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(71) Applicant: **ANTHROGENESIS CORPORATION** [US/US]; 7 Powder Horn Drive, Warren, NJ 07059 (US).

(72) Inventors: **KANG, Lin**; 1106 Timber Oaks Road, Edison, NJ 08820 (US). **ZHANG, Xiaokui**; 10 Mounthaven Drive, Livingston, NJ 07039 (US). **HARRIS, Jeffrey**; 17 Steeple View Court, Lambertville, NJ 08530 (US). **JANKOVIC, Vladimir**; 10 River Road, Apt. 15b, New York, NY 10044 (US).

(74) Agents: **GEORGE, Nikolaos, C.** et al.; Jones Day, 250 Vesey Street, New York, NY 10281-1047 (US).

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(54) Title: METHODS OF TREATING HEMATOLOGICAL DISORDERS, SOLID TUMORS, OR INFECTIOUS DISEASES USING NATURAL KILLER CELLS

(57) Abstract: Provided herein are methods of treating a hematological disorder, a solid tumor, or an infectious disease in a subject in need thereof using natural killer cells in combination with a second agent, or using natural killer cells with genetic modifications for target specificity and/or homing specificity.

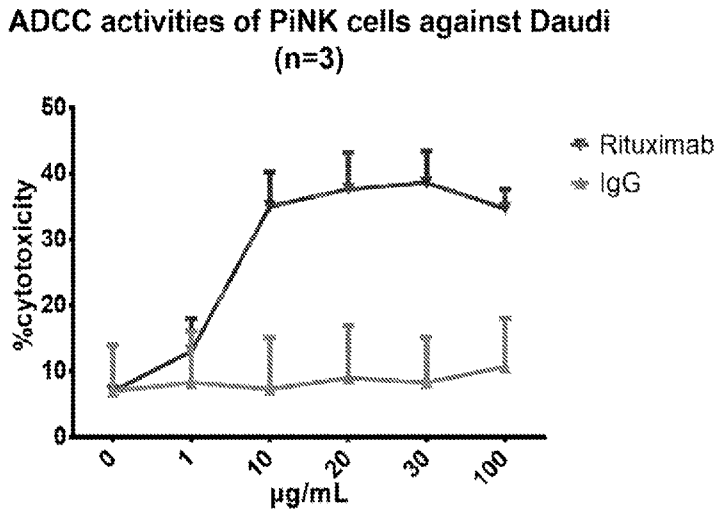


Fig. 1

WO 2016/109668 A1

METHODS OF TREATING HEMATOLOGICAL DISORDERS, SOLID TUMORS, OR INFECTIOUS DISEASES USING NATURAL KILLER CELLS

[0001] This application claims benefit of U.S. Provisional Patent Application No. 62/098,547, filed December 31, 2014, and U.S. Provisional Patent Application No. 62/139,952, filed March 30, 2015, the disclosures of each of which are incorporated by reference herein in its entirety.

1. FIELD

[0002] Provided herein are methods of treating a hematological disorder, a solid tumor, or an infectious disease in a subject in need thereof using natural killer cells in combination with a second agent, or using natural killer cells with genetic modifications for target specificity and/or homing specificity.

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. NK cells possess two types of surface receptors, labeled "activating receptors" and "inhibitory receptors," that control the cells' cytotoxic activity.

[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. Activated and expanded NK cells and 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 great need for developing more efficacious NK cells and more efficacious therapeutic regimens that utilize NK cells.

3. SUMMARY OF THE INVENTION

[0007] The present invention provides methods of treating a disease (*e.g.*, a hematological

disorder, a solid tumor, or an infectious disease) in a subject in need thereof, using natural killer (NK) cells in combination with a second agent that can be used to treat the disease. Also provided herein are methods of treating a disease (*e.g.*, a hematological disorder, a solid tumor, or an infectious disease) in a subject in need thereof, using NK cells with genetic modifications (*e.g.*, NK cells that comprise a chimeric antigen receptor (CAR) and/or a homing receptor) for target specificity and/or homing specificity.

[0008] In one aspect, provided herein are methods of treating a cancer in a subject in need thereof, comprising: (a) administering to said subject an isolated population of natural killer (NK) cells or a pharmaceutical composition thereof; and (b) administering to said subject a second agent or a pharmaceutical composition thereof, wherein said second agent can be used to treat said cancer. In a specific embodiment, said cancer is multiple myeloma.

[0009] In certain embodiments, the second agent is an antibody or antigen binding fragment thereof that specifically binds to a tumor-associated antigen (TAA). In specific embodiments, the antibody is a monoclonal antibody. In specific embodiments, the TAA is selected from the group consisting of CD123, CLL-1, CD38, CS-1 (also referred to as SLAM7, SLAMF7, CD319, and CRACC), CD138, ROR1, FAP, MUC1, PSCA, EGFRvIII, EPHA2, and GD2. In a more specific embodiment, the second agent is an antibody that binds to CS-1. In more specific embodiments, the second agent is elotuzumab (HuLuc63, Bristol Myers-Squibb/AbbVie humanized anti-CS-1 monoclonal antibody).

[0010] In certain embodiments, the second agent is an antibody or antigen binding fragment thereof that specifically binds to a tumor microenvironment-associated antigen (TMAA). In specific embodiments, the antibody is a monoclonal antibody. In specific embodiments, the TMAA is selected from the group consisting of VEGF-A, EGF, PDGF, IGF, and bFGF.

[0011] In certain embodiments, the second agent is an antibody or antigen binding fragment thereof that specifically binds to and antagonizes the activity of an immune checkpoint protein. In specific embodiments, the antibody is a monoclonal antibody. In specific embodiments, the immune checkpoint protein is selected from the group consisting of CTLA-4, PD-1, PD-L1, PD-L2, and LAG-3.

[0012] In certain embodiments, the second agent is a bispecific killer cell engager (BiKE). In specific embodiments, the BiKE comprises a first single chain variable fragment (scFv) that specifically binds to a TAA. In further specific embodiments, the TAA is selected from the

group consisting of CD123, CLL-1, CD38, CS-1, CD138, ROR1, FAP, MUC1, PSCA, EGFRvIII, EPHA2, and GD2. In specific embodiments, the BiKE comprises a second scFv that specifically binds to CD16.

[0013] In certain embodiments, the second agent is an anti-inflammatory agent.

[0014] In certain embodiments, the second agent is an immunomodulatory agent. In specific embodiments, the second agent is lenalidomide or pomalidomide.

[0015] In certain embodiments, the second agent is a cytotoxic agent.

[0016] In certain embodiments, the second agent is a cancer vaccine.

[0017] In certain embodiments, the second agent is a chemotherapeutic.

[0018] In certain embodiments, the second agent is an HDAC inhibitor. In other specific embodiments, the second agent is romidepsin (ISTODAX®, Celgene).

[0019] In certain embodiments, the second agent is an siRNA.

[0020] In some embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered before the second agent or a pharmaceutical composition thereof. In some embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered after the second agent or a pharmaceutical composition thereof. In other embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered at the same time as the second agent or a pharmaceutical composition thereof.

[0021] In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is performed with a devise, a matrix, or a scaffold. In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection. 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 NK cells is by injection by syringe. In specific embodiments, administration of NK cells by injection is aided by laparoscopy, endoscopy, ultrasound, computed tomography, magnetic resonance, or radiology.

[0022] In specific embodiments, the step of administering to said subject a second agent or a pharmaceutical composition thereof is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In specific embodiments, the step of administering to said subject a second agent or a pharmaceutical composition thereof is performed with a devise, a matrix, or a scaffold.

[0023] In various embodiments, the NK cells are fucosylated on the cell surface.

[0024] In some embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered in a single dose. In other embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered in multiple doses.

[0025] In some embodiments, the second agent or a pharmaceutical composition thereof is administered in a single dose. In other embodiments, the second agent or a pharmaceutical composition thereof is administered in multiple doses.

[0026] In another aspect, provided herein are methods of treating a cancer in a subject in need thereof, comprising administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof, wherein the NK cells comprise a chimeric antigen receptor (CAR), wherein said CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and optionally a co-stimulatory domain. Also provided herein are methods of treating a cancer in a subject in need thereof, comprising administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof, wherein the NK cells comprise a homing receptor, and methods of treating a cancer in a subject in need thereof, comprising administering to said subject an isolated population of Natural Killer (NK) cells or a pharmaceutical composition thereof, wherein the NK cells comprise a chimeric antigen receptor (CAR) and a homing receptor, wherein said CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and optionally a co-stimulatory domain. In various embodiments, the CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and a co-stimulatory domain.

[0027] In specific embodiments, the NK cells comprising the CAR and/or the homing receptor are derived from CD34+ hematopoietic stem cells (HSCs) that are engineered to express the CAR and/or the homing receptor.

[0028] In various embodiments, the extracellular domain of the CAR is an antigen binding

domain. In specific embodiments, the antigen binding domain is an scFv domain. In certain embodiments, the antigen binding domain specifically binds to a TAA. In specific embodiments, the TAA is selected from the group consisting of CD123, CLL-1, CD38, CD20, and CS-1. In more specific embodiments, the antigen-binding domain comprises a single-chain Fv (scFv) or antigen-binding fragment derived from an antibody that binds CS-1. In more specific embodiments, the antigen-binding domain comprises a single-chain version of elotuzumab and/or an antigen-binding fragment of elotuzumab. In specific embodiments, the antigen-binding domain comprises a single-chain Fv (scFv) or antigen-binding fragment derived from an antibody that binds CD20.

[0029] In various embodiments, the intracellular stimulatory domain of the CAR is a CD3 zeta signaling domain.

[0030] In various embodiments, the co-stimulatory domain of the CAR comprises the intracellular domain of CD28, 4-1BB, PD-1, OX40, CTLA-4, NKp46, NKp44, NKp30, DAP10 or DAP12.

[0031] In various embodiments, the homing receptor is a chemotactic receptor. In specific embodiments, the chemotactic receptor is selected from the group consisting of CXCR4, VEGFR2, and CCR7.

[0032] In one embodiment, provided herein is a method of treating an individual having multiple myeloma, comprising administering to the individual (1) lenalidomide or pomalidomide and (2) NK cells that comprise a CAR ("CAR NK cells"), wherein said CAR NK cells are effective to treat multiple myeloma in said individual. In specific embodiments of the method of treating an individual with multiple myeloma, said CAR NK cells comprise a CAR extracellular domain, which extracellular domain is a CS-1 binding domain. In specific embodiments, the CS-1 binding domain comprises an scFv or antigen-binding fragment of an antibody that binds CS-1. In certain specific embodiments, the CS-1 binding domain comprises a single-chain version of elotuzumab and/or an antigen-binding fragment of elotuzumab.

[0033] In another embodiment, provided herein is a method of treating an individual having multiple myeloma, comprising administering to the individual (1) lenalidomide or pomalidomide; (2) elotuzumab; and (3) CAR NK cells, wherein said CAR NK cells are effective to treat multiple myeloma in said individual. In certain specific embodiments of the method of treating an individual with multiple myeloma, said CAR NK cells comprise a CAR extracellular

domain, which extracellular domain is a CS-1 binding domain. In specific embodiments, the CS-1 binding domain comprises an scFv or antigen-binding fragment of an antibody that binds CS-1.

[0034] In another embodiment, provided herein is a method of treating an individual having a blood cancer (*e.g.*, Burkitt's lymphoma), comprising administering to the individual (1) romidepsin and (2) CAR NK cells, wherein said CAR NK cells are effective to treat the blood cancer (*e.g.*, Burkitt's lymphoma) in said individual. In certain specific embodiments of the method of treating an individual with blood cancer (*e.g.*, Burkitt's lymphoma), said CAR NK cells comprise a CAR extracellular domain, which extracellular domain is a CD20 binding domain. In specific embodiments, the CD20 binding domain comprises an scFv or antigen-binding fragment of an antibody that binds CD20.

[0035] In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is performed with a devise, a matrix, or a scaffold. In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection. 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 NK cells is by injection by syringe. In specific embodiments, administration of NK cells by injection is aided by laparoscopy, endoscopy, ultrasound, computed tomography, magnetic resonance, or radiology.

[0036] In various embodiments, the NK cells are fucosylated on the cell surface.

[0037] In some embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered in a single dose. In other embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered in multiple doses.

[0038] In another aspect, provided herein are methods of treating a viral infection in a subject in need thereof, comprising: (a) administering to said subject an isolated population of natural killer (NK) cells or a pharmaceutical composition thereof; and (b) administering to said

subject a second agent or a pharmaceutical composition thereof, wherein said second agent can be used to treat said viral infection.

[0039] In certain embodiments, the second agent is an antibody or antigen binding fragment thereof that specifically binds to and antagonizes the activity of an immune checkpoint protein. In specific embodiments, the antibody is a monoclonal antibody. In specific embodiments, the immune checkpoint protein is selected from the group consisting of CTLA-4, PD-1, PD-L1, PD-L2, and LAG-3.

[0040] In certain embodiments, the second agent is a bispecific killer cell engager (BiKE).

[0041] In some embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered before the second agent or a pharmaceutical composition thereof. In some embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered after the second agent or a pharmaceutical composition thereof. In other embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered at the same time as the second agent or a pharmaceutical composition thereof.

[0042] In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is performed with a devise, a matrix, or a scaffold. In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection. 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 NK cells is by injection by syringe. In specific embodiments, administration of NK cells by injection is aided by laparoscopy, endoscopy, ultrasound, computed tomography, magnetic resonance, or radiology.

[0043] In specific embodiments, the step of administering to said subject a second agent or a pharmaceutical composition thereof is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In specific embodiments, the step of administering to said subject a second agent or a pharmaceutical composition thereof is

performed with a devise, a matrix, or a scaffold.

[0044] In various embodiments, the NK cells are fucosylated on the cell surface.

[0045] In some embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered in a single dose. In other embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered in multiple doses.

[0046] In some embodiments, the second agent or a pharmaceutical composition thereof is administered in a single dose. In other embodiments, the second agent or a pharmaceutical composition thereof is administered in multiple doses.

[0047] In another aspect, provided herein are methods of treating a viral infection in a subject in need thereof, comprising administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof, wherein the NK cells comprise a chimeric antigen receptor (CAR), wherein said CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and optionally a co-stimulatory domain. Also provided herein are methods of treating a viral infection in a subject in need thereof, comprising administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof, wherein the NK cells comprise a homing receptor, and methods of treating a viral infection in a subject in need thereof, comprising administering to said subject an isolated population of Natural Killer (NK) cells or a pharmaceutical composition thereof, wherein the NK cells comprise a chimeric antigen receptor (CAR) and a homing receptor, wherein said CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and optionally a co-stimulatory domain. In various embodiments, the CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and a co-stimulatory domain.

[0048] In specific embodiments, the NK cells comprising the CAR and/or the homing receptor are derived from CD34+ hematopoietic stem cells (HSCs) that are engineered to express the CAR and/or the homing receptor.

[0049] In various embodiments, the extracellular domain of the CAR is an antigen binding domain. In specific embodiments, the antigen binding domain is an scFv domain.

[0050] In various embodiments, the intracellular stimulatory domain of the CAR is a CD3 zeta signaling domain.

[0051] In various embodiments, the co-stimulatory domain of the CAR comprises the intracellular domain of CD28, 4-1BB, PD-1, OX40, CTLA-4, NKp46, NKp44, NKp30, DAP10 or DAP12.

[0052] In various embodiments, the homing receptor is a chemotactic receptor. In specific embodiments, the chemotactic receptor is selected from the group consisting of CXCR4, VEGFR2, and CCR7.

[0053] In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is performed with a devise, a matrix, or a scaffold. In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection. 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 NK cells is by injection by syringe. In specific embodiments, administration of NK cells by injection is aided by laparoscopy, endoscopy, ultrasound, computed tomography, magnetic resonance, or radiology.

[0054] In various embodiments, the NK cells are fucosylated on the cell surface.

[0055] In some embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered in a single dose. In other embodiments, the isolated population of NK cells or a pharmaceutical composition thereof is administered in multiple doses.

[0056] The present invention also provides kits for treating a disease (*e.g.*, a hematological disorder, a solid tumor, or an infectious disease) in a subject in need thereof, which comprise an isolated population of NK cells and a second agent that can be used to treat the disease.

[0057] In one aspect, provided herein are kits for treating a cancer in a subject in need thereof, comprising: (a) an isolated population of NK cells or a pharmaceutical composition thereof; and (b) a second agent or a pharmaceutical composition thereof, wherein said second agent can be used to treat said cancer. The second agent can be any that may be used in the methods of treating a cancer as provided above.

[0058] In another aspect, provided herein are kits for treating a viral infection in a subject in need thereof, comprising: (a) an isolated population of NK cells or a pharmaceutical composition thereof; and (b) a second agent or a pharmaceutical composition thereof, wherein said second agent can be used to treat said viral infection. The second agent can be any that may be used in the methods of treating a viral infection as provided above.

[0059] In various embodiments of the methods or kits provided herein, the NK cells are placental intermediate natural killer (PiNK) cells. In certain embodiments, the PiNK cells are derived from placental cells. In specific embodiments, the placental cells are obtained from placental perfusate. In specific embodiments, the placental cells are obtained from placental tissue that has been mechanically and/or enzymatically disrupted.

[0060] In various embodiments of the methods or kits provided herein, the NK cells are activated NK cells. In certain embodiments, the activated NK cells are produced by a process comprising: (a) seeding a population of hematopoietic stem or progenitor cells in a first medium comprising interleukin-15 (IL-15) and, optionally, one or more of stem cell factor (SCF) and interleukin-7 (IL-7), wherein said IL-15 and optional SCF and IL-7 are not comprised within an undefined component of said medium, such that the population expands, and a plurality of hematopoietic stem or progenitor cells within said population of hematopoietic stem or progenitor cells differentiate into NK cells during said expanding; and (b) expanding the cells from the step (a) in a second medium comprising interleukin-2 (IL-2), to produce a population of activated NK cells. In certain embodiments, the activated NK cells are produced by a process comprising: expanding a population of hematopoietic stem or progenitor cells in a first medium comprising one or more of stem cell factor (SCF), interleukin-7 (IL-7) and interleukin-15 (IL-15), and wherein said SCF, IL-7 and IL-15 are not comprised within an undefined component of said medium, and wherein a plurality of hematopoietic stem or progenitor cells within said population of hematopoietic stem or progenitor cells differentiate into NK cells during said expanding; and wherein a second step of said method comprises expanding the cells from the first step in a second medium comprising interleukin-2 (IL-2), to produce activated NK cells.

[0061] In specific embodiments, the first medium further comprises one or more of Fms-like-tyrosine kinase 3 ligand (Flt3-L), thrombopoietin (Tpo), interleukin-2 (IL-2), or heparin. In further specific embodiments, the first medium further comprises fetal bovine serum or human serum. In further specific embodiments, the SCF is present at a concentration of about 1 to about

150 ng/mL in the first medium. In further specific embodiments, the Flt3-L is present at a concentration of about 1 to about 150 ng/mL in the first medium. In further specific embodiments, the IL-2 is present at a concentration of about 50 to about 1500 IU/mL in the first medium. In further specific embodiments, the IL-7 is present at a concentration of about 1 to about 150 ng/mL in the first medium. In further specific embodiments, the IL-15 is present at a concentration 1 to about 150 ng/mL in the first medium. In further specific embodiments, the Tpo is present at a concentration of about 1 to about 150 ng/mL in the first medium. In further specific embodiments, the heparin is present at a concentration of about 0.1 to about 30 U/mL in the first medium.

[0062] In specific embodiments, said IL-2 in the second step above is present at a concentration 50 to about 1500 IU/mL in the second medium.

[0063] In specific embodiments, said second medium additionally comprises one or more of fetal calf serum (FCS), transferrin, insulin, ethanolamine, oleic acid, linoleic acid, palmitic acid, bovine serum albumin (BSA) and phytohemagglutinin.

[0064] In specific embodiments, the hematopoietic stem or progenitor cells are $CD34^+$.

[0065] In specific embodiments, the hematopoietic stem or progenitor cells comprise hematopoietic stem or progenitor cells from human placental perfusate and hematopoietic stem or progenitor cells from umbilical cord, wherein said placental perfusate and said umbilical cord blood are from the same placenta.

[0066] In specific embodiments, the feeder cells in step (b) above comprise mitomycin C-treated peripheral blood mononuclear cells (PBMC), K562 cells or tissue culture-adherent stem cells.

[0067] In specific embodiments, the NK cells are $CD3^-CD56^+CD16^-$. In a further specific embodiment, the NK cells are additionally $CD94^+CD117^+$. In another further specific embodiment, the NK cells are additionally $CD161^-$. In another further specific embodiment, the NK cells are additionally $NKG2D^+$. In another further specific embodiment, the NK cells are additionally $NKp46^+$. In another further specific embodiment, the NK cells are additionally $CD226^+$.

[0068] In various embodiments of the methods or kits provided herein, the NK cells are Three-Step Process NK (TSPNK) cells. In specific embodiments, the TSPNK cells are NK progenitor cells. In certain embodiments, the TSPNK cells are produced by a process

comprising: (a) culturing hematopoietic stem cells or progenitor cells in a first medium comprising Flt3L, TPO, SCF, IL-7, G-CSF, IL-6 and GM-CSF; (b) subsequently culturing said cells in a second medium comprising Flt3L, SCF, IL-15, and IL-7, IL-17 and IL-15, G-CSF, IL-6 and GM –CSF; and (c) subsequently culturing said cells in a third medium comprising SCF, IL-15, IL-7, IL-2, G-CSF, IL-6 and GM-CSF.

[0069] In specific embodiments, the duration of culturing step (a) is 7-9 days, the duration of culturing step (b) is 5-7 days, and the duration of culturing step (c) is 5-9 days. In specific embodiments, the duration of culturing step (a) is 7-9 days, the duration of culturing step (b) is 5-7 days, and the duration of culturing step (c) is 21-35 days.

[0070] In specific embodiments, the hematopoietic stem or progenitor cells used in the process are CD34+.

[0071] In specific embodiments, the hematopoietic stem or progenitor cells comprise hematopoietic stem or progenitor cells from human placental perfusate and hematopoietic stem or progenitor cells from umbilical cord, wherein said placental perfusate and said umbilical cord blood are from the same placenta.

[0072] In specific embodiments, CD34- cells comprise more than 80% of the TSPNK cells at the end of step (a) of the process of producing TSPNK cells above.

[0073] In specific embodiments, the TSPNK cells comprise no more than 40% CD3- CD56+ cells.

[0074] In specific embodiments, the TSPNK cells comprise cells which are CD52+ CD117+.

[0075] In various embodiments of the methods or kits described herein, the NK cells are produced by a process 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 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 80% of the natural killer cells are viable.

[0076] The cancer in any one of the methods or kits provided herein can be a hematological

cancer or a solid tumor.

[0077] In preferred embodiment of any one of the methods or kits provided herein, the subject is a human.

3.1. Terminology

[0078] As used herein, "natural killer cell" or "NK cells" without further modification, includes natural killer cells derived from any tissue source, and include mature natural killer cells as well as natural killer progenitor cells. In some embodiments, NK cells are placental intermediate natural killer (PiNK) cells as described in Section 5.1.1. In some embodiments, NK cells are activated NK cells as described in Section 5.1.2. In some embodiments, NK cells are Three-Step Process NK (TSPNK) cells as described in Section 5.1.3. Natural killer cells can be derived from any tissue source, and include mature natural killer cells as well as NK progenitor cells.

[0079] As used herein, the term "NK progenitor cell population" refers to a population of cells comprising cells of the natural killer cell lineage that have yet to develop into mature NK cells, as indicated by, *e.g.*, the level(s) of expression one or more phenotypic markers, *e.g.*, CD56, CD16, and KIRs. In one embodiment, the NK progenitor cell population comprises cells with low CD16 and high CD56.

[0080] As used herein, "PiNK" and "PiNK cells" refer to placental intermediate natural killer cells that are obtained from human placenta, *e.g.*, human placental perfusate or placental tissue that has been mechanically and/or enzymatically disrupted. The cells are $CD56^+$ and $CD16^-$, *e.g.*, as determined by flow cytometry, *e.g.*, fluorescence-activated cell sorting using antibodies to CD56 and CD16.

[0081] 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.

[0082] As used herein, "placental perfusate cells" means nucleated cells, *e.g.*, total nucleated cells, isolated from, or isolatable from, placental perfusate.

[0083] 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.

[0084] As used herein, the term "hematopoietic cells" includes hematopoietic stem cells and hematopoietic progenitor cells.

[0085] 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, human serum (*e.g.*, human serum AB) and fetal serum (*e.g.*, fetal bovine serum or fetal calf serum).

[0086] 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.

[0087] 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.

[0088] As used herein, "cancer" refers to a hematological cancer or a solid tumor.

4. BRIEF DESCRIPTION OF FIGURES

[0089] **Fig. 1** depicts the antibody-dependent cellular cytotoxicity (ADCC) activities of PiNK cells against Daudi cells at different concentrations of rituximab.

[0090] **Fig. 2** depicts the expression of PD-L1 and CS-1 on the MM cells lines MM285, MM293, RPMI8226, and OPM2. Cells were stained with anti-PD-L1 APC (Biolegend, Cat# 329708), anti-CS1 PE-Cy7 (Biolegend, Cat# 331816), and 7-AAD (BD Bioscience, Cat# 559925) according to the manufacturer's protocol. Data were acquired on BD LSRFortessa (BD Biosciences) and analyzed using FLOWJO® software (Tree Star). Data were expressed as % positive cells gated under 7-AAD- single cells. Setting of the % positive gate was done using unstained sample as control. The left-most peak in the panels indicates the control, whereas the right-most peak indicates the sample. The percentage of cells positive for PD-L1 was as follows:

71.6% MM285, 70.7% MM293, 66.2% OPM-2, and 94.4% RPMI8226. The percentage of cells positive for CS-1 was as follows: 31.8% MM285, 58.8% MM293, 93.4% OPM-2, and 29.5% RPMI8226.

[0091] **Fig. 3** depicts the 24-hour cytotoxicity assay of three-stage NK cells against the indicated MM cell lines and primary MM samples at a 3:1 effector-to-target ratio. The number of viable target cells ($PKH26^+TO\text{-}PRO\text{-}3^-$) in each sample was quantified by flow cytometry using counting beads following the protocol provided by the manufacturer (Invitrogen, Cat# C36950). Counting beads were introduced in this assay in order to account for any potential proliferation of tumor cells during the prolonged 24 hour culture. After incubation for 24 hours at 37° C and 5% CO_2, cells were harvested, followed by staining with 1 μM TO-PRO-3 to identify the dead cells. Results are depicted as mean ± standard deviation of the mean.

[0092] **Fig. 4** depicts the 24-hour cytotoxicity assay of three-stage NK cells against OPM2 cells at a 3:1 effector-to-target ratio, along with the following additional conditions: IL-15 (5 ng/mL) (Invitrogen, Cat# PHC9153); IL-2 (200 IU/mL) (Invitrogen, Cat# PHC0023); anti-PD-L1 (10ng/mL) (Affymetrix, Cat# 16-5983-82); anti-IgG (10ng/mL) (Affymetrix, Cat# 16-4714-82); REVLIMID® (lenalidomide; 1uM), or DMSO (0.1%) in 48-well plates. Target cells alone were plated as controls. After incubation for 24 hours at 37° C and 5% CO_2, cells were harvested, followed by staining with 1 μM TO-PRO-3 to identify the dead cells. Results are depicted as mean ± standard deviation of the mean.

5. DETAILED DESCRIPTION

[0093] Provided herein are methods of treating a disease (*e.g.*, a hematological disorder, a solid tumor, or an infectious disease) in a subject in need thereof, using natural killer (NK) cells in combination with a second agent that can be used to treat the disease. Also provided herein are methods of treating a disease (*e.g.*, a hematological disorder, a solid tumor, or an infectious disease) in a subject in need thereof, using NK cells with genetic modifications (*e.g.*, NK cells that comprise a chimeric antigen receptor (CAR) and/or a homing receptor) for target specificity and/or homing specificity. Kits for treating a disease (*e.g.*, a hematological disorder, a solid tumor, or an infectious disease) in a subject in need thereof, which comprise an isolated population of NK cells and a second agent that can be used to treat the disease, or which comprise an isolated population of NK cells with genetic modifications (*e.g.*, NK cells that comprise a chimeric antigen receptor (CAR) and/or a homing receptor) are also provided herein.

5.1. NK Cells

[0094] Described herein are NK cells, including PiNK cells, activated NK cells, TSPNK cells, and NK cells produced by the three-stage method.

5.1.1. Placental Intermediate Natural Killer (PiNK) Cells

[0095] In some embodiments, natural killer cells are placental intermediate natural killer (PiNK) cells (see also U.S. Patent No. 8,263,065, the disclosure of which is hereby incorporated by reference in its entirety). In various embodiments, PiNK cells are derived from placental cells. In specific embodiments, the placental cells are obtained from placental perfusate, *e.g.*, human placental perfusate. In specific embodiments, the placental cells are obtained from placental tissue that has been mechanically and/or enzymatically disrupted.

[0096] PiNK cells are characterized as being $CD56^{+}CD16^{-}$, *i.e.*, displaying the CD56 cellular marker and lacking the CD16 cellular marker, *e.g.*, as determined by flow cytometry, *e.g.*, fluorescence-activated cell sorting using antibodies against CD16 and CD56, as described above.

[0097] In certain embodiments, the PiNK cells are $CD3^{-}$.

[0098] In other embodiments, the PiNK cells do not exhibit one or more cellular markers exhibited by fully mature natural killer cells (*e.g.*, CD16), or exhibit such one or more markers at a detectably reduced level compared to fully mature natural killer cells, or exhibit one or more cellular markers associated with natural killer cell precursors but not fully mature natural killer cells. In a specific embodiment, a PiNK cell described herein expresses NKG2D, CD94 and/or NKp46 at a detectably lower level than a fully mature NK cell. In another specific embodiment, a plurality of PiNK cells described herein expresses, in total, NKG2D, CD94 and/or NKp46 at a detectably lower level than an equivalent number of fully mature NK cells.

[0099] In certain embodiments, PiNK cells express one or more of the microRNAs hsa-miR-100, hsa-miR-127, hsa-miR-211, hsa-miR-302c, hsa-miR-326, hsa-miR-337, hsa-miR-497, hsa-miR-512-3p, hsa-miR-515-5p, hsa-miR-517b, hsa-miR-517c, hsa-miR-518a, hsa-miR-518e, hsa-miR-519d, hsa-miR-520g, hsa-miR-520h, hsa-miR-564, hsa-miR-566, hsa-miR-618, and/or hsa-miR-99a at a detectably higher level than peripheral blood natural killer cells.

[00100] Because the post-partum placenta comprises tissue and cells from the fetus and from the mother placental perfusate, depending upon the method of collection, PiNK cells can comprise fetal cells only, or a substantial majority of fetal cells (*e.g.*, greater than about 90%, 95%, 98% or 99%), or can comprise a mixture of fetal and maternal cells (*e.g.*, the fetal cells

comprise less than about 90%, 80%, 70%, 60%, or 50% of the total nucleated cells of the perfusate). In one embodiment, the PiNK cells are derived only from fetal placental cells, *e.g.*, cells obtained from closed-circuit perfusion of the placenta (*see* above) wherein the perfusion produces perfusate comprising a substantial majority, or only, fetal placental cells. In another embodiment, the PiNK cells are derived from fetal and maternal cells, *e.g.*, cells obtained by perfusion by the pan method (*see* above), wherein the perfusion produced perfusate comprising a mix of fetal and maternal placental cells. Thus, in one embodiment, the NK cells are a population of placenta-derived intermediate natural killer cells, the substantial majority of which have the fetal genotype. In another embodiment, the NK cells are a population of placenta-derived intermediate natural killer cells that comprise natural killer cells having the fetal genotype and natural killer cells having the maternal phenotype.

5.1.2. Activated NK Cells

[00101] In some embodiments, natural killer cells are activated NK cells (*i.e.*, Two-Step NK cells, or TSNK cells) (see also U. S. Patent Application Publication No. 2012/0148553, the disclosure of which is hereby incorporated by reference in its entirety), which are NK cells produced by any method/process described below in Section 5.2.4.

[00102] In a specific embodiment, the activated NK cells are $CD3^{-}CD56^{+}$. In a specific embodiment, the activated NK cells are $CD3^{-}CD56^{+}CD16^{-}$. In another specific embodiment, the activated NK cells are additionally $CD94^{+}CD117^{+}$. In another specific embodiment, the activated NK cells are additionally $CD161^{-}$. In another specific embodiment, the activated NK cells are additionally $NKG2D^{+}$. In another specific embodiment, the activated NK cells are additionally $NKp46^{+}$. In another specific embodiment, the activated NK cells are additionally $CD226^{+}$.

[00103] In certain embodiments, greater than 50%, 60%, 70%, 80%, 90%, 92%, 94%, 96%, 98% of said activated NK cells are $CD56^{+}$ and $CD16^{-}$. In other embodiments, at least 50%, 60%, 70%, 80%, 82%, 84%, 86%, 88% or 90% of said activated NK cells are $CD3^{-}$ and $CD56^{+}$. In other embodiments, at least 50%, 52%, 54%, 56%, 58% or 60% of said activated NK cells are $NKG2D^{+}$. In other embodiments, fewer than 30%, 20%, 10%, 9%, 8%, 7%, 6%, 5%, 4% or 3% of said cells are $NKB1^{+}$. In certain other embodiments, fewer than 30%, 20%, 10%, 8%, 6%, 4% or 2% of said activated NK cells are $NKAT2^{+}$. In certain other embodiments, fewer than 30%, 20%, 10%, 8%, 6%, 4% or 2% of said activated NK cells are $CD56^{+}$ and $CD16^{+}$. In more

specific embodiments, at least 10%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65% or 70% of said $CD3^-$, $CD56^+$ activated NK cells are $NKp46^+$. In other more specific embodiments, at least 10%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80% or 85% of said $CD3^-$, $CD56^+$ activated NK cells are $CD117^+$. In other more specific embodiments, at least 10%, 20%, 25%, 30%, 35%, 40%, 45% or 50% of said $CD3^-$, $CD56^+$ activated NK cells are $CD94^+$. In other more specific embodiments, at least 10%, 20%, 25%, 30%, 35%, 40%, 45% or 50% of said $CD3^-$, $CD56^+$ activated NK cells are $CD161^-$. In other more specific embodiments, at least 10%, 12%, 14%, 16%, 18% or 20% of said $CD3^-$, $CD56^+$ activated NK cells are $CD226^+$. In more specific embodiments, at least 20%, 25%, 30%, 35% or 40% of said $CD3^-$, $CD56^+$ activated NK cells are $CD7^+$. In more specific embodiments, at least 30%, 35%, 40%, 45%, 50%, 55% or 60% of said $CD3^-$, $CD56^+$ activated NK cells are $CD5^+$.

[00104] Activated NK cells can have a fetal genotype or a maternal genotype. For example, because the post-partum placenta, as a source of hematopoietic cells suitable for producing activated NK cells, comprises tissue and cells from the fetus and from the mother, placental perfusate can comprise fetal cells only, or a substantial majority of fetal cells (*e.g.*, greater than about 90%, 95%, 98% or 99%), or can comprise a mixture of fetal and maternal cells (*e.g.*, the fetal cells comprise less than about 90%, 80%, 70%, 60%, or 50% of the total nucleated cells of the perfusate). In one embodiment, the activated NK cells are derived only from fetal placental hematopoietic cells, *e.g.*, cells obtained from closed-circuit perfusion of the placenta wherein the perfusion produces perfusate comprising a substantial majority, or only, fetal placental hematopoietic cells. In another embodiment, the activated NK cells are derived from fetal and maternal cells, *e.g.*, cells obtained by perfusion by the pan method (*see* above), wherein the perfusion produced perfusate comprising a mix of fetal and maternal placental cells. Thus, in one embodiment, the activated NK cells are derived from a population of placenta-derived intermediate natural killer cells, the substantial majority of which have the fetal genotype. In another embodiment, the activated NK cells are derived from a population of placenta-derived intermediate natural killer cells that comprise natural killer cells having the fetal genotype and natural killer cells having the maternal phenotype.

[00105] In certain embodiments, the activated NK cells or populations enriched for activated NK cells can be assessed by detecting one or more functionally relevant markers, for example, CD94, CD161, NKp44, DNAM-1, 2B4, NKp46, CD94, KIR, and the NKG2 family of activating

receptors (*e.g.*, NKG2D).

[00106] Optionally, the cytotoxic activity of isolated or enriched natural killer cells can be assessed, *e.g.*, in a cytotoxicity assay using tumor cells, *e.g.*, cultured K562, LN-18, U937, WERI-RB-1, U-118MG, HT-29, HCC2218, KG-1, or U266 tumor cells, or the like as target cells.

5.1.3. Three-Step Process NK (TSPNK) Cells

[00107] In some embodiments, natural killer cells are Three-Step Process NK (TSPNK) cells, which are NK cells produced by any method/process described below in Section 5.2.5. In specific embodiments, the TSPNK cells are NK progenitor cells (see also U. S. Patent Application Publication No. 2012/0148553, the disclosure of which is hereby incorporated by reference in its entirety).

5.1.3.1. TSPNK Cells

[00108] In one embodiment, said isolated TSPNK cell population produced by a three-step process described herein comprises a greater percentage of CD3–CD56+ cells than an NK progenitor cell population produced by a three-step process described herein, *e.g.*, an NK progenitor cell population produced by the same three-step process 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 TSPNK cell population. In a specific embodiment, said TSPNK cell population comprises about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% CD3–CD56+ cells. In another specific embodiment, said TSPNK cell population comprises no less than 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% CD3–CD56+ cells. In another specific embodiment, said TSPNK cell population comprises between 65%-70%, 70%-75%, 75%-80%, 80%-85%, 85%-90%, 90%-95%, or 95%-99% CD3–CD56+ cells. In another specific embodiment, said TSPNK cell population produced by a three-step process described herein is produced using a three-step process that comprises a long third culture step, *e.g.*, a third culture step of 18-20, 19-21, 20-22, or 21-23 days.

[00109] In certain embodiments, said $CD3^{-}CD56^{+}$ cells in said TSPNK cell population comprises $CD3^{-}CD56^{+}$ cells that are additionally $CD117^{+}$, wherein said TSPNK cell population comprises a lesser percentage of $CD3^{-}CD56^{+}CD117^{+}$ cells than an NK progenitor cell population produced by a three-step process described herein, *e.g.*, an NK progenitor cell

population produced by the same three-step process 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 TSPNK cell population.

[00110] In certain embodiments, said $CD3^-CD56^+$ cells in said TSPNK cell population comprises $CD3^-CD56^+$ cells that are additionally $CD161^+$, wherein said TSPNK cell population comprises a lesser percentage of $CD3^-CD56^+CD161^+$ cells than an NK progenitor cell population produced by a three-step process described herein, *e.g.*, an NK progenitor cell population produced by the same three-step process 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 TSPNK cell population.

[00111] In certain embodiments, said $CD3^-CD56^+$ cells in said TSPNK cell population comprises $CD3^-CD56^+$ cells that are additionally $NKp46^+$, wherein said TSPNK cell population comprises a greater percentage of $CD3^-CD56^+NKp46^+$ cells than an NK progenitor cell population produced by a three-step process described herein, *e.g.*, an NK progenitor cell population produced by the same three-step process 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 TSPNK cell population.

[00112] In certain embodiments, said $CD3^-CD56^+$ cells in said TSPNK cell population comprises $CD3^-CD56^+$ cells that are additionally CD16-, wherein said TSPNK cell population comprises a greater percentage of $CD3^-CD56^+$CD16- cells than an NK progenitor cell population produced by a three-step process described herein, *e.g.*, an NK progenitor cell population produced by the same three-step process 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 TSPNK cell population. In another embodiment, the TSPNK cells produced using the three-step process described herein possess longer telomeres than peripheral blood (PB) derived NK cells.

[00113] In one embodiment, a TSPNK cell population produced by a three-step process described herein comprises cells which are CD117+. In a specific embodiment, said TSPNK cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% $CD117^+$ cells. In one embodiment, a TSPNK cell population produced by a three-step process described herein comprises cells which

are NKG2D+. In a specific embodiment, said TSPNK cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% $NKG2D^+$ cells. In one embodiment, a TSPNK cell population produced by a three-step process described herein comprises cells which are NKp44+. In a specific embodiment, said TSPNK cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% $NKp44^+$ cells. In one embodiment, a TSPNK cell population produced by a three-step process described herein comprises cells which are CD52+. In a specific embodiment, said TSPNK cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% $CD52^+$ cells. In a particular embodiment, said TSPNK cell population produced by a three-step process described herein comprises cells which are CD52+ CD117+. In one embodiment, a TSPNK cell population produced by a three-step process described herein comprises cells which are CD244+. In a specific embodiment, said TSPNK cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% $CD244^+$ cells. In a particular embodiment, said TSPNK cell population produced by a three-step process described herein comprises cells which are CD244+ CD117+. In one embodiment, a TSPNK cell population produced by a three-step process described herein comprises cells which are LFA-1+. In a specific embodiment, said TSPNK cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% LFA-1+ cells. In one embodiment, a TSPNK cell population produced by a three-step process described herein comprises cells which are CD94+. In a specific embodiment, said TSPNK cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% CD94+ cells.

5.1.3.2. NK Progenitor Cells

[00114] In one embodiment, said isolated NK progenitor cell population comprises a low percentage of CD3–CD56+ cells as compared to the percentage of CD3–CD56+ cells associated with non-progenitor NK cell populations, such as non-progenitor NK cell populations produced by the three-step methods described herein, *e.g.*, the NK progenitor cell population comprises about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% CD3–CD56+ cells. In another specific embodiment, said NK progenitor cell population comprises no more than 5%, 10%,

15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% CD3–CD56+ cells. In another specific embodiment, said NK progenitor cell population comprises between 0%-5%, 5%-10%, 10%-15%, 15%-20%, 20%-25%, 25%-30%, 30%-35%, 35%-40%, 40%-45%, or 45%-50% CD3–CD56+ cells. In some embodiments, said NK progenitor cell populations, *e.g.*, a NK progenitor cell populations that comprise a low percentage of CD3–CD56+ cells as compared to the percentage of CD3–CD56+ cells associated with non-progenitor NK cell populations, comprise no more than 1%, no more than 2%, no more than 3%, no more than 4%, no more than 5%, no more than 10%, or no more than 15% CD3–CD56+ cells. In another specific embodiment, said NK progenitor cell populations produced by a three-step process described herein are produced using a three-step process that comprises a short third culture step, *e.g.*, a third culture step of 4-6, 5-7, 6-8, or 7-9 days.

