wherein when two R<1> substituents are present on the same nitrogen atom, then each R<1> substituent is independently selected from the list of R<1> values described thereafter,

and wherein n is an integer equal to either 0, 1 , 2, 3, 4, or 5,

and wherein, when (R<1>) and R<1> are attached to a nitrogen atom, optionally they join together with the nitrogen atom to form a 3 to 7-membered ring which optionally includes one or more other heteroatom selected from N, 0 and S, optionally the ring is substituted with one or more R<1> or R<A>;

L is

1) -Ci-6 alkyl,

2) -C2-6 alkenyl,

3) -C2-6 alkynyl,

4) -C3-7 cycloalkyi,

5) -C3-7 cycloalkenyl,

6) heterocyclyl,

7) -Ci-6 alkyl-C3-7 cycloalkyi,

8) -Ci-6 alkyl-heterocyclyl,

9) aryl, or

10) heteroaryl,

and wherein the alkyl, the alkenyl, the alkynyl, the cycloalkyi, the cycloalkenyl, the heterocyclyl, the aryl and the heteroaryl groups are each independently optionally substituted with one or two R<A> substituent;

Ri is

1) -H,

2) -C1-6 alkyl,

3) -C2-6 alkenyl,

4) -C2-6 alkynyl, 5) -C3-7 cycloalkyl,

6) -C3-7 cycloalkenyl,

7) -C1-5 perfluorinated,

8) -heterocydyl,

9) -aryl,

10) -heteroaryl,

1 1) -benzyl, or

12) 5-[(3aS,4S,6aR)-2-oxohexahydro-1 H-thieno[3,4-d]imidazol-4-yl]pentanoyl,

and wherein the alkyi, the alkenyl, the alkynyl, the cycloalkenyl, the perfluorinated alkyi, the heterocydyl, the aryl, the heteroaryl and the benzyl groups are each independently optionally substituted with 1 , 2 or 3 R<A> or R<1> substituents;

R2 is

1) -H,

2) -C1-6 alkyi,

3) -SR ,

4) -C(0)R1,

5) -S(0)R1,

6) -S(0)2R<1>,

7) -benzyl optionally substituted with 1 , 2 or 3 R<A> or R<1> substituents,

8) -L-heteroaryl optionally substituted with one or more R<A> or R<1> substituents attached on either one or both the L and the heteroaryl groups,

9) -L-heterocyclyl optionally substituted with one or more R<A> or R<1> substituents attached on either one or both the L and the heterocydyl groups,

10) -L-aryl optionally substituted with one or more R<A> or R<1> substituents attached on either one or both the L and the aryl groups,

11) -heteroaryl optionally substituted with one or more R<A> or R<1> substituents, or

12) -aryl optionally substituted with one or more R<A> or R<1> substituents,

and wherein each substituent is optionally attached to the L group if it is not already present;