[00115] In certain embodiments, said $CD3^{-}CD56^{+}$ cells in said NK progenitor cell populations are additionally $CD117^{+}$. In a specific embodiment, about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of said $CD3^{-}CD56^{+}$ cells in said NK progenitor cell populations are $CD117^{+}$. In another specific embodiment, no less than 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of said $CD3^{-}CD56^{+}$ cells in said NK progenitor cell populations are $CD117^{+}$. In another specific embodiment, between 65%-70%, 70%-75%, 75%-80%, 80%-85%, 85%-90%, 90%-95%, or 95%-99% of said $CD3^{-}CD56^{+}$ cells in said NK progenitor cell populations are $CD117^{+}$.

[00116] In certain embodiments, said CD3–CD56+ cells in said NK progenitor cell populations are additionally CD161+. In a specific embodiment, about 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% of said CD3–CD56+ cells in said NK progenitor cell populations are CD161+. In another specific embodiment, no less than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% of said CD3–CD56+ cells in said NK progenitor cell populations are CD161+. In another specific embodiment, between 40%-45%, 45%-50%, 50%-55%, 55%-60%, 60%-65%, 65%-70%, or 70%-75% of said CD3–CD56+ cells in said NK progenitor cell populations are CD161+.

[00117] In certain embodiments, said CD3–CD56+ cells in said NK progenitor cell populations are additionally NKp46+. In a specific embodiment, about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more of said CD3–CD56+ cells in said NK progenitor cell populations are NKp46+. In a more specific embodiment, about 25%, 30%, 35%, 40%, 45%, 50%, or 55% of said CD3–CD56+ cells in said NK progenitor cell

populations are NKp46+. In another specific embodiment, no more than 25%, 30%, 35%, 40%, 45%, 50%, or 55% of said CD3–CD56+ cells in said NK progenitor cell populations are NKp46+. In another specific embodiment, between 25%-30%, 30%-35%, 35%-40%, 40%-45%, 45%-50%, 50%-55%, 55%-60%, 60%-65%, 65%-70%, 70%-75%, 75%-80%, 80%-85%, 85%-90% or more of said CD3–CD56+ cells in said NK progenitor cell populations are NKp46+. In a more specific embodiment, between 25%-30%, 30%-35%, 35%-40%, 40%-45%, 45%-50%, or 50%-55% of said CD3–CD56+ cells in said NK progenitor cell populations are NKp46+.

[00118] In certain embodiments, said NK progenitor cell population contains cells that are $CD56^{+}CD16^{-}$. In certain embodiments, $CD3^{-}CD56^{+}$ cells in said NK progenitor cell populations are $CD16^{-}$. In certain embodiments, $CD3^{-}CD56^{+}$ cells in said NK progenitor cell populations are $CD16^{+}$. In a specific embodiment, said NK progenitor cell populations comprise no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% $CD16^{+}$ cells. In another specific embodiment, said NK progenitor cell populations comprise between 0%-5%, 5%-10%, 10%-15%, 15%-20%, or 20%-25% $CD16^{+}$ cells. In some embodiments, said NK progenitor cell populations comprise no more than 1%, no more than 2%, no more than 3%, no more than 4%, no more than 5%, no more than 10%, or no more than 15% $CD16^{+}$ cells.

[00119] In certain embodiments, said CD3–CD56+ cells in said NK progenitor cell populations are additionally CD16-. In certain embodiments, said CD3–CD56+ cells in said NK progenitor cell populations are additionally CD117+ and CD161+. In certain embodiments, said CD3–CD56+ cells in said NK progenitor cell populations are additionally CD16-, CD117+ and CD161+. In certain embodiments, said CD3–CD56+ cells in said NK progenitor cell populations are additionally CD16-, CD117+, CD161+, and NKp46+.

[00120] In one embodiment, an NK progenitor cell population produced by a three-step process described herein comprises no more than about 40% CD3–CD56+ cells. In one embodiment, an NK progenitor cell population produced by a three-step process described herein comprises cells which are CD117+. In a specific embodiment, said NK progenitor cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% CD117+ cells. In one embodiment, an NK progenitor cell population produced by a three-step process described herein comprises cells which are CD52+. In a specific embodiment, said NK progenitor cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%,

75%, 80%, 85%, or 90% CD52+ cells. In a particular embodiment, said NK progenitor cell population produced by a three-step process described herein comprises cells which are CD52+ CD117+. In one embodiment, an NK progenitor cell population produced by a three-step process described herein comprises cells which are CD244+. In a specific embodiment, said NK progenitor cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% CD244+ cells. In a particular embodiment, said NK progenitor cell population produced by a three-step process described herein comprises cells which are CD244+ CD117+. In one embodiment, an NK progenitor cell population produced by a three-step process described herein comprises cells which are LFA-1+. In a specific embodiment, said NK progenitor cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% LFA-1+ cells. In one embodiment, an NK progenitor cell population produced by a three-step process described herein comprises cells which are CD94+. In a specific embodiment, said NK progenitor cell populations comprise no more than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% CD94+ cells.

[00121] In particular embodiments, an NK progenitor cell population produced by a three-step process described herein comprises a greater proportion of CD56- cells than CD56+ cells. In particular embodiments, an NK progenitor cell population produced by a three-step process described herein differentiates in vivo or ex vivo into a population with an increased proportion of CD56+ cells.

[00122] In a specific embodiment, an NK progenitor cell population produced by a three-step process described herein comprises a low percentage of $CD34^{-}CD117^{+}$ cells as compared to the percentage of $CD34^{-}CD117^{+}$ cells associated with a non-progenitor NK cell population, *e.g.*, the NK progenitor cell population comprises about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% $CD34^{-}CD117^{+}$ cells. In another specific embodiment, said NK progenitor cell population comprises no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% $CD34^{-}CD117^{+}$ cells. In another specific embodiment, said NK progenitor cell population comprises between 0%-5%, 5%-10%, 10%-15%, 15%-20%, 20%-25%, 25%-30%, 30%-35%, 35%-40%, 40%-45%, or 45%-50% $CD34^{-}CD117^{+}$ cells. In some embodiments, said NK progenitor cell population comprises no more than 1%, no more than 2%, no more than 3%, no more than 4%, no more than 5%, no more than 10%, or no more than 15% $CD34^{-}CD117^{+}$ cells.

In another specific embodiment, said NK progenitor cell population produced by a three-step process described herein is produced using a three-step process that comprises a short third culture step, *e.g.*, a third culture step of 4-6, 5-7, 6-8, or 7-9 days.

[00123] In a specific embodiment, an NK progenitor cell population produced by a three-step process described herein comprises a low percentage of CD161+ cells as compared to the percentage of $CD161^+$ cells associated with a non-progenitor NK cell population, *e.g.*, the NK progenitor cell population comprises about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% $CD161^+$ cells. In another specific embodiment, said NK progenitor cell population comprises no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% $CD161^+$ cells. In another specific embodiment, said NK progenitor cell population comprises between 0%-5%, 5%-10%, 10%-15%, 15%-20%, 20%-25%, 25%-30%, 30%-35%, 35%-40%, 40%-45%, or 45%-50% $CD161^+$ cells. In some embodiments, said NK progenitor cell population comprises no more than 1%, no more than 2%, no more than 3%, no more than 4%, no more than 5%, no more than 10%, or no more than 15% $CD161^+$ cells. In another specific embodiment, said NK progenitor cell population produced by a three-step process described herein is produced using a three-step process that comprises a short third culture step, *e.g.*, a third culture step of 4-6, 5-7, 6-8, or 7-9 days.

[00124] In a specific embodiment, an NK progenitor cell population produced by a three-step process described herein comprises a low percentage of $NKp46^+$ cells as compared to the percentage of $NKp46^+$ cells associated with a non-progenitor NK cell population, *e.g.*, the NK progenitor cell population comprises about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% $NKp46^+$ cells. In another specific embodiment, said NK progenitor cell population comprises no more than 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% $NKp46^+$ cells. In another specific embodiment, said NK progenitor cell population comprises between 0%-5%, 5%-10%, 10%-15%, 15%-20%, 20%-25%, 25%-30%, 30%-35%, 35%-40%, 40%-45%, or 45%-50% $NKp46^+$ cells. In some embodiments, said NK progenitor cell population comprises no more than 1%, no more than 2%, no more than 3%, no more than 4%, no more than 5%, no more than 10%, or no more than 15% $NKp46^+$ cells. In another specific embodiment, said NK progenitor cell population produced by a three-step process described herein is produced using a three-step process that comprises a short third culture step, *e.g.*, a third culture step of 4-6, 5-7, 6-8, or 7-9 days.

[00125] In a specific embodiment, an NK progenitor cell population produced by a three-step process described herein comprises a low percentage of $CD56^{+}CD16-$ cells as compared to the percentage of $CD56^{+}CD16-$ cells associated with a non-progenitor NK cell population, *e.g.*, the NK progenitor cell population comprises about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% $CD56^{+}CD16-$ cells. In another specific embodiment, said NK progenitor cell population comprises no more than 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% $CD56^{+}CD16-$ cells. In another specific embodiment, said NK progenitor cell population comprises between 0%-5%, 5%-10%, 10%-15%, 15%-20%, 20%-25%, 25%-30%, 30%-35%, 35%-40%, 40%-45%, or 45%-50% $CD56^{+}CD16-$ cells. In some embodiments, said NK progenitor cell population comprises no more than 1%, no more than 2%, no more than 3%, no more than 4%, no more than 5%, no more than 10%, or no more than 15% $CD56^{+}CD16-$ cells. In another specific embodiment, said NK progenitor cell population produced by a three-step process described herein is produced using a three-step process that comprises a short third culture step, *e.g.*, a third culture step of 4-6, 5-7, 6-8, or 7-9 days.

[00126] In one embodiment, an NK progenitor cell population produced by a three-step process described herein comprises cells that are CD52+CD117+. In a specific embodiment, an NK progenitor cell population produced by a three-step process described herein comprises a higher percentage of $CD52^{+}CD117+$ cells as compared to the percentage of $CD52^{+}CD117+$ cells associated with a hematopoietic progenitor cell population. In a specific embodiment, an NK progenitor cell population produced by a three-step process described herein comprises a higher percentage of $CD52^{+}CD117+$ cells as compared to the percentage of $CD52^{+}CD117+$ cells associated with a non-progenitor NK cell population, *e.g.*, the NK progenitor cell population comprises about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more $CD52^{+}CD117+$ cells. In another specific embodiment, said NK progenitor cell population comprises no less than 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% $CD52^{+}CD117+$ cells. In another specific embodiment, said NK progenitor cell population comprises between 50%-55%, 55%-60%, 60%-65%, 65%-70%, 70%-75%, 75%-80%, 80%-85%, 85%-90%, 90%-95% or more $CD52^{+}CD117+$ cells. In another specific embodiment, said NK progenitor cell population which comprises $CD52^{+}CD117+$ cells produced by a three-step process described herein is produced using a three-step process that comprises a short third culture step, *e.g.*, a third culture step of 4-6, 5-7, 6-8, or 7-9 days. In a specific embodiment, said NK progenitor cell population which comprises

$CD52^{+}CD117+$ cells is produced using a three-step process that comprises a total of 12 days or more, 13 days or more, 14 days or more, 15 days or more, 16 days or more, 17 days or more, 18 days or more, 19 days or more, 20 days or more, or 21 days or more of culture. In a specific embodiment, said NK progenitor cell population which comprises $CD52^{+}CD117+$ cells is produced using a three-step process that comprises a total of at least 12 days, 13 days, or 14 days of culture but not more than 21-25 days, 25-30 days, or 30-35 days of culture. In a specific embodiment, said NK progenitor cell population which comprises $CD52^{+}CD117+$ cells is produced using a three-step process that comprises a total of 21 days of culture.

[00127] In a specific embodiment, the NK progenitor cells described herein possess a greater ability to engraft bone marrow (*e.g.*, in vivo) than non-progenitor NK cells, *e.g.*, non-progenitor NK cells produced using a comparable method. For example, in certain embodiments, NK progenitor cells produced using a three-step process that comprises a short third culture step, *e.g.*, a third culture step of 4-6, 5-7, 6-8, or 7-9 days engraft bone marrow (*e.g.*, in vivo) at a higher efficiency than non-progenitor NK cells produced using a three-step process that comprises a longer third culture step, *e.g.*, a third culture step of 18-20, 19-21, 20-22, or 21-23 days. In another embodiment, the NK progenitor cells described herein possess longer telomeres than peripheral blood (PB) derived NK cells.

5.1.4. NK Cells Produced by Three-Stage Method

[00128] In one embodiment, provided herein is an isolated NK cell population, wherein said NK cells are produced according to the three-stage method described below.

[00129] 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.

[00130] 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+.

[00131] 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+.

5.1.5. Cell Combinations and Cell/Perfusate Combinations

[00132] The NK cells, *e.g.,* activated NK cells and/or TSPNK cells can further be combined with placental perfusate, placental perfusate cells and/or adherent placental cells in the present invention.

5.1.5.1. Combinations of NK Cells and Perfusate or Perfusate Cells

[00133] In specific embodiments, the natural killer cells comprise $CD56^{+}CD16^{-}$ PiNK cells in combination with $CD56^{+}CD16^{+}$ natural killer cells. In more specific embodiments, the $CD56^{+}CD16^{+}$ natural killer cells can be isolated from placenta, or from another source, *e.g.*, peripheral blood, umbilical cord blood, bone marrow, or the like. Thus, in various other embodiments, PiNK cells can be combined with $CD56^{+}CD16^{+}$ natural killer cells, *e.g.*, in ratios of, for example, about 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 about 9:1. As used in this context, "isolated" means that the cells have been removed from their normal environment, *e.g.*, the placenta.

[00134] In various specific embodiments, the isolated population of NK cells comprises at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or at least about 99%

PiNK cells. In another embodiment, the plurality of PiNK cells comprises, or consists of, PiNK cells that have not been expanded; *e.g.*, are as collected from placental perfusate. In another embodiment, the plurality of PiNK cells comprises, or consists of, PiNK cells that have been expanded. Methods of expanding natural killer cells are described elsewhere herein, and have been described, *e.g.*, in Ohno *et al.*, U.S. Patent Application Publication No. 2003/0157713; *see also* Yssel *et al.*, *J. Immunol. Methods* 72(1):219-227 (1984) and Litwin *et al.*, J. Exp. Med. 178(4):1321-1326 (1993).

[00135] In specific embodiments, the isolated population of NK cells is a population of placental cells comprising PiNK cells. In a specific embodiment, the isolated population of NK cells is total nucleated cells from placental perfusate, *e.g.*, placental perfusate cells, comprising autologous, isolated PiNK cells. In various other embodiments, activated NK cells can be combined with, *e.g.*, NK cells, wherein said NK cells have been isolated from a tissue source and have not been expanded, NK cells isolated from a tissue source and expanded, or NK cells produced by a different method, *e.g.*, $CD56^+CD16^+$ natural killer cells, *e.g.*, in ratios of, for example, about 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 about 9:1. As used in this context, "isolated" means that the cells have been removed from their normal tissue environment.

[00136] In specific embodiments, activated NK cells can also be combined with, *e.g.*, NK cells, wherein said NK cells have been isolated from a tissue source and have not been expanded, NK cells isolated from a tissue source and expanded, or NK cells produced by a different method, *e.g.*, $CD56^+CD16^+$ natural killer cells, *e.g.*, in ratios of, for example, about 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 about 9:1. As used in this context, "isolated" means that the cells have been removed from their normal tissue environment.

[00137] In one embodiment, for example, a volume of placental perfusate supplemented with NK cells produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells), is used. 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 produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells). In another embodiment, placental perfusate cells are supplemented with NK cells produced using the

processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells). In certain other embodiments, when placental perfusate cells are combined with NK cells produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells), 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 produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells), are combined with a plurality of placental perfusate cells and/or combined natural killer cells, the NK 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 produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells), 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 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.

[00138] 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.

[00139] Pooled placental perfusate that is obtained from two or more sources, *e.g.*, two or more placentas, and combined, *e.g.*, pooled, can further be used in the present invention. 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.

[00140] Similarly, 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, can also be used in the present invention. 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.*

[00141] NK cells produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells), and combinations of such cells with placental perfusate and/or placental perfusate cells can be assayed to determine the degree or amount of tumor/infection suppression (that is, the potency) to be expected from, *e.g.*, a given number of the NK cells, 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/infected cells under conditions in which the tumor/infected cells would otherwise proliferate, and the rate of proliferation of the tumor/infected 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/infected 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/infection spread, *e.g.*, by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or the like.

[00142] In certain embodiments, NK cells produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells), 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 or NK cell populations, or NK progenitor cell populations in combination with other NK 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 any of the other cells.

[00143] In the above embodiments, the NK cells or combinations of NK cells with 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).

[00144] 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.

5.1.5.2. Combination of NK Cells from Matched Perfusate and Cord Blood

[00145] Natural Killer Cells can be further obtained from combinations of matched units of placental perfusate and umbilical cord blood in the present invention, and are referred to herein as combined natural killer cells. "Matched units," as used herein, indicates that the NK cells are obtained from placental perfusate cells, and umbilical cord blood cells, wherein the umbilical cord blood cells are obtained from umbilical cord blood from the placenta from which the placental perfusate is obtained, *i.e.*, the placental perfusate cells and umbilical cord blood cells, and thus the natural killer cells from each, are from the same individual.

[00146] In certain embodiments, the combined placental killer cells comprise only, or substantially only, natural killer cells that are $CD56^+$ and $CD16^-$. In certain other embodiments, the combined placental killer cells comprise NK cells that are $CD56^+$ and $CD16^-$, and NK cells that are $CD56^+$ and $CD16^+$. In certain specific embodiments, the combined placental killer cells comprise at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 99.5% $CD56^+CD16^-$ natural killer cells (PiNK cells).

[00147] In one embodiment, the combined natural killer cells have not been cultured. In a specific embodiment, the combined natural killer cells comprise a detectably higher number of $CD3^-CD56^+CD16^-$ natural killer cells than an equivalent number of natural killer cells from peripheral blood. In another specific embodiment, the combined natural killer cells comprise a detectably lower number of $CD3^-CD56^+CD16^-$ natural killer cells than an equivalent number of natural killer cells from peripheral blood. In another specific embodiment, the combined natural killer cells comprise a detectably higher number of $CD3^-CD56^+KIR2DL2/L3^+$ natural killer cells than an equivalent number of natural killer cells from peripheral blood. In another specific

embodiment, the combined natural killer cells comprise a detectably lower number of $CD3^-$ $CD56^+$ $NKp46^+$ natural killer cells than an equivalent number of natural killer cells from peripheral blood. In another specific embodiment, the combined natural killer cells comprise a detectably lower number of $CD3^-CD56^+$ $NKp30^+$ natural killer cells than an equivalent number of natural killer cells from peripheral blood. In another specific embodiment, the combined natural killer cells comprise a detectably lower number of $CD3^-CD56^+2B4^+$ natural killer cells than an equivalent number of natural killer cells from peripheral blood. In another specific embodiment, the combined natural killer cells comprise a detectably lower number of $CD3^-$ $CD56^+CD94^+$ natural killer cells than an equivalent number of natural killer cells from peripheral blood.

[00148] In another embodiment, the combined natural killer cells have been cultured, *e.g.*, for 21 days. In a specific embodiment, the combined natural killer cells comprise a detectably lower number of $CD3^-CD56^+KIR2DL2/L3^+$ natural killer cells than an equivalent number of natural killer cells from peripheral blood. In another specific embodiment, the combined natural killer cells have not been cultured. In another specific embodiment, the combined natural killer cells comprise a detectably higher number of $CD3^-CD56^+NKp44^+$ natural killer cells than an equivalent number of natural killer cells from peripheral blood. In a specific embodiment, the combined natural killer cells comprise a detectably higher number of $CD3^-CD56^+NKp30^+$ natural killer cells than an equivalent number of natural killer cells from peripheral blood.

[00149] In another embodiment, the combined natural killer cells express a detectably higher amount of granzyme B than an equivalent number of peripheral blood natural killer cells.

[00150] Combined natural killer cells can further be combined with umbilical cord blood. In various embodiments, cord blood is combined with combined natural killer cells at 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 combined natural killer cells per milliliter of cord blood.

5.1.5.3. Combinations of NK Cells with Adherent Placental Stem Cells

[00151] In other embodiments, the NK cells produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells) produced using the three-step process 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. "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 not trophoblasts, embryonic germ cells or embryonic stem cells. In certain embodiments, adherent placental stem cells are used as feeder cells during the processes (*e.g.*, two-step method) as described above.

[00152] The NK cells produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells), 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.

[00153] 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.

[00154] 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.

[00155] 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.

[00156] In certain embodiments, the isolated adherent placental cells described herein detectably suppress cancer cell proliferation or tumor growth.

[00157] 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.

[00158] 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.

[00159] In another embodiment, the isolated adherent placental cells are $CD73^+$, $CD105^+$, $CD200^+$. In a specific embodiment, 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.

[00160] 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-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.

[00161] In another embodiment, the isolated adherent placental cells are $CD73^+$, $CD105^+$ and HLA-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-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-4^+ and $CD200^+$.

[00162] 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 $CD45^-$.

[00163] 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.

[00164] 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, 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.

[00165] 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)).

[00166] 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).

[00167] 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.

5.1.5.4. Compositions Comprising Adherent Placental Cell Conditioned Media

[00168] Also can be used in the present invention is a composition comprising NK cells produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells) produced using the three-step process described herein, and additionally conditioned medium, wherein said composition is tumor suppressive, or is effective in the treatment of cancer or viral infection. 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.

[00169] Such conditioned medium can be combined with any of, or any combination of NK cells produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells), placental perfusate, 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.

[00170] Thus, in one embodiment, used in the present invention is a composition comprising NK cells produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells), 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.

[00171] In another embodiment, used in the present invention is a composition comprising NK cells produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells), 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.

[00172] 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 1×10^5 to about 5×10^5 isolated adherent placental cells and about 1×10^5 tumor cells; about 1×10^6 to about 5×10^6 isolated adherent placental cells and about 1×10^6 tumor cells; about 1×10^7 to about 5×10^7 isolated adherent placental cells and about 1×10^7 tumor cells; or about 1×10^8 to about 5×10^8 isolated adherent placental cells and about 1×10^8 tumor cells.

5.2. Methods of Producing NK Cells

[00173] NK cells 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.

[00174] One important source of natural killer cells and cells that can be used to derive natural killer cells as described above is the placenta, for example, full-term placenta, e.g., full-term human placenta. 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 each of which are hereby

incorporated in their entireties.

5.2.1. Cell Collection Composition

[00175] 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, *e.g.*, NK 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.

[00176] 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.

[00177] 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.*).

[00178] 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.

[00179] 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.

[00180] 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).

5.2.2. Collection and Handling of Placenta

[00181] 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.

[00182] 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.

[00183] 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.

[00184] 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.

5.2.3. Placental Perfusion

[00185] 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 each of which are hereby incorporated by reference herein in their entireties.

[00186] 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.

[00187] 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).

[00188] 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.

[00189] 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.

[00190] 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.

[00191] 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.

[00192] 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.

[00193] 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.

[00194] 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.

[00195] 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.

5.2.4. Placental Perfusate and Placental Perfusate Cells

[00196] 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, *e.g.*, NK 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.

5.2.5. Hematopoietic Cells

[00197] In various embodiments, NK cells are produced from hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells.

[00198] Hematopoietic cells as used herein can be any hematopoietic cells able to differentiate into NK 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. 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 one embodiment, hematopoietic cells from placental perfusate comprise at least about 90%, 95%, 98%, 99% or 99.5% fetal cells.

[00199] In another specific embodiment, the hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells, are obtained from placental perfusate, umbilical cord blood or peripheral blood. In another specific embodiment, the hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells, 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%).

[00200] 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.

[00201] In another specific embodiment, the hematopoietic cells, *e.g.*, hematopoietic stem cells or progenitor cells, 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.

[00202] 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 $CD34^+CD38^-CD33^-CD117^-CD235^-CD36^-$.

[00203] 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 $CD34^+ALDH^+$.

[00204] 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, and/or hsa-miR-520a.

[00205] In certain embodiments, the hematopoietic cells are $CD34^-$.

[00206] 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, preferably all, of lineage markers CD2, CD3, CD11b, CD11c, CD14, CD16, CD19, CD24, CD56, CD66b and glycophorin A.

[00207] 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 are discussed in detail below.

[00208] Hematopoietic cells as used 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.

[00209] Hematopoietic cells as used 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.

[00210] Hematopoietic cells as used 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 NK cells comprise hematopoietic cells from placenta, *e.g.*, placenta perfusate. In various embodiments, the hematopoietic cells used to produce NK cells 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 a preferred 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.

5.2.5.1. Placental Hematopoietic Stem Cells

[00211] In certain embodiments, the hematopoietic cells are placental hematopoietic cells. As used herein, "placental hematopoietic cells" means hematopoietic cells obtained from the placenta itself, and not from placental blood or from umbilical cord blood. 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.

[00212] 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 $CD34^+CD45^+$.

5.2.6. Methods of Producing PiNK Cells

[00213] In various embodiments, PiNK cells are derived from placental cells. In specific embodiments, the placental cells are obtained from placental perfusate, *e.g.*, human placental perfusate. In specific embodiments, the placental cells are obtained from placental tissue that has been mechanically and/or enzymatically disrupted.

5.2.6.1. Obtaining PiNK Cells from Placental Perfusate

[00214] In one embodiment, PiNK cells are collected by obtaining placental perfusate, then contacting the placental perfusate with a composition that specifically binds to $CD56^+$ cells, *e.g.*, an antibody against CD56, followed by isolating of $CD56^+$ cells on the basis of said binding to form a population of $CD56^+$ cells. The population of $CD56^+$ cells comprises an isolated

population of natural killer cells. In a specific embodiment, $CD56^+$ cells are contacted with a composition that specifically binds to $CD16^+$ cells, *e.g.*, an antibody against CD16, and the $CD16^+$ cells are excluded from the population of $CD56^+$ cells. In another specific embodiment, $CD3^+$ cells are also excluded from the population of $CD56^+$ cells.

[00215] In one embodiment, PiNK cells are obtained from placental perfusate as follows. Post-partum human placenta is exsanguinated and perfused, *e.g.*, with about 200-800 mL of perfusion solution, through the placental vasculature only. In a specific embodiment, the placenta is drained of cord blood and flushed, *e.g.*, with perfusion solution, through the placental vasculature to remove residual blood prior to said perfusing. The perfusate is collected and processed to remove any residual erythrocytes. Natural killer cells in the total nucleated cells in the perfusate can be isolated on the basis of expression of CD56 and CD16. In certain embodiments, the isolation of PiNK cells comprises isolation using an antibody to CD56, wherein the isolated cells are $CD56^+$. In another embodiment, the isolation of PiNK cells comprises isolation using an antibody to CD16, wherein the isolated cells are $CD16^-$. In another embodiment, the isolation of PiNK cells comprises isolation using an antibody to CD56, and exclusion of a plurality of non-PiNK cells using an antibody to CD16, wherein the isolated cells comprise $CD56^+$, $CD16^-$ cells.

[00216] Cell separation can be accomplished by any method known in the art, *e.g.*, fluorescence-activated cell sorting (FACS), or, preferably, magnetic cell sorting using microbeads conjugated with specific antibodies. Magnetic cell separation can be performed and automated using, *e.g*, an AUTOMACS™ Separator (Miltenyi).

[00217] In another aspect, the process of isolating placental natural killer cells (*e.g.*, PiNK cells) comprises obtaining a plurality of placental cells, and isolating natural killer cells from said plurality of placental cells. In a specific embodiment, the placental cells are, or comprise, placental perfusate cells, *e.g.*, total nucleated cells from placental perfusate. In another specific embodiment, said plurality of placental cells are, or comprise, placental cells obtained by mechanical and/or enzymatic digestion of placental tissue. In another embodiment, said isolating is performed using one or more antibodies. In a more specific embodiment, said one or more antibodies comprises one or more of antibodies to CD3, CD16 or CD56. In a more specific embodiment, said isolating comprises isolating $CD56^+$ cells from $CD56^-$ cells in said plurality of placental cells. In a more specific embodiment, said isolating comprises isolating $CD56^+$, $CD16^-$

placental cells, *e.g.*, placental natural killer cells, *e.g.*, PiNK cells, from placental cells that are $CD56^-$ or $CD16^+$. In a more specific embodiment, said isolating comprises isolating $CD56^+$, $CD16^-$, $CD3^-$ placental cells from placental cells that are $CD56^-$, $CD16^+$, or $CD3^+$. In another embodiment, said process of isolating placental natural killer cells results in a population of placental cells that is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or at least 99% $CD56^+$, $CD16^-$ natural killer cells.

[00218] In certain embodiments, the placental natural killer cells, *e.g.*, PiNK cells, have been expanded in culture. In certain other embodiments, the placental perfusate cells have been expanded in culture. In a specific embodiment, said placental perfusate cells have been expanded in the presence of a feeder layer and/or in the presence of at least one cytokine. In a more specific embodiment, said feeder layer comprises K562 cells or peripheral blood mononuclear cells. In another more specific embodiment, said at least one cytokine is interleukin-2. In specific embodiments, the PiNK cells have been cultured, *e.g.*, expanded in culture, for at least, about, or at most 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 a specific embodiment, the PiNK cells are cultured for about 21 days.

5.2.6.2. Disruption and Digestion of Placental Tissue to Obtain PiNK Cells

[00219] Placental natural killer cells, *e.g.*, PiNK cells, can also be obtained from placental tissue that has been mechanically and/or enzymatically disrupted.

[00220] Placental tissue can be disrupted using one or more tissue-degrading enzymes, *e.g.*, a metalloprotease, a serine protease, a neutral protease, 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. Typically after digestion, the digested tissue is passed through a strainer or filter to remove partially-digested cell clumps, leaving a substantially single-celled suspension.

[00221] After a suspension of placental cells is obtained, natural killer cells can be isolated using, *e.g.*, antibodies to CD3 and CD56. In a specific embodiment, placental natural killer cells are isolated by selecting for cells that are $CD56^+$ to produce a first cell population; contacting said first cell population with antibodies specific for CD3 and/or CD16; and removing cells from said first cell population that are $CD3^+$ or $CD56^+$, thereby producing a second population of cells

that is substantially $CD56^+$ and $CD3^-$, $CD56^+$ and $CD16^-$, or $CD56^+$, $CD3^-$ and $CD16^-$.

[00222] In one embodiment, magnetic beads are used to isolate placental natural killer cells from a suspension of placental cells. 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. 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.

5.2.7. Methods of Producing Activated NK Cells

[00223] Activated NK cells may be produced from hematopoietic cells, which are described above. In certain embodiment, the activated NK cells are produced from expanded hematopoietic cells, *e.g.*, hematopoietic stem cells and/or hematopoietic progenitor cells. In a specific embodiment, the hematopoietic cells are expanded and differentiated, continuously, in a first medium without the use of feeder cells. The cells are then cultured in a second medium in the presence of feeder cells. Such isolation, expansion and differentiation can be performed in a central facility, which provides expanded hematopoietic cells for shipment to decentralized expansion and differentiation at points of use, *e.g.*, hospital, military base, military front line, or the like.

[00224] In some embodiments, production of activated NK cells comprises expanding a population of hematopoietic cells. During cell expansion, a plurality of hematopoietic cells within the hematopoietic cell population differentiate into NK cells.

[00225] In one embodiment, the process of producing a population of activated natural killer (NK) cells comprises: (a) seeding a population of hematopoietic stem or progenitor cells in a first medium comprising interleukin-15 (IL-15) and, optionally, one or more of stem cell factor (SCF) and interleukin-7 (IL-7), wherein said IL-15 and optional SCF and IL-7 are not comprised within an undefined component of said medium, such that the population expands, and a

plurality of hematopoietic stem or progenitor cells within said population of hematopoietic stem or progenitor cells differentiate into NK cells during said expanding; and (b) expanding the cells from step (a) in a second medium comprising interleukin-2 (IL-2), to produce a population of activated NK cells.

[00226] In another embodiment, activated NK cells as described herein are produced by a two-step process of expansion/ differentiation and maturation of NK cells. The first and second steps comprise culturing the cells in media with a unique combination of cellular factors. In certain embodiments, the process involves (a) culturing and expanding a population of hematopoietic cells in a first medium, wherein a plurality of hematopoietic stem or progenitor cells within the hematopoietic cell population differentiate into NK cells; and (b) expanding the NK cells from step (a) in a second medium, wherein the NK cells are further expanded and differentiated, and wherein the NK cells are maturated (*e.g.*, activated or otherwise possessing cytotoxic activity). In certain embodiments, the process includes no intermediary steps between step (a) and (b), no additional culturing steps prior to step (a), and/or no additional steps (*e.g.*, maturation step) after step (b).

5.2.7.1. First Step

[00227] In certain embodiments, the process of producing activated NK cells comprises a first step of culturing and expanding a population of hematopoietic cells in a first medium, wherein a plurality of hematopoietic stem or progenitor cells within the hematopoietic cell population differentiate into NK cells.

[00228] Without wishing to be bound by any parameter, mechanism or theory, culture of the hematopoietic cells as described herein results in continuous expansion of the hematopoietic cells and differentiation of NK cells from said cells. In certain embodiments, hematopoietic cells, *e.g.*, stem cells or progenitor cells, used in the processes described herein are expanded and differentiated in the first step using a feeder layer. In other embodiments, hematopoietic cells, *e.g.*, stem cells or progenitor cells, are expanded and differentiated in the first step without the use of a feeder layer.

[00229] Feeder cell-independent expansion and differentiation of hematopoietic cells can take place in any container compatible with cell culture and expansion, *e.g.*, flask, tube, beaker, dish, multiwell plate, bag or the like. In a specific embodiment, feeder cell-independent expansion of hematopoietic cells takes place in a bag, *e.g.*, a flexible, gas-permeable fluorocarbon culture bag

(for example, from American Fluoroseal). In a specific embodiment, the container in which the hematopoietic cells are expanded is suitable for shipping, *e.g.*, to a site such as a hospital or military zone wherein the expanded NK cells are further expanded and differentiated.

[00230] In certain embodiments, hematopoietic cells are expanded and differentiated, *e.g.*, in a continuous fashion, in a first culture medium. In one embodiment, the first culture medium is an animal-component free medium. Exemplary animal component-free media useful in the processes described herein include, but are not limited to, Basal Medium Eagle (BME), Dulbecco's Modified Eagle's Medium (DMEM), Glasgow Minimum Essential Medium (GMEM), Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DMEM/F-12), Minimum Essential Medium (MEM), Iscove's Modified Dulbecco's Medium (IMDM), Nutrient Mixture F-10 Ham (Ham's F-10), Nutrient Mixture F-12 Ham (Ham's F-12), RPMI-1640 Medium, Williams' Medium E, STEMSPAN® (Cat. No. Stem Cell Technologies, Vancouver, Canada), Glycostem Basal Growth Medium (GBGM®), AIM-V® medium (Invitrogen), X-VIVO™ 10 (Lonza), X-VIVO™ 15 (Lonza), OPTMIZER (Invitrogen), STEMSPAN® H3000 (STEMCELL Technologies), CELLGRO COMPLETE™ (Mediatech), or any modified variants or combinations thereof. In a specific embodiment of any of the embodiments herein, the medium is not GBGM®.

[00231] In preferred embodiments, the first culture medium comprises one or more of medium supplements (*e.g.*, nutrients, cytokines and/or factors). Medium supplements suitable for use in the processes described herein include, for example without limitation, serum such as human serum AB, fetal bovine serum (FBS) or fetal calf serum (FCS), vitamins, 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), heparin, or O-acetyl-carnitine (also referred to as acetylcarnitine, O-acetyl-L-carnitine or OAC). In a specific embodiment, the medium used herein comprises human serum AB. In another specific embodiment, the medium used herein comprises FBS. In another specific embodiment, the medium used herein comprises OAC.

[00232] In certain embodiments, the first medium does not comprise one or more of, granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony stimulating

factor (GM-CSF), interleukin-6 (IL-6), macrophage inflammatory Protein 1 α (MIP1α), or leukemia inhibitory factor (LIF).

[00233] Thus, in one aspect, described herein is a two-step process of producing NK cells, wherein said first step comprises expanding and differentiating a population of hematopoietic cells in a first culture medium in the absence of feeder cells, wherein a plurality of hematopoietic cells within said population of hematopoietic cells differentiate into NK cells during said expanding, and wherein the medium comprises SCF at a concentration of about 1 to about 150 ng/mL, IL-2 at a concentration of about 50 to about 1500 IU/mL, IL-7 at a concentration of about 1 to about 150 ng/mL, IL-15 at a concentration 1 to about 150 ng/mL and heparin at a concentration of about 0.1 to about 30 IU/mL, and wherein said SCF, IL-2, IL-7, IL-15 and heparin are not comprised within an undefined component of said medium (*e.g.*, serum). In certain embodiments, said medium comprises one or more of O-acetyl-carnitine (also referred to as acetylcarnitine, O-acetyl-L-carnitine or OAC), or a compound that affects acetyl-CoA cycling in mitodronia, thiazovivin, Y-27632, pyintegrin, Rho kinase (ROCK) inhibitors, caspase inhibitors or other anti-apoptotic compounds/peptides, NOVA-RS (Sheffield Bio-Science) or other small-molecule growth enhancers. In certain embodiments, said medium comprises nicotinamide. In certain embodiments, said medium comprises about 0.5 mM-10 mM OAC. In one embodiment, said medium comprises Stemspan® H3000, and/or DMEM:F12 and about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mM OAC. In a specific embodiment, said medium is GBGM®. In another specific embodiment, the medium is not GBGM®. In another specific embodiment, said medium comprises Stemspan® H3000 and about 5 mM of OAC. In another specific embodiment, said medium comprises DMEM:F12 and about 5 mM of OAC. The OAC can be added anytime during the culturing processes described herein. In certain embodiments, said OAC is added to the first medium and/or during the first culturing step. In some embodiments, said OAC is added to the first medium on Day 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 , 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 of the culture. In a specific embodiment, said OAC is added to the first medium on Day 7 of the first culturing step. In a more specific embodiment, said OAC is added to the first medium on Day 7 of the culture and is present throughout the first and second culturing steps. In certain embodiments, said OAC is added to the second medium and/or during the second culturing step. In some embodiments, said OAC is added to the second medium on Day 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 of the culture.

[00234] In another specific embodiment, said medium is IMDM supplemented with about 5-20% BSA, about 1-10 μg/mL recombinant human insulin, about 10-50 μg/mL iron saturated human transferrin and about 10-50 μM β-mercaptoethanol. In another specific embodiment, said medium does not comprise one or more, or any, of IL-11, IL-3, homeobox-B4 (HoxB4), and/or methylcellulose.

[00235] In other specific embodiments, said medium comprises SCF at a concentration of about 0.1 to about 500 ng/mL; about 5 to about 100 ng/mL; or about 20 ng/mL. In other specific embodiments, said medium comprises IL-2 at a concentration of about 10 to about 2000 IU/mL; or about 100 to about 500 IU/mL; or about 200 IU/mL. In other specific embodiments, said medium comprises IL-7 at a concentration of about 0.1 to about 500 ng/mL; about 5 to about 100 ng/mL; or about 20 ng/mL. In other specific embodiments, said medium comprises IL-15 at a concentration of about 0.1 to about 500 ng/mL; about 5 to about 100 ng/mL; or about 10 ng/mL. In other specific embodiments, said medium comprises heparin at concentration of about 0.05 to about 100 U/mL; or about 0.5 to about 20 U/ml; or about 1.5 U/mL.

[00236] In yet other specific embodiment, said medium further comprises Fms-like-tyrosine kinase 3 ligand (Flt-3L) at a concentration of about 1 to about 150 ng/mL, thrombopoietin (Tpo) at a concentration of about 1 to about 150 ng/mL, or a combination of both. In other specific embodiments, said medium comprises Flt-3L at a concentration of about 0.1 to about 500 ng/mL; about 5 to about 100 ng/mL; or about 20 ng/mL. In other specific embodiments, said medium comprises Tpo at a concentration of about 0.1 to about 500 ng/mL; about 5 to about 100 ng/mL; or about 20 ng/mL.

[00237] In a more specific embodiment, the first culture medium is GBGM®, which comprises about 20 ng/mL SCF, about 20 ng/mL IL-7, about 10 ng/mL IL-15. In another more specific embodiment, the first culture medium is GBGM®, which comprises about 20 ng/mL SCF, about 20 ng/mL Flt3-L, about 200 IU/mL IL-2, about 20 ng/mL IL-7, about 10 ng/mL IL-15, about 20 ng/mL Tpo, and about 1.5 U/mL heparin. In another specific embodiment, said first culture medium further comprises 10% human serum (*e.g.*, human serum AB) or fetal serum (*e.g.*, FBS). In a specific embodiment of any of the embodiments herein, the medium is not GBGM®.

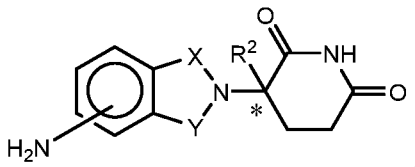
[00238] In another embodiment, hematopoietic cells are expanded by culturing said cells, *e.g.*, in said first medium, in contact with an immunomodulatory compound, *e.g.*, a TNF-α inhibitory

compound, for a time and in an amount sufficient to cause a detectable increase in the proliferation of the hematopoietic cells over a given time, compared to an equivalent number of hematopoietic cells not contacted with the immunomodulatory compound. *See*, *e.g.*, U.S. Patent Application Publication No. 2003/0235909, the disclosure of which is hereby incorporated by reference in its entirety. In certain embodiments, the immunomodulatory compound is an amino-substituted isoindoline. In a preferred 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 preferred embodiment, the immunomodulatory compound is pomalidomide, or lenalidomide.

[00239] Specific examples of immunomodulatory compounds include, but are not limited to, cyano and carboxy derivatives of substituted styrenes such as those disclosed in U.S. patent no. 5,929,117; 1-oxo-2-(2,6-dioxo-3-fluoropiperidin-3yl) isoindolines and 1,3-dioxo-2-(2,6-dioxo-3-fluoropiperidine-3-yl) isoindolines such as those described in U.S. patent no. 5,874,448; the tetra substituted 2-(2,6-dioxopiperdin-3-yl)-1-oxoisoindolines described in U.S. patent no. 5,798,368; 1-oxo and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines (*e.g.*, 4-methyl derivatives of thalidomide and EM-12), including, but not limited to, those disclosed in U.S. patent no. 5,635,517; and a class of non-polypeptide cyclic amides disclosed in U.S. patent nos. 5,698,579 and 5,877,200; analogs and derivatives of thalidomide, including hydrolysis products, metabolites, derivatives and precursors of thalidomide, such as those described in U.S. patent nos. 5,593,990, 5,629,327, and 6,071,948 to D'Amato; aminothalidomide, as well as analogs, hydrolysis products, metabolites, derivatives and precursors of aminothalidomide, and substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindoles such as those described in U.S. patent nos. 6,281,230 and 6,316,471; isoindole-imide compounds such as those described in U.S. patent application no. 09/972,487 filed on October 5, 2001, U.S. patent application no. 10/032,286 filed on December 21, 2001, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106). The entireties of each of the patents and patent applications identified herein are incorporated herein by reference. Immunomodulatory compounds do not include thalidomide.

[00240] In another embodiment, immunomodulatory compounds include, but are not limited to, 1-oxo-and 1,3 dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines substituted with amino in the

benzo ring as described in U.S. Patent no. 5,635,517 which is incorporated herein by reference. These compounds have the structure



,

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.

[00241] In another embodiment, specific immunomodulatory compounds include, but are not limited to:

1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline;

1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline;

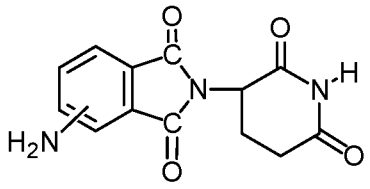
1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline;

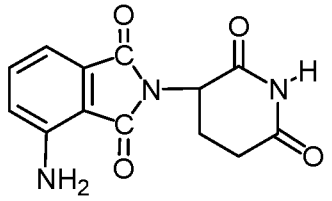
1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline;

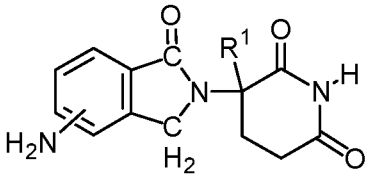
1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline; and

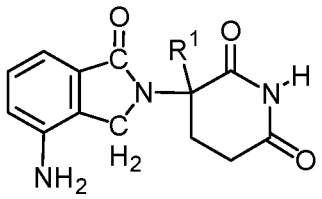
1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline.

[00242] Other specific immunomodulatory compounds belong to a class of substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindoles, such as those described in U.S. patent nos. 6,281,230; 6,316,471; 6,335,349; and 6,476,052, and International Patent Application No. PCT/US97/13375 (International Publication No. WO 98/03502), each of which is incorporated herein by reference. Compounds representative of this class are of the formulas:

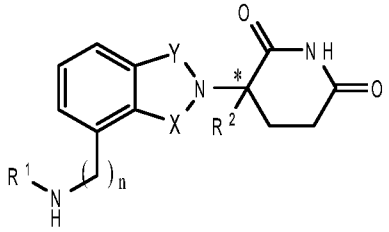








wherein R^1 is hydrogen or methyl. In a separate embodiment, the invention encompasses the use of enantiomerically pure forms (*e.g.* optically pure (R) or (S) enantiomers) of these compounds. Still other specific immunomodulatory compounds belong to a class of isoindole-imides disclosed in U.S. patent application nos. 10/032,286 and 09/972,487, and International Application No. PCT/US01/50401 (International Publication No. WO 02/059106), each of which are incorporated herein by reference. In one representative 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 $(C_1\text{-}C_8)$alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl, benzyl, aryl, or $(C_2\text{-}C_5)$heteroaryl;

each occurrence of R^6 is independently H, $(C_1\text{-}C_8)$alkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl, benzyl, aryl, $(C_2\text{-}C_5)$heteroaryl, or $(C_0\text{-}C_8)$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 specific compounds of the above formula, when n is 0 then R^1 is $(C_3\text{-}C_7)$cycloalkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl, benzyl, aryl, $(C_0\text{-}C_4)$alkyl–$(C_1\text{-}C_6)$heterocycloalkyl, $(C_0\text{-}C_4)$alkyl-$(C_2\text{-}C_5)$heteroaryl, C(O)R^3, C(O)OR^4, $(C_1\text{-}C_8)$alkyl–N$(R^6)_2$, $(C_1\text{-}C_8)$alkyl–OR^5, $(C_1\text{-}C_8)$alkyl–C(O)OR^5, C(S)NHR^3, or $(C_1\text{-}C_8)$alkyl–O(CO)R^5;

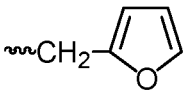
R^2 is H or $(C_1\text{-}C_8)$alkyl; and

R^3 is $(C_1\text{-}C_8)$alkyl, $(C_3\text{-}C_7)$cycloalkyl, $(C_2\text{-}C_8)$alkenyl, $(C_2\text{-}C_8)$alkynyl, benzyl, aryl, $(C_0\text{-}C_4)$alkyl–$(C_1$ –$C_6)$heterocycloalkyl, $(C_0\text{-}C_4)$alkyl–$(C_2\text{-}C_5)$heteroaryl, $(C_5\text{-}C_8)$alkyl–N$(R^6)_2$; $(C_0\text{-}C_8)$alkyl–NH–C(O)O–R^5; $(C_1\text{-}C_8)$alkyl–OR^5, $(C_1\text{-}C_8)$alkyl–C(O)OR^5, $(C_1\text{-}C_8)$alkyl–O(CO)R^5, or C(O)OR^5; and the other variables have the same definitions.

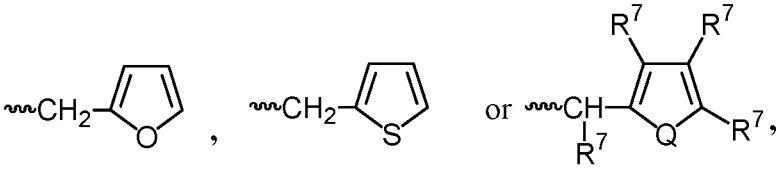
In other specific compounds of the above formula, R^2 is H or $(C_1\text{-}C_4)$alkyl.

In other specific compounds of the above formula, R^1 is $(C_1\text{-}C_8)$alkyl or benzyl.

In other specific compounds of the above formula, R^1 is H, $(C_1\text{-}C_8)$alkyl, benzyl, CH_2OCH_3, $CH_2CH_2OCH_3$, or



In another embodiment of the compounds of the above formula, R^1 is



wherein Q is O or S, and each occurrence of R^7 is independently H, $(C_1\text{-}C_8)$alkyl, benzyl, CH_2OCH_3, or $CH_2CH_2OCH_3$.

In other specific compounds of the above formula, R^1 is C(O)R^3.

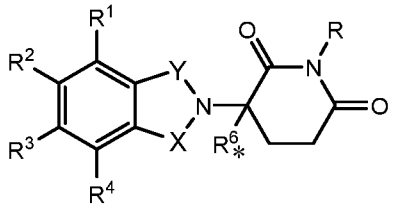
In other specific compounds of the above formula, R^3 is $(C_0\text{-}C_4)$alkyl–$(C_2\text{-}C_5)$heteroaryl, $(C_1\text{-}C_8)$alkyl, aryl, or $(C_0\text{-}C_4)$alkyl–OR^5.

In other specific compounds of the above formula, heteroaryl is pyridyl, furyl, or thienyl.

In other specific compounds of the above formula, R^1 is $C(O)OR^4$.

In other specific compounds of the above formula, the H of C(O)NHC(O) can be replaced with $(C_1\text{-}C_4)$alkyl, aryl, or benzyl.

[00243] 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 $R^7\text{-}CHR^{10}\text{-}N(R^8R^9)$;

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.

[00244] In a specific embodiment, expansion of the hematopoietic cells is performed in IMDM supplemented with 20% BITS (bovine serum albumin, recombinant human insulin and

transferrin), SCF, Flt-3 ligand, IL-3, and 4-(Amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione (10 μM in 0.05% DMSO). In a more specific embodiment, about 5 x 10^7 hematopoietic cells, *e.g.*, $CD34^+$ cells, are expanded in the medium to from about 5 x 10^{10} cells to about 5 x 10^{12} cells, which are resuspended in 100 mL of IMDM to produce a population of expanded hematopoietic cells. The population of expanded hematopoietic cells is preferably cryopreserved to facilitate shipping.

[00245] In various specific embodiments, at least 50%, 55%, 60%, 65%, 70%. 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% of the hematopoietic cells are differentiated to NK cells.

[00246] In certain embodiments, the process of expansion and differentiation of the hematopoietic cells, as described herein, comprises maintaining the cell population comprising said hematopoietic cells at between about 2 x 10^4 and about 2 x 10^5 cells per milliliter during expansion and differentiation. In certain other embodiments, the process of expansion and differentiation of the hematopoietic cells, as described herein, comprises maintaining the cell population comprising said hematopoietic cells at no more than about 1 x 10^5 cells per milliliter.

[00247] The time for expansion and differentiation of hematopoietic cells into NK cells can be, for example, from about 3 days to about 120 days. In one embodiment, the differentiation time is about 7 days to about 75 days. In another embodiment, the differentiation time is about 14 days to about 50 days. In a specific embodiment, the differentiation time is about 21 days to about 28 days.

5.2.7.2. Second Step

[00248] The hematopoietic cells, *e.g.*, stem cells or progenitor cells, and natural killer cells, resulting from the first step, are further expanded and differentiated in a second step, *e.g.*, without the use of feeder layer or in the presence of feeder cells. Culture of the cells as described herein results in continuous expansion, differentiation as well as maturation of the NK cells from the first step. In the second step, the NK cells are expanded, differentiated and maturated, in a continuous fashion, in a second culture medium, *e.g.*, comprising different cytokines and/or bioactive molecules than said first medium. In certain embodiments, the second culture medium is an animal component-free medium. Exemplary animal component-free cell culture media are described in the disclosure.

[00249] Thus, in one aspect, described herein is a process of producing activated NK cells, comprising expanding the NK cells from the first step, described above, in a second medium in

the presence of feeder cells and in contact with interleukin-2 (IL-2). In specific embodiments, said second medium comprises cell growth medium comprising IL-2, *e.g.*, 10 IU/mL to 1000 IU/mL, and one or more of: human serum (*e.g.*, human serum AB), fetal bovine serum (FBS) or fetal calf serum (FCS), *e.g.*, 5%-15% FCS v/v; transferrin, *e.g.*, 10 μg/mL to 50 μg/mL; insulin, *e.g.*, 5 μg/mL to 20 μg/mL; ethanolamine, *e.g.*, 5 x 10^{-4} to 5 x 10^{-5} M; oleic acid, *e.g.*, 0.1 μg/mL to 5 μg/mL; linoleic acid, *e.g.*, 0.1 μg/mL to 5 μg/mL; palmitic acid, *e.g.*, 0.05 μg/mL to 2 μg/mL; bovine serum albumin (BSA), *e.g.*, 1 μg/mL to 5 μg/mL; and/or phytohemagglutinin, *e.g.*, 0.01 μg/mL to 1 μg/mL. In a more specific embodiment, said second medium comprises cell growth medium comprising FBS or FCS, *e.g.*, 10% FCS v/v, IL-2, transferrin, insulin, ethanolamine, oleic acid, linoleic acid, palmitic acid, bovine serum albumin (BSA) and phytohemagglutinin. In a more specific embodiment, said second medium comprises Iscove's Modified Dulbecco's Medium (IMDM), 10% FBS or FCS, 400 IU IL-2, 35 μg/mL transferrin, 5 μg/mL insulin, 2 x 10^{-5} M ethanolamine, 1 μg/mL oleic acid, 1 μg/mL linoleic acid (Sigma-Aldrich), 0.2 μg/mL palmitic acid (Sigma-Aldrich), 2.5 μg/mL BSA (Sigma-Aldrich) and 0.1 μg/mL phytohemagglutinin.

[00250] In certain embodiments, the second medium does not comprise one or more of, granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony stimulating factor (GM-CSF), interleukin-6 (IL-6), macrophage inflammatory Protein 1 α (MIP1α), or leukemia inhibitory factor (LIF).

[00251] Feeder cells, when used, can be established from various cell types. Examples of these cell types include, without limitation, fibroblasts, stem cells (*e.g.*, tissue culture-adherent placental stem cells), blood cells (*e.g.*, peripheral blood mononuclear cells (PBMC)), and cancerous cells (*e.g.*, chronic myelogenous leukemia (CML) cells such as K562). In a specific embodiment, said culturing in said second medium comprises culturing using feeder cells, *e.g.*, K562 cells and/or peripheral blood mononuclear cells (PBMCs), *e.g.*, at the time the cells are started in said second medium, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days thereafter. In certain embodiments, feeder cells are optionally from a different species as the cells they are supporting. For example, human NK cells can be supported by mouse embryonic fibroblasts (from primary culture or a telomerized line).

[00252] In certain embodiments, feeder cells are optionally inactivated by irradiation (*e.g.*, γ-irradiation) or treatment with an anti-mitotic agent such as mitomycin C, to prevent them from

outgrowing the cells they are supporting, but permit synthesis of important factors that support the NK cells. For example, cells can be irradiated at a dose to inhibit proliferation but permit synthesis of important factors that support human embryonic stem (hES) cells (about 4000 rads gamma irradiation).

[00253] Culture of NK cells for the second step can take place in any container compatible with cell culture and expansion, *e.g.*, flask, tube, beaker, dish, multiwell plate, bag or the like. In a specific embodiment, feeder cell-dependent culture of NK cells takes place in a bag, *e.g.*, a flexible, gas-permeable fluorocarbon culture bag (for example, from American Fluoroseal). In a specific embodiment, the container in which the NK cells are cultured is suitable for shipping, *e.g.*, to a site such as a hospital or military zone wherein the expanded NK cells are further expanded, differentiated and maturated.

[00254] Differentiation of the cells from step 1 into activated NK cells can be assessed by detecting NK cell-specific markers, *e.g.*, by flow cytometry. NK cell-specific markers include, but are not limited to, CD56, CD94, CD117 and NKp46. Differentiation can also be assessed by the morphological characteristics of NK cells, *e.g.*, large size, high protein synthesis activity in the abundant endoplasmic reticulum (ER), and/or preformed granules.

[00255] The time for expansion and differentiation of cells from step 1 into activated NK cells can be, for example, from about 3 days to about 120 days. In one embodiment, the differentiation time is about 7 days to about 75 days. In another embodiment, the differentiation time is about 14 days to about 50 days. In a specific embodiment, the differentiation time is about 10 days to about 21 days.

[00256] Differentiation of hematopoietic cells into NK cells can be assessed by detecting markers, *e.g.*, CD56, CD94, CD117, NKG2D, DNAM-1 and NKp46, by, for example, flow cytometry. Differentiation can also be assessed by the morphological characteristics of NK cells, *e.g.*, large size, high protein synthesis activity in the abundant endoplasmic reticulum (ER), and/or preformed granules. Maturation of NK cells (*e.g.*, activated NK cells) can be assessed by detecting one or more functionally relevant makers, for example, CD94, CD161, NKp44, DNAM-1, 2B4, NKp46, CD94, KIR, and the NKG2 family of activating receptors (*e.g.*, NKG2D). Maturation of NK cells (*e.g.*, activated NK cells) can also be assessed by detecting specific markers during different developmental stages. For example, in one embodiment, pro-NK cells are $CD34^+$, $CD45RA^+$, $CD10^+$, $CD117^-$ and/or $CD161^-$. In another embodiment, pre-

NK cells are $CD34^+$, $CD45RA^+$, $CD10^-$, $CD117^+$, and/or $CD161^-$. In another embodiment, immature NK cells are $CD34^-$, $CD117^+$, $CD161^+$, $NKp46^-$ and/or $CD94/NKG2A^-$. In another embodiment, $CD56^{bright}$ NK cells are $CD117^+$, $NKp46^+$, $CD94/NKG2A^+$, $CD16^-$, and/or $KIR^{+/-}$. In another embodiment, $CD56^{dim}$ NK cells are $CD117^-$, $NKp46^+$, $CD94/NKG2A^{+/-}$, CD16+, and/or KIR^+. In a specific embodiment, maturation of NK cells (*e.g.*, activated NK cells) is determined by the percentage of NK cells (*e.g.*, activated NK cells) that are $CD161^-$, $CD94^+$ and/or $NKp46^+$. In a more specific embodiment, at least 10%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65% or 70% of mature NK cells (*e.g.*, activated NK cells) are $NKp46^+$. In another more specific embodiment, at least 10%, 20%, 25%, 30%, 35%, 40%, 45% or 50% of mature NK cells (*e.g.*, activated NK cells) are $CD94^+$. In another more specific embodiment, at least 10%, 20%, 25%, 30%, 35%, 40%, 45% or 50% of mature NK cells (*e.g.*, activated NK cells) are $CD161^-$.

[00257] In certain embodiments, the differentiation of hematopoietic cells into NK cells are assessed by detecting the expression level of, *e.g.*, CD3, CD7 or CD127, CD10, CD14, CD15, CD16, CD33, CD34, CD56, CD94, CD117, CD161, NKp44, NKp46, NKG2D, DNAM-1, 2B4 or TO-PRO-3, using, *e.g.*, antibodies to one or more of these cell markers. Such antibodies can be conjugated to a detectable label, for example, as fluorescent label, *e.g.*, FITC, R-PE, PerCP, PerCP-Cy5.5, APC, APC-Cy7 or APC-H7.

5.2.8. Methods of Producing TSPNK Cells

[00258] TSPNK cells may be produced from hematopoietic cells, which are described above. In certain embodiment, the TSPNK cells are produced from expanded hematopoietic cells, *e.g.*, hematopoietic stem cells and/or hematopoietic progenitor cells.

[00259] In one embodiment, the TSPNK cells are produced by a three-step process. In certain embodiments, the process of expansion and differentiation of the hematopoietic cells, as described herein, to produce NK progenitor cell populations or NK cell populations according to a three-step process 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, *e.g.*, between about 2×10^4 and about 2×10^5 cells per milliliter, during expansion and differentiation. In certain other embodiments, the process of expansion and differentiation of the hematopoietic cells, as described herein, comprises maintaining the cell population comprising said hematopoietic cells at no more than about 1×10^5 cells per milliliter. In certain other

embodiments, the process of expansion and differentiation of the hematopoietic cells, as described herein, comprises maintaining the cell population comprising said hematopoietic cells at no more than about 1 x 10^5 cells per milliliter, 2 x 10^5 cells per milliliter, 3 x 10^5 cells per milliliter, 4 x 10^5 cells per milliliter, 5 x 10^5 cells per milliliter, 6 x 10^5 cells per milliliter, 7 x 10^5 cells per milliliter, 8 x 10^5 cells per milliliter, 9 x 10^5 cells per milliliter, 1 x 10^6 cells per milliliter, 2 x 10^6 cells per milliliter, 3 x 10^6 cells per milliliter, 4 x 10^6 cells per milliliter, 5 x 10^6 cells per milliliter, 6 x 10^6 cells per milliliter, 7 x 10^6 cells per milliliter, 8 x 10^6 cells per milliliter, or 9 x 10^6 cells per milliliter.

[00260] In a certain embodiment, the three-step process comprises a first step ("step 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. In certain embodiments, the first medium contains one or more factors that promote expansion of hematopoietic progenitor cells, one or more factors for initiation of lymphoid differentiation within the expanding hematopoietic progenitor population, and/or one or more factors that mimic stromal feeder support. In certain embodiments, the first medium comprises one or more cytokines (for example, Flt3L, TPO, SCF). In certain embodiments, the first medium comprises IL-7. In certain embodiments, the first medium comprises sub-ng/mL concentrations of G-CSF, IL-6 and/or GM-CSF. In a specific embodiment, the first medium comprises the cytokines Flt3L, TPO, and SCF, IL-7, and sub-ng/mL concentrations of G-CSF, IL-6 and GM-CSF. In specific embodiments, in the first medium, CD34+ cells undergo expansion into lineage specific progenitors, which then become CD34-. In certain embodiments, this expansion occurs rapidly. In certain embodiments, the CD34- cells comprise more than 50%, more than 55%, more than 60%, more than 65%, more than 70%, more than 75%, more than 80%, or more of the total population at the end of step 1. In a more specific embodiment, CD34- cells comprise more than 80% of the total population at the end of step 1.

[00261] In certain embodiments, subsequently, in "step 2" said cells are cultured in a second medium for a specified time period, *e.g.*, as described herein. In certain embodiments, the second medium contains factors that may promote further expansion of lymphoid progenitors, factors that may contribute to development along the NK lineage, and/or factors that mimic stromal feeder support. In certain embodiments, the second medium comprises one or more cytokines (*e.g.*, Flt3L, SCF, IL-15, and/or IL-7). In certain embodiments, the second medium comprises

IL-17 and/or IL-15. In certain embodiments, the second medium comprises sub-ng/mL concentrations of G-CSF, IL-6 and/or GM-CSF. In a specific embodiment, the second medium comprises the cytokines Flt3L, SCF, IL-15, and IL-7, IL-17 and IL-15, and sub-ng/mL concentrations of G-CSF, IL-6 and GM-CSF.

[00262] In certain embodiments, subsequently, in "step 3" said cells are cultured in a third medium for a specified time period, *e.g.*, as described herein. In certain embodiments, the third medium comprises factors that promote differentiation and functional activation of CD56+CD3-CD16- cells, which may be NK progenitor cells. In one embodiment, such factors comprise IL2 and IL12 and IL18, IL12 and IL15, IL12 and IL18, IL2 and IL12 and IL15 and IL18, or IL2 and IL15 and IL18. In certain embodiments, the third medium comprises factors that mimic stromal feeder support. In certain embodiments, the third medium comprises one or more cytokines (*e.g.*, SCF, IL-15, IL-7, IL-2). In certain embodiments, the third medium comprises sub-ng/mL concentrations of G-CSF, IL-6 and/or GM-CSF. In a specific embodiment, the third medium comprises the cytokines SCF, IL-15, IL-7, IL-2, and sub-ng/mL concentrations of G-CSF, IL-6 and GM-CSF.

[00263] In specific embodiments, the three-step process is used to produce NK cell (*e.g.*, mature NK cell) populations. In specific embodiments, the three-step process is used to produce NK progenitor cell populations. In certain embodiments, the three-step process is conducted in the absence of stromal feeder cell support. In certain embodiments, the three-step process is conducted in the absence of exogenously added steroids (*e.g.*, cortisone, hydrocortisone, or derivatives thereof).

[00264] In certain embodiments, the first medium used in the three-step processes described herein may contain any of the components of the first or second medium described in Section 5.2.4 in connection with the two-step method. In certain embodiments, said first medium used in the three-step process comprises medium comprising one or more of: animal serum, *e.g.*, human serum (*e.g.*, human serum AB), fetal bovine serum (FBS) or fetal calf serum (FCS), *e.g.*, 1% to 20 % v/v serum, *e.g.*, 5% to 20% v/v serum; stem cell factor (SCF), *e.g.*, 1 ng/mL to 50 ng/mL SCF; FMS-like tyrosine kinase-3 ligand (Flt-3 ligand), *e.g.*, 1 ng/ml to 30 ng/mL Flt-3 ligand; interleukin-7 (IL-7), *e.g.*, 1 ng/mL to 50 ng/mL IL-7; thrombopoietin (TPO), *e.g.*, 1 ng/mL to 100 ng/mL, for example, 1 ng/mL to 50 ng/mLTPO; interleukin-2 (IL-2), *e.g.*, up to 2000 IU/mL, for example, 50 IU/mL to 500 IU/mL; and/or heparin, *e.g.*, low-weight heparin (LWH),

e.g., 0.1 IU/mL to 10 IU/mL heparin. 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, said first medium additionally comprises OAC. In certain embodiments, said first medium additionally comprises interleukin-6 (IL-6), leukemia inhibitory factor (LIF), G-CSF, GM-CSF, and/or MIP-1α. In certain embodiments, said first medium additionally comprises one or more anti-oxidants, *e.g.*, holo-transferrin, insulin solution, reduced glutathione, sodium selenite, ethanolamine, ascorbic acid, b-mercaptoethanol, O-acetyl-L-carnitine, N-acetylcysteine, (+/-) lipoic acid, nicotinamide, or resveratrol. 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 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 a specific embodiment of any of the embodiments herein, the medium is not GBGM®.

[00265] In certain embodiments, the second medium used in the three-step processes described herein may contain any of the components of the first or second medium described in Section 5.2.4 in connection with the two-step method. In certain embodiments, said second medium used in the three-step process comprises medium comprising one or more of: animal serum, *e.g.*, human serum (*e.g.*, human serum AB), FBS or FCS, *e.g.*, 5% to 20% v/v serum; SCF, *e.g.*, 1 ng/mL to 50 ng/mL SCF; Flt-3 ligand, *e.g.*, 1 ng/ml to 30 ng/mL Flt-3 ligand; IL-7, *e.g.*, 1 ng/mL to 50 ng/mL IL-7; interleukin-15 (IL-15), *e.g.*, 1 ng/mL to 50 ng/mL IL-15; and/or heparin, *e.g.*, LWH, *e.g.*, 0.1 IU/mL to 10 IU/mL heparin. 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, said second medium additionally

comprises OAC. In certain embodiments, said second medium additionally comprises interleukin-6 (IL-6), leukemia inhibitory factor (LIF), G-CSF, GM-CSF, and/or MIP-1α. In certain embodiments, said second medium additionally comprises one or more anti-oxidants, *e.g.*, holo-transferrin, insulin solution, reduced glutathione, sodium selenite, ethanolamine, ascorbic acid, b-mercaptoethanol, O-acetyl-L-carnitine, N-acetylcysteine, (+/-) lipoic acid, nicotinamide, or resveratrol. 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 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 a specific embodiment of any of the embodiments herein, the medium is not GBGM®.

[00266] In certain embodiments, the third medium used in the three-step processes described herein may contain any of the components of the first or second medium described in Section 5.2.4 in connection with the two-step method. In certain embodiments, said third medium used in the three-step process comprises medium comprising one or more of: animal serum, *e.g.*, human serum (*e.g.*, human serum AB), FBS or FCS, *e.g.*, 5% to 20% v/v serum; SCF, *e.g.*, 1 ng/mL to 50 ng/mL SCF; Flt-3 ligand, *e.g.*, 1 ng/ml to 30 ng/mL Flt-3 ligand; IL-7, *e.g.*, 1 ng/mL to 50 ng/mL IL-7; IL-15, *e.g.*, 1 ng/mL to 50 ng/mL IL-15; and interleukin-2 (IL-2), *e.g.*, in the range from 0 to 2000 IU/mL, for example, 50 IU/mL to 1000 IU/mL IL-2. 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, said third medium additionally comprises OAC. In certain embodiments, said third medium additionally comprises interleukin-6 (IL-6), leukemia inhibitory factor (LIF), G-CSF, GM-CSF, and/or MIP-1α. In certain embodiments, said third medium additionally comprises one or more

anti-oxidants, *e.g.*, holo-transferrin, insulin solution, reduced glutathione, sodium selenite, ethanolamine, ascorbic acid, b-mercaptoethanol, O-acetyl-L-carnitine, N-acetylcysteine, (+/-) lipoic acid, nicotinamide, or resveratrol. 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 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 a specific embodiment of any of the embodiments herein, the medium is not GBGM®.

[00267] In certain embodiments, in the three-step processes 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 cultured in said first medium 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 cultured in said first medium and said second medium 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.

[00268] In certain embodiments, in the three-step processes described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for 2-12 days, 3-11 days, for example, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, or 9-11 days, before said culturing in said second medium. In certain embodiments, cells cultured in said first medium are cultured in said second medium for 1-10 days, for example, 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, or 7-9 days, before said culturing in said third medium. In certain embodiments, cells cultured in said first medium and said second medium are cultured in said third medium for 2-27 days, for example, 3-25 days, *e.g.*, for 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, 18-20, 19-21, 20-22, 21-23, 22-24, or 23-25 days.

[00269] In a specific embodiment, in the three-step processes described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for 9 days before said culturing in said second medium; cultured in said second medium for 5 days before said culturing in said third medium; and cultured in said third medium for 7 days, *i.e.*, the cells are cultured a total of 21 days.

[00270] In a specific embodiment, in the three-step processes described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for 7-9 days before said culturing in said second medium; cultured in said second medium for 5-7 days before said culturing in said third medium; and cultured in said third medium for 21-35 days, *i.e.*, the cells are cultured a total of 35 days. In a more specific embodiment, in the three-step processes described herein, said hematopoietic stem or progenitor cells are cultured in said first medium for 9 days before said culturing in said second medium; cultured in said second medium for 5 days before said culturing in said third medium; and cultured in said third medium for 21 days, *i.e.*, the cells are cultured a total of 35 days.

5.2.9. Methods of Producing Three-Stage NK Cells

[00271] Production of NK cells and NK cell populations by the three-stage method comprises expanding a population of hematopoietic cells. During cell expansion, a plurality of hematopoietic cells within the hematopoietic cell population differentiate into NK 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 80%, of the natural killer cells are viable with 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-.

[00272] In one embodiment, provided herein is a three-stage method of producing NK cell

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

[00273] 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.

[00274] In certain aspects, said first population of cells are initially inoculated into said second medium from 5 x 10^4 to 5 x 10^5 cells/mL. In a specific aspect, said first population of cells is initially inoculated into said second medium at about 1 x 10^5 cells/mL.

[00275] In certain aspects said second population of cells is initially inoculated into said third medium from 1 x 10^5 to 5 x 10^6 cells/mL. In certain aspects, said second population of cells is initially inoculated into said third medium from 1 x 10^5 to 1 x 10^6 cells/mL. In a specific aspect, said second population of cells is initially inoculated into said third medium at about 5 x 10^5 cells/mL. In a more specific aspect, said second population of cells is initially inoculated into said third medium at about 5 x 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 x 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.

[00276] 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.

[00277] 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.

[00278] 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.

[00279] In a specific embodiment, the three-stage method is used to produce NK 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).

[00280] 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, 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 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 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 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 a specific embodiment of any of the embodiments herein, the medium is not GBGM®.

[00281] 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, 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 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 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 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 a specific embodiment of any of the embodiments herein, the medium is not GBGM®.

[00282] In certain embodiments, the third medium used in the three-stage method comprises medium comprising 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 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 a specific embodiment of any of the embodiments herein, the medium is not GBGM®.

[00283] 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.

[00284] 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.

[00285] 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.

[00286] 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.

[00287] 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.

[00288] 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.

[00289] 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.

[00290] 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.

[00291] In certain aspects, said hematopoietic stem or progenitor cells are initially inoculated into said first medium from 1 x 10^4 to 1 x 10^5 cells/mL. In a specific aspect, said hematopoietic stem or progenitor cells are initially inoculated into said first medium at about 3 x 10^4 cells/mL.

[00292] In certain aspects, said first population of cells are initially inoculated into said second medium from 5 x 10^4 to 5 x 10^5 cells/mL. In a specific aspect, said first population of cells is initially inoculated into said second medium at about 1 x 10^5 cells/mL.

[00293] In certain aspects said second population of cells is initially inoculated into said third medium from 1 x 10^5 to 5 x 10^6 cells/mL. In certain aspects, said second population of cells is initially inoculated into said third medium from 1 x 10^5 to 1 x 10^6 cells/mL. In a specific aspect, said second population of cells is initially inoculated into said third medium at about 5 x 10^5 cells/mL. In a more specific aspect, said second population of cells is initially inoculated into said third medium at about 5 x 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 x 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.

5.2.10. Isolation of Cells

[00294] Methods of isolating natural killer cells are known in the art and can be used to isolate the natural killer cells, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells) produced using the three-step process, described herein. NK cells can be isolated or enriched by staining cells from a tissue source, *e.g.*, peripheral blood, with antibodies to CD56 and CD3, and selecting for $CD56^+CD3^-$ cells. NK cells, *e.g.*, activated NK cells or TSPNK cells, can be

isolated using a commercially available kit, for example, the NK Cell Isolation Kit (Miltenyi Biotec). NK cells, *e.g.*, activated NK cells or TSPNK cells, 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.*, activated NK cells or TSPNK cells. For example, NK cells, *e.g.*, activated NK cells or TSPNK cells, 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 $CD16^+$ and $CD16^-$ cells.

[00295] Cell separation can be accomplished by, *e.g.*, flow cytometry, fluorescence-activated cell sorting (FACS), or, preferably, 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.

[00296] In some embodiments, the purity of the isolated or enriched natural killer cells can be confirmed by detecting one or more of CD56, CD3 and CD16.

5.2.11. Preservation of Cells/Perfusate

[00297] Cells, *e.g.*, NK cells produced using the methods described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells) produced using the three-step process described herein, or placental perfusate cells comprising hematopoietic stem cells or progenitor cells, or placental perfusate, 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.

[00298] 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.

[00299] 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 °C and about 25 °C 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 °C and 10 °C, or between about 2 °C and about 5 °C, 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.

[00300] 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.

[00301] In another embodiment, 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.

[00302] 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.

[00303] Cells, *e.g.*, placental perfusate cells, hematopoietic cells, *e.g.*, $CD34^+$ hematopoietic stem cells; NK cells produced using the processes described herein, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells); isolated adherent placental cells provided herein can be cryopreserved, *e.g.*, in cryopreservation medium in small containers, *e.g.*, ampoules or septum vials. In specific embodiments, cells are or have been cryoprerved at a concentration of about $1 \times 10^4 - 5 \times 10^8$ cells per mL. In specific embodiments, cells are or have been 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 or have been 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. In certain embodiments, NK cells have been cryopreserved before administration. In certain embodiments, NK cells have not been cryopreserved before administration.

[00304] 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.

[00305] Cells can be cryopreserved by any of a variety of methods known in the art, 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 or second 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 TSPNK cells (*e.g.*, NK progenitor cells) are made using a three-step process 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 progenitor cells are made using a three-step process described herein, and said cells are cryopreserved after being cultured in a first medium for 9 days; after being cultured in a second medium for 5 days; and after being cultured in a third medium for 7 days.

[00306] In one aspect, a population of NK cells, *e.g.*, activated NK cells, are produced by a process comprising: (a) seeding a population of hematopoietic stem or progenitor cells in a first medium comprising interleukin-15 (IL-15) and, optionally, one or more of stem cell factor (SCF) and interleukin-7 (IL-7), wherein said IL-15 and optional SCF and IL-7 are not comprised within an undefined component of said medium, such that the population expands, and a plurality of hematopoietic stem or progenitor cells within said population of hematopoietic stem or progenitor cells differentiate into NK cells during said expanding; (b) expanding the cells from step (a) in a second medium comprising interleukin-2 (IL-2), to produce a population of activated NK cells, and (c) cryopreserving the NK cells from step (b) in a cryopreservation

medium. In a specific embodiment, said step (c) 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 between step (a) and (b), and between step (b) and (c), and/or no additional culturing steps prior to step (a).

[00307] In another embodiment, the cryopreserving of a population of NK cells, *e.g.*, activated NK cells or TSPNK cells (*e.g.*, NK progenitor cells), comprises: (a) expanding a population of hematopoietic stem or progenitor cells in a first medium comprising one or more of stem cell factor (SCF), IL-2, interleukin-7 (IL-7), interleukin-15 (IL-15) and heparin, and wherein said SCF, IL-2, IL-7 and IL-15 are not comprised within an undefined component of said medium, and wherein a plurality of hematopoietic stem or progenitor cells within said population of hematopoietic stem or progenitor cells differentiate into NK cells during said expanding; (b) expanding the cells from step (a) in a second medium comprising interleukin-2 (IL-2), to produce activated NK cells; and (c) cryopreserving the NK cells from step (b) in a cryopreservation medium. In a specific embodiment, said step (c) 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 between step (a) and (b), and between step (b) and (c).

[00308] Cells are preferably cooled in a controlled-rate freezer, *e.g.*, at about 0.1, 0.3, 0.5, 1, or 2 °C/min during cryopreservation. A preferred cryopreservation temperature is about -80 °C to about -180 °C, preferably 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 preferably are thawed at a temperature of about 25 °C to about 40 °C, preferably 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.

[00309] Suitable thawing medium includes, but is not limited to, normal saline, plasmalyte culture medium including, for example, growth medium, *e.g.*, RPMI medium. In preferred 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.

[00310] 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.

5.3. Genetically Modified NK Cells

[00311] In another aspect, NK cells can be genetically modified to enhance target specificity and/or homing specificity.

[00312] In some embodiments, the genetically modified NK cells are NK cells that comprise a chimeric antigen receptor (CAR). CAR is an artificial membrane-bound protein that directs an immune cell (*e.g.*, a T lymphocyte) to an antigen, and stimulates the immune cell to kill a cell displaying the antigen. See, *e.g.*, Eshhar, U.S. Patent No. 7,741,465; U.S. Patent Application Publication No. 2012/0093842; International Application Publication No. WO 2014/100385; and International Application Publication No. WO 2014/124143. At a minimum, the CAR comprises an extracellular domain that binds to an antigen, *e.g.*, an antigen on a cell, a transmembrane domain, and an intracellular (cytoplasmic) signaling domain (*i.e.*, intracellular stimulatory domain) that transmits a primary activation signal to an immune cell. All other conditions being satisfied, when the CAR is expressed on the surface of, *e.g.*, a T lymphocyte, for example, a primary T lymphocyte, and the extracellular domain of the CAR binds to an antigen, the intracellular signaling domain transmits a signal to the T lymphocyte to activate and/or proliferate, and, if the antigen is present on a cell surface, to kill the cell expressing the antigen. Because some immune cells, *e.g.*, T lymphocytes and NK cells, require two signals, a primary activation signal and a costimulatory signal, in order to maximally activate, CARs can also optionally comprise a costimulatory domain such that binding of the antigen to the extracellular domain results in transmission of both a primary activation signal and a costimulatory signal.

[00313] Adaptive immune responses are initiated in secondary lymphoid organs, including the lymph nodes. B cells and T cells are sequestered in distinct regions of the lymph nodes, termed the "B cell zone," located in the outer cortex of the lymph node, or follicles, and the "T cell zone," which is more diffusely distributed in the area surrounding the follicles (also known as the paracortex) respectively. B cells and T cells express receptors that allow them to home to these respective zones so that they can be exposed to antigen. Intact antigens are present in the B cell zone, whereas in the T cell zone, antigens are presented by antigen-presenting cells, such as dendritic cells. Intact antigens, such as tumor antigens, are also present at the site of the tumor.

[00314] In some embodiments, the genetically modified NK cells are NK cells that comprise a

homing receptor, which causes a cell comprising said homing receptor to home to a particular anatomical zone, a particular tissue, or a particular type of cell, *e.g.*, B cell zone of the lymph nodes, gastrointestinal tract, or skin.

[00315] In certain embodiments, the genetically modified NK cells are NK cells that comprise both a CAR and a homing receptor as described herein.

[00316] Without wishing to be bound by any particular mechanism or theory, it is thought that when the genetically modified cells herein express homing receptors that cause a cell expressing said homing receptor to home to a particular zone, they are more likely to be exposed to native antigen, where the cells, for example, cells expressing a CAR, are capable of being activated.

[00317] The NK cells that comprise a CAR and/or a homing receptor can be generated by any method known in the art. In some embodiments, the NK cells comprising a CAR and/or a homing receptor are first produced as described in Section 5.2 (*e.g.*, by a two-step process or by a three-step process), and are then engineered to express the CAR and/or the homing receptor by introducing the NK cells to (*e.g.*, by transfection) one or more vectors comprising the nucleic acid sequence(s) encoding the CAR and/or the homing receptor. In some embodiments, the cells (*e.g.*, CD34+ hematopoietic stem cells), from whom NK cells can be produced, are first engineered to express a CAR and/or a homing receptor by introducing to the cells (*e.g.*, by transfection) one or more vectors comprising the nucleic acid sequence(s) encoding the CAR and/or the homing receptor, and are then used to derive NK cells comprising the CAR and/or the homing receptor by any process described in Section 5.2 (*e.g.*, a two-step process or a three-step process).

5.3.1. General CAR Structure and Intracellular Domain

[00318] In certain embodiments, the intracellular domain of the CAR is or comprises an intracellular domain or motif of a protein that is expressed on the surface of immune cells and triggers activation and/or proliferation of said NK cells. Such a domain or motif is able to transmit a primary antigen-binding signal that is necessary for the activation of a NK cell in response to the antigen's binding to the CAR's extracellular portion. Typically, this domain or motif comprises, or is, an ITAM (immunoreceptor tyrosine-based activation motif). ITAM-containing polypeptides suitable for CARs include, for example, the zeta CD3 chain (CD3ζ) or ITAM-containing portions thereof. In a specific embodiment, the intracellular domain is a CD3ζ intracellular signaling domain. In other specific embodiments, the intracellular domain is from a

lymphocyte receptor chain, a TCR/CD3 complex protein, an Fc receptor subunit or an IL-2 receptor subunit.

[00319] In certain embodiments, the CAR additionally comprises one or more co-stimulatory domains or motifs, *e.g.*, as part of the intracellular domain of the polypeptide. The one or more co-stimulatory domains or motifs can be, or comprise, one or more of a co-stimulatory CD27 polypeptide sequence, a co-stimulatory CD28 polypeptide sequence, a co-stimulatory OX40 (CD134) polypeptide sequence, a co-stimulatory 4-1BB (CD137) polypeptide sequence, a co-stimulatory inducible T-cell costimulatory (ICOS) polypeptide sequence, a co-stimulatory PD-1 polypeptide sequence, a co-stimulatory CTLA-4 polypeptide sequence, a co-stimulatory NKp46 polypeptide sequence, a co-stimulatory NKp44 polypeptide sequence, a co-stimulatory NKp30 polypeptide sequence, a co-stimulatory NKG2D polypeptide sequence, a co-stimulatory DAP10 polypeptide sequence, a co-stimulatory DAP12 polypeptide sequence, or other costimulatory domain or motif.