R<A> is

1) -halogen,

2) -CFs, 3) -OH,

4) -OR<1>,

5) -L-OH,

6) -L-OR<1>,

7) -OCFs,

8) -SH,

9) -SR1 ,

10) -CN,

11) -NO2,

12) -NH2,

13) -NHR<1>,

14) -NR<1>R<1>,

15) -L-NH2,

16) -L-NHR<1>,

17) -L-NR<4>R<1>,

18) -L-SR<1>,

19) -L-S(0)R<1>,

20) -L-S(0)2R<1>,

21) -C(0)OH,

22) -C(0)OR<1>,

23) -C(0)NH2,

24) -C(0)NHR<1>,

25) -C(0)N(R<1>)R<1>,

26) -NHC(0)R<1>,

27) -NR1C(0)R<1>,

28) -NHC(0)OR<1>,

29) -NR1C(0)0R<1>,

30) -OC(0)NH2,

31) -OC(0)NHR<1>,

32) -OC(0)N(R)R<1>,

33) -OC(0)R<1>,

34) -C(0)R1, 35) -NHC(0)NH2,

36) -NHC(0)NHR1,

37) -NHC(0)N(R)R<1>,

38) -NR C(0)NH2,

39) -NR C(0)NHR<1>,

40) -NR1C(0)N(R1)R1,

41) -NHS(0)2R<1>,

42) -NR S(0)2R<1>,

43) -S(0)2NH2,

44) -S(0)2NHR<1>,

45) -S(0)2N(R)R<1>,

46) -S(0)R<1>,

47) -S(0)2R<1>,

48) -0S(0)2R<1>,

49) -S(0)20R<1>,

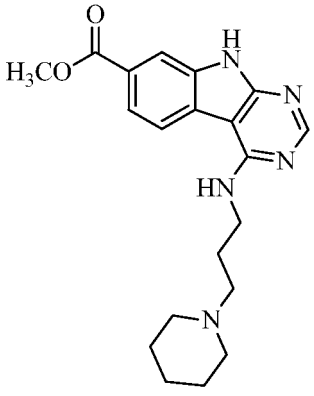
50) -benzyl,

51) -N3, or

52) -C(-N=N-)(CF3),

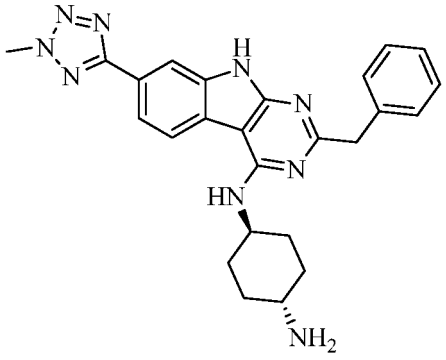
and wherein the benzyl group is optionally substituted with 1 , 2 or 3 R<A> or R<1> substituents.

52. The method of claim 50, wherein said pyrimido(4,5-b)indole derivative has the chemical structure



(UM729).

53. The method of claim 50, wherein said pyrimido(4,5-b)indole derivative has the chemical structure



(UM171).

54. The method of any of claims 1-53, wherein said first medium additionally comprises one or more of LMWH, Flt-3 Ligand (Flt-3L), stem cell factor (SCF), IL-6, IL-7, granulocyte colony-stimulating factor (G-CSF), or granulocyte-macrophage-stimulating factor (GM-CSF).

55. The method of any of claims 1-54, wherein said first medium does not comprise LMWH.

56. The method of claim 55, wherein said first medium does not comprise a desulphated glycosaminoglycan.

57. The method of claim 54, wherein said first medium comprises each of LMWH, Flt-3L, SCF, IL-6, IL-7, G-CSF, and GM-CSF.

58. The method of claim 55 or 56, wherein said first medium comprises each of Flt-3L, SCF, IL-6, IL-7, G-CSF, and GM-CSF.

59. The method of claim 54 or claim 57, wherein in the first medium the LMWH is present at a concentration of from 1U/mL to 10U/mL; the Flt-3L is present at a concentration of from 1 ng/mL to 50 ng/mL; the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL.

60. The method of claim 54 or claim 57, wherein in the first medium the LMWH is present in the first medium at a concentration of from 4U/mL to 5U/mL; the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a

concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL.

61. The method of claim 54 or claim 57, wherein in the first medium the LMWH is present in the first medium at a concentration of about 4.5U/mL; the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about .25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL.

62. The method of claim 55 or claim 58, wherein in the first medium the Flt-3L is present at a concentration of from 1 ng/mL to 50 ng/mL; the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL.

63. The method of claim 55 or claim 58, wherein in the first medium the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL.

64. The method of claim 55 or claim 58, wherein in the first medium the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about .25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL.

65. The method of any of claims 1-64, wherein said second medium additionally comprises one or more of LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF.

66. The method of any of claims 1-65, wherein said second medium does not comprise LMWH.

67. The method of claim 66, wherein said second medium does not comprise a desulphated glycosaminoglycan.

68. The method of any of claims 1-65, wherein said second medium additionally comprises each of LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF.

69. The method of any of claims 1-66, wherein said second medium additionally comprises each of Flt-3, SCF, IL-6, IL-7, G-CSF, and GM-CSF.

70. The method of claim 65 or claim 68, wherein in the second medium the LMWH is present at a concentration of from 1U/mL to 10U/mL; the Flt-3L is present at a concentration of from 1 ng/mL to 50 ng/mL; the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL.