[00320] The transmembrane region can be any transmembrane region that can be incorporated into a functional CAR, typically a transmembrane region from a CD4 or a CD8 molecule.

5.3.2. CAR Extracellular Domain

[00321] The extracellular domain of the polypeptide binds to an antigen of interest. In certain embodiments, the extracellular domain comprises a receptor, or a portion of a receptor, that binds to said antigen. The extracellular domain may be, *e.g.*, a receptor, or a portion of a receptor, that binds to said antigen. In certain embodiments, the extracellular domain comprises, or is, an antibody or an antigen-binding portion thereof. In specific embodiments, the extracellular domain comprises, or is, a single-chain Fv domain. The single-chain Fv domain can comprise, for example, a V_L linked to V_H by a flexible linker, wherein said V_L and V_H are from an antibody that binds said antigen.

[00322] The antigen to which the extracellular domain of the polypeptide binds can be any antigen of interest, *e.g.*, can be an antigen on a tumor cell or an antigen on an infected cell. The tumor cell may be, *e.g.*, a cell in a solid tumor, or a cell of a blood cancer. The antigen can be any antigen that is expressed on a cell of any tumor or cancer type, *e.g.*, cells of a lymphoma, a lung cancer, a breast cancer, a prostate cancer, an adrenocortical carcinoma, a thyroid carcinoma, a nasopharyngeal carcinoma, a melanoma, *e.g.*, a malignant melanoma, a skin carcinoma, a colorectal carcinoma, a desmoid tumor, a desmoplastic small round cell tumor, an endocrine

tumor, an Ewing sarcoma, a peripheral primitive neuroectodermal tumor, a solid germ cell tumor, a hepatoblastoma, a neuroblastoma, a non-rhabdomyosarcoma soft tissue sarcoma, an osteosarcoma, a retinoblastoma, a rhabdomyosarcoma, a Wilms tumor, a glioblastoma, a myxoma, a fibroma, a lipoma, or the like. In more specific embodiments, said lymphoma can be chronic lymphocytic leukemia (small lymphocytic lymphoma), B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, Waldenström macroglobulinemia, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, extranodal marginal zone B cell lymphoma, MALT lymphoma, nodal marginal zone B cell lymphoma, follicular lymphoma, mantle cell lymphoma, diffuse large B cell lymphoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, Burkitt's lymphoma, T lymphocyte prolymphocytic leukemia, T lymphocyte large granular lymphocytic leukemia, aggressive NK cell leukemia, adult T lymphocyte leukemia/lymphoma, extranodal NK/T lymphocyte lymphoma, nasal type, enteropathy-type T lymphocyte lymphoma, hepatosplenic T lymphocyte lymphoma, blastic NK cell lymphoma, mycosis fungoides, Sezary syndrome, primary cutaneous anaplastic large cell lymphoma, lymphomatoid papulosis, angioimmunoblastic T lymphocyte lymphoma, peripheral T lymphocyte lymphoma (unspecified), anaplastic large cell lymphoma, Hodgkin lymphoma, a non-Hodgkin lymphoma, or multiple myeloma.

[00323] In certain embodiments, the antigen is a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA). In various specific embodiments, without limitation, the tumor-associated antigen or tumor-specific antigen is Her2, prostate stem cell antigen (PSCA), alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen-125 (CA-125), CA19-9, calretinin, MUC-1, epithelial membrane protein (EMA), epithelial tumor antigen (ETA), tyrosinase, melanoma-associated antigen (MAGE), CD19, CD20, CD34, CD45, CD99, CD117, chromogranin, cytokeratin, desmin, glial fibrillary acidic protein (GFAP), gross cystic disease fluid protein (GCDFP-15), HMB-45 antigen, high molecular weight melanoma-associated antigen (HMW-MAA), protein melan-A (MART-1), myo-D1, muscle-specific actin (MSA), neurofilament, neuron-specific enolase (NSE), placental alkaline phosphatase, synaptophysis, thyroglobulin, thyroid transcription factor-1, the dimeric form of the pyruvate kinase isoenzyme type M2 (tumor M2-PK), an abnormal ras protein, or an abnormal p53 protein.

[00324] In certain embodiments, the TAA or TSA is a cancer/testis (CT) antigen, *e.g.*, BAGE,

CAGE, CTAGE, FATE, GAGE, HCA661, HOM-TES-85, MAGEA, MAGEB, MAGEC, NA88, NY-ESO-1, NY-SAR-35, OY-TES-1, SPANXB1, SPA17, SSX, SYCP1, or TPTE.

[00325] In certain other embodiments, the TAA or TSA is a carbohydrate or ganglioside, *e.g.*, fuc-GM1, GM2 (oncofetal antigen-immunogenic-1; OFA-I-1); GD2 (OFA-I-2), GM3, GD3, and the like.

[00326] In certain other embodiments, the TAA or TSA is alpha-actinin-4, Bage-1, BCR-ABL, Bcr-Abl fusion protein, beta-catenin, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, Casp-8, cdc27, cdk4, cdkn2a, CEA, coa-1, dek-can fusion protein, EBNA, EF2, Epstein Barr virus antigens, ETV6-AML1 fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAAO205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin class I, OS-9, pml-RARα fusion protein, PTPRK, K-ras, N-ras, triosephosphate isomerase, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, TRP2-Int2, gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, RAGE, GAGE-1, GAGE-2, p15(58), RAGE, , SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, 13-Catenin, Mum-1, p16, TAGE, PSMA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, 13HCG, BCA225, BTAA, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90, TAAL6, TAG72, TLP, TPS, CD19, CD22, CD27, CD30, CD70, GD2 (ganglioside G2), EGFRvIII (epidermal growth factor variant III), sperm protein 17 (Sp17), mesothelin, PAP (prostatic acid phosphatase), prostein, TARP (T cell receptor gamma alternate reading frame protein), Trp-p8, STEAP1 (six-transmembrane epithelial antigen of the prostate 1), an abnormal ras protein, or an abnormal p53 protein. In another specific embodiment, said tumor-associated antigen or tumor-specific antigen is integrin αvβ3 (CD61), galactin, K-Ras (V-Ki-ras2 Kirsten rat sarcoma viral oncogene), or Ral-B.

[00327] In specific embodiments, the TAA or TSA is CD20, CD123, CLL-1, CD38, CS-1, CD138, ROR1, FAP, MUC1, PSCA, EGFRvIII, EPHA2, or GD2. In further specific embodiments, the TAA or TSA is CD123, CLL-1, CD38, or CS-1. In a specific embodiment, the extracellular domain of the CAR binds CS-1. In a further specific embodiment, the extracellular domain comprises a single-chain version of elotuzumab and/or an antigen-binding

fragment of elotuzumab. In a specific embodiment, the extracellular domain of the CAR binds CD20. In a more specific embodiment, the extracellular domain of the CAR is an scFv or antigen-binding fragment thereof binds to CD20.

[00328] Other tumor-associated and tumor-specific antigens are known to those in the art.

[00329] Antibodies, and scFvs, that bind to TSAs and TAAs are known in the art, as are nucleotide sequences that encode them.

[00330] In certain specific embodiments, the antigen is an antigen not considered to be a TSA or a TAA, but which is nevertheless associated with tumor cells, or damage caused by a tumor. In specific embodiments, the antigen is a tumor microenvironment-associated antigen (TMAA). In certain embodiments, for example, the TMAA is, *e.g.*, a growth factor, cytokine or interleukin, *e.g.*, a growth factor, cytokine, or interleukin associated with angiogenesis or vasculogenesis. Such growth factors, cytokines, or interleukins can include, *e.g.*, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), or interleukin-8 (IL-8). Tumors can also create a hypoxic environment local to the tumor. As such, in other specific embodiments, the TMAA is a hypoxia-associated factor, *e.g.*, HIF-1α, HIF-1β, HIF-2α, HIF-2β, HIF-3α, or HIF-3β. Tumors can also cause localized damage to normal tissue, causing the release of molecules known as damage associated molecular pattern molecules (DAMPs; also known as alarmins). In certain other specific embodiments, therefore, the TMAA is a DAMP, *e.g.*, a heat shock protein, chromatin-associated protein high mobility group box 1 (HMGB1), S100A8 (MRP8, calgranulin A), S100A9 (MRP14, calgranulin B), serum amyloid A (SAA), or can be a deoxyribonucleic acid, adenosine triphosphate, uric acid, or heparin sulfate. In specific embodiments, the TMAA is VEGF-A, EGF, PDGF, IGF, or bFGF.

[00331] In a specific embodiment, in which the cancer a gastrointestinal cancer, for example, liver cancer, stomach cancer, esophageal cancer, gallbladder cancer, colorectal cancer, anal cancer, or pancreatic cancer, the antigen is an antigen specific for or associated with a gastrointestinal cancer. In a specific embodiment, NK cells comprise a gastrointestinal homing receptor and also comprise a CAR with an extracellular domain that binds to an antigen associated with a gastrointestinal cancer. In a specific embodiment, the extracellular domain of the CAR binds CEA. In other specific embodiments, the extracellular domain of the CAR binds Her2, CA242, MUC1, CA125, or CA19-9.

[00332] In a specific embodiment, in which the cancer is a skin cancer, for example, melanoma, squamous cell carcinoma, or basal cell carcinoma, the antigen is an antigen specific for or associated with a skin cancer. In a specific embodiment, NK cells comprise a skin homing receptor and also comprise a CAR with an extracellular domain that binds to an antigen associated with a skin cancer. In a specific embodiment, the extracellular domain of the CAR binds HMW-MAA. In other specific embodiments, the extracellular domain of the CAR binds Her2, GD2, GD3, CEA, or SPAG9.

[00333] In certain embodiments, the extracellular domain is joined to said transmembrane domain by a linker, spacer or hinge polypeptide sequence, *e.g.*, a sequence from CD28.

5.3.3. Circulatory System Homing Receptors

[00334] In certain embodiments, the homing receptor causes a cell comprising said homing receptor to home to the circulatory system. Such a receptor is referred to herein as a "circulatory system homing receptor." In various embodiments, the circulatory system homing receptor is a chemotactic receptor. In specific embodiment, the chemotactic receptor is CXCR4, VEGFR2, or CCR7.

[00335] In one embodiment, the homing receptor causes a cell comprising said homing receptor to home to the bone marrow. Such a receptor is referred to herein as a "bone marrow homing receptor." In specific embodiments, the bone marrow homing receptor is CXCR4, for example, human CXCR4. GenBank™ accession numbers NM_001008540.1 and NM_003467.2 provide exemplary nucleotide sequences for human CXCR4. GenBank™ accession numbers NP_001008540.1 and NP_003458.1 provide exemplary amino acid sequences for human CXCR4. Exemplary nucleotide and amino acid sequences for human homing receptors can be found in Table 1.

[00336] In another embodiment, the homing receptor causes a cell comprising said homing receptor to home to a secondary lymphoid organ, *e.g.*, a lymph node. Such a receptor is referred to herein as a "secondary lymphoid organ homing receptor." In specific embodiments, the secondary lymphoid organ homing receptor is CCR7, for example, human CCR7. GenBank™ accession numbers NM_001301714.1, NM_001301716.1, NM_001301717.1, NM_001301718.1 and NM_001838.3 provide exemplary nucleotide sequences for human CCR7. GenBank™ accession numbers NP_001288643.1, NP_001288645.1 NP_001288646.1, NP_001288647.1 and NP_001829.1 provide exemplary amino acid sequences for human CCR7. Exemplary nucleotide

and amino acid sequences for human homing receptors can be found in Table 1.

[00337] In another embodiment, the homing receptor causes a cell comprising said homing receptor to home to the vascular endothelium. Such a receptor is referred to herein as a "vascular endothelium homing receptor." In specific embodiments, the vascular endothelium homing receptor is VEGFR2, for example, human VEGFR2. GenBank™ accession number NM_002253.2 provides exemplary nucleotide sequences for human VEGFR2. GenBank™ accession number NP_002244.1 provides exemplary amino acid sequences for human VEGFR2. Exemplary nucleotide and amino acid sequences for human homing receptors can be found in Table 1.

[00338] In another embodiment, the homing receptor causes a cell comprising said homing receptor to home to the B cell zone of the lymph nodes, *e.g.*, the follicles of the lymph node. Such a receptor is referred to herein as a "B cell zone homing receptor." In specific embodiments, the B cell zone homing receptor is CXCR5, for example, human CXCR5. GenBank™ accession numbers NM_001716.4 and NM_032966.2 provide exemplary nucleotide sequences for human CXCR5. GenBank™ accession numbers NP_116743.1 and NP_001707.1 provide exemplary amino acid sequences for human CXCR5. Exemplary nucleotide and amino acid sequences for human homing receptors can be found in Table 1.

[00339] In some embodiments, the step of engineering a NK cell to comprise a circulatory system homing receptor comprises a step of introducing to the cells one or more vectors comprising the receptor nucleic acid sequence(s), *i.e.*, the nucleic acid sequence (s) encoding the receptor(s). In specific embodiments, the vector comprises the nucleic acid sequence for human CXCR4, CCR7, VEGFR2 or CXCR5. In a certain embodiment, the step of engineering a NK cell to comprise a circulatory system homing receptor is performed by any method known to one of skill in the art.

[00340] Also described herein is a method of generating genetically engineered NK cells that home to the circulatory system, comprising a step of engineering a NK cell to comprise a circulatory system homing receptor, *e.g.*, CXCR4, CCR7, VEGFR2 or CXCR5, wherein said circulatory system homing receptor is expressed by the cell at a sufficient level or sufficient amount to cause the cell to home to the circulatory system. In some embodiments, the step of engineering a NK cell to comprise a circulatory system homing receptor comprises a step of introducing to the cells one or more vectors comprising the receptor nucleic acid sequence(s),

i.e., the nucleic acid sequence (s) encoding the receptor(s). In specific embodiments, the vector comprises the nucleic acid sequence for human CXCR4, CCR7, VEGFR2 or CXCR5. In a certain embodiment, the step of engineering a NK cell to comprise a circulatory system homing receptor is performed by any method known to one of skill in the art.

5.3.4. Gastrointestinal Homing Receptors

[00341] In one embodiment, the homing receptor causes a cell comprising said homing receptor to home to the gastrointestinal tract, *e.g.*, gastrointestinal organs, tissues, or cells. Such a receptor that causes a cell to home to the gastrointestinal tract is referred to herein as a "gastrointestinal homing receptor." In certain embodiments, the gastrointestinal homing receptor is CCR9 or integrin α4β7, for example, human CCR9 or human integrin α4β7. GenBank™ accession numbers NM_031200.2 and NM001256369.1 provide exemplary nucleotide sequences for human CCR9. GenBank™ accession numbers NP_112477.1 and NP_001243298.1 provide exemplary amino acid sequences for human CCR9. GenBank™ accession numbers NM_000885.4 and NM_000889.2 provide exemplary nucleotide sequences for human α4 and human β7, respectively. GenBank™ accession numbers NP_000876.3 and NP_000880.1 provide exemplary amino acid sequences for human α4 and human β7, respectively. Exemplary nucleotide and amino acid sequences for human homing receptors can be found in Table 1. In some embodiments, the NK cells further comprise a second gastrointestinal homing receptor. In some embodiments, the NK cells comprise a first gastrointestinal homing receptor, wherein the first gastrointestinal homing receptor is CCR9, and further comprise a second gastrointestinal homing receptor, wherein the second gastrointestinal homing receptor is integrin α4β7. In other specific embodiments, the NK cells comprise the gastrointestinal-homing receptor CXCR3.

[00342] In certain embodiments, the NK cells comprising one or more gastrointestinal homing receptors are expanded, activated, or both expanded and activated in the presence of a Vitamin A metabolite. In specific embodiments, the expansion, activation, or both expansion and activation occurs *in vivo*, *in vitro*, or *ex vivo*. In specific embodiments, the Vitamin A metabolite is retinoic acid. In certain embodiments, the NK cells comprising one or more gastrointestinal homing receptors additionally comprise a B cell zone homing receptor. In specific embodiments, the B cell zone homing receptor is CXCR5.

[00343] Also described herein are methods of generating genetically modified NK cells that home to the gastrointestinal tract, *e.g.*, gastrointestinal organs, skin, or tissue. In certain

embodiments, NK cells comprising one or more homing receptors that that cause a cell comprising the one or more receptors to home to the gastrointestinal tract, *e.g.*, CCR9 or integrin α4β7, are generated by a method comprising a step of engineering a NK cell to express one or more gastrointestinal homing receptors. In some embodiments, the step of engineering a NK cell to comprise one or more gastrointestinal homing receptors comprises introducing to the cells one or more vectors comprising a nucleic acid sequence encoding the homing receptor. In specific embodiments, the vector comprises the nucleic acid sequence for human CCR9, the nucleic acid sequence for human integrin α4β7, or both.

[00344] In certain embodiments, NK cells that home to the gastrointestinal tract are generated by a method comprising a step of treating the cells with a molecule that induces the expression of one or more gastrointestinal homing receptors, *e.g.*, CCR9 or α4β7. In specific embodiments, the molecule is Vitamin A.

[00345] In certain embodiments, the method for generating the genetically modified NK cells that comprise one or more receptors that that cause a cell comprising the one or more receptors to home to the gastrointestinal tract comprises a step of expanding the cells, which step is carried out in the presence of a vitamin A metabolite. In certain embodiments, the method for generating the genetically modified NK cells that comprise one or more receptors homing to the gastrointestinal tract comprises a step of activating the cells, which step is carried out in the presence of a vitamin A metabolite. In certain embodiments, both the expanding and activating steps are carried out in the presence of a vitamin A metabolite. In certain embodiments the vitamin A metabolite is retinoic acid. In a certain embodiment, the step of engineering a NK cell to comprise a gastrointestinal homing receptor is performed by any method known to one of skill in the art.

5.3.5. Skin Homing Receptors

[00346] In one embodiment, the homing receptor causes a cell comprising said homing receptor to home to the skin, *e.g.*, skin tissue, or skin cells. In certain embodiments, the skin homing receptor is CCR10, CCR8, CCR4, or CLA, for example, human CCR10, human CCR8, human CCR4, or human CLA. GenBank™ accession numbers NM_016602.2 and AF215981.1 provide exemplary nucleotide sequences for human CCR10. GenBank™ accession numbers NP_057686.2 and P46092.3 provide exemplary amino acid sequences for human CCR10. GenBank™ accession numbers NM_005201.3 and BC107159.1 provide exemplary nucleotide

sequences for human CCR8. GenBank™ accession numbers NP_005192.1 and AAI07160.1 provide exemplary amino acid sequences for human CCR8. GenBank™ accession number NM_005508.4 provides an exemplary nucleotide sequence for human CCR4. GenBank™ accession number P51679.1 provides an exemplary amino acid sequence for human CCR4. GenBank™ accession numbers NM_001206609.1 and NM_003006.4 provide exemplary nucleotide sequences for human CLA. GenBank™ accession numbers NP_001193538.1 and NP_002997.2 provide exemplary amino acid sequences for human CLA. Exemplary nucleotide and amino acid sequences for human homing receptors can be found in Table 1. In some embodiments, the NK cells further comprise a second skin homing receptor. In some embodiments, the NK cells comprise a first skin homing receptor, wherein the first skin homing receptor is CCR10, and further comprise a second skin homing receptor, wherein the second skin homing receptor is CLA. In some embodiments, the NK cells comprise a first skin homing receptor, wherein the first skin homing receptor is CCR10, and further comprise a second skin homing receptor, wherein the second skin homing receptor is CCR4. In some embodiments, the NK cells comprise a first skin homing receptor, wherein the first skin homing receptor is CCR4, and further comprise a second skin homing receptor, wherein the second skin homing receptor is CLA. In some embodiments, the NK cells further comprise a third skin homing receptor. In some embodiments, the NK cells comprise a first skin homing receptor, wherein the first skin homing receptor is CCR10, further comprise a second skin homing receptor, wherein the second skin homing receptor is CCR4, and further comprise a third skin homing receptor, wherein the third skin homing receptor is CLA. In some embodiments, the NK cells comprise a first skin homing receptor, wherein the first skin homing receptor is CCR8, and further comprise a second skin homing receptor, wherein the second skin homing receptor is CLA, CCR4, or CCR10. In some embodiments, the NK cells comprise a first skin homing receptor, wherein the first skin homing receptor is CCR8, further comprise a second skin homing receptor, wherein the second skin homing receptor is CLA, CCR4, or CCR10, and further comprise a third skin homing receptor, wherein the third skin homing receptor is distinct from the second skin homing receptor, and is selected from the group consisting of CLA, CCR4, and CCR10. In some embodiments, the NK cells further comprise a third skin homing receptor. In some embodiments, the NK cells comprise a first skin homing receptor, wherein the first skin homing receptor is CCR10, further comprise a second skin homing receptor, wherein the second skin

homing receptor is CCR4, further comprise a third skin homing receptor, wherein the third skin homing receptor is CLA, and further comprise a fourth skin homing receptor, wherein the fourth skin homing receptor is CCR8. In certain embodiments, the NK cells comprise one or more skin homing receptors. In other specific embodiments, the NK cells comprise the skin-homing receptor CCR6.

[00347] In certain embodiments, the NK cells comprising one or more skin homing receptors are expanded, activated, or both expanded and activated in the presence of a Vitamin D metabolite. In specific embodiments, the expansion, activation, or both expansion and activation occurs *in vivo*, *in vitro*, or *ex vivo*. In specific embodiments, the Vitamin D metabolite is 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$). In certain embodiments, the NK cells comprising one or more skin homing receptors are expanded, activated, or both expanded and activated in the presence of IL-12. In specific embodiments, the expansion, activation, or both expansion and activation occurs *in vivo*, *in vitro*, or *ex vivo*. In more specific embodiments, the NK cells comprising one or more skin homing receptors are expanded, activated, or both expanded and activated in the presence of a Vitamin D metabolite and IL-12. In specific embodiments, the expansion, activation, or both expansion and activation occurs *in vivo*, *in vitro*, or *ex vivo*. In certain embodiments, the NK cells comprising one or more skin homing receptors additionally comprise a B cell zone homing receptor. In specific embodiments, the B cell zone homing receptor is CXCR5.

[00348] Also described herein are methods of generating genetically modified NK cells that home to the skin, *e.g.*, skin tissue or cells. In certain embodiments, NK cells that home to the skin are generated by a method comprising a step of engineering the NK cells to comprise a skin homing receptor, *e.g.*, CCR4, CCR8, CCR10, or CLA. In some embodiments, the step of engineering the NK cells to comprise a skin homing receptor comprises introducing into the cells one or more vectors comprising the receptor nucleic acid sequence(s), *i.e.*, the nucleic acid sequence(s) encoding the receptor(s). In specific embodiments, the vector comprises the nucleic acid sequence for human CCR10, the nucleic acid sequence for human CLA, or both. In specific embodiments, the vector comprises the nucleic acid sequence for human CCR4, and optionally the nucleic acid sequence for human CLA. In specific embodiments, the vector comprises the nucleic acid sequence for human CCR4 and the nucleic acid sequence for human CCR10. In specific embodiments, the vector comprises the nucleic acid sequence for human CCR10, the

nucleic acid sequence for human CCR4, and the nucleic acid sequence for human CLA. In specific embodiments, the vector comprises the nucleic acid sequence for human CCR8. In specific embodiments, the vector comprises the nucleic acid sequence for human CCR8, and optionally the nucleic acid sequence for human CLA. In specific embodiments, the vector comprises the nucleic acid sequence for human CCR8 and the nucleic acid sequence for human CCR10. In specific embodiments, the vector comprises the nucleic acid sequence for human CCR8, the nucleic acid sequence for human CCR4, and the nucleic acid sequence for human CLA. In specific embodiments, the vector comprises the nucleic acid sequence for human CCR8, the nucleic acid sequence for human CCR10, and the nucleic acid sequence for human CLA. In specific embodiments, the vector comprises the nucleic acid sequence for human CCR8, the nucleic acid sequence for human CCR4, and the nucleic acid sequence for human CCR10. In specific embodiments, the vector comprises the nucleic acid sequence for human CCR8, the nucleic acid sequence for human CCR4, the nucleic acid for CCR10, and the nucleic acid sequence for human CLA.

[00349] In certain embodiments, cells, *e.g.*, NK cells, that home to the skin are generated by a method comprising a step of treating the cells, *e.g.*, NK cells, with a molecule that induces, *e.g.*, increases, the expression of one or more skin homing receptors, *e.g.*, CCR4, CCR10, CCR8, or CLA. In specific embodiments, the molecule is Vitamin D. In certain embodiments, the induction of expression of skin homing receptors is aided by treating the cells, *e.g.*, NK cells, with IL-12, *e.g.*, contacting the cells with IL-12 in an amount and for a time sufficient to increase expression of one or more of CCR4, CCR8, CCR10, or CLA by said cells.

[00350] In certain embodiments, the method for generating the NK cells that comprise one or more homing receptors that cause a cell comprising the one or more receptors to home to the skin, comprises a step of expanding the cells, which step is carried out in the presence of a vitamin D metabolite and, optionally, IL-12. In certain embodiments, the method for generating the NK cells that comprise one or more receptors that that cause a cell comprising the one or more receptors to home to the gastrointestinal tract, comprises a step of activating the cells, which step is carried out in the presence of a vitamin D metabolite, and, optionally, IL-12. In certain embodiments, both the expanding and activating steps are carried out in the presence of a vitamin D metabolite, and, optionally, IL-12. In certain embodiments the vitamin D metabolite is $1,25(OH)_2D_3$. In a certain embodiment, the step of engineering a NK cell to comprise a skin

homing receptor is performed by any method known to one of skill in the art.

[00351] Table 1. Exemplary nucleotide and amino acid sequences for human homing receptors.

SEQ ID NO:	GenBank Accession Number and Description	Sequence
1	NM_001008540.1 Exemplary nucleic acid sequence encoding human CXCR4 isoform a	1 tttttttct tccctctagt gggcggggca gaggagttag ccaagatgtg actttgaaac 61 cctcagcgtc tcagtgccct tttgttctaa acaaagaatt ttgtaattgg ttctaccaaa 121 gaaggatata atgaagtcac tatgggaaaa gatggggagg agagttgtag gattctacat 181 taattctctt gtgcccttag cccactactt cagaatttcc tgaagaaagc aagcctgaat 241 tggtttttta aattgcttta aaaatttttt ttaactgggt taatgcttgc tgaattggaa 301 gtgaatgtcc attcctttgc ctcttttgca gatatacact tcagataact acaccgagga 361 aatgggctca ggggactatg actccatgaa ggaaccctgt ttccgtgaag aaaatgctaa 421 tttcaataaa atcttcctgc ccaccatcta ctccatcatc ttcttaactg gcattgtggg 481 caatggattg gtcatcctgg tcatgggtta ccagaagaaa ctgagaagca tgacggacaa 541 gtacaggctg cacctgtcag tggccgacct cctctttgtc atcacgcttc ccttctgggc 601 agttgatgcc gtggcaaact ggtactttgg gaacttccta tgcaaggcag tccatgtcat 661 ctacacagtc aacctctaca gcagtgtcct catcctggcc ttcatcagtc tggaccgcta 721 cctggccatc gtccacgcca ccaacagtca gaggccaagg aagctgttgg ctgaaaaggt 781 ggtctatgtt ggcgtctgga tccctgccct cctgctgact attcccgact tcatctttgc 841 caacgtcagt gaggcagatg acagatatat ctgtgaccgc ttctacccca atgacttgtg 901 ggtggttgtg ttccagtttc agcacatcat ggttggcctt atcctgcctg gtattgtcat 961 cctgtcctgc tattgcatta tcatctccaa gctgtcacac tccaagggcc accagaagcg 1021 caaggccctc aagaccacag tcatcctcat cctggctttc ttcgcctgtt ggctgcctta 1081 ctacattggg atcagcatcg actccttcat cctcctggaa atcatcaagc aagggtgtga 1141 gtttgagaac actgtgcaca agtggatttc catcaccgag gccctagctt tcttccactg 1201 ttgtctgaac cccatcctct atgctttcct tggagccaaa tttaaaacct ctgcccagca 1261 cgcactcacc tctgtgagca gagggtccag cctcaagatc ctctccaaag gaaagcgagg 1321 tggacattca tctgtttcca ctgagtctga gtcttcaagt tttcactcca gctaacacag 1381 atgtaaaaga ctttttttta tacgataaat aacttttttt taagttacac atttttcaga 1441 tataaaagac tgaccaatat tgtacagttt ttattgcttg ttggattttt gtcttgtgtt 1501 tctttagttt ttgtgaagtt taattgactt atttatataa atttttttg tttcatattg 1561 atgtgtgtct aggcaggacc tgtggccaag ttcttagttg ctgtatgtct cgtggtagga 1621 ctgtagaaaa gggaactgaa cattccagag cgtgtagtga atcacgtaaa gctagaaatg 1681 atccccagct gtttatgcat agataatctc tccattcccg tggaacgttt ttcctgttct 1741 taagacgtga ttttgctgta gaagatggca cttataacca aagcccaaag tggtatagaa 1801 atgctggttt ttcagttttc aggagtgggt tgatttcagc acctacagtg tacagtcttg 1861 tattaagttg ttaataaaag tacatgttaa acttaaaaaa aaaaaaaaaa aa
2	NM_003467.2 Exemplary nucleic acid sequence encoding human CXCR4 isoform b	1 aacttcagtt tgttggctgc ggcagcaggt agcaaagtga cgccgagggc ctgagtgctc 61 cagtagccac cgcatctgga gaaccagcgg ttaccatgga ggggatcagt atatacactt 121 cagataacta caccgaggaa atgggctcag gggactatga ctccatgaag gaaccctgtt 181 tccgtgaaga aaatgctaat ttcaataaaa tcttcctgcc caccatctac tccatcatct 241 tcttaactgg cattgtgggc aatggattgg tcatcctggt catgggttac cagaagaaac 301 tgagaagcat gacggacaag tacaggctgc acctgtcagt ggccgacctc ctctttgtca 361 tcacgcttcc cttctgggca gttgatgccg tggcaaactg gtactttggg aacttcctat 421 gcaaggcagt ccatgtcatc tacacagtca acctctacag cagtgtcctc atcctggcct 481 tcatcagtct ggaccgctac ctggccatcg tccacgccac caacagtcag aggccaagga

SEQ ID NO:	GenBank Accession Number and Description	Sequence
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3	NP_001008540.1 Exemplary amino acid sequence for human CXCR4 isoform a	1 msiplpllqi ytsdnyteem gsgdydsmke pcfreenanf nkiflptiys iifltgivgn 61 glvilvmgyq kklrsmtdky rlhlsvadll fvitlpfwav davanwyfgn flckavhviy 121 tvnlyssvli lafisldryl aivhatnsqr prkllaekvv yvgvwipall ltipdfifan 181 vseaddryic drfypndlwv vvfqfqhimv glilpgivil scyciiiskl shskghqkrk 241 alkttvilil affacwlpyy igisidsfil leiikqgcef entvhkwisi tealaffhcc 301 lnpilyaflg akfktsaqha ltsvsrgssl kilskgkrgg hssvsteses ssfhss
4	NP_003458.1 Exemplary amino acid sequence for human CXCR4 isoform b	1 megisiytsd nyteemgsgd ydsmkepcfr eenanfnkif lptiysiifl tgivgnglvi 61 lvmgyqkklr smtdkyrlhl svadllfvit lpfwavdava nwyfgnflck avhviytvnl 121 yssvlilafi sldrylaivh atnsqrprkl laekvvyvgv wipallltip dfifanvsea 181 ddryicdrfy pndlwvvvfq fqhimvglil pgivilscyc iiisklshsk ghqkrkalkt 241 tvililaffa cwlpyyigis idsfilleii kqgcefentv hkwisiteal affhcclnpi 301 lyaflgakfk tsaqhaltsv srgsslkils kgkrgghssv stesesssfh ss
5	NM_001301714.1 Exemplary nucleic acid sequence encoding human CCR7 isoform b	1 cacttcctcc ccagacaggg gtagtgcgag gccgggcaca gccttcctgt gtggttttac 61 cgcccagaga gcgtcatgga cctgggtatg cctgtgtcaa gatgaggtca cggacgatta 121 catcggagac aacaccacag tggactacac tttgttcgag tctttgtgct ccaagaagga 181 cgtgcggaac tttaaagcct ggttcctccc tatcatgtac tccatcattt gtttcgtggg 241 cctactgggc aatgggctgg tcgtgttgac ctatatctat ttcaagaggc tcaagaccat 301 gaccgatacc tacctgctca acctggcggt ggcagacatc ctcttcctcc tgacccttcc 361 cttctgggcc tacagcgcgg ccaagtcctg ggtcttcggt gtccacttttt gcaagctcat 421 ctttgccatc tacaagatga gcttcttcag tggcatgctc ctacttcttt gcatcagcat 481 tgaccgctac gtggccatcg tccaggctgt ctcagctcac cgccaccgtg cccgcgtcct 541 tctcatcagc aagctgtcct gtgtgggcat ctggatacta gccacagtgc tctccatccc 601 agagctcctg tacagtgacc tccagaggag cagcagtgag caagcgatgc gatgctctct 661 catcacagag catgtggagg cctttatcac catccaggtg gcccagatgg tgatcggctt 721 tctggtcccc ctgctggcca tgagcttctg ttaccttgtc atcatccgca ccctgctcca

SEQ ID NO:	GenBank Accession Number and Description	Sequence
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6	NM_001301716.1 Exemplary nucleic acid sequence encoding human CCR7 isoform c precursor	1 ctctagatga gtcagtggag ggcgggtgga gcgttgaacc gtgaagagtg tggttgggcg 61 taaacgtgga cttaaactca ggagctaagg ggtaattcag tgaaaaaggg gaatgagcgg 121 tggggagctc tgttgcaaca gggtccaatc gcagcaggac tacaaatgcc cgagcgcagg 181 ctgggaacga ggggacagcg gctgcctgtc cccagaatag aaaatgcagc taggaagccc 241 tctttgagtg gacagcggag gactggactg ccaggccaag catcaggggc ttcatcctca 301 gggccggtta gagcccctga ggatttagga ggaagggaaa ccaatgaaaa gcgtgctggt 361 ggtggctctc cttgtcattt tccaggtatg cctgtgtcaa gatgaggtca cggacgatta 421 catcggagac aacaccacag tggactacac tttgttcgag tctttgtgct ccaagaagga 481 cgtgcggaac tttaaagcct ggttcctccc tatcatgtac tccatcattt gtttcgtggg 541 cctactgggc aatgggctgg tcgtgttgac ctatatctat ttcaagaggc tcaagaccat 601 gaccgatacc tacctgctca acctggcggt ggcagacatc ctcttcctcc tgacccttcc 661 cttctgggcc tacagcgcgg ccaagtcctg ggtcttcggt gtccacttttt gcaagctcat 721 ctttgccatc tacaagatga gcttcttcag tggcatgctc ctacttcttt gcatcagcat 781 tgaccgctac gtggccatcg tccaggctgt ctcagctcac cgccaccgtg cccgcgtcct 841 tctcatcagc aagctgtcct gtgtgggcat ctggatacta gccacagtgc tctccatccc 901 agagctcctg tacagtgacc tccagaggag cagcagtgag caagcgatgc gatgctctct 961 catcacagag catgtggagg cctttatcac catccaggtg gcccagatgg tgatcggctt 1021 tctggtcccc ctgctggcca tgagcttctg ttaccttgtc atcatccgca ccctgctcca 1081 ggcacgcaac tttgagcgca acaaggccat caaggtgatc atcgctgtgg tcgtggtctt 1141 catagtcttc cagctgccct acaatggggt ggtcctggcc cagacggtgg ccaacttcaa 1201 catcaccagt agcacctgtg agctcagtaa gcaactcaac atcgcctacg acgtcaccta 1261 cagcctggcc tgcgtccgct gctgcgtcaa ccctttcttg tacgccttca tcggcgtcaa 1321 gttccgcaac gatctcttca agctcttcaa ggacctgggc tgcctcagcc aggagcagct 1381 ccggcagtgg tcttcctgtc ggcacatccg gcgctcctcc atgagtgtgg aggccgagac

SEQ ID NO:	GenBank Accession Number and Description	Sequence
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7	NM_001301717.1 Exemplary nucleic acid sequence encoding human CCR7 isoform c precursor	1 ctctagatga gtcagtggag ggcgggtgga gcgttgaacc gtgaagagtg tggttgggcg 61 taaacgtgga cttaaactca ggagctaagg gggaaaccaa tgaaaagcgt gctggtggtg 121 gctctccttg tcattttcca ggtatgcctg tgtcaagatg aggtcacgga cgattacatc 181 ggagacaaca ccacagtgga ctacactttg ttcgagtctt tgtgctccaa gaaggacgtg 241 cggaacttta aagcctggtt cctccctatc atgtactcca tcatttgttt cgtgggccta 301 ctgggcaatg ggctggtcgt gttgacctat atctatttca agaggctcaa gaccatgacc 361 gatacctacc tgctcaacct ggcggtggca gacatcctct tcctcctgac ccttcccttc 421 tgggcctaca gcgcggccaa gtcctgggtc ttcggtgtcc acttttgcaa gctcatcttt 481 gccatctaca agatgagctt cttcagtggc atgctcctac ttctttgcat cagcattgac 541 cgctacgtgg ccatcgtcca ggctgtctca gctcaccgcc accgtgcccg cgtccttctc 601 atcagcaagc tgtcctgtgt gggcatctgg atactagcca cagtgctctc catcccagag 661 ctcctgtaca gtgacctcca gaggagcagc agtgagcaag cgatgcgatg ctctctcatc 721 acagagcatg tggaggcctt tatcaccatc caggtggccc agatggtgat cggctttctg 781 gtccccctgc tggccatgag cttctgttac cttgtcatca tccgcaccct gctccaggca 841 cgcaactttg agcgcaacaa ggccatcaag gtgatcatcg ctgtggtcgt ggtcttcata 901 gtcttccagc tgccctacaa tggggtggtc ctggcccaga cggtggccaa cttcaacatc 961 accagtagca cctgtgagct cagtaagcaa ctcaacatcg cctacgacgt cacctacagc 1021 ctggcctgcg tccgctgctg cgtcaaccct ttcttgtacg ccttcatcgg cgtcaagttc 1081 cgcaacgatc tcttcaagct cttcaaggac ctgggctgcc tcagccagga gcagctccgg 1141 cagtggtctt cctgtcggca catccggcgc tcctccatga gtgtggaggc cgagaccacc 1201 accaccttct ccccataggc gactcttctg cctggactag agggacctct cccagggtcc 1261 ctggggtggg gatagggagc agatgcaatg actcaggaca tccccccgcc aaaagctgct 1321 cagggaaaag cagctctccc ctcagagtgc aagcccctgc tccagaagat agcttcaccc 1381 caatcccagc tacctcaacc aatgccaaaa aaagacaggg ctgataagct aacaccagac 1441 agacaacact gggaaacaga ggctattgtc ccctaaacca aaaactgaaa gtgaaagtcc 1501 agaaactgtt cccacctgct ggagtgaagg ggccaaggag ggtgagtgca aggggcgtgg 1561 gagtggcctg aagagtcctc tgaatgaacc ttctggcctc ccacagactc aaatgctcag 1621 accagctctt ccgaaaacca ggccttatct ccaagaccag agatagtggg gagacttctt 1681 ggcttggtga ggaaaagcgg acatcagctg gtcaaacaaa ctctctgaac ccctccctcc 1741 atcgttttct tcactgtcct ccaagccagc gggaatggca gctgccacgc cgccctaaaa

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		1801 gcacactcat cccctcactt gccgcgtcgc cctcccaggc tctcaacagg ggagagtgtg 1861 gtgtttcctg caggccaggc cagctgcctc cgcgtgatca aagccacact ctgggctcca 1921 gagtggggat gacatgcact cagctcttgg ctccactggg atgggaggag aggacaaggg 1981 aaatgtcagg ggcggggagg gtgacagtgg ccgcccaagg cccacgagct tgttctttgt 2041 tctttgtcac agggactgaa aacctctcct catgttctgc tttcgattcg ttaagagagc 2101 aacattttac ccacacacag ataaagtttt cccttgagga aacaacagct ttaaaagaaa 2161 aagaaaaaaa aagtctttgg taaatggcaa aaaaaaaaaa aaaaaaaaaa aaa
8	NM_001301718.1 Exemplary nucleic acid sequence encoding human CCR7 isoform c precursor	1 aggagaaggt gccttaaaca ggttcccacg catttcctgg cgctattgag cttggagctg 61 ccaagggcct gccttcactt gtggcatcgc agttactgac tctccagtgg gccaggccct 121 acctagctgg gacctgaggg tcaggatacg ggaagagggc tactgccgcc ctgacttgta 181 gggaaaccaa tgaaaagcgt gctggtggtg gctctccttg tcattttcca ggtatgcctg 241 tgtcaagatg aggtcacgga cgattacatc ggagacaaca ccacagtgga ctacactttg 301 ttcgagtctt tgtgctccaa gaaggacgtg cggaacttta aagcctggtt cctccctatc 361 atgtactcca tcatttgttt cgtgggccta ctgggcaatg ggctggtcgt gttgacctat 421 atctatttca agaggctcaa gaccatgacc gatacctacc tgctcaacct ggcggtggca 481 gacatcctct tcctcctgac ccttcccttc tgggcctaca gcgcggccaa gtcctgggtc 541 ttcggtgtcc acttttgcaa gctcatcttt gccatctaca agatgagctt cttcagtggc 601 atgctcctac ttctttgcat cagcattgac cgctacgtgg ccatcgtcca ggctgtctca 661 gctcaccgcc accgtgcccg cgtccttctc atcagcaagc tgtcctgtgt gggcatctgg 721 atactagcca cagtgctctc catcccagag ctcctgtaca gtgacctcca gaggagcagc 781 agtgagcaag cgatgcgatg ctctctcatc acagagcatg tggaggcctt tatcaccatc 841 caggtggccc agatggtgat cggctttctg gtccccctgc tggccatgag cttctgttac 901 cttgtcatca tccgcaccct gctccaggca cgcaactttg agcgcaacaa ggccatcaag 961 gtgatcatcg ctgtggtcgt ggtcttcata gtcttccagc tgccctacaa tggggtggtc 1021 ctggcccaga cggtggccaa cttcaacatc accagtagca cctgtgagct cagtaagcaa 1081 ctcaacatcg cctacgacgt cacctacagc ctggcctgcg tccgctgctg cgtcaaccct 1141 ttcttgtacg ccttcatcgg cgtcaagttc cgcaacgatc tcttcaagct cttcaaggac 1201 ctgggctgcc tcagccagga gcagctccgg cagtggtctt cctgtcggca catccggcgc 1261 tcctccatga gtgtggaggc cgagaccacc accaccttct ccccataggc gactcttctg 1321 cctggactag agggacctct cccagggtcc ctggggtggg gatagggagc agatgcaatg 1381 actcaggaca tccccccgcc aaaagctgct cagggaaaag cagctctccc ctcagagtgc 1441 aagcccctgc tccagaagat agcttcaccc caatcccagc tacctcaacc aatgccaaaa 1501 aaagacaggg ctgataagct aacaccagac agacaacact gggaaacaga ggctattgtc 1561 ccctaaacca aaaactgaaa gtgaaagtcc agaaactgtt cccacctgct ggagtgaagg 1621 ggccaaggag ggtgagtgca aggggcgtgg gagtggcctg aagagtcctc tgaatgaacc 1681 ttctggcctc ccacagactc aaatgctcag accagctctt ccgaaaacca ggccttatct 1741 ccaagaccag agatagtggg gagacttctt ggcttggtga ggaaaagcgg acatcagctg 1801 gtcaaacaaa ctctctgaac ccctccctcc atcgttttct tcactgtcct ccaagccagc 1861 gggaatggca gctgccacgc cgccctaaaa gcacactcat cccctcactt gccgcgtcgc 1921 cctcccaggc tctcaacagg ggagagtgtg gtgtttcctg caggccaggc cagctgcctc 1981 cgcgtgatca aagccacact ctgggctcca gagtggggat gacatgcact cagctcttgg 2041 ctccactggg atgggaggag aggacaaggg aaatgtcagg ggcggggagg gtgacagtgg 2101 ccgcccaagg cccacgagct tgttctttgt tctttgtcac agggactgaa aacctctcct 2161 catgttctgc tttcgattcg ttaagagagc aacattttac ccacacacag ataaagtttt 2221 cccttgagga aacaacagct ttaaaagaaa aagaaaaaaa aagtctttgg taaatggcaa 2281 aaaaaaaaaa aaaaaaaaaa aaa

SEQ ID NO:	GenBank Accession Number and Description	Sequence
9	NM_001838.3 Exemplary nucleic acid sequence encoding human CCR7 isoform a precursor	1 cacttcctcc ccagacaggg gtagtgcgag gccgggcaca gccttcctgt gtggttttac 61 cgcccagaga gcgtcatgga cctggggaaa ccaatgaaaa gcgtgctggt ggtggctctc 121 cttgtcattt tccaggtatg cctgtgtcaa gatgaggtca cggacgatta catcggagac 181 aacaccacag tggactacac tttgttcgag tctttgtgct ccaagaagga cgtgcggaac 241 tttaaagcct ggttcctccc tatcatgtac tccatcattt gtttcgtggg cctactgggc 301 aatgggctgg tcgtgttgac ctatatctat ttcaagaggc tcaagaccat gaccgatacc 361 tacctgctca acctggcggt ggcagacatc ctcttcctcc tgacccttcc cttctgggcc 421 tacagcgcgg ccaagtcctg ggtcttcggt gtccacttttgcaagctcat ctttgccatc 481 tacaagatga gcttcttcag tggcatgctc ctacttcttt gcatcagcat tgaccgctac 541 gtggccatcg tccaggctgt ctcagctcac cgccaccgtg cccgcgtcct tctcatcagc 601 aagctgtcct gtgtgggcat ctggatacta gccacagtgc tctccatccc agagctcctg 661 tacagtgacc tccagaggag cagcagtgag caagcgatgc gatgctctct catcacagag 721 catgtggagg cctttatcac catccaggtg gcccagatgg tgatcggctt tctggtcccc 781 ctgctggcca tgagcttctg ttaccttgtc atcatccgca ccctgctcca ggcacgcaac 841 tttgagcgca acaaggccat caaggtgatc atcgctgtgg tcgtggtctt catagtcttc 901 cagctgccct acaatggggt ggtcctggcc cagacggtgg ccaacttcaa catcaccagt 961 agcacctgtg agctcagtaa gcaactcaac atcgcctacg acgtcaccta cagcctggcc 1021 tgcgtccgct gctgcgtcaa ccctttcttg tacgccttca tcggcgtcaa gttccgcaac 1081 gatctcttca agctcttcaa ggacctgggc tgcctcagcc aggagcagct ccggcagtgg 1141 tcttcctgtc ggcacatccg gcgctcctcc atgagtgtgg aggccgagac caccaccacc 1201 ttctccccat aggcgactct tctgcctgga ctagagggac ctctcccagg gtccctgggg 1261 tggggatagg gagcagatgc aatgactcag gacatccccc cgccaaaagc tgctcaggga 1321 aaagcagctc tcccctcaga gtgcaagccc ctgctccaga agatagcttc accccaatcc 1381 cagctacctc aaccaatgcc aaaaaaagac agggctgata agctaacacc agacagacaa 1441 cactgggaaa cagaggctat tgtcccctaa accaaaaact gaaagtgaaa gtccagaaac 1501 tgttcccacc tgctggagtg aaggggccaa ggagggtgag tgcaaggggc gtgggagtgg 1561 cctgaagagt cctctgaatg aaccttctgg cctcccacag actcaaatgc tcagaccagc 1621 tcttccgaaa accaggcctt atctccaaga ccagagatag tggggagact tcttggcttg 1681 gtgaggaaaa gcggacatca gctggtcaaa caaactctct gaaccccctcc ctccatcgtt 1741 ttcttcactg tcctccaagc cagcgggaat ggcagctgcc acgccgccct aaaagcacac 1801 tcatcccctc acttgccgcg tcgccctccc aggctctcaa caggggagag tgtggtgttt 1861 cctgcaggcc aggccagctg cctccgcgtg atcaaagcca cactctgggc tccagagtgg 1921 ggatgacatg cactcagctc ttggctccac tgggatggga ggagaggaca agggaaatgt 1981 caggggcggg gagggtgaca gtggccgccc aaggcccacg agcttgttct ttgttctttg 2041 tcacagggac tgaaaacctc tcctcatgtt ctgctttcga ttcgttaaga gagcaacatt 2101 ttacccacac acagataaag ttttcccttg aggaaacaac agctttaaaa gaaaaagaaa 2161 aaaaaagtct ttggtaaatg gcaaaaaaaa aaaaaaaaaa aaaaaaa
10	NP_001288643.1 Exemplary amino acid sequence for human CCR7 isoform b	1 mysiicfvgl lgnglvvlty iyfkrlktmt dtyllnlava dilflltlpf waysaakswv 61 fgvhfcklif aiykmsffsg mllllcisid ryvaivqavs ahrhrarvll isklscvgiw 121 ilatvlsipe llysdlqrss seqamrcsli tehveafiti qvaqmvigfl vpllamsfcy 181 lviirtllqa rnfernkaik viiavvvvfi vfqlpyngvv laqtvanfni tsstcelskq 241 lniaydvtys lacvrccvnp flyafigvkf rndlfklfkd lgclsqeqlr qwsscrhirr 301 ssmsveaett ttfsp
11	NP_001288645.1 Exemplary amino	1 mksvlvvall vifqvclcqd evtddyigdn ttvdytlfes lcskkdvrnf kawflpimys 61 iicfvgllgn glvvltyiyf krlktmtdty llnlavadil flltlpfway saakswvfgv 121 hfcklifaiy kmsffsgmll llcisidryv aivqavsahr hrarvllisk lscvgiwila

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	acid sequence for human CCR7 isoform c precursor	181 tvlsipelly sdlqrssseq amrcsliteh veafitiqva qmvigflvpl lamsfcylvi 241 irtllqarnf ernkaikvii avvvvfivfq lpyngvvlaq tvanfnitss tcelskqlni 301 aydvtyslac vrccvnpfly afigvkfrnd lfklfkdlgc lsqeqlrqws scrhirrssm 361 sveaettttf sp
12	NP_001288646.1 Exemplary amino acid sequence for human CCR7 isoform c precursor	1 mksvlvvall vifqvclcqd evtddyigdn ttvdytlfes lcskkdvrnf kawflpimys 61 iicfvgllgn glvvltyiyf krlktmtdty llnlavadil flltlpfway saakswvfgv 121 hfcklifaiy kmsffsgmll llcisidryv aivqavsahr hrarvllisk lscvgiwila 181 tvlsipelly sdlqrssseq amrcsliteh veafitiqva qmvigflvpl lamsfcylvi 241 irtllqarnf ernkaikvii avvvvfivfq lpyngvvlaq tvanfnitss tcelskqlni 301 aydvtyslac vrccvnpfly afigvkfrnd lfklfkdlgc lsqeqlrqws scrhirrssm 361 sveaettttf sp
13	NP_001288647.1 Exemplary amino acid sequence for human CCR7 isoform c precursor	1 mksvlvvall vifqvclcqd evtddyigdn ttvdytlfes lcskkdvrnf kawflpimys 61 iicfvgllgn glvvltyiyf krlktmtdty llnlavadil flltlpfway saakswvfgv 121 hfcklifaiy kmsffsgmll llcisidryv aivqavsahr hrarvllisk lscvgiwila 181 tvlsipelly sdlqrssseq amrcsliteh veafitiqva qmvigflvpl lamsfcylvi 241 irtllqarnf ernkaikvii avvvvfivfq lpyngvvlaq tvanfnitss tcelskqlni 301 aydvtyslac vrccvnpfly afigvkfrnd lfklfkdlgc lsqeqlrqws scrhirrssm 361 sveaettttf sp
14	NP_001829.1 Exemplary amino acid sequence for human CCR7 isoform a precursor	1 mdlgkpmksv lvvallvifq vclcqdevtd dyigdnttvd ytlfeslcsk kdvrnfkawf 61 lpimysiicf vgllgnglvv ltyiyfkrlk tmtdtyllnl avadilfllt lpfwaysaak 121 swvfgvhfck lifaiykmsf fsgmllllci sidryvaivq avsahrhrar vllisklscv 181 giwilatvls ipellysdlq rssseqamrc slitehveaf itiqvaqmvi gflvpllams 241 fcylviirtl lqarnfernk aikviiavvv vfivfqlpyn gvvlaqtvan fnitsstcel 301 skqlniaydv tyslacvrcc vnpflyafig vkfrndlfkl fkdlgclsqe qlrqwsscrh 361 irrssmsvea ettttfsp
15	NM_002253.2 Exemplary nucleic acid sequence encoding human VEGFR2 precursor	1 actgagtccc gggaccccgg gagagcggtc aatgtgtggt cgctgcgttt cctctgcctg 61 cgccgggcat cacttgcgcg ccgcagaaag tccgtctggc agcctggata tcctctccta 121 ccggcacccg cagacgcccc tgcagccgcg gtcggcgccc gggctcccta gccctgtgcg 181 ctcaactgtc ctgcgctgcg gggtgccgcg agttccacct ccgcgcctcc ttctctagac 241 aggcgctggg agaaagaacc ggctcccgag ttctgggcat ttcgcccggc tcgaggtgca 301 ggatgcagag caaggtgctg ctggccgtcg ccctgtggct ctgcgtggag acccgggccg 361 cctctgtggg tttgcctagt gtttctcttg atctgcccag gctcagcata caaaaagaca 421 tacttacaat taaggctaat acaactcttc aaattacttg caggggacag agggacttgg 481 actggctttg gcccaataat cagagtggca gtgagcaaag ggtggaggtg actgagtgca 541 gcgatggcct cttctgtaag acactcacaa ttccaaaagt gatcggaaat gacactggag 601 cctacaagtg cttctaccgg gaaactgact tggcctcggt catttatgtc tatgttcaag 661 attacagatc tccatttatt gcttctgtta gtgaccaaca tggagtcgtg tacattactg 721 agaacaaaaa caaaactgtg gtgattccat gtctcgggtc catttcaaat ctcaacgtgt 781 cactttgtgc aagataccca gaaaagagat ttgttcctga tggtaacaga atttcctggg 841 acagcaagaa gggctttact attcccagct acatgatcag ctatgctggc atggtcttct 901 gtgaagcaaa aattaatgat gaaagttacc agtctattat gtacatagtt gtcgttgtag 961 ggtataggat ttatgatgtg gttctgagtc cgtctcatgg aattgaacta tctgttggag 1021 aaaagcttgt cttaaattgt acagcaagaa ctgaactaaa tgtggggatt gacttcaact 1081 gggaataccc ttcttcgaag catcagcata agaaacttgt aaaccgagac ctaaaaaccc

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16	NP_002244.1 Exemplary nucleic acid sequence encoding human VEGFR2 precursor	1 mqskvllava lwlcvetraa svglpsvsld lprlsiqkdi ltikanttlq itcrgqrdld 61 wlwpnnqsgs eqrvevtecs dglfcktlti pkvigndtga ykcfyretdl asviyvyvqd 121 yrspfiasvs dqhgvvyite nknktvvipc lgsisnlnvs lcarypekrf vpdgnriswd 181 skkgftipsy misyagmvfc eakindesyq simyivvvvg yriydvvlsp shgielsvge 241 klvlnctart elnvgidfnw eypsskhqhk klvnrdlktq sgsemkkfls tltidgvtrs 301 dqglytcaas sglmtkknst fvrvhekpfv afgsgmeslv eatvgervri pakylgyppp 361 eikwykngip lesnhtikag hvltimevse rdtgnytvil tnpiskekqs hvvslvvyvp 421 pqigekslis pvdsyqygtt qtltctvyai ppphhihwyw qleeecanep sqavsvtnpy 481 pceewrsved fqggnkievn knqfaliegk nktvstlviq aanvsalykc eavnkvgrge 541 rvisfhvtrg peitlqpdmq pteqesvslw ctadrstfen ltwyklgpqp lpihvgelpt 601 pvcknldtlw klnatmfsns tndilimelk naslqdqgdy vclaqdrktk krhcvvrqlt 661 vlervaptit gnlenqttsi gesievscta sgnpppqimw fkdnetlved sgivlkdgnr 721 nltirrvrke deglytcqac svlgcakvea ffiiegaqek tnleiiilvg taviamffwl

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17	NM_001716.4 Exemplary nucleic acid sequence encoding human CXCR5	1 aaaaaaaaaa agtgatgagt tgtgaggcag gtcgcggccc tactgcctca ggagacgatg 61 cgcagctcat ttgcttaaat ttgcagctga cggctgccac ctctctagag gcacctggcg 121 gggagcctct caacataaga cagtgaccag tctggtgact cacagccggc acagccatga 181 actacccgct aacgctggaa atggacctcg agaacctgga ggacctgttc tgggaactgg 241 acagattgga caactataac gacacctccc tggtggaaaa tcatctctgc cctgccacag 301 aggggcccct catggcctcc ttcaaggccg tgttcgtgcc cgtggcctac agcctcatct 361 tcctcctggg cgtgatcggc aacgtcctgg tgctggtgat cctggagcgg caccggcaga 421 cacgcagttc cacggagacc ttcctgttcc acctggccgt ggccgacctc ctgctggtct 481 tcatcttgcc ctttgccgtg gccgagggct ctgtgggctg ggtcctgggg accttcctct 541 gcaaaactgt gattgccctg cacaaagtca acttctactg cagcagcctg ctcctggcct 601 gcatcgccgt ggaccgctac ctggccattg tccacgccgt ccatgcctac cgccaccgcc 661 gcctcctctc catccacatc acctgtggga ccatctggct ggtgggcttc ctccttgcct 721 tgccagagat tctcttcgcc aaagtcagcc aaggccatca caacaactcc ctgccacgtt 781 gcaccttctc ccaagagaac caagcagaaa cgcatgcctg gttcacctcc cgattcctct 841 accatgtggc gggattcctg ctgcccatgc tggtgatggg ctggtgctac gtgggggtag 901 tgcacaggtt gcgccaggcc cagcggcgcc ctcagcggca gaaggcagtc agggtggcca 961 tcctggtgac aagcatcttc ttcctctgct ggtcacccta ccacatcgtc atcttcctgg 1021 acaccctggc gaggctgaag gccgtggaca atacctgcaa gctgaatggc tctctccccg 1081 tggccatcac catgtgtgag ttcctgggcc tggcccactg ctgcctcaac cccatgctct 1141 acactttcgc cggcgtgaag ttccgcagtg acctgtcgcg gctcctgacg aagctgggct 1201 gtaccggccc tgcctccctg tgccagctct tccctagctg gcgcaggagc agtctctctg 1261 agtcagagaa tgccacctct ctcaccacgt tctaggtccc agtgtcccct tttattgctg 1321 cttttccttg gggcaggcag tgatgctgga tgctccttcc aacaggagct gggatcctaa 1381 gggctcaccg tggctaagag tgtcctagga gtatcctcat ttggggtagc tagaggaacc 1441 aacccccatt tctagaacat ccctgccagc tcttctgccg gccctggggc taggctggag 1501 cccagggagc ggaaagcagc tcaaaggcac agtgaaggct gtccttaccc atctgcaccc 1561 ccctgggctg agagaacctc acgcacctcc catcctaatc atccaatgct caagaaacaa 1621 cttctacttc tgcccttgcc aacggagagc gcctgcccct cccagaacac actccatcag 1681 cttaggggct gctgacctcc acagcttccc ctctctcctc ctgcccacct gtcaaacaaa 1741 gccagaagct gagcaccagg ggatgagtgg aggttaaggc tgaggaaagg ccagctggca 1801 gcagagtgtg gccttcggac aactcagtcc ctaaaaacac agacattctg ccaggccccc 1861 aagcctgcag tcatcttgac caagcaggaa gctcagactg gttgagttca ggtagctgcc 1921 cctggctctg accgaaacag cgctgggtcc accccatgtc accggatcct gggtggtctg 1981 caggcagggc tgactctagg tgcccttgga ggccagccag tgacctgagg aagcgtgaag 2041 gccgagaagc aagaaagaaa cccgacagag ggaagaaaag agctttcttc ccgaacccca 2101 aggagggaga tggatcaatc aaacccggcg gtcccctccg ccaggcgaga tggggtgggg 2161 tggagaactc ctagggtggc tgggtccagg ggatgggagg ttgtgggcat tgatggggaa

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18	NM_032966.2 Exemplary nucleic acid sequence encoding human CXCR5	1 ccactctaag gaatgcggtc cctttgacag gcgaaaaact gaagttggaa aagacaaagt 61 gatttgttca aaattgaaat ttgaaacttg acatttggtc agtgggccct atgtaggaaa 121 aaacctccaa gagagctagg gttcctctca gagaggaaag acaggtcctt aggtcctcac 181 cctcccgtct ccttgccctt gcagttctgg gaactggaca gattggacaa ctataacgac 241 acctccctgg tggaaaatca tctctgccct gccacagagg ggcccctcat ggcctccttc 301 aaggccgtgt tcgtgcccgt ggcctacagc ctcatcttcc tcctgggcgt gatcggcaac 361 gtcctggtgc tggtgatcct ggagcggcac cggcagacac gcagttccac ggagaccttc 421 ctgttccacc tggccgtggc cgacctcctg ctggtcttca tcttgccctt tgccgtggcc 481 gagggctctg tgggctgggt cctggggacc ttcctctgca aaactgtgat tgccctgcac 541 aaagtcaact tctactgcag cagcctgctc ctggcctgca tcgccgtgga ccgctacctg 601 gccattgtcc acgccgtcca tgcctaccgc caccgccgcc tcctctccat ccacatcacc 661 tgtgggacca tctggctggt gggcttcctc cttgccttgc cagagattct cttcgccaaa 721 gtcagccaag gccatcacaa caactccctg ccacgttgca ccttctccca agagaaccaa 781 gcagaaacgc atgcctggtt cacctcccga ttcctctacc atgtggcggg attcctgctg 841 cccatgctgg tgatgggctg gtgctacgtg ggggtagtgc acaggttgcg ccaggcccag 901 cggcgccctc agcggcagaa ggcagtcagg gtggccatcc tggtgacaag catcttcttc 961 ctctgctggt caccctacca catcgtcatc ttcctggaca ccctggcgag gctgaaggcc 1021 gtggacaata cctgcaagct gaatggctct ctccccgtgg ccatcaccat gtgtgagttc 1081 ctgggcctgg cccactgctg cctcaacccc atgctctaca ctttcgccgg cgtgaagttc 1141 cgcagtgacc tgtcgcggct cctgacgaag ctgggctgta ccggccctgc ctccctgtgc 1201 cagctcttcc ctagctggcg caggagcagt ctctctgagt cagagaatgc cacctctctc 1261 accacgttct aggtcccagt gtccccttt attgctgctt ttccttgggg caggcagtga 1321 tgctggatgc tccttccaac aggagctggg atcctaaggg ctcaccgtgg ctaagagtgt 1381 cctaggagta tcctcatttg gggtagctag aggaaccaac ccccatttct agaacatccc 1441 tgccagctct tctgccggcc ctggggctag gctggagccc agggagcgga aagcagctca 1501 aaggcacagt gaaggctgtc cttacccatc tgcacccccc tgggctgaga gaacctcacg 1561 cacctcccat cctaatcatc caatgctcaa gaaacaactt ctacttctgc ccttgccaac 1621 ggagagcgcc tgcccctccc agaacacact ccatcagctt aggggctgct gacctccaca 1681 gcttcccctc tctcctcctg cccacctgtc aaacaaagcc agaagctgag caccagggga 1741 tgagtggagg ttaaggctga ggaaaggcca gctggcagca gagtgtggcc ttcggacaac 1801 tcagtcccta aaaacacaga cattctgcca ggcccccaag cctgcagtca tcttgaccaa 1861 gcaggaagct cagactggtt gagttcaggt agctgccccc ggctctgacc gaaacagcgc 1921 tgggtccacc ccatgtcacc ggatcctggg tggtctgcag gcagggctga ctctaggtgc 1981 ccttggaggc cagccagtga cctgaggaag cgtgaaggcc gagaagcaag aaagaaaccc 2041 gacagaggga agaaaagagc tttcttcccg aaccccaagg agggagatgg atcaatcaaa

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19	NP_116743.1 Exemplary amino acid sequence for human CXCR5 precursor	1 masfkavfvp vayslifllg vignvlvlvi lerhrqtrss tetflfhlav adlllvfilp 61 favaegsvgw vlgtflcktv ialhkvnfyc sslllaciav drylaivhav hayrhrrlls 121 ihitcgtiwl vgfllalpei lfakvsqghh nnslprctfs qenqaethaw ftsrflyhva 181 gfllpmlvmg wcyvgvvhrl rqaqrrpqrq kavrvailvt sifflcwspy hivifldtla 241 rlkavdntck lngslpvait mceflglahc clnpmlytfa gvkfrsdlsr lltklgctgp 301 aslcqlfpsw rrsslsesen atslttf
20	NP_001707.1 Exemplary amino acid sequence for human CXCR5 precursor	1 mnypltlemd lenledlfwe ldrldnyndt slvenhlcpa tegplmasfk avfvpvaysl 61 ifllgvignv lvlvilerhr qtrsstetfl fhlavadlll vfilpfavae gsvgwvlgtf 121 lcktvialhk vnfycsslll aciavdryla ivhavhayrh rrllsihitc gtiwlvgfll 181 alpeilfakv sqghhnnslp rctfsqenqa ethawftsrf lyhvagfllp mlvmgwcyvg 241 vvhrlrqaqr rpqrqkavrv ailvtsiffl cwspyhivif ldtlarlkav dntcklngsl 301 pvaitmcefl glahcclnpm lytfagvkfr sdlsrlltkl gctgpaslcq lfpswrrssl 361 sesenatslt tf
21	NM_031200.2 Exemplary nucleic acid sequence encoding human CCR9	1 gcttcctttc tcgtgttgtt atcgggtagc tgcctgctca gaacccacaa agcctgcccc 61 tcatcccagg cagagagcaa cccagctctt tccccagaca ctgagagctg gtggtgcctg 121 ctgtcccagg gagagttgca tcgccctcca cagagcaggc ttgcatctga ctgacccacc 181 atgacaccca cagacttcac aagccctatt cctaacatgg ctgatgacta tggctctgaa 241 tccacatctt ccatggaaga ctacgttaac ttcaacttca ctgacttcta ctgtgagaaa 301 aacaatgtca ggcagtttgc gagccatttc ctcccaccct tgtactggct cgtgttcatc 361 gtgggtgcct tgggcaacag tcttgttatc cttgtctact ggtactgcac aagagtgaag 421 accatgaccg acatgttcct tttgaatttg gcaattgctg acctcctctt tcttgtcact 481 cttcccttct gggccattgc tgctgctgac cagtggaagt tccagacctt catgtgcaag 541 gtggtcaaca gcatgtacaa gatgaacttc tacagctgtg tgttgctgat catgtgcatc 601 agcgtggaca ggtacattgc cattgcccag gccatgagag cacatacttg gagggagaaa 661 aggcttttgt acagcaaaat ggtttgcttt accatctggg tattggcagc tgctctctgc 721 atcccagaaa tcttatacag ccaaatcaag gaggaatccg gcattgctat ctgcaccatg 781 gtttacccta gcgatgagag caccaaactg aagtcagctg tcttgaccct gaaggtcatt 841 ctggggttct tccttccctt cgtggtcatg gcttgctgct ataccatcat cattcacacc 901 ctgatacaag ccaagaagtc ttccaagcac aaagccctaa aagtgaccat cactgtcctg 961 accgtctttg tcttgtctca gtttccctac aactgcattt tgttggtgca gaccattgac 1021 gcctatgcca tgttcatctc caactgtgcc gtttccacca acattgacat ctgcttccag

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22	NM001256369.1 Exemplary nucleic acid sequence encoding human CCR9	1 gcttcctttc tcgtgttgtt atcgggtagc tgcctgctca gaacccacaa agcctgcccc 61 tcatcccagg cagagagcaa cccagctctt tccccagaca ctgagagctg gtggtgcctg 121 ctgtcccagg gagagttgca tcgccctcca cagagcaggc ttgcatctga ctgacccacc 181 atgacaccca cagacttcac atctcctcca ggccccgctc cagatcacct tccctcgctg 241 gcccaggaat ccatctcctt ccaggacctt agcccaggac taacacaagc cctattccta 301 acatggctga tgactatggc tctgaatcca catcttccat ggaagactac gttaacttca 361 acttcactga cttctactgt gagaaaaaca atgtcaggca gtttgcgagc catttcctcc 421 caccettgta ctggctcgtg ttcatcgtgg gtgccttggg caacagtctt gttatccttg 481 tctactggta ctgcacaaga gtgaagacca tgaccgacat gttccttttg aatttggcaa 541 ttgctgacct cctctttctt gtcactcttc ccttctgggc cattgctgct gctgaccagt 601 ggaagttcca gaccttcatg tgcaaggtgg tcaacagcat gtacaagatg aacttctaca 661 gctgtgtgtt gctgatcatg tgcatcagcg tggacaggta cattgccatt gcccaggcca 721 tgagagcaca tacttggagg gagaaaaggc ttttgtacag caaaatggtt tgctttacca 781 tctgggtatt ggcagctgct ctctgcatcc cagaaatctt atacagccaa atcaaggagg 841 aatccggcat tgctatctgc accatggttt accctagcga tgagagcacc aaactgaagt 901 cagctgtctt gaccctgaag gtcattctgg ggttcttcct tcccttcgtg gtcatggctt 961 gctgctatac catcatcatt cacaccctga tacaagccaa gaagtcttcc aagcacaaag 1021 ccctaaaagt gaccatcact gtcctgaccg tctttgtctt gtctcagttt ccctacaact 1081 gcattttgtt ggtgcagacc attgacgcct atgccatgtt catctccaac tgtgccgttt 1141 ccaccaacat tgacatctgc ttccaggtca cccagaccat cgccttcttc cacagttgcc 1201 tgaaccctgt tctctatgtt tttgtgggtg agagattccg ccgggatctc gtgaaaaccc 1261 tgaagaactt gggttgcatc agccaggccc agtgggtttc atttacaagg agagagggaa

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24	NP_001243298.1 Exemplary amino acid sequence for human CCR9 precursor	1 maddygsest ssmedyvnfn ftdfyceknn vrqfashflp plywlvfivg algnslvilv 61 ywyctrvktm tdmfllnlai adllflvtlp fwaiaaadqw kfqtfmckvv nsmykmnfys 121 cvllimcisv dryiaiaqam rahtwrekrl lyskmvcfti wvlaaalcip eilysqikee 181 sgiaictmvy psdestklks avltlkvilg fflpfvvmac cytiiihtli qakksskhka 241 lkvtitvltv fvlsqfpync illvqtiday amfisncavs tnidicfqvt qtiaffhscl 301 npvlyvfvge rfrrdlvktl knlgcisqaq wvsftrregs lklssmllet tsgalsl
25	NM_000885.4 Exemplary nucleic acid sequence encoding human α4	1 ataacgtctt tgtcactaaa atgttcccca ggggccttcg gcgagtcttt ttgtttggtt 61 ttttgttttt aatctgtggc tcttgataat ttatctagtg gttgcctaca cctgaaaaac 121 aagacacagt gtttaactat caacgaaaga actggacggc tccccgccgc agtcccactc 181 cccgagtttg tggctggcat ttgggccacg ccgggctggg cggtcacagc gaggggcgcg 241 cagtttgggg tcacacagct ccgcttctag gccccaacca ccgttaaaag gggaagcccg 301 tgccccatca ggtccgctct tgctgagccc agagccatcc cgcgctctgc gggctgggag 361 gcccgggcca ggacgcgagt cctgcgcagc cgaggttccc cagcgccccc tgcagccgcg 421 cgtaggcaga gacggagccc ggccctgcgc ctccgcacca cgcccgggac cccacccagc 481 ggcccgtacc cggagaagca gcgcgagcac ccgaagctcc cggctggcgg cagaaaccgg

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26	NM_000889.2	1 aaatcttccc caccctgggg agtgtcactt cctcctctgc cgtctcccag atcagtacac 61 aaaggctgct gctgccgcca gaggaaggac tgctctgcac gcacctatgt ggaaactaaa

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	Exemplary nucleic acid sequence encoding human β7	121 gcccagagag aaagtctgac ttgccccaca gccagtgagt gactgcagca gcaccagaat 181 ctggtctgtt tcctgtttgg ctcttctacc actacggctt gggatctcgg gcatggtggc 241 tttgccaatg gtccttgttt tgctgctggt cctgagcaga ggtgagagtg aattggacgc 301 caagatccca tccacagggg atgccacaga atggcggaat cctcacctgt ccatgctggg 361 gtcctgccag ccagcccccc cctgccagaa gtgcatcctc tcacacccca gctgtgcatg 421 gtgcaagcaa ctgaacttca ccgcgtcggg agaggcggag gcgcggcgct gcgcccgacg 481 agaggagctg ctggctcgag gctgcccgct ggaggagctg gaggagcccc gcggccagca 541 ggaggtgctg caggaccagc cgctcagcca gggcgcccgc ggagagggtg ccacccagct 601 ggcgccgcag cgggtccggg tcacgctgcg gcctggggag ccccagcagc tccaggtccg 661 cttccttcgt gctgagggat acccggtgga cctgtactac cttatggacc tgagctactc 721 catgaaggac gacctggaac gcgtgcgcca gctcgggcac gctctgctgg tccggctgca 781 ggaagtcacc cattctgtgc gcattggttt tggttccttt gtggacaaaa cggtgctgcc 841 ctttgtgagc acagtaccct ccaaactgcg ccacccctgc cccacccggc tggagcgctg 901 ccagtcacca ttcagctttc accatgtgct gtccctgacg ggggacgcac aagccttcga 961 gcgggaggtg gggcgccaga gtgtgtccgg caatctggac tcgcctgaag gtggcttcga 1021 tgccattctg caggctgcac tctgccagga gcagattggc tggagaaatg tgtcccggct 1081 gctggtgttc acttcagacg acacattcca tacagctggg gacgggaagt tgggcggcat 1141 tttcatgccc agtgatgggc actgccactt ggacagcaat ggcctctaca gtcgcagcac 1201 agagtttgac taccttctg tgggtcaggt agcccaggcc ctctctgcag caaatatcca 1261 gcccatcttt gctgtcacca gtgccgcact gcctgtctac caggagctga gtaaactgat 1321 tcctaagtct gcagttgggg agctgagtga ggactccagc aacgtggtac agctcatcat 1381 ggatgcttat aatagcctgt cttccaccgt gacccttgaa cactcttcac tccctcctgg 1441 ggtccacatt tcttacgaat cccagtgtga gggtcctgag aagagggagg gtaaggctga 1501 ggatcgagga cagtgcaacc acgtccgaat caaccagacg gtgactttct gggtttctct 1561 ccaagccacc cactgcctcc cagagcccca tctcctgagg ctccgggccc ttggcttctc 1621 agaggagctg attgtggagt tgcacacgct gtgtgactgt aattgcagtg acacccagcc 1681 ccaggctccc cactgcagtg atggccaggg acacctacaa tgtggtgtat gcagctgtgc 1741 ccctggccgc ctaggtcggc tctgtgagtg ctctgtggca gagctgtcct ccccagacct 1801 ggaatctggg tgccgggctc ccaatggcac agggcccctg tgcagtggaa agggtcactg 1861 tcaatgtgga cgctgcagct gcagtggaca gagctctggg catctgtgcg agtgtgacga 1921 tgccagctgt gagcgacatg agggcatcct ctgcggaggc tttggtcgct gccaatgtgg 1981 agtatgtcac tgtcatgcca accgcacggg cagagcatgc gaatgcagtg gggacatgga 2041 cagttgcatc agtcccgagg gagggctctg cagtgggcat ggacgctgca aatgcaaccg 2101 ctgccagtgc ttggacggct actatggtgc tctatgcgac caatgcccag gctgcaagac 2161 accatgcgag agacaccggg actgtgcaga gtgtggggcc ttcaggactg gcccactggc 2221 caccaactgc agtacagctt gtgcccatac caatgtgacc ctggccttgg cccctatctt 2281 ggatgatggc tggtgcaaag agcggaccct ggacaaccag ctgttcttct tcttggtgga 2341 ggatgacgcc agaggcacgg tcgtgctcag agtgagaccc caagaaaagg gagcagacca 2401 cacgcaggcc attgtgctgg gctgcgtagg gggcatcgtg gcagtggggc tggggctggt 2461 cctggcttac cggctctcgg tggaaatcta tgaccgccgg gaatacagtc gctttgagaa 2521 ggagcagcaa caactcaact ggaagcagga cagtaatcct ctctacaaaa gtgccatcac 2581 gaccaccatc aatcctcgct ttcaagaggc agacagtccc actctctgaa ggagggaggg 2641 acacttaccc aaggctcttc tccttggagg acagtgggaa ctggagggtg agaggaaggg 2701 tgggtctgta agaccttggt aggggactaa ttcactggcg aggtgcggcc accaccctac 2761 ttcattttca gagtgacacc caagagggct gcttcccatg cctgcaacct tgcatccatc 2821 tgggctaccc cacccaagta tacaataaag tcttacctca gaccacaaaa aaaaaaaa
27	NP_000876.3	1 mawearrepg prraavretv mlllclgvpt grpynvdtes allyqgphnt lfgysvvlhs

SEQ ID NO:	GenBank Accession Number and Description	Sequence
	Exemplary amino acid sequence for human α4 precursor	61 hganrwllvg aptanwlana svinpgaiyr crigknpgqt ceqlqlgspn gepcgktcle 121 erdnqwlgvt lsrqpgengs ivtcghrwkn ifyiknenkl ptggcygvpp dlrtelskri 181 apcyqdyvkk fgenfascqa gissfytkdl ivmgapgssy wtgslfvyni ttnkykafld 241 kqnqvkfgsy lgysvgaghf rsqhttevvg gapqheqigk ayifsideke lnilhemkgk 301 klgsyfgasv cavdlnadgf sdllvgapmq stireegrvf vyinsgsgav mnametnlvg 361 sdkyaarfge sivnlgdidn dgfedvaiga pqeddlqgai yiyngradgi sstfsqrieg 421 lqiskslsmf gqsisgqida dnngyvdvav gafrsdsavl lrtrpvvivd aslshpesvn 481 rtkfdcveng wpsvcidltl cfsykgkevp gyivlfynms ldvnrkaesp prfyfssngt 541 sdvitgsiqv ssreancrth qafmrkdvrd iltpiqieaa yhlgphvisk rsteefpplq 601 pilqqkkekd imkktinfar fcahencsad lqvsakigfl kphenktyla vgsmktlmln 661 vslfnagdda yettlhvklp vglyfikile leekqincev tdnsgvvqld csigyiyvdh 721 lsridisfll dvsslsraee dlsitvhatc eneeemdnlk hsrvtvaipl kyevkltvhg 781 fvnptsfvyg sndenepetc mvekmnltfh vintgnsmap nvsveimvpn sfspqtdklf 841 nildvqtttg echfenyqrv caleqqksam qtlkgivrfl sktdkrllyc ikadphclnf 901 lcnfgkmesg keasvhiqle grpsilemde tsalkfeira tgfpepnprv ielnkdenva 961 hvlleglhhq rpkryftivi isssllgli vlllisyvmw kagffkrqyk silqeenrrd 1021 swsyinsksn dd
28	NP_000880.1 Exemplary amino acid sequence for human β7 precursor	1 mvalpmvlvl llvlsrgese ldakipstgd atewrnphls mlgscqpaps cqkcilshps 61 cawckqlnft asgeaearrc arreellarg cpleeleepr gqqevlqdqp lsqgargega 121 tqlapqrvrv tlrpgepqql qvrflraegy pvdlyylmdl sysmkddler vrqlghallv 181 rlqevthsvr igfgsfvdkt vlpfvstvps klrhpcptrl ercqspfsfh hvlsltgdaq 241 aferevgrqs vsgnldspeg gfdailqaal cqeqigwrnv srllvftsdd tfhtagdgkl 301 ggifmpsdgh chldsnglys rstefdypsv gqvaqalsaa niqpifavts aalpvyqels 361 klipksavge lsedssnvvq limdaynsls stvtlehssl ppgvhisyes qcegpekreg 421 kaedrgqcnh vrinqtvtfw vslqathclp ephllrlral gfseelivel htlcdcncsd 481 tqpqaphcsd gqghlqcgvc scapgrlgrl cecsvaelss pdlesgcrap ngtgplcsgk 541 ghcqcgrcsc sgqssghlce cddascerhe gilcggfgrc qcgvchchan rtgracecsg 601 dmdscispeg glcsghgrck cnrcqcldgy ygalcdqcpg cktpcerhrd caecgafrtg 661 platncstac ahtnvtlala pilddgwcke rtldnqlfff lveddargtv vlrvrpqekg 721 adhtqaivlg cvggivavgl glvlayrlsv eiydrreysr fekeqqqlnw kqdsnplyks 781 aitttinprf qeadsptl
29	NM_016602.2 Exemplary nucleic acid sequence encoding human CCR10	1 agagatgggg acggaggcca cagagcaggt ttcctggggc cattactctg gggatgaaga 61 ggacgcatac tcggctgagc cactgccgga gctttgctac aaggccgatg tccaggcctt 121 cagccgggcc ttccaaccca gtgtctccct gaccgtggct gcgctgggtc tggccggcaa 181 tggcctggtc ctggccaccc acctggcagc ccgacgcgca gcgcgctcgc ccacctctgc 241 ccacctgctc cagctggccc tggccgacct cttgctggcc ctgactctgc ccttcgcggc 301 agcaggggct cttcagggct ggagtctggg aagtgccacc tgccgcacca tctctggcct 361 ctactcggcc tccttccacg ccggcttcct cttcctggcc tgtatcagcg ccgaccgcta 421 cgtggccatc gcgcgagcgc tcccagccgg gccgcggccc tccactcccg gccgcgcaca 481 cttggtctcc gtcatcgtgt ggctgctgtc actgctcctg gcgctgcctg cgctgctctt 541 cagccaggat gggcagcggg aaggccaacg acgctgtcgc ctcatcttcc ccgagggcct 601 cacgcagacg gtgaaggggg cgagcgccgt ggcgcaggtg gccctgggct tcgcgctgcc 661 gctgggcgtc atggtagcct gctacgcgct tctgggccgc acgctgctgg ccgccagggg 721 gcccgagcgc cggcgtgcgc tgcgcgtcgt ggtggctctg gtggcggcct tcgtggtgct 781 gcagctgccc tacagcctcg ccctgctgct ggatactgcc gatctactgg ctgcgcgcga 841 gcggagctgc cctgccagca aacgcaagga tgtcgcactg ctggtgacca gcggcttggc