71. The method of claim 65 or claim 68, wherein in the second medium the LMWH is present in the second medium at a concentration of from 4U/mL to 5U/mL; the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL.

72. The method of claim 65 or claim 68, wherein in the second medium the LMWH is present in the second medium at a concentration of about 4.5U/mL; the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about 0.25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL.

73. The method of claim 66 or claim 69, wherein in the second medium the Flt-3L is present at a concentration of from 1 ng/mL to 50 ng/mL; the SCF is present at a concentration of from 1 ng/mL to 50 ng/mL; the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL.

74. The method of claim 66 or claim 69, wherein in the second medium the Flt-3L is present at a concentration of from 20 ng/mL to 30 ng/mL; the SCF is present at a concentration of from 20 ng/mL to 30 ng/mL; the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL.

75. The method of claim 66 or claim 69, wherein in the second medium the Flt-3L is present at a concentration of about 25 ng/mL; the SCF is present at a concentration of about 27 ng/mL; the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 25 ng/mL; the G-CSF is present at a concentration of about 0.25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL.

76. The method of any of claims 1-75, wherein said third medium additionally comprises one or more of IL-6, IL-7, G-CSF, or GM-CSF.

77. The method of claim 76, wherein said third medium comprises each of IL-6, IL-7, G-CSF, and GM-CSF.

78. The method of claim 76 or claim 77, wherein in the third medium the IL-6 is present at a concentration of from 0.01 ng/mL to 0.1 ng/mL; the IL-7 is present at a concentration of from 1 ng/mL to 50 ng/mL; the G-CSF is present at a concentration of from 0.01 ng/mL to 0.50 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.1 ng/mL.

79. The method of claim 76 or claim 77, wherein in the third medium the IL-6 is present at a concentration of from 0.04 ng/mL to 0.06 ng/mL; the IL-7 is present at a concentration of from 20 ng/mL to 30 ng/mL; the G-CSF is present at a concentration of from 0.20 ng/mL to 0.30 ng/mL; and the GM-CSF is present at a concentration of from 0.005 ng/mL to 0.5 ng/mL.

80. The method of claim 76 or claim 77, wherein in the third medium the IL-6 is present at a concentration of about 0.05 ng/mL; the IL-7 is present at a concentration of about 20 ng/mL; the G-CSF is present at a concentration of about 0.25 ng/mL; and the GM-CSF is present at a concentration of about 0.01 ng/mL.

81. The method of any one of claims 6-11 and 19-33, wherein the third medium comprises 100 ng/mL IL-7, 1000 ng/mL IL-2, 20 ng/mL IL-15, and 10 μM SR1 and lacks SCF.

82. The method of any one of claims 6-11 and 19-33, wherein the third medium comprises 20 ng/mL IL-7, 1000 ng/mL IL-2, 20 ng/mL IL-15, and 10 μM SR1 and lacks SCF.

83. The method of any one of claims 6-11 and 19-33, wherein the third medium comprises 20 ng/mL IL-7, 20 ng/mL IL-15, and 10 μM SR1 and lacks SCF.

84. The method of any one of claims 12-33, wherein the third medium comprises 100 ng/mL IL-7, 22 ng/mL SCF, 1000 ng/mL IL-2, and 20 ng/mL IL-15 and lacks SR1.

85. The method of any one of claims 12-33, wherein the third medium comprises 22 ng/mL SCF, 1000 ng/mL IL-2, and 20 ng/mL IL-15 and lacks SR1.

86. The method of any one of claims 12-33, wherein the third medium comprises 20 ng/mL IL-7, 22 ng/mL SCF, 1000 ng/mL IL-2, and 20 ng/mL IL-15 and lacks SR1.

87. The method of any one of claims 12-33, wherein the third medium comprises 20 ng/mL IL-7, 22 ng/mL SCF, and 1000 ng/mL IL-2 and lacks SR1.

88. The method of any of claims 54-87, wherein said LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and/or GM-CSF are not comprised within an undefined component of the first medium, second medium or third medium.

89. The method of any of claims 54-87, wherein said LMWH, Flt-3, SCF, IL-6, IL-7, G-CSF, and/or GM-CSF are not comprised within serum.