SEQ ID NO:	GenBank Accession Number and Description	Sequence
		901 cctcgcccgc tgtggcctca atcccgttct ctacgccttc ctgggcctgc gcttccgcca 961 ggacctgcgg aggctgctac ggggtgggag ctgcccctca gggcctcaac cccgccgcgg 1021 ctgcccccgc cggccccgcc tttcttcctg ctcagctccc acggagaccc acagtctctc 1081 ctgggacaac tagggctgcg aatctagagg agggggcagg ctgagggtcg tgggaaaggg 1141 gagtaggtgg gggaacactg agaaagaggc agggacctaa agggactacc tctgtgcctt 1201 gccacattaa attgataaca tggaaatgag atgcaaccca acaa
30	AF215981.1 Exemplary nucleic acid sequence encoding human CCR10	1 agagatgggg acggaggcca cagagcaggt ttcctggggc cattactctg gggatgaaga 61 ggacgcatac tcggctgagc cactgccgga gctttgctac aaggccgatg tccaggcctt 121 cagccgggcc ttccaaccca gtgtctccct gaccgtggct gcgctgggtc tggccggcaa 181 tggcctggtc ctggccaccc acctggcagc ccgacgcgca gcgcgctcgc ccacctctgc 241 ccacctgctc cagctggccc tggccgacct cttgctggcc ctgactctgc ccttcgcggc 301 agcaggggct cttcagggct ggagtctggg aagtgccacc tgccgcacca tctctggcct 361 ctactcggcc tccttccacg ccggcttcct cttcctggcc tgtatcagcg ccgaccgcta 421 cgtggccatc gcgcgagcgc tcccagccgg gccgcggccc tccactcccg gccgcgcaca 481 cttggtctcc gtcatcgtgt ggctgctgtc actgctcctg gcgctgcctg cgctgctctt 541 cagccaggat gggcagcggg aaggccaacg acgctgtcgc ctcatcttcc ccgagggcct 601 cacgcagacg gtgaaggggg cgagcgccgt ggcgcaggtg gccctgggct tcgcgctgcc 661 gctgggcgtc atggtagcct gctacgcgct tctgggccgc acgctgctgg ccgccagggg 721 gcccgagcgc cggcgtgcgc tgcgcgtcgt ggtggctctg gtggcggcct tcgtggtgct 781 gcagctgccc tacagcctcg ccctgctgct ggatactgcc gatctactgg ctgcgcgcga 841 gcggagctgc cctgccagca aacgcaagga tgtcgcactg ctggtgacca gcggcttggc 901 cctcgcccgc tgtggcctca atcccgttct ctacgccttc ctgggcctgc gcttccgcca 961 ggacctgcgg aggctgctac ggggtgggag ctcgccctca gggcctcaac cccgccgcgg 1021 ctgcccccgc cggccccgcc tttcttcctg ctcagctccc acggagaccc acagtctctc 1081 ctgggacaac tagggctgcg aatctagagg agggggcagg ctgagggtcg tgggaaaggg 1141 gagtaggtgg gggaacactg agaaagaggc agggacctaa agggactacc tctgtgcctt 1201 gccacattaa attgataaca tggaaatgaa aaaaaaaaaa aaaa
31	NP_057686.2 Exemplary amino acid sequence for human CCR10 precursor	1 mgteateqvs wghysgdeed aysaeplpel cykadvqafs rafqpsvslt vaalglagng 61 lvlathlaar raarsptsah llqlaladll laltlpfaaa galqgwslgs atcrtisgly 121 sasfhagflf lacisadryv aiaralpagp rpstpgrahl vsvivwllsl llalpallfs 181 qdgqregqrr crlifpeglt qtvkgasava qvalgfalpl gvmvacyall grtllaargp 241 errralrvvv alvaafvvlq lpyslalllld tadllaarer scpaskrkdv allvtsglal 301 arcglnpvly aflglrfrqd lrrllrggsc psgpqprrgc prrprlsscs aptethslsw 361 dn
32	P46092.3 Exemplary amino acid sequence for human CCR10 precursor	1 mgteateqvs wghysgdeed aysaeplpel cykadvqafs rafqpsvslt vaalglagng 61 lvlathlaar raarsptsah llqlaladll laltlpfaaa galqgwslgs atcrtisgly 121 sasfhagflf lacisadryv aiaralpagp rpstpgrahl vsvivwllsl llalpallfs 181 qdgqregqrr crlifpeglt qtvkgasava qvalgfalpl gvmvacyall grtllaargp 241 errralrvvv alvaafvvlq lpyslalllld tadllaarer scpaskrkdv allvtsglal 301 arcglnpvly aflglrfrqd lrrllrggsc psgpqprrgc prrprlsscs aptethslsw 361 dn
33	NM_005201.3 Exemplary nucleic	1 tttgtagtgg gaggatacct ccagagaggc tgctgctcat tgagctgcac tcacatgagg 61 atacagactt tgtgaagaag gaattggcaa cactgaaacc tccagaacaa aggctgtcac 121 taaggtcccg ctgccttgat ggattataca cttgacctca gtgtgacaac agtgaccgac

SEQ ID NO:	GenBank Accession Number and Description	Sequence
	acid sequence encoding human CCR8	181 tactactacc ctgatatctt ctcaagcccc tgtgatgcgg aacttattca gacaaatggc 241 aagttgctcc ttgctgtctt ttattgcctc ctgtttgtat tcagtcttct gggaaacagc 301 ctggtcatcc tggtccttgt ggtctgcaag aagctgagga gcatcacaga tgtatacctc 361 ttgaacctgg ccctgtctga cctgcttttt gtcttctcct tccccttca gacctactat 421 ctgctggacc agtgggtgtt tgggactgta atgtgcaaag tggtgtctgg cttttattac 481 attggcttct acagcagcat gtttttcatc accctcatga gtgtggacag gtacctggct 541 gttgtccatg ccgtgtatgc cctaaaggtg aggacgatca ggatgggcac aacgctgtgc 601 ctggcagtat ggctaaccgc cattatggct accatcccat tgctagtgtt ttaccaagtg 661 gcctctgaag atggtgttct acagtgttat tcattttaca atcaacagac tttgaagtgg 721 aagatcttca ccaacttcaa aatgaacatt ttaggcttgt tgatcccatt caccatcttt 781 atgttctgct acattaaaat cctgcaccag ctgaagaggt gtcaaaacca caacaagacc 841 aaggccatca ggttggtgct cattgtggtc attgcatctt tacttttctg ggtcccattc 901 aacgtggttc ttttcctcac ttccttgcac agtatgcaca tcttggatgg atgtagcata 961 agccaacagc tgacttatgc cacccatgtc acagaaatca tttcctttac tcactgctgt 1021 gtgaaccctg ttatctatgc ttttgttggg gagaagttca agaaacacct ctcagaaata 1081 tttcagaaaa gttgcagcca aatcttcaac tacctaggaa gacaaatgcc tagggagagc 1141 tgtgaaaagt catcatcctg ccagcagcac tcctcccgtt cctccagcgt agactacatt 1201 ttgtgaggat caatgaagac taaatataaa aaacattttc ttgaatggca tgctagtagc 1261 agtgagcaaa ggtgtgggtg tgaaaggttt ccaaaaaaag ttcagcatga aggatgccat 1321 atatgttgtt gccaacactt ggaacacaat gactaaagac atagttgtgc atgcctggca 1381 caacatcaag cctgtgattg tgtttattga tgatgttgaa caagtggtaa ctttaaagga 1441 ttctgtatgc caagtgaaaa aaaaagatgt ctgacctcct tacatat
34	BC107159.1 Exemplary nucleic acid sequence encoding human CCR8	1 ctttgtgaag aaggaattgg caacactgaa acctccagaa caaaggctgt cactaaggtc 61 ccgctgcctt gatggattat acacttgacc tcagtgtgac aacagtgacc gactactact 121 accctgatat cttctcaagc ccctgtgatg cggaacttat tcagacaaat ggcaagttgc 181 tccttgctgt cttttattgc ctcctgtttg tattcagtct tctgggaaac agcctggtca 241 tcctggtcct tgtggtctgc aagaagctga ggagcatcac agatgtatac ctcttgaacc 301 tggccctgtc tgacctgctt tttgtcttct ccttcccctt tcagacctac tatctgctgg 361 accagtgggt gtttgggact gtaatgtgca aagtggtgtc tggcttttat tacattggct 421 tctacagcag catgtttttc atcaccctca tgagtgtgga caggtacctg gctgttgtcc 481 atgccgtgta tgccctaaag gtgaggacga tcaggatggg cacaacgctg tgcctggcag 541 tatggctaac cgccattatg gctaccatcc cattgctagt gttttaccaa gtggcctctg 601 aagatggtgt tctacagtgt tattcatttt acaatcaaca gactttgaag tggaagatct 661 tcaccaactt caaaatgaac attttaggct tgttgatccc attcaccatc tttatgttct 721 gctacattaa aatcctgcac cagctgaaga ggtgtcaaaa ccacaacaag accaaggcca 781 tcaggttggt gctcattgtg gtcattgcat ctttacttttt ctgggtccca ttcaacgtgg 841 ttcttttcct cacttccttg cacagtatgc acatcttgga tggatgtagc ataagccaac 901 agctgactta tgccacccat gtcacagaaa tcatttcctt tactcactgc tgtgtgaacc 961 ctgttatcta tgcttttgtt ggggagaagt tcaagaaaca cctctcagaa atatttcaga 1021 aaagttgcag ccaaatcttc aactacctag gaagacaaat gcctagggag agctgtgaaa 1081 agtcatcatc ctgccagcag cactcctccc gttcctccag cgtagactac attttgtgag 1141 gatcaatgaa gactaaatat aaaaaacatt ttcttgaatg gcatgctagt agcagtgagc 1201 aaaggtgtgg gtgtgaaagg tttccaaaaa aagttcagca tgaaggatgc cgtgtgtgtt 1261 gttgccaaca cttggaacac gatgactggg gacgtggttg tgcatgcctg gcacaacatc 1321 aagcctgtga ttgtgtttat tgatgatgtt gaacaagtgg tggctttgga ggattctgta 1381 tgccaagtga aaggggagat gtctgacctc cttcatatag

SEQ ID NO:	GenBank Accession Number and Description	Sequence
35	NP_005192.1 Exemplary amino acid sequence for human CCR8 precursor	1 mdytldlsvt tvtdyyypdi fsspcdaeli qtngklllav fycllfvfsl lgnslvilvl 61 vvckklrsit dvyllnlals dllfvfsfpf qtyylldqwv fgtvmckvvs gfyyigfyss 121 mffitlmsvd rylavvhavy alkvrtirmg ttlclavwlt aimatipllv fyqvasedgv 181 lqcysfynqq tlkwkiftnf kmnilgllip ftifmfcyik ilhqlkrcqn hnktkairlv 241 livviaslll wvpfnvvlfl tslhsmhild gcsisqqlty athvteiisf thccvnpviy 301 afvgekfkkh lseifqkscs qifnylgrqm presceksss cqqhssrsss vdyil
36	AAI07160.1 Exemplary amino acid sequence for human CCR8 precursor	1 mdytldlsvt tvtdyyypdi fsspcdaeli qtngklllav fycllfvfsl lgnslvilvl 61 vvckklrsit dvyllnlals dllfvfsfpf qtyylldqwv fgtvmckvvs gfyyigfyss 121 mffitlmsvd rylavvhavy alkvrtirmg ttlclavwlt aimatipllv fyqvasedgv 181 lqcysfynqq tlkwkiftnf kmnilgllip ftifmfcyik ilhqlkrcqn hnktkairlv 241 livviaslll wvpfnvvlfl tslhsmhild gcsisqqlty athvteiisf thccvnpviy 301 afvgekfkkh lseifqkscs qifnylgrqm presceksss cqqhssrsss vdyil
37	NM_005508.4 Exemplary nucleic acid sequence encoding human CCR4	1 gctcacagga agccacgcac ccttgaaagg caccgggtcc ttcttagcat cgtgcttcct 61 gagcaagcct ggcattgcct cacagacctt cctcagagcc gctttcagaa aagcaagctg 121 cttctggttg ggcccagacc tgccttgagg agcctgtaga gttaaaaaat gaaccccacg 181 gatatagcag acaccaccct cgatgaaagc atatacagca attactatct gtatgaaagt 241 atccccaagc cttgcaccaa agaaggcatc aaggcatttg gggagctctt cctgccccca 301 ctgtattcct tggtttttgt atttggtctg cttggaaatt ctgtggtggt tctggtcctg 361 ttcaaataca agcggctcag gtccatgact gatgtgtacc tgctcaacct tgccatctcg 421 gatctgctct tcgtgttttc cctccctttt tggggctact atgcagcaga ccagtgggtt 481 tttgggctag gtctgtgcaa gatgatttcc tggatgtact tggtgggctt ttacagtggc 541 atattctttg tcatgctcat gagcattgat agatacctgg caattgtgca cgcggtgttt 601 tccttgaggg caaggacctt gacttatggg gtcatcacca gtttggctac atggtcagtg 661 gctgtgttcg cctcccttcc tggctttctg ttcagcactt gttatactga gcgcaaccat 721 acctactgca aaaccaagta ctctctcaac tccacgacgt ggaaggttct cagctccctg 781 gaaatcaaca ttctcggatt ggtgatcccc ttagggatca tgctgttttg ctactccatg 841 atcatcagga ccttgcagca ttgtaaaaat gagaagaaga acaaggcggt gaagatgatc 901 tttgccgtgg tggtcctctt ccttgggttc tggacacctt acaacatagt gctcttccta 961 gagaccctgg tggagctaga agtccttcag gactgcacct ttgaaagata cttggactat 1021 gccatccagg ccacagaaac tctggctttt gttcactgct gccttaatcc catcatctac 1081 ttttttctgg gggagaaatt tcgcaagtac atcctacagc tcttcaaaac ctgcaggggc 1141 ctttttgtgc tctgccaata ctgtgggctc ctccaaattt actctgctga caccccagc 1201 tcatcttaca cgcagtccac catggatcat gatctccatg atgctctgta gaaaaatgaa 1261 atggtgaaat gcagagtcaa tgaactttcc acattcagag cttacttaaa attgtatttt 1321 agtaagagat tcctgagcca gtgtcaggag gaaggcttac acccacagtg gaaagacagc 1381 ttctcatcct gcaggcagct ttttctctcc cactagacaa gtccagcctg gcaagggttc 1441 acctgggctg aggcatcctt cctcacacca ggcttgcctg caggcatgag tcagtctgat 1501 gagaactctg agcagtgctt gaatgaagtt gtaggtaata ttgcaaggca aagactattc 1561 ccttctaacc tgaactgatg ggtttctcca gagggaattg cagagtactg gctgatggag 1621 taaatcgcta ccttttgctg tggcaaatgg gccctct
38	P51679.1 Exemplary amino acid sequence for human CCR4	1 mnptdiadtt ldesiysnyy lyesipkpct kegikafgel flpplyslvf vfgllgnsvv 61 vlvlfkykrl rsmtdvylln laisdllfvf slpfwgyyaa dqwvfglglc kmiswmylvg 121 fysgiffvml msidrylaiv havfslrart ltygvitsla twsvavfasl pgflfstcyt 181 ernhtycktk yslnsttwkv lssleinilg lviplgimlf cysmiirtlq hcknekknka 241 vkmifavvvl flgfwtpyni vlfletlvel evlqdctfer yldyaiqate tlafvhccln

SEQ ID NO:	GenBank Accession Number and Description	Sequence
	precursor	301 piiyfflgek frkyilqlfk tcrglfvlcq ycgllqiysa dtpsssytqs tmdhdlhdal
39	NM_001206609.1 Exemplary nucleic acid sequence encoding human CLA	1 aatcatccga gaaccttgga gggtggacag tgccccttt acagatgaga aaactgaggc 61 ttgaaggga gaagcagctg cctctggcgg catggcttct ggctgcagga tgcccatgga 121 gttcgtggtg accctaggcc tgtgtctcgg cttcctttgc tgaacttgaa caggaagatg 181 gcagtggggg ccagtggtct agaaggagat aagatggctg gtgccatgcc tctgcaactc 241 ctcctgttgc tgatcctact gggccctggc aacagcttgc agctgtggga cacctgggca 301 gatgaagccg agaaagcctt gggtcccctg cttgcccggg accggagaca ggccaccgaa 361 tatgagtacc tagattatga tttcctgcca gaaacggagc ctccagaaat gctgaggaac 421 agcactgaca ccactcctct gactgggcct ggaacccctg agtctaccac tgtggagcct 481 gctgcaaggc gttctactgg cctggatgca ggaggggcag tcacagagct gaccacggag 541 ctggccaaca tggggaacct gtccacggat tcagcagcta tggagataca gaccactcaa 601 ccagcagcca cggaggcaca gaccactcaa ccagtgccca cggaggcaca gaccactcca 661 ctggcagcca cagaggcaca gacaactcga ctgacggcca cggaggcaca gaccactcca 721 ctggcagcca cagaggcaca gaccactcca ccagcagcca cggaagcaca gaccactcaa 781 cccacaggcc tggaggcaca gaccactgca ccagcagcca tggaggcaca gaccactgca 841 ccagcagcca tggaagcaca gaccactcca ccagcagcca tggaggcaca gaccactcaa 901 accacagcca tggaggcaca gaccactgca ccagaagcca cggaggcaca gaccactcaa 961 cccacagcca cggaggcaca gaccactcca ctggcagcca tggaggccct gtccacagaa 1021 cccagtgcca cagaggccct gtccatggaa cctactacca aaagaggtct gttcataccc 1081 ttttctgtgt cctctgttac tcacaagggc attcccatgg cagccagcaa tttgtccgtc 1141 aactacccag tgggggcccc agaccacatc tctgtgaagc agtgcctgct ggccatccta 1201 atcttggcgc tggtggccac tatcttcttc gtgtgcactg tggtgctggc ggtccgcctc 1261 tcccgcaagg gccacatgta ccccgtgcgt aattactccc ccaccgagat ggtctgcatc 1321 tcatccctgt tgcctgatgg gggtgagggg ccctctgcca cagccaatgg gggcctgtcc 1381 aaggccaaga gcccgggcct gacgccagag cccagggagg accgtgaggg ggatgacctc 1441 accctgcaca gcttcctccc ttagctcact ctgccatctg ttttggcaag accccacctc 1501 cacgggctct cctgggccac ccctgagtgc ccagacccca ttccacagct ctgggcttcc 1561 tcggagaccc ctggggatgg ggatcttcag ggaaggaact ctggccaccc aaacaggaca 1621 agagcagcct ggggccaagc agacgggcaa gtggagccac ctctttcctc cctccgcgga 1681 tgaagcccag ccacatttca gccgaggtcc aaggcaggag gccatttact tgagacagat 1741 tctctccttt ttcctgtccc ccatcttctc tgggtccctc taacatctcc catggctctc 1801 cccgcttctc ctggtcactg gagtctcctc cccatgtacc caaggaagat ggagctcccc 1861 catcccacac gcactgcact gccattgtct tttggttgcc atggtcacca aacaggaagt 1921 ggacattcta agggaggagt actgaagagt gacggacttc tgaggctgtt tcctgctgct 1981 cctctgactt ggggcagctt gggtcttctt gggcacctct ctgggaaaac ccagggtgag 2041 gttcagcctg tgagggctgg gatgggtttc gtgggcccaa gggcagacct ttctttggga 2101 ctgtgtggac caaggagctt ccatctagtg acaagtgacc cccagctatc gcctcttgcc 2161 ttcccctgtg gccactttcc agggtggact ctgtcttgtt cactgcagta tcccaactgc 2221 aggtccagtg caggcaataa atatgtgatg gacaaacgat agcggaatcc ttcaaggttt 2281 caaggctgtc tccttcaggc agccttcccg gaattctcca tccctcagtg caggatgggg 2341 gctggtcctc agctgtctgc cctcagcccc tggcccccca ggaagcctct ttcatgggct 2401 gttaggttga cttcagtttt gcctcttgga caacaggggg tcttgtacat ccttgggtga 2461 ccaggaaaag ttcaggctat ggggggccaa agggagggct gccccttccc caccagtgac

SEQ ID NO:	GenBank Accession Number and Description	Sequence
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40	NM_003006.4 Exemplary nucleic acid sequence encoding human CLA	1 acacacagcc attgggggtt gctcggatcc gggactgccg caggggtgc cacagcagtg 61 cctggcagcg tgggctggga ccttgtcact aaagcagaga agccacttct tctgggccca 121 cgaggcagct gtcccatgct ctgctgagca cggtggtgcc atgcctctgc aactcctcct 181 gttgctgatc ctactgggcc ctggcaacag cttgcagctg tgggacacct gggcagatga 241 agccgagaaa gccttgggtc ccctgcttgc ccgggaccgg agacaggcca ccgaatatga 301 gtacctagat tatgatttcc tgccagaaac ggagcctcca gaaatgctga ggaacagcac 361 tgacaccact cctctgactg ggcctggaac ccctgagtct accactgtgg agcctgctgc 421 aaggcgttct actggcctgg atgcaggagg ggcagtcaca gagctgacca cggagctggc 481 caacatgggg aacctgtcca cggattcagc agctatggag atacagacca ctcaaccagc 541 agccacggag gcacagacca ctcaaccagt gcccacggag gcacagacca ctccactggc 601 agccacagag gcacagacaa ctcgactgac ggccacggag gcacagacca ctccactggc 661 agccacagag gcacagacca ctccaccagc agccacggaa gcacagacca ctcaacccac 721 aggcctggag gcacagacca ctgcaccagc agccatggag gcacagacca ctgcaccagc 781 agccatggaa gcacagacca ctccaccagc agccatggag gcacagacca ctcaaaccac 841 agccatggag gcacagacca ctgcaccaga agccacggag gcacagacca ctcaacccac 901 agccacggag gcacagacca ctccactggc agccatggag gccctgtcca cagaacccag 961 tgccacagag gccctgtcca tggaacctac taccaaaaga ggtctgttca tacccttttc 1021 tgtgtcctct gttactcaca agggcattcc catggcagcc agcaatttgt ccgtcaacta 1081 cccagtgggg gccccagacc acatctctgt gaagcagtgc ctgctggcca tcctaatctt 1141 ggcgctggtg gccactatct tcttcgtgtg cactgtggtg ctggcggtcc gcctctcccg 1201 caagggccac atgtaccccg tgcgtaatta ctccccacc gagatggtct gcatctcatc 1261 cctgttgcct gatgggggtg aggggccctc tgccacagcc aatgggggcc tgtccaaggc 1321 caagagcccg ggcctgacgc cagagcccag ggaggaccgt gaggggatg acctcaccct 1381 gcacagcttc ctcccttagc tcactctgcc atctgttttg gcaagacccc acctccacgg 1441 gctctcctgg gccacccctg agtgcccaga ccccattcca cagctctggg cttcctcgga 1501 gacccctggg gatggggatc ttcagggaag gaactctggc cacccaaaca ggacaagagc 1561 agcctggggc caagcagacg ggcaagtgga gccacctctt tcctccctcc gcggatgaag 1621 cccagccaca tttcagccga ggtccaaggc aggaggccat ttacttgaga cagattctct 1681 ccttttttcct gtcccccatc ttctctgggt ccctctaaca tctcccatgg ctctccccgc 1741 ttctcctggt cactggagtc tcctccccat gtacccaagg aagatggagc tcccccatcc 1801 cacacgcact gcactgccat tgtcttttgg ttgccatggt caccaaacag gaagtggaca 1861 ttctaaggga ggagtactga agagtgacgg acttctgagg ctgtttcctg ctgctcctct 1921 gacttggggc agcttgggtc ttcttgggca cctctctggg aaaacccagg gtgaggttca 1981 gcctgtgagg gctgggatgg gtttcgtggg cccaagggca gacctttctt tgggactgtg 2041 tggaccaagg agcttccatc tagtgacaag tgacccccag ctatcgcctc ttgccttccc 2101 ctgtggccac tttccagggt ggactctgtc ttgttcactg cagtatccca actgcaggtc 2161 cagtgcaggc aataaatatg tgatggacaa acgatagcgg aatccttcaa ggtttcaagg 2221 ctgtctcctt caggcagcct tcccggaatt ctccatccct cagtgcagga tgggggctgg 2281 tcctcagctg tctgccctca gcccctggcc ccccaggaag cctctttcat gggctgttag 2341 gttgacttca gttttgcctc ttggacaaca gggggtcttg tacatccttg ggtgaccagg 2401 aaaagttcag gctatggggg gccaaaggga gggctgcccc ttccccacca gtgaccactt 2461 tattccactt cctccattac ccagttttgg cccacagagt ttggtccccc ccaaacctcg 2521 gaccaatatc cctctaaaca tcaatctatc ctcctgttaa agaaaaaaaa aaa
41	NP_001193538.1	1 mavgasgleg dkmagamplq lllllillgp gnslqlwdtw adeaekalgp llardrrqat

SEQ ID NO:	GenBank Accession Number and Description	Sequence
	Exemplary amino acid sequence for human CLA precursor	61 eyeyldydfl peteppemlr nstdttpltg pgtpesttve paarrstgld aggavteltt 121 elanmgnlst dsaameiqtt qpaateaqtt qpvpteaqtt plaateaqtt rltateaqtt 181 plaateaqtt ppaateaqtt qptgleaqtt apaameaqtt apaameaqtt ppaameaqtt 241 qttameaqtt apeateaqtt qptateaqtt plaamealst epsatealsm epttkrglfi 301 pfsvssvthk gipmaasnls vnypvgapdh isvkqcllai lilalvatif fvctvvlavr 361 lsrkghmypv rnysptemvc issllpdgge gpsatanggl skakspgltp epredregdd 421 ltlhsflp
42	NP_002997.2 Exemplary amino acid sequence for human CLA precursor	1 mplqllllli llgpgnslql wdtwadeaek algpllardr rqateyeyld ydflpetepp 61 emlrnstdtt pltgpgtpes ttvepaarrs tgldaggavt elttelanmg nlstdsaame 121 iqttqpaate aqttqpvpte aqttplaate aqttrltate aqttplaate aqttppaate 181 aqttqptgle aqttapaame aqttapaame aqttppaame aqttqttame aqttapeate 241 aqttqptate aqttplaame alstepsate alsmepttkr glfipfsvss vthkgipmaa 301 snlsvnypvg apdhisvkqc llaililalv atiffvctvv lavrlsrkgh mypvrnyspt 361 emvcissllp dggegpsata ngglskaksp gltpepredr egddltlhsf lp

5.3.6. Polynucleotide for Generating CAR and/or Homing Receptor

[00352] Described herein are polynucleotide sequences (*i.e.*, nucleic acid sequences) that encode the chimeric receptors and homing receptors. The polynucleotides may be contained within any polynucleotide vector suitable for the transformation of immune cells, *e.g.*, NK cells. For example, NK cells may be transformed using synthetic vectors, lentiviral or retroviral vectors, autonomously replicating plasmids, a virus (*e.g.*, a retrovirus, lentivirus, adenovirus, or herpes virus), or the like, containing polynucleotides encoding the first and second polypeptides (*e.g.*, chimeric receptors). Lentiviral vectors suitable for transformation of NK cells include, but are not limited to, *e.g.*, the lentiviral vectors described in U.S. Patent Nos. 5,994,136; 6,165,782; 6,428,953; 7,083,981; and 7,250,299, the disclosures of which are hereby incorporated by reference in their entireties. HIV vectors suitable for transformation of NK cells include, but are not limited to, *e.g.*, the vectors described in U.S. Patent No. 5,665,577, the disclosure of which is hereby incorporated by reference in its entirety.

[00353] Nucleic acids useful in the production of the polypeptides described herein, *e.g.*, within a NK cell, include DNA, RNA, or nucleic acid analogs. Nucleic acid analogs can be modified at the base moiety, sugar moiety, or phosphate backbone, and can include deoxyuridine substitution for deoxythymidine, 5-methyl-2'-deoxycytidine or 5-bromo-2'-deoxycytidine substitution for deoxycytidine. Modifications of the sugar moiety can include modification of the

2' hydroxyl of the ribose sugar to form 2'-O-methyl or 2'-O-allyl sugars. The deoxyribose phosphate backbone can be modified to produce morpholino nucleic acids, in which each base moiety is linked to a six membered, morpholino ring, or peptide nucleic acids, in which the deoxyphosphate backbone is replaced by a pseudopeptide backbone and the four bases are retained. See, for example, Summerton and Weller (1997) Antisense Nucleic Acid Drug Dev. 7:187-195; and Hyrup et al. (1996) Bioorgan. Med. Chain. 4:5-23. In addition, the deoxyphosphate backbone can be replaced with, for example, a phosphorothioate or phosphorodithioate backbone, a phosphoroamidite, or an alkyl phosphotriester backbone.

[00354] A nucleic acid encoding a polypeptide described herein may be introduced into host cells as part of a vector, such as, *e.g.*, an expression vector. In addition, a polypeptide described herein may be produced by transfecting a host cell with a nucleic acid encoding such a polypeptide, and such nucleic acid may be part of a vector. In a specific embodiment, the vector is an expression vector that is capable of directing the expression of a nucleic acid encoding a polypeptide described herein. Non-limiting examples of expression vectors include, but are not limited to, plasmids and viral vectors, such as replication defective retroviruses, adenoviruses, adeno-associated viruses, Newcastle disease virus, vaccinia virus and baculoviruses. Standard molecular biology techniques may be used to introduce a nucleic acid encoding a polypeptide described herein into an expression vector.

[00355] An expression vector comprises a nucleic acid encoding a polypeptide described herein in a form suitable for expression of the nucleic acid in a host cell or non-human subject. In a specific embodiment, an expression vector includes one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid to be expressed. Within an expression vector, "operably linked" is intended to mean that a nucleic acid of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleic acid (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). Regulatory sequences include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Regulatory sequences include those which direct constitutive expression of a nucleic acid in many types of host cells, those which direct expression of the nucleic acid only in certain host cells (*e.g.*, tissue-specific regulatory sequences), and those which direct the expression of the nucleic acid upon stimulation with a particular agent (*e.g.*, inducible regulatory sequences). It will be appreciated

by those skilled in the art that the design of the expression vector can depend on such factors as, *e.g.*, the choice of the host cell to be transformed, the level of expression of protein desired, *etc.*

[00356] An expression vector can be introduced into host cells via conventional transformation or transfection techniques. Such techniques include, but are not limited to, calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, and electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al., 1989, Molecular Cloning - A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, New York, and other laboratory manuals. In certain embodiments, a host cell is transiently transfected with an expression vector containing a nucleic acid encoding a polypeptide described herein. In other embodiments, a host cell is stably transfected with an expression vector containing a nucleic acid encoding a polypeptide described herein.

[00357] Cells containing any of the polynucleotide may be selected using one or more selectable markers.

5.4. Methods of Treating Hematological Disorders or Solid Tumors

[00358] Provided herein are methods of treating a hematological disorder or a solid tumor using NK cells or genetically modified NK cells (*e.g.,* NK cells comprising a CAR and/or a homing receptor) as described above.

5.4.1. NK Combination Therapies

[00359] In one aspect, provided herein are methods of treating a hematological disorder or a solid tumor in a subject in need thereof, comprising: (a) administering to said subject an isolated population of natural killer (NK) cells or a pharmaceutical composition thereof, or an isolated population of genetically modified NK cells (*e.g.,* NK cells comprising a CAR and/or a homing receptor) or a pharmaceutical composition thereof; and (b) administering to said subject a second agent or a pharmaceutical composition thereof. The second agent can be any pharmaceutically acceptable agent that can be used to treat the hematological disorder or the solid tumor, and includes, but is not limited to, an antibody (*e.g.,* a monoclonal antibody), a bispecific killer cell engager (BiKE), an anti-inflammatory agent, an immunomodulatory agent (*e.g.*, an immunmodulatory compound as described in section 5.2.7.1), a cytotoxic agent, a cancer vaccine, a chemotherapeutic agent, an HDAC inhibitor, or an siRNA.

5.4.1.1. NK Combinations with Antibodies

[00360] In certain embodiments, the second agent is an antibody or antigen-binding fragment thereof.

[00361] As used herein, the terms "antibody" and "immunoglobulin" and "Ig" are terms of art and can be used interchangeably herein and refer to a molecule with an antigen binding site that specifically binds an antigen.

[00362] Antibodies can include, for example, monoclonal antibodies, recombinantly produced antibodies, monospecific antibodies, multispecific antibodies (including bispecific antibodies), human antibodies, humanized antibodies, such as composite human antibodies or deimmunized antibodies, murine antibodies (*e.g.*, mouse or rat antibodies), chimeric antibodies, synthetic antibodies, and tetrameric antibodies comprising two heavy chain and two light chain molecules. In specific embodiments, antibodies can include, but are not limited to an antibody light chain monomer, an antibody heavy chain monomer, an antibody light chain dimer, an antibody heavy chain dimer, an antibody light chain- antibody heavy chain pair, intrabodies, heteroconjugate antibodies, single domain antibodies, and monovalent antibodies. In a specific embodiment, antibodies can include antigen-binding fragments or epitope binding fragments such as, but not limited to, single chain antibodies or single-chain Fvs (scFv) (*e.g.*, including monospecific, bispecific, *etc.*), camelized antibodies, affybodies, Fab fragments, F(ab') fragments, $F(ab')_2$ fragments, and disulfide-linked Fvs (sdFv). In specific embodiments, antibodies described herein refer to monoclonal antibodies.

[00363] Antibodies can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA or IgY), any class, (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 or IgA_2), or any subclass (*e.g.*, IgG_{2a} or IgG_{2b}) of immunoglobulin molecule. In certain embodiments, antibodies described herein are IgG antibodies, or a class (*e.g.*, human IgG_1, IgG_2, or IgG_4) or subclass thereof. In certain embodiments, antibodies described herein are IgG_2 antibodies (*e.g.*, human IgG_2) or a subclass thereof (*e.g.*, human IgG_{2a} or human IgG_{2b}, or a mixture thereof). In certain embodiments, antibodies described herein are IgG_1 antibodies (*e.g.*, human IgG_1) or a subclass thereof. In certain embodiments, IgG_1 antibodies described herein comprise one or more amino acid substitutions and/or deletions in the constant region.

[00364] As used herein, the term "monoclonal antibody" is a well known term of art that refers to an antibody obtained from a population of homogenous or substantially homogeneous

antibodies. The term "monoclonal" is not limited to any particular method for making the antibody. Generally, a population of monoclonal antibodies can be generated by cells, a population of cells, or a cell line. In specific embodiments, a "monoclonal antibody," as used herein, is an antibody produced by a single cell or cell line wherein the antibody immunospecifically binds to an epitope as determined, *e.g.,* by ELISA or other antigen-binding or competitive binding assay known in the art. In particular embodiments, a monoclonal antibody can be a chimeric antibody or a humanized antibody. In certain embodiments, a monoclonal antibody is a monovalent antibody or multivalent (*e.g.*, bivalent) antibody.

[00365] In specific embodiments, the antibody or antigen-binding fragment thereof specifically binds to a tumor-associated antigen (TAA), which is described in Section 5.3.2. In a further specific embodiment, the antibody or antigen-binding fragment thereof binds to CS-1. In a more specific embodiment, the antibody or antigen-binding fragment thereof is elotuzumab, or an antigen-binding fragment thereof. In a further specific embodiment, the antibody or antigen-binding fragment thereof binds to CD20.

[00366] In specific embodiments, the antibody or antigen-binding fragment thereof specifically binds to a tumor microenvironment-associated antigen (TMAA), which is described in Section 5.3.2.

[00367] In specific embodiments, the antibody or antigen-binding fragment thereof specifically binds to and antagonizes the activity of an immune checkpoint protein. In more specific embodiments, the immune checkpoint protein is CTLA-4, PD-1, PD-L1, PD-L2, or LAG-3. In more specific embodiments, the immune checkpoint-related protein is BTLA, KIR, TIM-3, A2aR, B7-H3, or B7-H4. In other specific embodiments, the antibody or antigen-binding fragment thereof specifically binds to and antagonizes the activity of a costimulatory signaling protein. In more specific embodiments, the costimulatory signaling protein is ICOS, CD28, 4-1BB, OX40, CD27, or CD40.

5.4.1.2. NK Combinations with Bispecific Killer Cell Engagers

[00368] In certain embodiments, the second agent is a bispecific killer cell engager (BiKE).

[00369] BiKEs are reagents that contain two single chain variable fragments (scFvs) and specifically engage both target cells (*e.g.*, tumor cells or infected cells) and NK cells to mediate target cell killing. They are used to colocalize target cells (*e.g.*, tumor cells or infected cells) with NK cells, and thereby triggering NK-cell mediated antibody-dependent cellular cytotoxicity

(ADCC). BiKEs can be generated by any method known in the art, for example, as described in Gleanson, M. K., et al., Mol Cancer Ther, 11: 2674-2684 (2012); Vallera, D. A., et al., Cancer Biother Radiopharm, 28: 274-282 (2013); Wiernik, A., et al., Clin Cancer Res, 19: 3844-3855 (2013); Reiners, K. S., et al., Mol Ther, 21: 895-903 (2013); Singer, H., et al., J Immunother, 33: 599-608 (2010); or Gleason, M. K., et al., Blood, 123: 3016-3026 (2014). One scFv of BiKE specifically binds to an antigen on the surface of target cells (*e.g.*, tumor cells or infected cells), and the other scFv specifically binds to a receptor (*e.g.*, an Fc receptor, such as CD16) on NK cells.

[00370] In specific embodiments, the BiKE comprises a first scFv that specifically binds to a TAA, which is described in Section 5.3.2. In further specific embodiments, the BiKE comprises a second scFv that specifically binds to CD16.

5.4.1.3. NK Combinations with Other Anti Cancer Agents

[00371] Other anticancer agents that can be administered as the second agent are well-known in the art and include anti-inflammatory agents, immumodulatory agents, cytotoxic agents, cancer vaccines, chemotherapeutics, HDAC inhibitors, 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); CC-122; CC-486 (oral azacididine); 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; lenalidomide; 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; pomalidomide; 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; thalidomide; 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.

[00372] Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 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; 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; 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-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.

[00373] In a specific embodiment, the anticancer agent that is administered as the second agent is thalidomide, lenalidomide, pomalidomide, CC-122, azacitidine, decitabine or CC-486 (oral azacididine). In a more specific embodiment, the anticancer agent that is administered as the second agent is lenalidomide or pomalidomide. In a specific embodiment, the anticancer agent that is administered as the second agent is an immunmodulatory compound (*e.g.*, an immunmodulatory compound as described in section 5.2.7.1). In a specific embodiment, the anticancer agent that is administered as the second agent is romidepsin.

5.4.2. Treatments Using Genetically Modified NK Cells

[00374] In another aspect, provided herein are methods of treating a hematological disorder or a solid tumor in a subject in need thereof, comprising administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof, wherein the NK cells are genetically modified (*e.g.*, comprising a chimeric antigen receptor (CAR) and/or a homing receptor, wherein said CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and optionally a co-stimulatory domain).

[00375] The genetically modified NK cells (e.g., NK cells comprising a CAR and/or a homing receptor) are described in Section 5.3.

5.4.3. Hematological Disorders and Solid Tumors

[00376] In specific embodiments, the hematological disorder is a hematological hyperproliferative disorder. In specific embodiments, the hematological disorder is a hematological cancer, *e.g.*, a leukemia or a lymphoma. In more specific embodiments, the hematological 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 hematological cancer is acute myelogenous leukemia (AML). In another specific embodiment, the hematological cancer is chronic lymphocytic leukemia (CLL). In another specific embodiment, the hematological cancer is multiple myeloma or myelodysplastic syndrome.

[00377] The solid tumor can be, but is not limited to, *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 solid tumor is prostate cancer.

[00378] In certain embodiments, the individual having a hematological 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 hematological cancer or said solid tumor. In certain other embodiments, the bone marrow transplant was in treatment of a condition other than said hematological cancer or said solid tumor. 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 graft-versus-host disease (GVHD) has manifested.

[00379] In certain specific embodiments, the individual having a hematological cancer or solid tumor 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 hematological cancer or said solid tumor. 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 hematological cancer or solid tumor, for example, a blood cancer, exhibits a Philadelphia chromosome.

[00380] In certain other embodiments, a hematological cancer or a solid tumor, in said individual is refractory to one or more anticancer drugs. In a specific embodiment, the hematological cancer or solid tumor is refractory to GLEEVEC® (imatinib mesylate).

[00381] In certain embodiments, a hematological cancer or a solid tumor, 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 activated NK, or TSPNK cells described herein, and/or combinations thereof, and optionally an immunomodulatory compound (*e.g.*, an immunmodulatory compound as described in section 5.2.7.1), are added as adjunct treatments or as a combination therapy with said anticancer drug. In certain other embodiments, the individual having a hematological cancer or a solid tumor, 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.

[00382] In certain embodiments, NK cells are isolated from a tumor lesion, *e.g.*, are tumor-infiltrating lymphocytes; such NK cells are expected to be specific for a tumor-associated antigen (TAA) or a tumor microenvironment-associated antigen (TMAA).

[00383] In one embodiment, provided herein is a method of treating an individual having multiple myeloma, comprising administering to the individual (1) lenalidomide or pomalidomide and (2) CAR NK cells, wherein said CAR NK cells are effective to treat multiple myeloma in said individual. In a specific embodiment, said CAR 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 CAR NK cells have been produced by a two or three-stage method described herein for producing NK cells. In another embodiment, said lenalidomide or pomalidomide, and

CAR NK cells are administered separately from each other. In certain specific embodiments of the method of treating an individual with multiple myeloma, said CAR NK cells comprise a CAR extracellular domain, which extracellular domain is a CS-1 binding domain. In specific embodiments, the CS-1 binding domain comprises an scFv or antigen-binding fragment of an antibody that binds CS-1. In certain specific embodiments, the CS-1 binding domain comprises a single-chain version of elotuzumab and/or an antigen-binding fragment of elotuzumab.

[00384] In one embodiment, provided herein is a method of treating an individual having multiple myeloma, comprising administering to the individual (1) lenalidomide or pomalidomide; (2) elotuzumab; and (3) CAR NK cells, wherein said CAR NK cells are effective to treat multiple myeloma in said individual. In a specific embodiment, said CAR 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 CAR NK cells have been produced by a two or three-stage method described herein for producing NK cells. In another embodiment, said lenalidomide or pomalidomide, elotuzumab, and/or CAR NK cells are administered separately from each other. In certain specific embodiments of the method of treating an individual with multiple myeloma, said CAR NK cells comprise a CAR extracellular domain, which extracellular domain is a CS-1 binding domain. In specific embodiments, the CS-1 binding domain comprises an scFv or antigen-binding fragment of an antibody that binds CS-1.

[00385] In another embodiment, provided herein is a method of treating an individual having a blood cancer (*e.g.*, Burkitt's lymphoma), comprising administering to the individual (1) romidepsin and (2) CAR NK cells, wherein said CAR NK cells are effective to treat the blood cancer (*e.g.*, Burkitt's lymphoma) in said individual. In certain specific embodiments of the method of treating an individual with blood cancer (*e.g.*, Burkitt's lymphoma), said CAR NK cells comprise a CAR extracellular domain, which extracellular domain is a CD20 binding domain. In specific embodiments, the CD20 binding domain comprises an scFv or antigen-binding fragment of an antibody that binds CD20.

5.5. Methods of Treating Infectious Diseases

[00386] Provided herein are methods of treating an infectious disease using NK cells or genetically modified NK cells (*e.g.,* NK cells comprising a CAR and/or a homing receptor) as described above.

5.5.1. Treatment of Infectious Diseases Using NK Combination Therapies

[00387] In another aspect, provided herein are methods of treating an infectious disease in a subject in need thereof, comprising: (a) administering to said subject an isolated population of natural killer (NK) cells or a pharmaceutical composition thereof, or an isolated population of genetically modified NK cells (*e.g.,* NK cells comprising a CAR and/or a homing receptor) or a pharmaceutical composition thereof; and (b) administering to said subject a second agent or a pharmaceutical composition thereof. The second agent can be any pharmaceutically acceptable agent that can be used to treat the infectious disease, and includes, but is not limited to, an antibody (*e.g.,* a monoclonal antibody), a bispecific killer cell engager (BiKE), or an antiviral agent.

5.5.1.1. Antibodies that Binds to an Immune Checkpoint Protein

[00388] In certain embodiments, the second agent is an antibody or antigen-binding fragment thereof (see Section 5.4.1.1 for description of antibodies). In specific embodiments, the antibody specifically binds to and antagonizes activity of an immune checkpoint protein, immune checkpoint-related protein, or costimulatory signaling protein as described in Section 5.4.1.1.

5.5.1.2. Bispecific Killer Cell Engager

[00389] In certain embodiments, the second agent is a BiKE, as described in Section 5.4.1.2.

5.5.1.3. Antiviral Agent

[00390] In certain embodiments, the second agent is an antiviral agent, which includes, but is 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; or non-nucleoside reverse transcriptase inhibitors such as nevirapine, or efavirenz.

5.5.2. Treatment of Infectious Diseases Using Genetically Modified NK Cells

[00391] In another aspect, provided herein are methods of treating an infectious disease in a subject in need thereof, comprising administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof, wherein the NK cells are genetically modified

(*e.g.*, comprising a chimeric antigen receptor (CAR) and/or a homing receptor comprise a chimeric antigen receptor (CAR), wherein said CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and optionally a co-stimulatory domain).

[00392] Genetically modified NK cells (e.g., NK cells comprising a CAR and/or a homing receptor) are described in Section 5.3.

5.5.3. Infectious Disease

[00393] In certain embodiments, the infectious disease is an infection caused by a virus, a bacterium, a fungus, or a helminth. In specific embodiments, the infectious disease is a viral infection.

[00394] 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.

[00395] 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.

[00396] 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).

[00397] In other embodiments, the NK cells are administered to the subject 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.

5.6. Administration

[00398] The NK cells, the genetically modified NK cells, or the second agent as described herein, may be administered to an individual, *e.g.*, an individual having tumor cells or infected cells, by any medically-acceptable route known in the art suitable to the administration of live cells or the second agent. In various embodiments, the cells may be surgically implanted, injected, infused, *e.g.*, by way of a catheter or syringe, or otherwise administered directly or indirectly to the site in need thereof. In various embodiments, the second agent may be injected, infused, *e.g.*, by way of a catheter or syringe, or otherwise administered directly or indirectly to the site in need thereof. In one embodiment, the cells or the second agent are administered to an individual intravenously. In another embodiment, the cells or the second agent are administered to the individual at the site of a tumor, *e.g.*, a solid tumor, or an infection. In a specific embodiment in which the individual has a tumor or an infection at more than one site, the cells or

the second agent are administered to at least two, or all, tumor/infection sites. In certain other embodiments, the cells or the second agent, or compositions thereof, are administered orally, nasally, intraarterially, parenterally, ophthalmically, intramuscularly, subcutaneously, intraperitoneally, intracerebrally, intraventricularly, intracerebroventricularly, intrathecally, intracisternally, intraspinally and/or perispinally. In specific embodiments, the cells or the second agent, or compositions thereof, are administered by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration. In certain specific embodiments, the cells or the second agent are delivered via intracranial or intravertebral needles and/or catheters with or without pump devices.

[00399] In specific embodiments, the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection. 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 NK cells is by injection by syringe. In specific embodiments, administration of NK cells by injection is aided by laparoscopy, endoscopy, ultrasound, computed tomography, magnetic resonance, or radiology.

[00400] The NK cells, the genetically modified NK cells, or the second agent, can be administered to an individual in a composition, *e.g.*, a matrix, hydrogel, scaffold, or the like.

[00401] In one embodiment, the cells are seeded onto a natural matrix, *e.g.*, a placental biomaterial such as an amniotic membrane material. Such an amniotic membrane material can be, *e.g.*, amniotic membrane dissected directly from a mammalian placenta; fixed or heat-treated amniotic membrane, substantially dry (*i.e.*, <20% H_2O) amniotic membrane, chorionic membrane, substantially dry chorionic membrane, substantially dry amniotic and chorionic membrane, and the like. Preferred placental biomaterials on which placental stem cells can be seeded are described in Hariri, U.S. Application Publication No. 2004/0048796, the disclosure of which is hereby incorporated by reference in its entirety.

[00402] In another embodiment, the cells are suspended in a hydrogel solution suitable for, *e.g.*, injection. Suitable hydrogels for such compositions include self-assembling peptides, such as RAD16. In one embodiment, a hydrogel solution comprising the cells can be allowed to harden, for instance in a mold, to form a matrix having cells dispersed therein for implantation. The cells in such a matrix can also be cultured so that the cells are mitotically expanded prior to

implantation. The hydrogel can be, for example, an organic polymer (natural or synthetic) that is cross-linked via covalent, ionic, or hydrogen bonds to create a three-dimensional open-lattice structure that entraps water molecules to form a gel. Hydrogel-forming materials include polysaccharides such as alginate and salts thereof, peptides, polyphosphazines, and polyacrylates, which are crosslinked ionically, or block polymers such as polyethylene oxide-polypropylene glycol block copolymers which are crosslinked by temperature or pH, respectively. In some embodiments, the hydrogel or matrix is biodegradable.

[00403] In some embodiments, the formulation used in the present invention comprises an *in situ* polymerizable gel (*see.*, *e.g.*, U.S. Patent Application Publication 2002/0022676; Anseth *et al.*, *J. Control Release*, 78(1-3):199-209 (2002); Wang *et al.*, *Biomaterials*, 24(22):3969-80 (2003).

[00404] In some embodiments, the polymers are at least partially soluble in aqueous solutions, such as water, buffered salt solutions, or aqueous alcohol solutions, that have charged side groups, or a monovalent ionic salt thereof. Examples of polymers having acidic side groups that can be reacted with cations are poly(phosphazenes), poly(acrylic acids), poly(methacrylic acids), copolymers of acrylic acid and methacrylic acid, poly(vinyl acetate), and sulfonated polymers, such as sulfonated polystyrene. Copolymers having acidic side groups formed by reaction of acrylic or methacrylic acid and vinyl ether monomers or polymers can also be used. Examples of acidic groups are carboxylic acid groups, sulfonic acid groups, halogenated (preferably fluorinated) alcohol groups, phenolic OH groups, and acidic OH groups.

[00405] The cells can be seeded onto a three-dimensional framework or scaffold and implanted *in vivo*. Such a framework can be implanted in combination with any one or more growth factors, cells, drugs or other components that stimulate tissue formation or otherwise enhance or improve the practice of the methods described herein.

[00406] Examples of scaffolds that can be used in the present invention include nonwoven mats, porous foams, or self assembling peptides. Nonwoven mats can be formed using fibers comprised of a synthetic absorbable copolymer of glycolic and lactic acids (*e.g.*, PGA/PLA) (VICRYL, Ethicon, Inc., Somerville, N.J.). Foams, composed of, *e.g.*, poly(ε-caprolactone)/poly(glycolic acid) (PCL/PGA) copolymer, formed by processes such as freeze-drying, or lyophilization (*see*, *e.g.*, U.S. Pat. No. 6,355,699), can also be used as scaffolds.

[00407] The cells can also be seeded onto, or contacted with, a physiologically-acceptable

ceramic material including, but not limited to, mono-, di-, tri-, alpha-tri-, beta-tri-, and tetra-calcium phosphate, hydroxyapatite, fluoroapatites, calcium sulfates, calcium fluorides, calcium oxides, calcium carbonates, magnesium calcium phosphates, biologically active glasses such as BIOGLASS®, and mixtures thereof. Porous biocompatible ceramic materials currently commercially available include SURGIBONE® (CanMedica Corp., Canada), ENDOBON® (Merck Biomaterial France, France), CEROS® (Mathys, AG, Bettlach, Switzerland), and mineralized collagen bone grafting products such as HEALOS™ (DePuy, Inc., Raynham, MA) and VITOSS®, RHAKOSS™, and CORTOSS® (Orthovita, Malvern, Pa.). The framework can be a mixture, blend or composite of natural and/or synthetic materials.

[00408] In another embodiment, cells can be seeded onto, or contacted with, a felt, which can be, *e.g.*, composed of a multifilament yarn made from a bioabsorbable material such as PGA, PLA, PCL copolymers or blends, or hyaluronic acid.

[00409] The cells can, in another embodiment, be seeded onto foam scaffolds that may be composite structures. Such foam scaffolds can be molded into a useful shape, such as that of a portion of a specific structure in the body to be repaired, replaced or augmented. In some embodiments, the framework is treated, *e.g.*, with 0.1M acetic acid followed by incubation in polylysine, PBS, and/or collagen, prior to inoculation of the cells described herein in order to enhance cell attachment. External surfaces of a matrix may be modified to improve the attachment or growth of cells and differentiation of tissue, such as by plasma-coating the matrix, or addition of one or more proteins (*e.g.*, collagens, elastic fibers, reticular fibers), glycoproteins, glycosaminoglycans (*e.g.*, heparin sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate, keratin sulfate, *etc.*), a cellular matrix, and/or other materials such as, but not limited to, gelatin, alginates, agar, agarose, and plant gums, and the like.

[00410] In some embodiments, the scaffold comprises, or is treated with, materials that render it non-thrombogenic. These treatments and materials may also promote and sustain endothelial growth, migration, and extracellular matrix deposition. Examples of these materials and treatments include but are not limited to natural materials such as basement membrane proteins such as laminin and Type IV collagen, synthetic materials such as EPTFE, and segmented polyurethaneurea silicones, such as PURSPAN™ (The Polymer Technology Group, Inc., Berkeley, Calif.). The scaffold can also comprise anti-thrombotic agents such as heparin; the scaffolds can also be treated to alter the surface charge (*e.g.*, coating with plasma) prior to

seeding with placental stem cells.

[00411] In specific embodiments, the NK cells, the genetically modified NK cells, or the second agent is administered with a pharmaceutical carrier. The pharmaceutical carrier can be any known in the art. In specific embodiments, the NK cells or the genetically modified NK cells are fucosylated on the cell surface.

[00412] Determination of the number of NK cells or genetically modified NK cells (e.g., NK cells comprising a CAR and/or a homing receptor), or the amount of the second agent can be performed independently. Such determination can be based on the condition of the subject and can be made by the physician.

[00413] In certain embodiments, the NK cells, the genetically modified NK cells, or the second agent, is 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. 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} cells. In other embodiments, cells 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} cells. In other embodiments, cells 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 cells. Cells can be administered to such an individual according to an approximate ratio between a number of NK cells or genetically modified NK cells and optionally placental perfusate cells, and a number of tumor/infected cells in said individual (*e.g.*, an estimated number). For example, NK cells or the genetically modified NK cells 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/infected cells in the individual. The number of tumor/infected cells in such an individual can be estimated, *e.g.*, by counting the number of tumor/infected 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.

[00414] In a specific embodiment, NK cells (or genetically modified NK cells) are supplemented with placental perfusate cells or placental perfusate. In a specific embodiment, 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 NK cells (or genetically modified NK 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 NK cells (or genetically modified NK cells) per milliliter, are supplemented with about, or at least 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 isolated placental perfusate 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 isolated placental perfusate cells per milliliter. In other more specific embodiments, 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 NK cells (or genetically modified NK 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 NK cells (or genetically modified NK cells) per milliliter 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.

[00415] In another specific embodiment, NK cells (or genetically modified NK 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 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.

[00416] In another specific embodiment, NK cells (or genetically modified NK 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 (or genetically modified NK cells). In certain embodiments, the tissue culture plastic-adherent placental cells are the multipotent adherent placental cells described in U.S. Patent No. 7,468,276 and U.S. Patent Application Publication No. 2007/0275362, 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 (*e.g.*, an immunmodulatory compound as described in section 5.2.7.1) or thalidomide.

[00417] In another specific embodiment, NK cells (or genetically modified NK cells) 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.

[00418] The NK cells, the genetically modified NK cells, or the second agent can be administered once (*i.e.*, in single dose) to an individual having a viral infection, a hematological disorder, or a solid tumor during a course of therapy; or can be administered multiple times (*i.e.*, in multiple doses), *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 wherein both NK cells (or genetically modified NK cells) and a second agent are used, the second agent and the NK cells (or genetically modified NK cells), 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 second agent can be administered before, after, or at the same time as the NK cells (or genetically modified NK cells). NK cells (or genetically modified NK cells) or a second agent can be administered without regard to whether the NK cells (or genetically modified NK cells) or the second agent have been administered to the individual in the past.

5.7. Patients

[00419] The patient referred to in this disclosure, can be, but is not limited to, a human or non-human vertebrate such as a wild, domestic or farm animal. In certain embodiments, the patient is

a mammal, *e.g.*, a human, a cow, a dog, a cat, a goat, a horse, a sheep, a pig, a rat, or a mouse. In one embodiment, the patient is a human patient.

5.8. Kits

[00420] Provided herein is a pharmaceutical pack or kit comprising one or more containers filled with a composition comprising NK cells or genetically modified NK cells (*e.g.*, NK cells comprising a CAR and/or a homing receptor) described above, and one or more containers filled with a composition comprising a second agent described above. Also provided herein is a pharmaceutical pack or kit comprising one or more containers filled with a composition comprising NK cells comprising a CAR and/or a homing receptor described above. 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.

[00421] The kits encompassed herein can be used in accordance with the methods of treating as provided herein, *e.g.*, methods of treating a hematological cancer, a solid tumor, or a viral infection

6. EXAMPLE

6.1. Example 1: Antibody-Dependent Cellular Cytotoxicity (ADCC) Using Rituximab

[00422] The Example presented herein demonstrates that co-administration of NK cells (here, PiNK cells) and an antibody specific for a cell surface antigen (in this case, CD20), e.g., a tumor-associated antigen increases NK antibody-dependent cell-mediated cytotoxicity (ADCC) of the NK cells.

[00423] The experiments presented herein utilize an anti-CD20 antibody, rituximab, and Daudi cells (Cat#: CCL-213, ATCC), which are high expressers of CD20. Daudi cells were harvested and labeled with PKH26 (Cat#: PKH26GL-1KT , Sigma-Aldrich) (Ferlazzo, G., et al., J Immunol, 172: 1455-1462 (2004); Lehmann, D., et al., Stem Cells Dev, 21: 2926-2938 (2012)), whose lipophilic aliphatic residue inserts into cell plasma membrane. The cells were washed and incubated with rituximab (and human IgG as an isotype control) at different concentrations as indicated in Fig. 1 for 1h at room temperature. After washing three times, 10^4 target cells were placed in 96-well U-bottom tissue culture plates and incubated with cultured NK cells at

various effector-target (E:T) ratios (50:1, 20:1, 10:1 and 2.5:1) in 200 µl RPMI 1640 supplemented with 10% FBS. The Cultures were incubated for 4h at 37°C in 5% CO_2. After incubation, cells were harvested and TO-PRO-3 (Catalog # T3605, Invitrogen), a membrane-impermeable DNA stain, was added to cultures to 0.25µM final concentration followed by FACS analysis using BD FACSCanto II. Cytotoxicity ("% cytotoxicity" in Fig. 1) is expressed as percentage of dead cells ($PKH26^+TO\text{-}PRO\text{-}3^+$) within the total $PKH26^+$ target tumor cells, subtracted by spontaneous cell death.

[00424] Incubating Daudi cells with rituximab increases the cytotoxicity of (PiNK) cells compared to human IgG controls, thereby indicating enhanced cytolytic activity of PiNK cells when accompanied by co-administration of the anti-CD20 antibody (Fig. 1).

6.2. Example 2: Cytotoxicity of Three-Stage NK cells Against Multiple Myeloma

[00425] **Phenotype characterization of MM cell lines and primary MM samples.** Primary multiple myeloma (MM) cells (Tissue Solution, donor IDs: MM285, MM293) or MM tumor cell lines: RPMI8226 (ATCC, Cat# CCL-155) and OPM2 (DSMZ, Cat# ACC-50) cells (1×10^6 each) were used for this assay. Cells were stained with anti-PD-L1 APC (Biolegend, Cat# 329708), anti-CS1 PE-Cy7 (Biolegend, Cat# 331816) and 7-AAD (BD Bioscience, Cat# 559925) according to the manufacturer's protocol. Data were acquired on BD LSRFortessa (BD Biosciences) and analyzed using FLOWJO® software (Tree Star). Data were expressed as % positive cells gated under 7-AAD- single cells. Setting of the % positive gate was done using unstained sample as control.

[00426] **Results.** The expression of PD-L1 and CS-1 on the MM cells lines is shown in Figure 2. The left-most peak in the panels of Figure 2 indicates the control, whereas the right-most peak indicates the sample. The percentage of cells positive for PD-L1 was as follows: 71.6% MM285, 70.7% MM293, 66.2% OPM-2, and 94.4% RPMI8226. The percentage of cells positive for CS-1 was as follows: 31.8% MM285, 58.8% MM293, 93.4% OPM-2, and 29.5% RPMI8226.

[00427] **24-hour Cytotoxicity assay of three-stage NK cells against MM cell lines and primary MM samples.** OPM2 cells were labeled with 10 µM PKH26 fluorescent dye (Sigma-Aldrich, Cat# PKH26-GL) prior to co-culture with three-stage NK cells from five different donors at an effector to target (E:T) ratio of 3:1 (3×10^5 and 1×10^5 three-stage NK and OPM2

cells, respectively) in 1 mL of RPMI1640 supplemented with 10% FBS and antibiotics (Basal medium), or the experimetnal conditions: IL-15 (5 ng/mL) (Invitrogen, Cat# PHC9153); IL-2 (200 IU/mL) (Invitrogen, Cat# PHC0023); anti-PD-L1 (10ng/mL) (Affymetrix, Cat# 16-5983-82); anti-IgG (10ng/mL) (Affymetrix, Cat# 16-4714-82); REVLIMID® (lenalidomide; 1uM), or DMSO (0.1%) in 48-well plates. Target cells alone were plated as controls. After incubation for 24 hours at 37° C and 5% CO_2, cells were harvested, followed by staining with 1 μM TO-PRO-3 to identify the dead cells. The number of viable target cells ($PKH26^+TO\text{-}PRO\text{-}3^-$) in each sample was quantified by flow cytometry using counting beads following the protocol provided by the manufacturer (Invitrogen, Cat# C36950). Counting beads were introduced in this assay in order to account for any potential proliferation of tumor cells during the prolonged 24 hour culture.

[00428] Briefly, the number of viable target cells in each sample was calculated as follows: (% $PKH26^+TO\text{-}PRO\text{-}3^-$ live targets) / (% counting beads) x (assigned bead count of the counting bead lot). Percent survival (% survival) in samples (target cells with co-cultures of three-stage NK cells) was calculated by dividing the absolute number of viable, $PKH26^+$, target cells remaining in co-cultures with three-stage NK cells after 24 hours with the absolute number of viable, $PKH26^+$, target cells remaining in culture of target cells alone. Percent cytotoxicity at 24 hours reported was calculated as: 100 - % survival. Results were depicted as mean ± standard deviation of the mean.

[00429] **Results**. Three-stage NK cells displayed cytotoxic activity against different MM cell lines. The three-stage NK cells exerted 20-60% specific lysis against four primary MM samples at an E:T ratio of 3:1 (Figure 3). Varying susceptibility of MM targets from different donors to NK killing was observed. In addition, initial assessment the cytotoxicity of three-stage NK cells against OPM2 indicated an enhancement of cytolytic activity by addition of the cytokines, immunomodulatory compounds, and monoclonal antibodies utilized in these experiments (Figure 4).

EQUIVALENTS

[00430] 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.

[00431] 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 treating a cancer in a subject in need thereof, comprising:
   (a) administering to said subject an isolated population of natural killer (NK) cells or a pharmaceutical composition thereof; and
   (b) administering to said subject a second agent or a pharmaceutical composition thereof, wherein said second agent can be used to treat said cancer.
2. The method of claim 1, wherein the second agent is an antibody or antigen binding fragment thereof that specifically binds to a tumor-associated antigen (TAA).
3. The method of claim 2, wherein the antibody is a monoclonal antibody.
4. The method of claim 2 or 3, wherein the TAA is selected from the group consisting of CD123, CLL-1, CD38, CS-1, CD138, ROR1, FAP, MUC1, PSCA, EGFRvIII, EPHA2, and GD2.
5. The method of claim 1, wherein the second agent is an antibody or antigen binding fragment thereof that specifically binds to a tumor microenvironment-associated antigen (TMAA).
6. The method of claim 5, wherein the antibody is a monoclonal antibody.
7. The method of claim 5 or 6, wherein the TMAA is selected from the group consisting of VEGF-A, EGF, PDGF, IGF, and bFGF.
8. The method of claim 1, wherein the second agent is an antibody or antigen binding fragment thereof that specifically binds to and antagonizes the activity of an immune checkpoint protein.
9. The method of claim 8, wherein the antibody is a monoclonal antibody.
10. The method of claim 8 or 9, wherein the immune checkpoint protein is selected from the group consisting of CTLA-4, PD-1, PD-L1, PD-L2, and LAG-3.
11. The method of claim 1, wherein the second agent is a bispecific killer cell engager (BiKE).
12. The method of claim 11, wherein the BiKE comprises a first single chain variable fragment (scFv) that specifically binds to a TAA.
13. The method of claim 12, wherein the TAA is selected from the group consisting of CD123, CLL-1, CD38, CS-1, CD138, ROR1, FAP, MUC1, PSCA, EGFRvIII, EPHA2, and GD2.

14. The method of any one of claims 11-13, wherein the BiKE comprises a second scFv that specifically binds to CD16
15. The method of claim 1, wherein the second agent is an anti-inflammatory agent.
16. The method of claim 1, wherein the second agent is an immunomodulatory agent.
17. The method of claim 1, wherein the second agent is a cytotoxic agent.
18. The method of claim 1, wherein the second agent is a cancer vaccine.
19. The method of claim 1, wherein the second agent is a chemotherapeutic.
20. The method of claim 1, wherein the second agent is an HDAC inhibitor.
21. The method of claim 1, wherein the second agent is an siRNA.
22. The method of any one of claims 1-21, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered before the second agent or a pharmaceutical composition thereof.
23. The method of any one of claims 1-21, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered after the second agent or a pharmaceutical composition thereof.
24. The method of any one of claims 1-21, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered at the same time as the second agent or a pharmaceutical composition thereof.
25. The method of any one of claims 1-24, wherein the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration.
26. The method of any one of claims 1-25, wherein the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is performed with a devise, a matrix, or a scaffold.
27. The method of any one of claims 1-26, wherein the step of administering to said subject a second agent or a pharmaceutical composition thereof is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration.

28. The method of any one of claims 1-27, wherein the step of administering to said subject a second agent or a pharmaceutical composition thereof is performed with a devise, a matrix, or a scaffold.
29. The method of any one of claims 1-28, wherein the NK cells are fucosylated on the cell surface.
30. The method of any one of claims 1-29, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered in a single dose.
31. The method of any one of claims 1-29, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered in multiple doses.
32. The method of any one of claims 1-31, wherein the second agent or a pharmaceutical composition thereof is administered in a single dose.
33. The method of any one of claims 1-31, wherein the second agent or a pharmaceutical composition thereof is administered in multiple doses.
34. A method of treating a cancer in a subject in need thereof, comprising administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof, wherein the NK cells comprise a chimeric antigen receptor (CAR), wherein said CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and optionally a co-stimulatory domain.
35. The method of claim 34, wherein the CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and a co-stimulatory domain.
36. The method of claim 34 or 35, wherein the NK cells comprising the CAR are derived from CD34+ hematopoietic stem cells (HSCs) that are engineered to express the CAR.
37. The method of any one of claims 34-36, wherein the extracellular domain is an antigen binding domain.
38. The method of claim 37, wherein the antigen binding domain is an scFv domain.
39. The method of claim 37 or 38, wherein the antigen binding domain specifically binds to a TAA.
40. The method of claim 39, wherein the TAA is selected from the group consisting of CD123, CLL-1, CD38, and CS-1.

41. The method of any one of claims 34-40, wherein the intracellular stimulatory domain is a CD3 zeta signaling domain.
42. The method of any one of claims 34-41, wherein the co-stimulatory domain comprises the intracellular domain of CD28, 4-1BB, PD-1, OX40, CTLA-4, NKp46, NKp44, NKp30, DAP10 or DAP12.
43. A method of treating a cancer in a subject in need thereof, comprising administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof, wherein the NK cells comprise a homing receptor.
44. The method of claim 43, wherein the NK cells comprising the homing receptor are derived from CD34+ hematopoietic stem cells (HSCs) that are engineered to express the homing receptor.
45. The method of claim 43 or 44, wherein the homing receptor is a chemotactic receptor,
46. The method of claim 45, wherein the chemotactic receptor is selected from the group consisting of CXCR4, VEGFR2, and CCR7.
47. A method of treating a cancer in a subject in need thereof, comprising administering to said subject an isolated population of Natural Killer (NK) cells or a pharmaceutical composition thereof, wherein the NK cells comprise a chimeric antigen receptor (CAR) and a homing receptor, wherein said CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and optionally a co-stimulatory domain.
48. The method of claim 47, wherein the CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and a co-stimulatory domain.
49. The method of claim 47 or 48, wherein the NK cells comprising the CAR and the homing receptor are derived from CD34+ hematopoietic stem cells (HSCs) that are engineered to express the CAR.
50. The method of any one of claims 47-49, wherein the NK cells comprising the CAR and the homing receptor are derived from CD34+ hematopoietic stem cells (HSCs) that are engineered to express the homing receptor.
51. The method of any one of claims 47-50, wherein the extracellular domain is an antigen binding domain.

52. The method of claim 51, wherein the antigen binding domain is an scFv domain.
53. The method of claim 51 or 52, wherein the antigen binding domain specifically binds to a TAA.
54. The method of claim 53, wherein the TAA is selected from the group consisting of CD123, CLL-1, CD38, and CS-1.
55. The method of any one of claims 47-54, wherein the intracellular stimulatory domain is a CD3 zeta signaling domain.
56. The method of any one of claims 47-55, wherein the co-stimulatory domain comprises the intracellular domain of CD28, 4-1BB, PD-1, OX40, CTLA-4, NKp46, NKp44, NKp30, DAP10 or DAP12.
57. The method of any one of claims 47-56, wherein the homing receptor is a chemotactic receptor,
58. The method of claim 57, wherein the chemotactic receptor is selected from the group consisting of CXCR4, VEGFR2, and CCR7.
59. The method of any one of claims 34-58, wherein the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration.
60. The method of any one of claims 34-59, wherein the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is performed with a devise, a matrix, or a scaffold.
61. The method of any one of claims 34-60, wherein the NK cells are fucosylated on the cell surface.
62. The method of any one of claims 34-61, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered in a single dose.
63. The method of any one of claims 34-61, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered in multiple doses.
64. The method of any one of claims 1-63, wherein the cancer is a hematological cancer.
65. The method of any one of claims 1-63, wherein the cancer is a solid tumor.
66. A method of treating a viral infection in a subject in need thereof, comprising:

(a) administering to said subject an isolated population of Natural Killer (NK) cells or a pharmaceutical composition thereof; and

(b) administering to said subject a second agent or a pharmaceutical composition thereof, wherein said second agent can be used to treat said viral infection.

67. The method of claim 66, wherein the second agent is an antibody or antigen binding fragment thereof that specifically binds to and antagonizes the activity of an immune checkpoint protein.

68. The method of claim 67, wherein the antibody is a monoclonal antibody.

69. The method of claim 67 or 68, wherein the immune checkpoint protein is selected from the group consisting of CTLA-4, PD-1, PD-L1, PD-L2, and LAG-3.

70. The method of claim 66, wherein the second agent is a bispecific killer cell engager (BiKE).

71. The method of any one of claims 66-70, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered before the second agent or a pharmaceutical composition thereof.

72. The method of any one of claims 66-70, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered after the second agent or a pharmaceutical composition thereof.

73. The method of any one of claims 66-70, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered at the same time as the second agent or a pharmaceutical composition thereof.

74. The method of any one of claims 66-73, wherein the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration.

75. The method of any one of claims 66-74, wherein the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is performed with a devise, a matrix, or a scaffold.

76. The method of any one of claims 66-75, wherein the step of administering to said subject a second agent or a pharmaceutical composition thereof is by injection, infusion,

intravenous (IV) administration, intrafemoral administration, or intratumor administration.

77. The method of any one of claims 66-76, wherein the step of administering to said subject a second agent or a pharmaceutical composition thereof is performed with a devise, a matrix, or a scaffold.

78. The method of any one of claims 66-77, wherein the NK cells are fucosylated on the cell surface.

79. The method of any one of claims 66-78, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered in a single dose.

80. The method of any one of claims 66-78, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered in multiple doses.

81. The method of any one of claims 66-80, wherein the second agent or a pharmaceutical composition thereof is administered in a single dose.

82. The method of any one of claims 66-80, wherein the second agent or a pharmaceutical composition thereof is administered in multiple doses.

83. A method of treating a viral infection in a subject in need thereof, comprising administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof, wherein the NK cells comprise a chimeric antigen receptor (CAR), wherein said CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and optionally a co-stimulatory domain.

84. The method of claim 83, wherein the CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and a co-stimulatory domain.

85. The method of claim 83 or 84, wherein the NK cells comprising the CAR are derived from CD34+ hematopoietic stem cells (HSCs) that are engineered to express the CAR.

86. The method of any one of claims 83-85, wherein the extracellular domain is an antigen binding domain.

87. The method of claim 86, wherein the antigen binding domain is an scFv domain.

88. The method of any one of claims 83-87, wherein the intracellular stimulatory domain is a CD3 zeta signaling domain.

89. The method of any one of claims 83-88, wherein the co-stimulatory domain comprises the intracellular domain of CD28, 4-1BB, PD-1, OX40, CTLA-4, NKp46, NKp44, NKp30, DAP10 or DAP12.

90. A method of treating a viral infection in a subject in need thereof, comprising administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof, wherein the NK cells comprise a homing receptor.

91. The method of claim 90, wherein the NK cells comprising the homing receptor are derived from CD34+ hematopoietic stem cells (HSCs) that are engineered to express the homing receptor.

92. The method of claim 90 or 91, wherein the homing receptor is a chemotactic receptor,

93. The method of claim 92, wherein the chemotactic receptor is selected from the group consisting of CXCR4, VEGFR2, and CCR7.

94. A method of treating a viral infection in a subject in need thereof, comprising administering to said subject an isolated population of Natural Killer (NK) cells or a pharmaceutical composition thereof, wherein the NK cells comprise a chimeric antigen receptor (CAR) and a homing receptor, wherein said CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and optionally a co-stimulatory domain.

95. The method of claim 94, wherein the CAR comprises an extracellular domain, a transmembrane domain, an intracellular stimulatory domain, and a co-stimulatory domain.

96. The method of claim 94 or 95, wherein the NK cells comprising the CAR and the homing receptor are derived from CD34+ hematopoietic stem cells (HSCs) that are engineered to express the CAR.

97. The method of any one of claims 94-96, wherein the NK cells are produced from CD34+ hematopoietic stem cells (HSCs) that are engineered to express the homing receptor.

98. The method of any one of claims 94-97, wherein the extracellular domain is an antigen binding domain.

99. The method of claim 98, wherein the antigen binding domain is an scFv domain.

100. The method of any one of claims 94-99, wherein the intracellular stimulatory domain is a CD3 zeta signaling domain.

101. The method of any one of claims 94-100, wherein the co-stimulatory domain comprises the intracellular domain of CD28, 4-1BB, PD-1, OX40, CTLA-4, NKp46, NKp44, NKp30, DAP10 or DAP12.
102. The method of any one of claims 94-101, wherein the homing receptor is a chemotactic receptor,
103. The method of claim 102, wherein the chemotactic receptor is selected from the group consisting of CXCR4, VEGFR2, and CCR7.
104. The method of any one of claims 83-103, wherein the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is by injection, infusion, intravenous (IV) administration, intrafemoral administration, or intratumor administration.
105. The method of any one of claims 83-104, wherein the step of administering to said subject an isolated population of NK cells or a pharmaceutical composition thereof is performed with a devise, a matrix, or a scaffold.
106. The method of any one of claims 83-105, wherein the NK cells are fucosylated on the cell surface.
107. The method of any one of claims 83-106, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered in a single dose.
108. The method of any one of claims 83-106, wherein the isolated population of NK cells or a pharmaceutical composition thereof is administered in multiple doses.
109. The method of any one of claims 1-13 and 15-108, wherein the NK cells are placental intermediate natural killer (PiNK) cells.
110. The method of any one of claims 1-108, wherein the NK cells are activated NK cells.
111. The method of any one of claims 1-108, wherein the NK cells are Three-Step Process NK (TSPNK) cells.
112. The method of claim 111, wherein the TSPNK cells are NK progenitor cells.
113. The method of claim 108, wherein the PiNK cells are derived from placental cells.
114. The method of claim 113, wherein the placental cells are obtained from placental perfusate.
115. The method of claim 113, wherein the placental cells are obtained from placental tissue that has been mechanically and/or enzymatically disrupted.

116. The method of claim 110, wherein the activated NK cells are produced by a process comprising:

(a) seeding a population of hematopoietic stem or progenitor cells in a first medium comprising interleukin-15 (IL-15) and, optionally, one or more of stem cell factor (SCF) and interleukin-7 (IL-7), wherein said IL-15 and optional SCF and IL-7 are not comprised within an undefined component of said medium, such that the population expands, and a plurality of hematopoietic stem or progenitor cells within said population of hematopoietic stem or progenitor cells differentiate into NK cells during said expanding; and

(b) expanding the cells from the step (a) in a second medium comprising interleukin-2 (IL-2), to produce a population of activated NK cells.

117. The method of claim 110, wherein the activated NK cells are produced by a process comprising: expanding a population of hematopoietic stem or progenitor cells in a first medium comprising one or more of stem cell factor (SCF), interleukin-7 (IL-7) and interleukin-15 (IL-15), and wherein said SCF, IL-7 and IL-15 are not comprised within an undefined component of said medium, and wherein a plurality of hematopoietic stem or progenitor cells within said population of hematopoietic stem or progenitor cells differentiate into NK cells during said expanding; and wherein a second step of said method comprises expanding the cells from the first step in a second medium comprising interleukin-2 (IL-2), to produce activated NK cells.

118. The method of claim 116, wherein the first medium further comprises one or more of Fms-like-tyrosine kinase 3 ligand (Flt3-L), thrombopoietin (Tpo), interleukin-2 (IL-2), or heparin.

119. The method of claim 118, wherein the first medium further comprises fetal bovine serum or human serum.

120. The method of claim 118, wherein the SCF is present at a concentration of about 1 to about 150 ng/mL in the first medium.

121. The method of claim 118, wherein the Flt3-L is present at a concentration of about 1 to about 150 ng/mL in the first medium.

122. The method of claim 118, wherein the IL-2 is present at a concentration of about 50 to about 1500 IU/mL in the first medium.

123. The method of claim 118, wherein the IL-7 is present at a concentration of about 1 to about 150 ng/mL in the first medium.

124. The method of claim 118, wherein the IL-15 is present at a concentration 1 to about 150 ng/mL in the first medium.

125. The method of claim 118, wherein the Tpo is present at a concentration of about 1 to about 150 ng/mL in the first medium.

126. The method of claim 118, wherein the heparin is present at a concentration of about 0.1 to about 30 U/mL in the first medium.

127. The method of claim 116, wherein said IL-2 in the second step is present at a concentration 50 to about 1500 IU/mL in the second medium.

128. The method of claim 116, wherein said second medium additionally comprises one or more of fetal calf serum (FCS), transferrin, insulin, ethanolamine, oleic acid, linoleic acid, palmitic acid, bovine serum albumin (BSA) and phytohemagglutinin.

129. The method of claim 116, wherein the hematopoietic stem or progenitor cells are $CD34^+$.

130. The method of claim 116, wherein the hematopoietic stem or progenitor cells comprise hematopoietic stem or progenitor cells from human placental perfusate and hematopoietic stem or progenitor cells from umbilical cord, wherein said placental perfusate and said umbilical cord blood are from the same placenta.

131. The method of claim 116, wherein the feeder cells in step (b) comprise mitomycin C-treated peripheral blood mononuclear cells (PBMC), K562 cells or tissue culture-adherent stem cells.

132. The method of claim 116, wherein the NK cells are $CD3^-CD56^+CD16^-$.

133. The method of claim 132, wherein the NK cells are additionally $CD94^+CD117^+$.

134. The method of claim 132, wherein the NK cells are additionally $CD161^-$.

135. The method of claim 132, wherein the NK cells are additionally $NKG2D^+$.

136. The method of claim 132, wherein the NK cells are additionally $NKp46^+$.

137. The method of claim 132, wherein the NK cells are additionally $CD226^+$.

138. The method of claim 111 or 112, wherein the TSPNK cells are produced by a process comprising:

(a) culturing hematopoietic stem cells or progenitor cells in a first medium comprising Flt3L, TPO, SCF, IL-7, G-CSF, IL-6 and GM-CSF;

(b) subsequently culturing said cells in a second medium comprising Flt3L, SCF, IL-15, and IL-7, IL-17 and IL-15, G-CSF, IL-6 and GM –CSF; and

(c) subsequently culturing said cells in a third medium comprising SCF, IL-15, IL-7, IL-2, G-CSF, IL-6 and GM-CSF.

139. The method of claim 138, wherein the duration of culturing step (a) is 7-9 days, wherein the duration of culturing step (b) is 5-7 days, and wherein the duration of culturing step (c) is 5-9 days.

140. The method of claim 138, wherein the duration of culturing step (a) is 7-9 days, wherein the duration of culturing step (b) is 5-7 days, and wherein the duration of culturing step (c) is 21-35 days.

141. The method of claim 138, 139, or 140 wherein the hematopoietic stem or progenitor cells used in the process are CD34+.

142. The method of claim 138, 139, or 140 wherein the hematopoietic stem or progenitor cells comprise hematopoietic stem or progenitor cells from human placental perfusate and hematopoietic stem or progenitor cells from umbilical cord, wherein said placental perfusate and said umbilical cord blood are from the same placenta.

143. The method of any one of claims 138-142, wherein CD34- cells comprise more than 80% of the TSPNK cells at the end of step (a).

144. The method of any one of claims 138-143, wherein the TSPNK cells comprise no more than 40% CD3- CD56+ cells.

145. The method of any one of claims 138-144, wherein the TSPNK cells comprise cells which are CD52+ CD117+.

146. The method of any one of claims 1-108, wherein the NK cells are produced by a process 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 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 80% of the natural killer cells are viable.

147. The method of any one of claims 1-146, wherein the subject is a human.

148. A kit for treating a cancer in a subject in need thereof, comprising:
(a) an isolated population of NK cells or a pharmaceutical composition thereof; and
(b) a second agent or a pharmaceutical composition thereof, wherein said second agent can be used to treat said cancer.

149. The kit of claim 148, wherein the second agent is an antibody or antigen binding fragment thereof that specifically binds to a tumor-associated antigen (TAA).

150. The kit of claim 149, wherein the antibody is a monoclonal antibody.

151. The kit of claim 149 or 150, wherein the TAA is selected from the group consisting of CD123, CLL-1, CD38, CS-1, CD138, ROR1, FAP, MUC1, PSCA, EGFRvIII, EPHA2, and GD2.

152. The kit of claim 148, wherein the second agent is an antibody or antigen binding fragment thereof that specifically binds to a tumor microenvironment-associated antigen (TMAA).

153. The kit of claim 152, wherein the antibody is a monoclonal antibody.

154. The kit of claim 152 or 153, wherein the TMAA is selected from the group consisting of VEGF-A, EGF, PDGF, IGF, and bFGF.

155. The kit of claim 148, wherein the second agent is an antibody or antigen binding fragment thereof that specifically binds to and antagonizes the activity of an immune checkpoint protein.

156. The kit of claim 155, wherein the antibody is a monoclonal antibody.

157. The kit of claim 155 or 156, wherein the immune checkpoint protein is selected from the group consisting of CTLA-4, PD-1, PD-L1, PD-L2, and LAG-3.

158. The method of claim 148, wherein the second agent is a bispecific killer cell engager (BiKE).

159. The kit of claim 158, wherein the BiKE comprises a first single chain variable fragment (scFv) that specifically binds to a TAA.
160. The kit of claim 159, wherein the TAA is selected from the group consisting of CD123, CLL-1, CD38, CS-1, CD138, ROR1, FAP, MUC1, PSCA, EGFRvIII, EPHA2, and GD2.
161. The kit of any one of claims 158-160, wherein the BiKE comprises a second scFv that specifically binds to CD16.
162. The kit of claim 148, wherein the second agent is an anti-inflammatory agent.
163. The kit of claim 148, wherein the second agent is an immunomodulatory agent.
164. The kit of claim 148, wherein the second agent is a cytotoxic agent.
165. The kit of claim 148, wherein the second agent is a cancer vaccine.
166. The kit of claim 148, wherein the second agent is a chemotherapeutic.
167. The kit of claim 148, wherein the second agent is an HDAC inhibitor.
168. The kit of claim 148, wherein the second agent is an siRNA.
169. The kit of any one of claims 148-168, wherein the cancer is a hematological cancer.
170. The method of any one of claims 148-168, wherein the cancer is a solid tumor.
171. A kit for treating a viral infection in a subject in need thereof, comprising:
(a) an isolated population of Natural Killer (NK) cells or a pharmaceutical composition thereof; and
(b) a second agent or a pharmaceutical composition thereof, wherein said second agent can be used to treat said viral infection.
172. The kit of claim 171, wherein the second agent is an antibody or antigen binding fragment thereof that specifically binds to and antagonizes the activity of an immune checkpoint protein.
173. The kit of claim 172, wherein the antibody is a monoclonal antibody.
174. The kit of claim 171 or 172, wherein the immune checkpoint protein is selected from the group consisting of CTLA-4, PD-1, PD-L1, PD-L2, and LAG-3.
175. The method of claim 171, wherein the second agent is a bispecific killer cell engager (BiKE).
176. The kit of any one of claims 148-160 and 162-175, wherein the NK cells are placenta intermediate natural killer (PiNK) cells.
177. The kit of any one of claims 148-175, wherein the NK cells are activated NK cells.