90. The method of any of claims 1-89, wherein any of said first medium, second medium or third medium comprises human serum-AB.

91. The method of claim 90, wherein any of said first medium, second medium or third medium comprises 1% to 20% human serum-AB.

92. The method of claim 90, wherein any of said first medium, second medium or third medium comprises 5% to 15% human serum-AB.

93. The method of claim 90, wherein any of said first medium, second medium or third medium comprises about 10% human serum-AB.

94. The method of any of claims 1-93, wherein any of said first medium, second medium or third medium comprises 2-mercaptoethanol.

95. The method of any of claims 1-94, wherein any of said first medium, second medium or third medium comprises gentamycin.

96. The method of any of claims 1-95, wherein said method comprises culturing the hematopoietic stem cells in the first medium for 7-13 days.

97. The method of claim 96, wherein said method comprises culturing the hematopoietic stem cells in the first medium for 8-12 days.

98. The method of claim 96, wherein said method comprises culturing the hematopoietic stem cells in the first medium for about 10 days.

99. The method of any of claims 1-95, wherein said method comprises culturing said first population of cells in said second medium for 2-6 days.

100. The method of any of claims 1-95, wherein said method comprises culturing said first population of cells in said second medium for 3-5 days.

101. The method of any of claims 1-95, wherein said method comprises culturing said first population of cells in said second medium for about 4 days.

102. The method of any of claims 1-101, wherein said method comprises culturing said second population of cells in said third medium for 10-30 days.

103. The method of any of claims 1-101, wherein said method comprises culturing said second population of cells in said third medium for 15-25 days.

104. The method of any of claims 1-101, wherein said method comprises culturing said second population of cells in said third medium for about 21 days.

105. The method of any of claims 1-104, wherein said culturing in said first medium, second medium and third medium are all done under static culture conditions.

106. The method of any of claims 1-104, wherein said culturing in at least one of said first medium, second medium or third medium are done in a spinner flask.

107. The method of any of claims 1-104, wherein said culturing in said first medium and said second medium is done under static culture conditions, and said culturing in said third medium is done in a spinner flask.

108. The method of any of claims 1-107, wherein said hematopoietic cells are initially inoculated into said first medium from 1×10^4 to 1×10^5 cells/mL.

109. The method of claim 108, wherein said hematopoietic cells are initially inoculated into said first medium at about 3×10^4 cells/mL.

110. The method of any of claims 1-109, wherein said first population of cells are initially inoculated into said second medium from 5×10^4 to 5×10^5 cells/mL.

111. The method of any of claim 110, wherein said first population of cells is initially inoculated into said second medium at about 1×10^5 cells/mL.

112. The method of any of claims 1-111, wherein said second population of cells is initially inoculated into said third medium from 1 x 10^5 to 5 x 10^6 cells/mL.

113. The method of claim 112, wherein said second population of cells is initially inoculated into said third medium from 1 x 10^5 to 1 x 10^6 cells/mL.

114. The method of claim 112, wherein said second population of cells is initially inoculated into said third medium at about 5 x 10^5 cells/mL.

115. The method of claim 112, wherein said second population of cells is initially inoculated into said third medium at about 3 x 10^5 cells/mL.

116. The method of any of claims 1-11, 19-83, and 88-115, wherein said method produces at least 5000-fold more natural killer cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium.

117. The method of claim 116, wherein said method produces at least 10,000-fold more natural killer cells.

118. The method of claim 116, wherein said method produces at least 50,000-fold more natural killer cells.

119. The method of claim 116, wherein said method produces at least 75,000-fold more natural killer cells.

120. The method of any of claims 12-80 and 84-115, wherein said method produces at least 5000-fold more ILC3 cells as compared to the number of hematopoietic stem cells initially inoculated into said first medium.

121. The method of claim 120, wherein said method produces at least 10,000-fold more ILC3 cells.

122. The method of claim 120, wherein said method produces at least 50,000-fold more ILC3 cells.

123. The method of claim 120, wherein said method produces at least 75,000-fold more ILC3 cells.

124. The method of any of claims 1-5, 19-83, and 88-119, wherein said method produces natural killer cells that comprise at least 20% CD56+CD3– natural killer cells.

125. The method of any of claims 1-5, 19-83, and 88-119, wherein said method produces natural killer cells that comprise at least 40% CD56+CD3– natural killer cells.

126. The method of any of claims 1-5, 19-83, and 88-119, wherein said method produces natural killer cells that comprise at least 60% CD56+CD3– natural killer cells.

127. The method of any of claims 1-5, 19-83, and 88-119, wherein said method produces natural killer cells that comprise at least 80% CD56+CD3– natural killer cells.

128. The method of any of claims 1-11, 19-83, and 88-119, wherein said method produces natural killer cells that comprise at least 20% CD56+CD3–CD11a+ natural killer cells.

129. The method of any of claims 1-11, 19-83, and 88-119, wherein said method produces natural killer cells that comprise at least 40% CD56+CD3– CD11a+ natural killer cells.

130. The method of any of claims 1-11, 19-83, and 88-119, wherein said method produces natural killer cells that comprise at least 60% CD56+CD3– CD11a+ natural killer cells.

131. The method of any of claims 1-11, 19-83, and 88-119, wherein said method produces natural killer cells that comprise at least 80% CD56+CD3– CD11a+ natural killer cells.

132. The method of any of claims 12-80, 84-115, and 120-123, wherein said method produces ILC3 cells that comprise at least 20% CD56+CD3– CD11a– ILC3 cells.

133. The method of any of claims 12-80, 84-115, and 120-123, wherein said method produces ILC3 cells that comprise at least 40% CD56+CD3– CD11a– ILC3 cells.

134. The method of any of claims 12-80, 84-115, and 120-123, wherein said method produces ILC3 cells that comprise at least 60% CD56+CD3– CD11a– ILC3 cells.

135. The method of any of claims 12-80, 84-115, and 120-123, wherein said method produces natural killer cells that comprise at least 80% CD56+CD3– CD11a– ILC3 cells.

136. The method of any of claims 1-11, 19-83, 88-119, and 124-131, wherein said natural killer cells exhibit at least 20% cytotoxicity against K562 cells when said natural killer cells and said K562 cells are co-cultured *in vitro* at a ratio of 10:1.

137. The method of claim 136, wherein said natural killer cells exhibit at least 35% cytotoxicity against the K562 cells.

138. The method of claim 136, wherein said natural killer cells exhibit at least 45% cytotoxicity against the K562 cells.

139. The method of claim 136, wherein said natural killer cells exhibit at least 60% cytotoxicity against the K562 cells.

140. The method of claim 136, wherein said natural killer cells exhibit at least 75% cytotoxicity against the K562 cells.

141. The method of any of claims 12-80, 84-115, 120-123, and 132-135, wherein said ILC3 cells exhibit at least 20% cytotoxicity against K562 cells when said ILC3 cells and said K562 cells are co-cultured *in vitro* at a ratio of 10:1.

142. The method of claim 141, wherein said ILC3 cells exhibit at least 35% cytotoxicity against the K562 cells.

143. The method of claim 141, wherein said ILC3 cells exhibit at least 45% cytotoxicity against the K562 cells.

144. The method of claim 141, wherein said ILC3 cells exhibit at least 60% cytotoxicity against the K562 cells.

145. The method of claim 141, wherein said ILC3 cells exhibit at least 75% cytotoxicity against the K562 cells.

146. The method of any of claims 1-5, 19-83, 88-119, 124-131, and 136-140, wherein viability of said natural killer cells is determined by 7-aminoactinomycin D (7AAD) staining.

147. The method of any of claims 1-5, 19-83, 88-119, 124-131, and 136-140, wherein viability of said natural killer cells is determined by annexin-V staining.

148. The method of any of claims 1-5, 19-83, 88-119, 124-131, and 136-140, wherein viability of said natural killer cells is determined by both 7-AAD staining and annexin-V staining.

149. The method of any of claims 1-5, 19-83, 88-119, 124-131, and 136-140, wherein viability of said natural killer cells is determined by trypan blue staining.

150. The method of any of claims 1-149, additionally comprising cryopreserving said population of cells after step (c) or step (d).

151. The method of any of claims 1-149, additionally comprising cryopreserving said population of cells after step (c).

152. The method of any of claims 1-149, wherein said first medium and/or said second medium lack leukemia inhibiting factor (LIF) and/or macrophage inflammatory protein-1 alpha (MIP-1α).

153. The method of any of claims 1-149, wherein said third medium lacks LIF, MIP-1α, and FMS-like tyrosine kinase-3 ligand (Flt-3L).

154. The method of any of claims 1-149, wherein said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L.

155. The method of any of claims 1-149, wherein none of the first medium, second medium or third medium comprises a desulphated glycosaminoglycan.

156. The method of any of claims 1-149, wherein the first medium, second medium or third medium comprises heparin.

157. The method of claim 156, wherein said heparin is low-molecular weight heparin (LMWH).

158. The method of any of claims 1-5, 19-83, 88-119, 124-131, 136-140, and 146-149, additionally comprising cryopreserving said natural killer cells after step (c) or step (d).

159. A population of natural killer cells produced by the method of any of claims 1-5, 19-83, 88-119, 124-131, 136-140, and 146-149.

160. A population of cells comprising natural killer cells, wherein the population of cells is produced by the method of any of claims 1-5, 19-83, 88-119, 124-131, 136-140, and 146-149.

161. A population of ILC3 cells produced by the method of any of claims 12-80, 84-115, 120-123, 132-135, and 141-145.

162. A population of cells comprising ILC3 cells, wherein the population of cells is produced by the method of any of claims 12-80, 84-115, 120-123, 132-135, and 141-145.

163. A population of cells comprising the third population of cells of any one of claims 1-8 and 10-15.

164. A population of cells comprising the fourth population of cells of claim 9 or claim 16.

165. A method of suppressing the proliferation of tumor cells comprising contacting the tumor cells with a plurality of natural killer cells, wherein the natural killer cells are produced by the method of any one of claims 1-5, 19-83, 88-119, 124-131, 136-140, and 146-149.

166. The method of claim 165, wherein said contacting takes place *in vitro*.

167. The method of claim 165, wherein said contacting takes place *in vivo*.

168. The method of claim 167, wherein said contacting takes place in a human individual.

169. The method of claim 167, wherein said method comprises administering said natural killer cells to said individual.

170. The method of any of claims 165-169, wherein said tumor cells are multiple myeloma cells.

171. The method of any of claims 165-169, wherein said tumor cells are acute myeloid leukemia (AML) cells.

172. The method of claim 171, wherein said individual has relapsed/refractory AML.

173. The method of claim 171, wherein said individual has AML that has failed at least one non-innate lymphoid cell (ILC) therapeutic against AML.

174. The method of claim 165, wherein said individual is 65 years old or greater, and is in first remission.

175. The method of any of claims 165-168, wherein said individual has been conditioned with fludarabine, cytarabine, or both, prior to administering said natural killer cells.

176. The method of any of claims 159-163, wherein said tumor cells are breast cancer cells, head and neck cancer cells, or sarcoma cells.

177. The method of any of claims 159-163, wherein said tumor cells are primary ductal carcinoma cells, leukemia cells, acute T cell leukemia cells, chronic myeloid lymphoma (CML) cells, chronic myelogenous leukemia (CML) cells, lung carcinoma cells, colon adenocarcinoma cells, histiocytic lymphoma cells, colorectal carcinoma cells, colorectal adenocarcinoma cells, or retinoblastoma cells.

178. The method of any of claims 165-169, wherein said tumor cells are solid tumor cells.

179. The method of any of claims 165-169, wherein said tumor cells are liver tumor cells.

180. The method of any of claims 165-169, wherein said tumor cells are lung tumor cells.

181. The method of any of claims 165-169, wherein said tumor cells are pancreatic tumor cells.

182. The method of any of claims 165-169, wherein said tumor cells are renal tumor cells.

183. The method of any of claims 165-169, wherein said tumor cells are glioblastoma multiforme (GBM) cells.

184. The method of any one of claims 165-183, wherein said natural killer cells are administered with an anti-CD33 antibody.

185. The method of any one of claims 165-183, wherein said natural killer cells are administered with an anti-CD20 antibody.

186. The method of any one of claims 165-183, wherein said natural killer cells are administered with an anti-CD138 antibody.

187. The method of any one of claims 165-183, wherein said natural killer cells are administered with an anti-CD32 antibody.

188. The method of any of claims 165-187, wherein said natural killer cells have been cryopreserved prior to said contacting or said administering.

189. The method of any of claims 165-187, wherein said natural killer cells have not been cryopreserved prior to said contacting or said administering.

190. A method of suppressing the proliferation of tumor cells comprising contacting the tumor cells with a plurality of ILC3 cells, wherein the ILC3 cells are produced by the method of any one of claims 12-80, 84-115, 120-123, 132-135, and 141-145.

191. The method of claim 190, wherein said contacting takes place in vitro.

192. The method of claim 190, wherein said contacting takes place in vivo.

193. The method of claim 192, wherein said contacting takes place in a human individual.

194. The method of claim 192, wherein said method comprises administering said natural killer cells to said individual.

195. The method of any of claims 190-194, wherein said tumor cells are multiple myeloma cells.

196. The method of any of claims 190-194, wherein said tumor cells are acute myeloid leukemia (AML) cells.

197. The method of claim 196, wherein said individual has relapsed/refractory AML.

198. The method of claim 196, wherein said individual has AML that has failed at least one non-innate lymphoid cell (ILC) therapeutic against AML.

199. The method of claim 196, wherein said individual is 65 years old or greater, and is in first remission.

200. The method of any of claims 196-199, wherein said individual has been conditioned with fludarabine, cytarabine, or both prior to administering said ILC3 cells.

201. The method of any of claims 190-194, wherein said tumor cells are breast cancer cells, head and neck cancer cells, or sarcoma cells.

202. The method of any of claims 190-194, wherein said tumor cells are primary ductal carcinoma cells, leukemia cells, acute T cell leukemia cells, chronic myeloid lymphoma (CML) cells, chronic myelogenous leukemia (CML) cells, lung carcinoma cells, colon adenocarcinoma cells, histiocytic lymphoma cells, colorectal carcinoma cells, colorectal adenocarcinoma cells, or retinoblastoma cells.

203. The method of any of claims 190-194, wherein said tumor cells are solid tumor cells.

204. The method of any of claims 190-194, wherein said tumor cells are liver tumor cells.

205. The method of any of claims 190-194, wherein said tumor cells are lung tumor cells.

206. The method of any of claims 190-194, wherein said tumor cells are pancreatic tumor cells.

207. The method of any of claims 190-194, wherein said tumor cells are renal tumor cells.

208. The method of any of claims 190-194, wherein said tumor cells are glioblastoma multiforme (GBM) cells.

209. The method of any one of claims 190-208, wherein said natural killer cells are administered with an anti-CD33 antibody.

210. The method of any one of claims 190-208, wherein said natural killer cells are administered with an anti-CD20 antibody.

211. The method of any one of claims 190-208, wherein said natural killer cells are administered with an anti-CD138 antibody.

212. The method of any one of claims 190-208, wherein said natural killer cells are administered with an anti-CD32 antibody.

213. The method of any of claims 190-212, wherein said ILC3 cells have been cryopreserved prior to said contacting or said administering.

214. The method of any of claims 190-212, wherein said ILC3 cells have not been cryopreserved prior to said contacting or said administering., A method of repairing the gastrointestinal tract after chemotherapy comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells are produced by the method of any one of claims 12-80, 84-115, 120-123, 132-135, and 141-145.

215. A method of protecting an individual against radiation comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells are produced by the method of any one of claims 12-80, 84-115, 120-123, 132-135, and 141-145.

216. The method of claim 210, wherein said ILC3 cells are used as an adjunct to bone marrow transplantation.

217. A method of reconstituting the thymus of an individual comprising administering to an individual a plurality of ILC3 cells, wherein the ILC3 cells are produced by the method of any one of claims 12-80, 84-115, 120-123, 132-135, and 141-145.

218. A population of natural killer cells that is CD56+CD3– CD117+CD11a+, wherein said natural killer cells express perforin and/or EOMES, and do not express one or more of RORγt, aryl hydrocarbon receptor, and IL1R1.

219. The population of claim 219, wherein said natural killer cells express perforin and EOMES, and do not express any of RORγt, aryl hydrocarbon receptor, or IL1R1.

220. The population of claim 219 or 220, wherein said natural killer cells additionally express T-bet, GZMB, NKp46, NKp30, and NKG2D.

221. The population of any one of claims 219-221, wherein said natural killer cells express CD94.

222. The population of any one of claims 219-221, wherein said natural killer cells do not express CD94.

223. A population of ILC3 cells that is CD56+CD3– CD117+CD11a-, wherein said ILC3 cells express one or more of RORγt, aryl hydrocarbon receptor, and IL1R1, and do not express one or more of CD94, perforin, and EOMES.

224. The population of claim 224, wherein said ILC3 cells express RORγt, aryl hydrocarbon receptor, and IL1R1, and do not express any of CD94, perforin, or EOMES.

225. The population of claim 224 or 225, wherein said ILC3 cells additionally express CD226 and/or 2B4.

226. The population of any one of claims 224-226, wherein said ILC3 cells additionally express one or more of IL-22, TNFα, and DNAM-1.

227. The population of any one of claims 224-227, wherein said ILC3 cells express CD226, 2B4, IL-22, TNFα, and DNAM-1.

228. A method of producing a cell population comprising natural killer cells and ILC3 cells, comprising the steps of:

(a) culturing hematopoietic stem or progenitor cells in a first medium comprising a stem cell mobilizing agent and thrombopoietin (Tpo) to produce a first population of cells;

(b) culturing the first population of cells in a second medium comprising a stem cell mobilizing agent and interleukin-15 (IL-15), and lacking Tpo, to produce a second population of cells;

(c) culturing the second population of cells in a third medium comprising IL-2 and IL-15, and lacking each of a stem cell mobilizing agent and LMWH, to produce a third population of cells; and

(d) separating CD11a+ cells and CD11a– cells from the third population of cells; and

(e) combining the CD11a+ cells with the CD11a– cells in a ratio of 50:1, 40:1, 30:1, 20:1, 10:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:10, 1:20, 1:30, 1:40, or 1:50 to produce a fourth population of cells.

229. The method of claim 229, wherein said first medium and/or said second medium lack LIF and/or MIP-1α.

230. The method of claim 229, wherein said third medium lacks LIF, MIP-1α, and Flt-3L.

231. The method of claim 229, wherein said first medium and said second medium lack LIF and MIP-1α, and said third medium lacks LIF, MIP-1α, and Flt3L.

232. The method of any of claims 229-232, wherein none of the first medium, second medium or third medium comprises a desulphated glycosaminoglycan.

233. The method of any of claims 229-232 wherein the first medium, second medium or third medium comprises heparin.

234. The method of claim 234, wherein said heparin is low-molecular weight heparin (LMWH).

235. The fourth population of cells of claim 229, wherein the CD11a+ cells and CD11a– cells are combined in a ratio of 50:1.

236. The fourth population of cells of claim 229, wherein the CD11a+ cells and CD11a– cells are combined in a ratio of 20:1.

237. The fourth population of cells of claim 229, wherein the CD11a+ cells and CD11a– cells are combined in a ratio of 10:1.

238. The fourth population of cells of claim 229, wherein the CD11a+ cells and CD11a– cells are combined in a ratio of 5:1.

239. The fourth population of cells of claim 229, wherein the CD11a+ cells and CD11a– cells are combined in a ratio of 1:1.

240. The fourth population of cells of claim 229, wherein the CD11a+ cells and CD11a– cells are combined in a ratio of 1:5.

241. The fourth population of cells of claim 229, wherein the CD11a+ cells and CD11a– cells are combined in a ratio of 1:10.

242. The fourth population of cells of claim 229, wherein the CD11a+ cells and CD11a– cells are combined in a ratio of 1:20.

243. The fourth population of cells of claim 229, wherein the CD11a+ cells and CD11a– cells are combined in a ratio of 1:50.