178. The kit of any one of claims 148-175, wherein the NK cells are TSPNK cells.

179. The kit of claim 178, wherein the TSPNK cells are NK progenitor cells.

180. The kit of claim 176, wherein the PiNK cells are derived from placental cells.

181. The kit of claim 180 wherein the placental cells are obtained from placental perfusate.

182. The kit of claim 181, wherein the placental cells are obtained from placental tissue that has been mechanically and/or enzymatically disrupted.

183. The kit of claim 177, wherein the activated NK cells are produced by a process comprising:

(a) seeding a population of hematopoietic stem or progenitor cells in a first medium comprising interleukin-15 (IL-15) and, optionally, one or more of stem cell factor (SCF) and interleukin-7 (IL-7), wherein said IL-15 and optional SCF and IL-7 are not comprised within an undefined component of said medium, such that the population expands, and a plurality of hematopoietic stem or progenitor cells within said population of hematopoietic stem or progenitor cells differentiate into NK cells during said expanding; and

(b) expanding the cells from the step (a) in a second medium comprising interleukin-2 (IL-2), to produce a population of activated NK cells.

184. The kit of claim 177, wherein the activated NK cells are produced by a process comprising: expanding a population of hematopoietic stem or progenitor cells in a first medium comprising one or more of stem cell factor (SCF), interleukin-7 (IL-7) and interleukin-15 (IL-15), and wherein said SCF, IL-7 and IL-15 are not comprised within an undefined component of said medium, and wherein a plurality of hematopoietic stem or progenitor cells within said population of hematopoietic stem or progenitor cells differentiate into NK cells during said expanding; and wherein a second step of said method comprises expanding the cells from the first step in a second medium comprising interleukin-2 (IL-2), to produce activated NK cells.

185. The kit of claim 183, wherein the first medium further comprises one or more of Fms-like-tyrosine kinase 3 ligand (Flt3-L), thrombopoietin (Tpo), interleukin-2 (IL-2), or heparin.

186. The kit of claim 185, wherein the first medium further comprises fetal bovine serum or human serum.

187. The kit of claim 185, wherein the SCF is present at a concentration of about 1 to about 150 ng/mL in the first medium.
188. The kit of claim 185, wherein the Flt3-L is present at a concentration of about 1 to about 150 ng/mL in the first medium.
189. The kit of claim 185, wherein the IL-2 is present at a concentration of about 50 to about 1500 IU/mL in the first medium.
190. The kit of claim 185, wherein the IL-7 is present at a concentration of about 1 to about 150 ng/mL in the first medium.
191. The kit of claim 185, wherein the IL-15 is present at a concentration 1 to about 150 ng/mL in the first medium.
192. The kit of claim 185, wherein the Tpo is present at a concentration of about 1 to about 150 ng/mL in the first medium.
193. The kit of claim 185, wherein the heparin is present at a concentration of about 0.1 to about 30 U/mL in the first medium.
194. The kit of claim 183, wherein said IL-2 in the second step is present at a concentration 50 to about 1500 IU/mL in the second medium.
195. The kit of claim 183, wherein said second medium additionally comprises one or more of fetal calf serum (FCS), transferrin, insulin, ethanolamine, oleic acid, linoleic acid, palmitic acid, bovine serum albumin (BSA) and phytohemagglutinin.
196. The kit of claim 183, wherein the hematopoietic stem or progenitor cells are $CD34^+$.
197. The kit of claim 183, wherein the hematopoietic stem or progenitor cells comprise hematopoietic stem or progenitor cells from human placental perfusate and hematopoietic stem or progenitor cells from umbilical cord, wherein said placental perfusate and said umbilical cord blood are from the same placenta.
198. The kit of claim 183, wherein the feeder cells in step (b) comprise mitomycin C-treated peripheral blood mononuclear cells (PBMC), K562 cells or tissue culture-adherent stem cells.
199. The kit of claim 183, wherein the NK cells are $CD3^-CD56^+CD16^-$.
200. The kit of claim 199, wherein the NK cells are additionally $CD94^+CD117^+$.
201. The kit of claim 199, wherein the NK cells are additionally $CD161^-$.
202. The kit of claim 199, wherein the NK cells are additionally $NKG2D^+$.

203. The kit of claim 199, wherein the NK cells are additionally $NKp46^+$.

204. The kit of claim 199, wherein the NK cells are additionally $CD226^+$.

205. The kit of claim 178 or 179, wherein the TSPNK cells are produced by a process comprising:

(a) culturing hematopoietic stem cells or progenitor cells in a first medium comprising Flt3L, TPO, SCF, IL-7, G-CSF, IL-6 and GM-CSF;

(b) subsequently culturing said cells in a second medium comprising Flt3L, SCF, IL-15, and IL-7, IL-17 and IL-15, G-CSF, IL-6 and GM –CSF; and

(c) subsequently culturing said cells in a third medium comprising SCF, IL-15, IL-7, IL-2, G-CSF, IL-6 and GM-CSF.

206. The kit of claim 205, wherein the duration of culturing step (a) is 7-9 days, wherein the duration of culturing step (b) is 5-7 days, and wherein the duration of culturing step (c) is 5-9 days.

207. The kit of claim 205, wherein the duration of culturing step (a) is 7-9 days, wherein the duration of culturing step (b) is 5-7 days, and wherein the duration of culturing step (c) is 21-35 days.

208. The kit of claim 205, 206, or 207 wherein the hematopoietic stem or progenitor cells used in the process are CD34+.

209. The kit of claim 205, 206, or 207 wherein the hematopoietic stem or progenitor cells comprise hematopoietic stem or progenitor cells from human placental perfusate and hematopoietic stem or progenitor cells from umbilical cord, wherein said placental perfusate and said umbilical cord blood are from the same placenta.

210. The kit of any one of claims 205-209, wherein CD34- cells comprise more than 80% of the TSPNK cells at the end of step (a).

211. The kit of any one of claims 205-210, wherein the TSPNK cells comprise no more than 40% CD3- CD56+ cells.

212. The kit of any one of claims 205-211, wherein the TSPNK cells comprise cells which are CD52+ CD117+.

213. The kit of any one of claims 148-175, wherein the NK cells are produced by a process 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 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 80% of the natural killer cells are viable.

214. The kit of any one of claims 147-213, wherein the subject is a human.

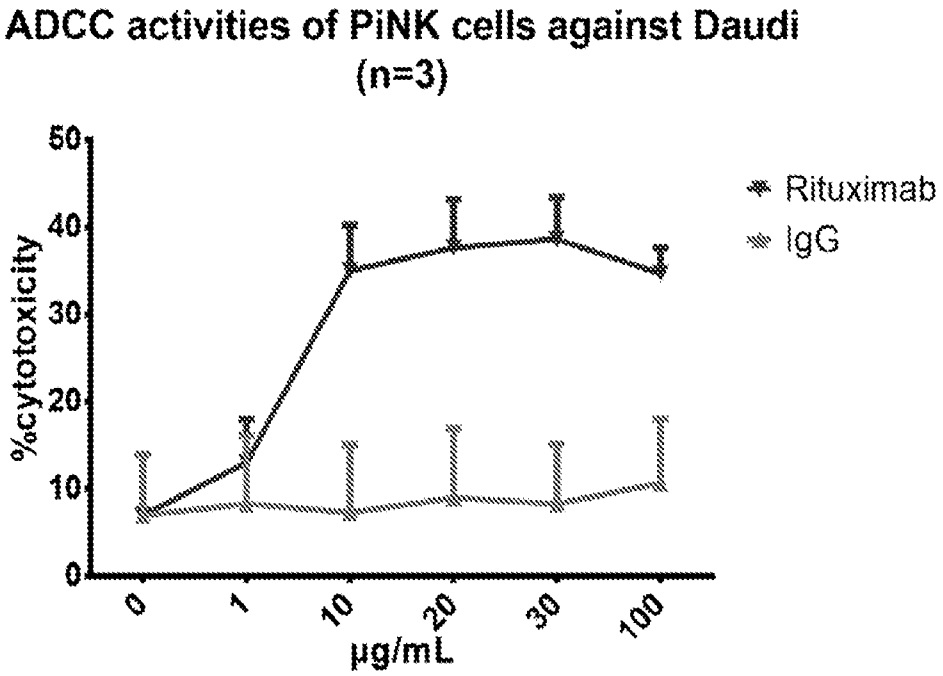
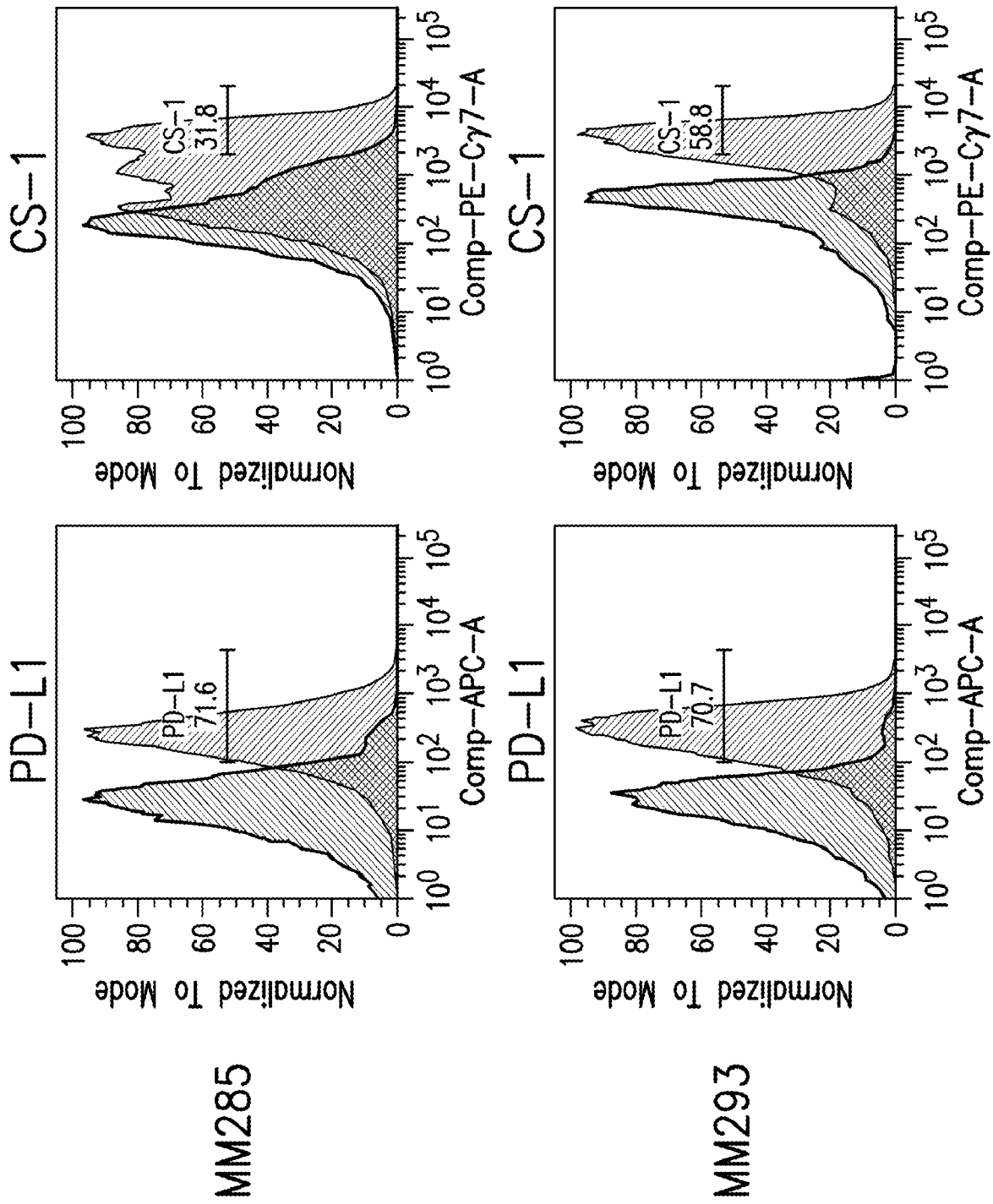

ADCC activities of PiNK cells against Daudi
(n=3)
%cytotoxicity
50
40
30
20
10
0
0
1
10
20
30
100
µg/mL
Rituximab
IgG

Fig. 1


MM285
MM293
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CS-1
PD-L1
71.6
CS-1
31.8
PD-L1
70.7
CS-1
58.8
Normalized To Mode
Comp-APC-A
Comp-PE-Cy7-A
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100
10^0
10^1
10^2
10^3
10^4
10^5

Fig. 2

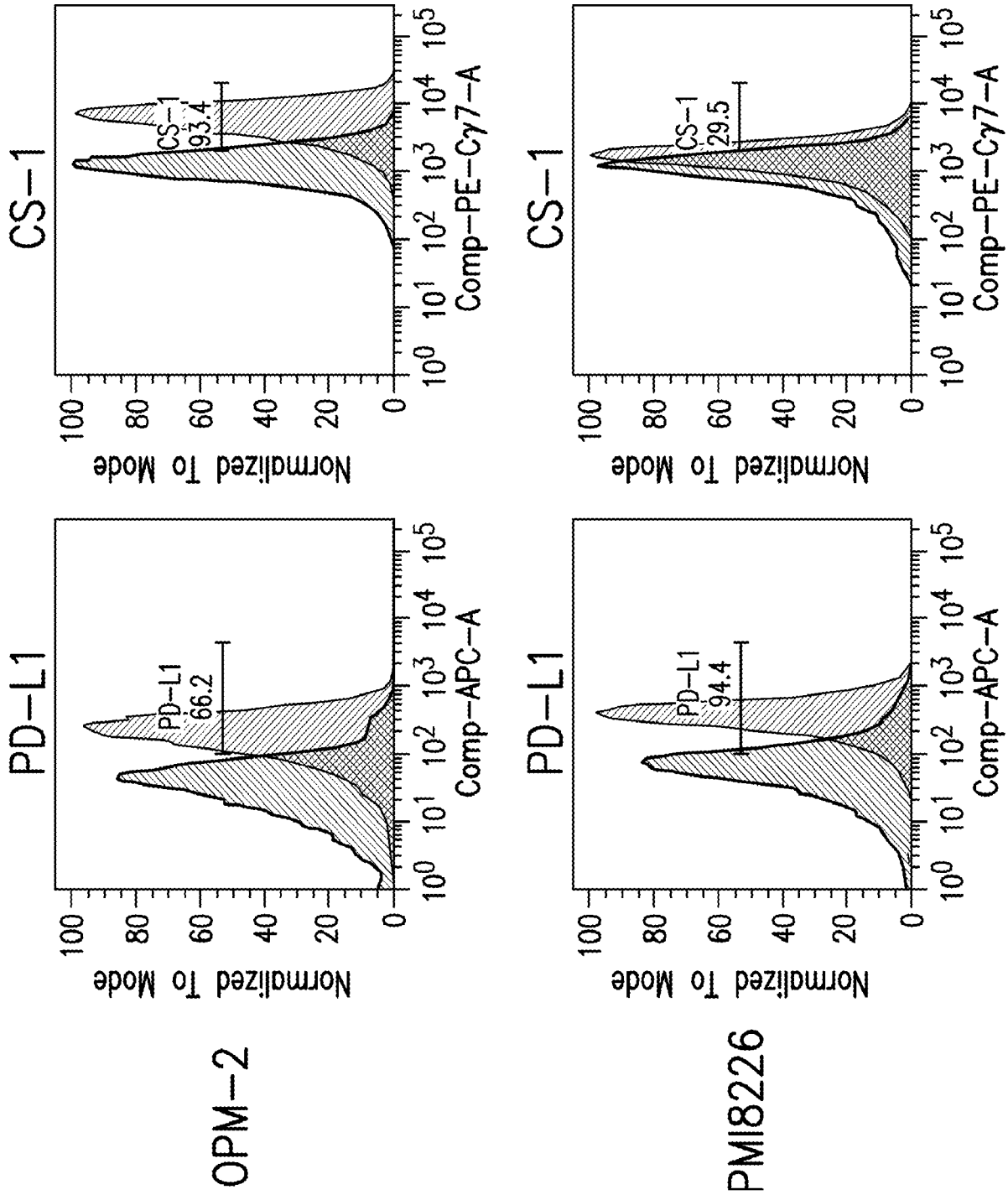

Fig. 2 (continued)

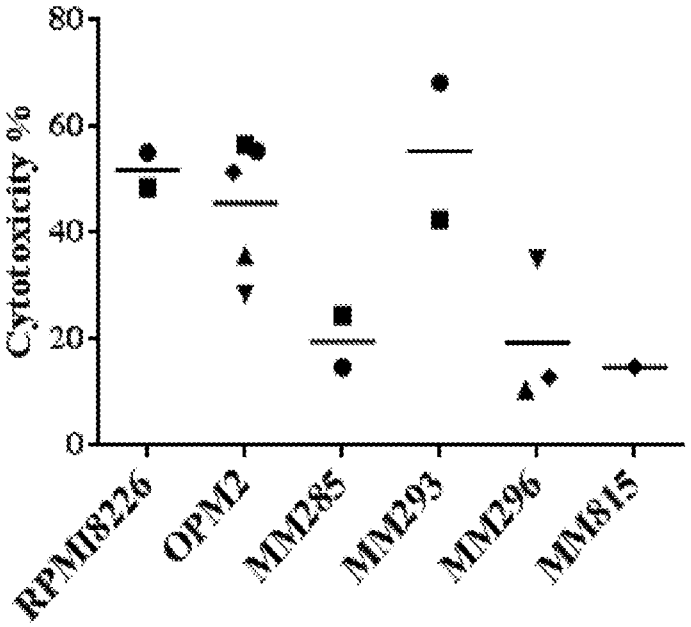
Cytotoxicity %
80
60
40
20
0
RPMI8226
OPM2
MM285
MM293
MM296
MM815

Fig. 3

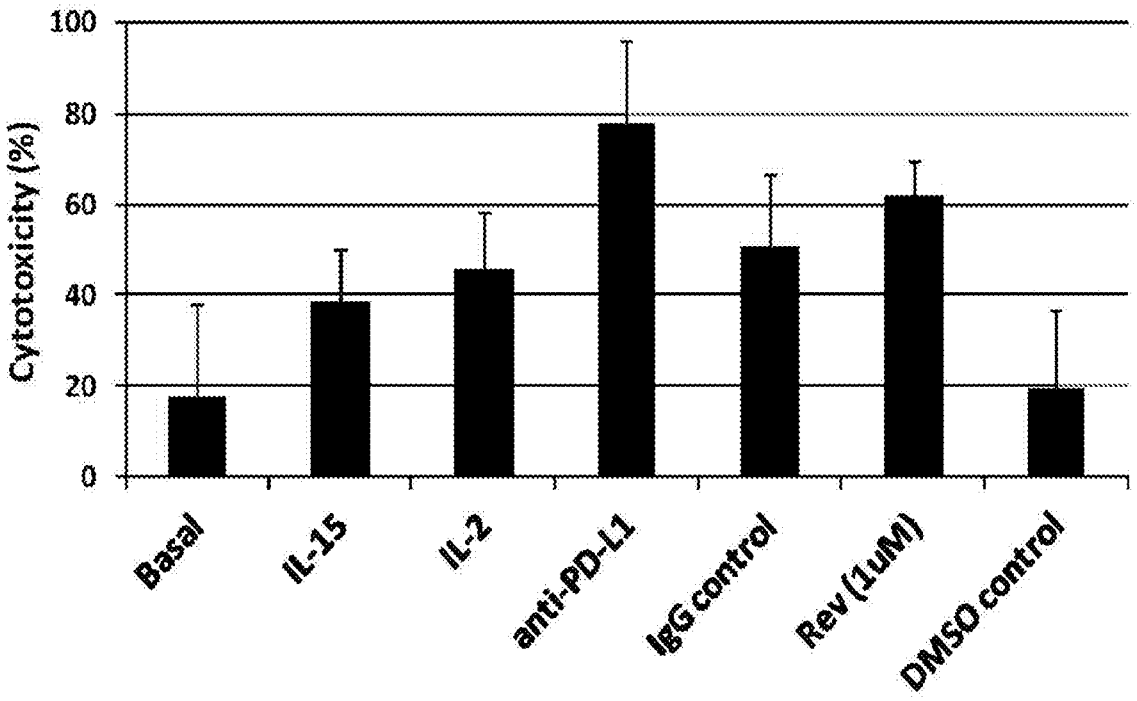
Cytotoxicity (%)
100
80
60
40
20
0
Basal
IL-15
IL-2
anti-PD-L1
IgG control
Rev (1uM)
DMSO control

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/068069

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 35/17 (2016.01)
CPC - A61K 35/17 (2016.02)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 35/17; C07K 14/705, 16/28, 19/00; C12N 5/0783 (2016.01)
CPC - A61K 2039/515, 2039/5156, 35/17, 39/0011, 39/39558; C07K 14/705, 14/70596, 16/28, 19/00, 2317/73, 2317/80, 2319/02, 2319/03; C12N 5/0646 (2016.02)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/328, 372.3; 530/387.3; 536/23.4 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Google Patents, Google Scholar.
Search terms used: NK, NKT, natural killer, chimeric antigen receptor, tumor, microenvironment, method, administration, therapeutic, treatment, injection, infusion, cells, siRNA, VEGFA, EGF, PDGF, IGF, bFGF, BiKE, bispecific, CD16, express, chemotaxis, chemotactic, homing, CXCR4, VEGFR2, CCR7, CTLA4, PD1, PDL1, LAG3, HDAC inhibitor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/0322183 A1 (UNIV PENNSYLVANIA) 30 October 2014 (30.10.2014) entire documnet	1-7, 15-19, 21, 34-36, 66, 83-85, 148-154, 162-166, 168, 171
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Y		8-14, 20, 47-49, 67-70, 94-96, 155-161, 167, 172-175
X	CANY et al. "Natural killer cells generated from cord blood hematopoietic progenitor cells efficiently target bone marrow-residing human leukemia cells in NOD/SCID/IL2Rg(null) mice," PLoS One, 05 June 2013 (05.06.2013), Vol. 8, No.6, e64384, Pg. 1-11. entire document	43-46, 90-93
--		----------
Y		47-49, 94-96
Y	BENSON et al. "The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody," Blood, 11 May 2010 (11.05.2010), Vol. 116, No. 13, Pgs. 2286-94. entire document	8-10, 67-69, 155-157, 172-174
Y	GLEASON et al. "Bispecific and trispecific killer cell engagers directly activate human NK cells through CD16 signaling and induce cytotoxicity and cytokine production," Mol Cancer Ther. 17 October 2012 (17.10.2012), Vol. 11, No. 12, Pgs. 2674-84. entire document	11-14, 70, 158-161, 175
Y	SONG et al. "Chimeric NKG2D CAR-expressing T cell-mediated attack of human ovarian cancer is enhanced by histone deacetylase inhibition," Hum Gene Ther. 01 March 2013 (01.03.2013), Vol. 24, No. 3, Pgs. 295-305. entire document	20, 167
A	WO 2013/123061 A1 (SEATTLE CHILDREN'S HOSPITAL D/B/A SEATTLE CHILDREN'S RESEARCH INSTITUTE) 22 August 2013 (22.08.2013) entire document	1-21, 34-36, 43-49, 66-70, 83-85, 90-96, 148-168, 171-175

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 March 2016	31 MAR 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/068069

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

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

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

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

3. ☒ Claims Nos.: 22-33, 37-42, 50-65, 71-82, 86-89, 97-147, 169, 170, 176-214
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(71)申请人 人类起源公司
地址 美国新泽西州

(72)发明人 康琳 张小葵 杰弗里·哈里斯 弗拉基米尔·扬科维奇

(74)专利代理机构 北京安信方达知识产权代理有限公司 11262
代理人 郑霞

(51)Int.Cl.
A61K 35/17(2006.01)

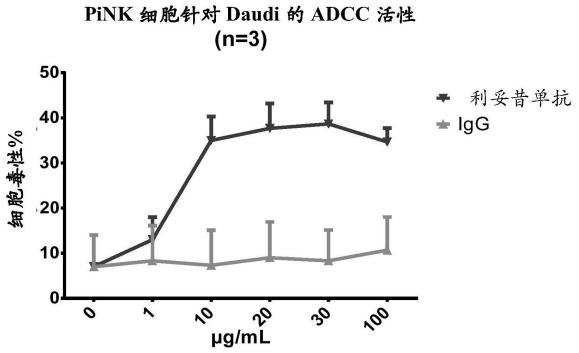
权利要求书11页 说明书99页 附图4页

(54)发明名称

使用自然杀伤细胞治疗血液病症、实体瘤或感染性疾病的方法

(57)摘要

本文提供使用与第二药剂组合的自然杀伤细胞，或使用具有针对靶特异性和/或归巢特异性的遗传修饰的自然杀伤细胞治疗有需要的对象的血液病症、实体瘤或感染性疾病的方法。



CN 107249604 A

1.一种治疗有需要的对象的癌症的方法,所述方法包括:

(a)给予所述对象分离的自然杀伤(NK)细胞群或其药物组合物;和

(b)给予所述对象第二药剂或其药物组合物,其中所述第二药剂可用于治疗所述癌症。

2.权利要求1的方法,其中所述第二药剂是与肿瘤相关抗原(TAA)特异性结合的抗体或其抗原结合片段。

3.权利要求2的方法,其中所述抗体是单克隆抗体。

4.权利要求2或3的方法,其中所述TAA选自CD123、CLL-1、CD38、CS-1、CD138、ROR1、FAP、MUC1、PSCA、EGFRvIII、EPHA2和GD2。

5.权利要求1的方法,其中所述第二药剂是与肿瘤微环境相关抗原(TMAA)特异性结合的抗体或其抗原结合片段。

6.权利要求5的方法,其中所述抗体是单克隆抗体。

7.权利要求5或6的方法,其中所述TMAA选自VEGF-A、EGF、PDGF、IGF和bFGF。

8.权利要求1的方法,其中所述第二药剂是与免疫检查点蛋白特异性结合并拮抗其活性的抗体或其抗原结合片段。

9.权利要求8的方法,其中所述抗体是单克隆抗体。

10.权利要求8或9的方法,其中所述免疫检查点蛋白选自CTLA-4、PD-1、PD-L1、PD-L2和LAG-3。

11.权利要求1的方法,其中所述第二药剂是双特异性杀伤细胞衔接器(BiKE)。

12.权利要求11的方法,其中所述BiKE包含与TAA特异性结合的第一单链可变片段(scFv)。

13.权利要求12的方法,其中所述TAA选自CD123、CLL-1、CD38、CS-1、CD138、ROR1、FAP、MUC1、PSCA、EGFRvIII、EPHA2和GD2。

14.权利要求11-13中任一项的方法,其中所述BiKE包含与CD16特异性结合的第二scFv。

15.权利要求1的方法,其中所述第二药剂是抗炎药。

16.权利要求1的方法,其中所述第二药剂是免疫调节剂。

17.权利要求1的方法,其中所述第二药剂是细胞毒性剂。

18.权利要求1的方法,其中所述第二药剂是癌症疫苗。

19.权利要求1的方法,其中所述第二药剂是化疗剂。

20.权利要求1的方法,其中所述第二药剂是HDAC抑制剂。

21.权利要求1的方法,其中所述第二药剂是siRNA。

22.权利要求1-21中任一项的方法,其中所述分离的NK细胞群或其药物组合物在第二药剂或其药物组合物之前给予。

23.权利要求1-21中任一项的方法,其中所述分离的NK细胞群或其药物组合物在第二药剂或其药物组合物之后给予。

24.权利要求1-21中任一项的方法,其中所述分离的NK细胞群或其药物组合物与第二药剂或其药物组合物在同一时间给予。

25.权利要求1-24中任一项的方法,其中给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。

26.权利要求1-25中任一项的方法，其中给予所述对象分离的NK细胞群或其药物组合物的步骤用装置、基质或支架进行。

27.权利要求1-26中任一项的方法，其中给予所述对象第二药剂或其药物组合物的步骤是通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。

28.权利要求1-27中任一项的方法，其中给予所述对象第二药剂或其药物组合物的步骤用装置、基质或支架进行。

29.权利要求1-28中任一项的方法，其中所述NK细胞在细胞表面上被岩藻糖基化。

30.权利要求1-29中任一项的方法，其中所述分离的NK细胞群或其药物组合物以单剂量给予。

31.权利要求1-29中任一项的方法，其中所述分离的NK细胞群或其药物组合物以多剂量给予。

32.权利要求1-31中任一项的方法，其中所述第二药剂或其药物组合物以单剂量给予。

33.权利要求1-31中任一项的方法，其中所述第二药剂或其药物组合物以多剂量给予。

34.一种治疗有需要的对象的癌症的方法，所述方法包括给予所述对象分离的NK细胞群或其药物组合物，其中所述NK细胞包含嵌合抗原受体(CAR)，其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和可选的共刺激结构域。

35.权利要求34的方法，其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和共刺激结构域。

36.权利要求34或35的方法，其中包含CAR的NK细胞来源于经工程改造表达CAR的CD34+造血干细胞(HSC)。

37.权利要求34-36中任一项的方法，其中所述胞外域是抗原结合域。

38.权利要求37的方法，其中所述抗原结合域是scFv结构域。

39.权利要求37或38的方法，其中所述抗原结合域与TAA特异性结合。

40.权利要求39的方法，其中所述TAA选自CD123、CLL-1、CD38和CS-1。

41.权利要求34-40中任一项的方法，其中所述胞内刺激结构域是CD3ζ信号转导结构域。

42.权利要求34-41中任一项的方法，其中所述共刺激结构域包含CD28、4-1BB、PD-1、OX40、CTLA-4、NKp46、NKp44、NKp30、DAP10或DAP12的胞内域。

43.一种治疗有需要的对象的癌症的方法，所述方法包括给予所述对象分离的NK细胞群或其药物组合物，其中所述NK细胞包含归巢受体。

44.权利要求43的方法，其中包含归巢受体的所述NK细胞来源于经工程改造表达归巢受体的CD34+造血干细胞(HSC)。

45.权利要求43或44的方法，其中所述归巢受体是趋化性受体。

46.权利要求45的方法，其中所述趋化性受体选自CXCR4、VEGFR2和CCR7。

47.一种治疗有需要的对象的癌症的方法，所述方法包括给予所述对象分离的自然杀伤(NK)细胞群或其药物组合物，其中所述NK细胞包含嵌合抗原受体(CAR)和归巢受体，其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和可选的共刺激结构域。

48.权利要求47的方法，其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和共刺激结构域。

49.权利要求47或48的方法，其中所述包含CAR和归巢受体的NK细胞来源于经工程改造表达CAR的CD34+造血干细胞(HSC)。

50.权利要求47-49中任一项的方法，其中包含CAR和归巢受体的NK细胞来源于经工程改造表达归巢受体的CD34+造血干细胞(HSC)。

51.权利要求47-50中任一项的方法，其中所述胞外域是抗原结合域。

52.权利要求51的方法，其中所述抗原结合域是scFv结构域。

53.权利要求51或52的方法，其中所述抗原结合域与TAA特异性结合。

54.权利要求53的方法，其中所述TAA选自CD123、CLL-1、CD38和CS-1。

55.权利要求47-54中任一项的方法，其中所述胞内刺激结构域是CD3ζ信号转导结构域。

56.权利要求47-55中任一项的方法，其中所述共刺激结构域包含CD28、4-1BB、PD-1、OX40、CTLA-4、NKp46、NKp44、NKp30、DAP10或DAP12的胞内域。

57.权利要求47-56中任一项的方法，其中所述归巢受体是趋化性受体。

58.权利要求57的方法，其中所述趋化性受体选自CXCR4、VEGFR2和CCR7。

59.权利要求34-58中任一项的方法，其中给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。

60.权利要求34-59中任一项的方法，其中给予所述对象分离的NK细胞群或其药物组合物的步骤用装置、基质或支架进行。

61.权利要求34-60中任一项的方法，其中所述NK细胞在细胞表面上被岩藻糖基化。

62.权利要求34-61中任一项的方法，其中所述分离的NK细胞群或其药物组合物以单剂量给予。

63.权利要求34-61中任一项的方法，其中所述分离的NK细胞群或其药物组合物以多剂量给予。

64.权利要求1-63中任一项的方法，其中所述癌症是血液癌症。

65.权利要求1-63中任一项的方法，其中所述癌症是实体瘤。

66.一种治疗有需要的对象的病毒感染的方法，所述方法包括：

(a)给予所述对象分离的自然杀伤(NK)细胞群或其药物组合物；和

(b)给予所述对象第二药剂或其药物组合物，其中所述第二药剂可用于治疗所述病毒感染。

67.权利要求66的方法，其中所述第二药剂是与免疫检查点蛋白特异性结合并拮抗其活性的抗体或其抗原结合片段。

68.权利要求67的方法，其中所述抗体是单克隆抗体。

69.权利要求67或68的方法，其中所述免疫检查点蛋白选自CTLA-4、PD-1、PD-L1、PD-L2和LAG-3。

70.权利要求66的方法，其中所述第二药剂是双特异性杀伤细胞衔接器(BiKE)。

71.权利要求66-70中任一项的方法，其中所述分离的NK细胞群或其药物组合物在第二药剂或其药物组合物之前给予。

72.权利要求66-70中任一项的方法，其中所述分离的NK细胞群或其药物组合物在第二药剂或其药物组合物之后给予。

73.权利要求66-70中任一项的方法，其中所述分离的NK细胞群或其药物组合物与第二药剂或其药物组合物在同一时间给予。

74.权利要求66-73中任一项的方法，其中给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。

75.权利要求66-74中任一项的方法，其中给予所述对象分离的NK细胞群或其药物组合物的步骤用装置、基质或支架进行。

76.权利要求66-75中任一项的方法，其中给予所述对象第二药剂或其药物组合物的步骤是通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。

77.权利要求66-76中任一项的方法，其中给予所述对象第二药剂或其药物组合物的步骤用装置、基质或支架进行。

78.权利要求66-77中任一项的方法，其中所述NK细胞在细胞表面上被岩藻糖基化。

79.权利要求66-78中任一项的方法，其中所述分离的NK细胞群或其药物组合物以单剂量给予。

80.权利要求66-78中任一项的方法，其中所述分离的NK细胞群或其药物组合物以多剂量给予。

81.权利要求66-80中任一项的方法，其中所述第二药剂或其药物组合物以单剂量给予。

82.权利要求66-80中任一项的方法，其中所述第二药剂或其药物组合物以多剂量给予。

83.一种治疗有需要的对象的病毒感染的方法，所述方法包括给予所述对象分离的NK细胞群或其药物组合物，其中所述NK细胞包含嵌合抗原受体(CAR)，其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和可选的共刺激结构域。

84.权利要求83的方法，其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和共刺激结构域。

85.权利要求83或84的方法，其中包含CAR的NK细胞来源于经工程改造表达CAR的CD34+造血干细胞(HSC)。

86.权利要求83-85中任一项的方法，其中所述胞外域是抗原结合域。

87.权利要求86的方法，其中所述抗原结合域是scFv结构域。

88.权利要求83-87中任一项的方法，其中所述胞内刺激结构域是CD3ζ信号转导结构域。

89.权利要求83-88中任一项的方法，其中所述共刺激结构域包含CD28、4-1BB、PD-1、OX40、CTLA-4、NKp46、NKp44、NKp30、DAP10或DAP12的胞内域。

90.一种治疗有需要的对象的病毒感染的方法，所述方法包括给予所述对象分离的NK细胞群或其药物组合物，其中所述NK细胞包含归巢受体。

91.权利要求90的方法，其中包含归巢受体的所述NK细胞来源于经工程改造表达归巢受体的CD34+造血干细胞(HSC)。

92.权利要求90或91的方法，其中所述归巢受体是趋化性受体。

93.权利要求92的方法，其中所述趋化性受体选自CXCR4、VEGFR2和CCR7。

94.一种治疗有需要的对象的病毒感染的方法，所述方法包括给予所述对象分离的自

然杀伤(NK)细胞群或其药物组合物,其中所述NK细胞包含嵌合抗原受体(CAR)和归巢受体,其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和可选的共刺激结构域。

95.权利要求94的方法,其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和共刺激结构域。

96.权利要求94或95的方法,其中包含CAR和归巢受体的NK细胞来源于经工程改造表达CAR的CD34+造血干细胞(HSC)。

97.权利要求94-96中任一项的方法,其中所述NK细胞自经工程改造表达归巢受体的CD34+造血干细胞(HSC)产生。

98.权利要求94-97中任一项的方法,其中所述胞外域是抗原结合域。

99.权利要求98的方法,其中所述抗原结合域是scFv结构域。

100.权利要求94-99中任一项的方法,其中所述胞内刺激结构域是CD3ζ信号转导结构域。

101.权利要求94-100中任一项的方法,其中所述共刺激结构域包含CD28、4-1BB、PD-1、OX40、CTLA-4、NKp46、NKp44、NKp30、DAP10或DAP12的胞内域。

102.权利要求94-101中任一项的方法,其中所述归巢受体是趋化性受体。

103.权利要求102的方法,其中所述趋化性受体选自CXCR4、VEGFR2和CCR7。

104.权利要求83-103中任一项的方法,其中给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。

105.权利要求83-104中任一项的方法,其中给予所述对象分离的NK细胞群或其药物组合物的步骤用装置、基质或支架进行。

106.权利要求83-105中任一项的方法,其中所述NK细胞在细胞表面上被岩藻糖基化。

107.权利要求83-106中任一项的方法,其中所述分离的NK细胞群或其药物组合物以单剂量给予。

108.权利要求83-106中任一项的方法,其中所述分离的NK细胞群或其药物组合物以多剂量给予。

109.权利要求1-13和15-108中任一项的方法,其中所述NK细胞是胎盘中间体自然杀伤(PiNK)细胞。

110.权利要求1-108中任一项的方法,其中所述NK细胞是活化NK细胞。

111.权利要求1-108中任一项的方法,其中所述NK细胞是三步法NK(TSPNK)细胞。

112.权利要求111的方法,其中所述TSPNK细胞是NK祖细胞。

113.权利要求108的方法,其中所述PiNK细胞来源于胎盘细胞。

114.权利要求113的方法,其中所述胎盘细胞获自胎盘灌注液。

115.权利要求113的方法,其中所述胎盘细胞获自经机械和/或酶破坏的胎盘组织。

116.权利要求110的方法,其中所述活化NK细胞通过包括以下的步骤产生:

(a)将造血干细胞群或祖细胞群接种在包含白介素-15(IL-15)和可选的干细胞因子(SCF)和白介素-7(IL-7)的一种或多种的第一培养基中,其中所述IL-15和可选的SCF和IL-7不包括在所述培养基的成分不确定的组分中,使得该群扩增,且所述造血干细胞群或祖细胞群内的大量造血干细胞或祖细胞在所述扩增期间分化成NK细胞;和

(b)使来自步骤(a)的细胞在包含白介素-2(IL-2)的第二培养基中扩增,以产生活化NK

细胞群。

117.权利要求110的方法，其中所述活化NK细胞通过包括以下的步骤产生：使造血干细胞群或祖细胞群在包含干细胞因子(SCF)、白介素-7(IL-7)和白介素-15(IL-15)的一种或多种的第一培养基中扩增，且其中所述SCF、IL-7和IL-15不包括在所述培养基的成分不确定的组分中，其中所述造血干细胞群或祖细胞群内的大量造血干细胞或祖细胞在所述扩增期间分化成NK细胞；且其中所述方法的第二步包括将来自第一步骤的细胞在包含白介素-2(IL-2)的第二培养基中培养，以产生活化NK细胞。

118.权利要求116的方法，其中所述第一培养基另包含Fms样酪氨酸激酶3配体(Flt3-L)、血小板生成素(Tpo)、白介素-2(IL-2)或肝素的一种或多种。

119.权利要求118的方法，其中所述第一培养基另包含胎牛血清或人血清。

120.权利要求118的方法，其中所述SCF以约1-约150ng/mL的浓度存在于第一培养基中。

121.权利要求118的方法，其中所述Flt3-L以约1-约150ng/mL的浓度存在于第一培养基中。

122.权利要求118的方法，其中所述IL-2以约50-约1500IU/mL的浓度存在于第一培养基中。

123.权利要求118的方法，其中所述IL-7以约1-约150ng/mL的浓度存在于第一培养基中。

124.权利要求118的方法，其中所述IL-15以1-约150ng/mL的浓度存在于第一培养基中。

125.权利要求118的方法，其中所述Tpo以约1-约150ng/mL的浓度存在于第一培养基中。

126.权利要求118的方法，其中所述肝素以约0.1-约30U/mL的浓度存在于第一培养基中。

127.权利要求116的方法，其中第二步中的所述IL-2以50-约1500IU/mL的浓度存在于第二培养基中。

128.权利要求116的方法，其中所述第二培养基另外包含胎牛血清(FCS)、运铁蛋白、胰岛素、乙醇胺、油酸、亚油酸、棕榈酸、牛血清白蛋白(BSA)和植物凝集素的一种或多种。

129.权利要求116的方法，其中所述造血干细胞或祖细胞是$CD34^+$。

130.权利要求116的方法，其中所述造血干细胞或祖细胞包含来自人胎盘灌注液的造血干细胞或祖细胞和来自脐带的造血干细胞或祖细胞，其中所述胎盘灌注液和所述脐带血来自同一胎盘。

131.权利要求116的方法，其中步骤(b)中的所述饲养细胞包含丝裂霉素C处理的外周血单核细胞(PBMC)、K562细胞或组织培养贴壁干细胞。

132.权利要求116的方法，其中所述NK细胞是$CD3^-CD56^+CD16-$。

133.权利要求132的方法，其中所述NK细胞另外是$CD94^+CD117^+$。

134.权利要求132的方法，其中所述NK细胞另外是$CD161^-$。

135.权利要求132的方法，其中所述NK细胞另外是$NKG2D^+$。

136.权利要求132的方法，其中所述NK细胞另外是$NKp46^+$。

137.权利要求132的方法，其中所述NK细胞另外是$CD226^+$。

138.权利要求111或112的方法，其中所述TSPNK细胞通过包括以下的步骤产生：

(a)将造血干细胞或祖细胞在包含Flt3L、TPO、SCF、IL-7、G-CSF、IL-6和GM-CSF的第一培养基中培养；

(b)随后将所述细胞在包含Flt3L、SCF、IL-15和IL-7、IL-17和IL-15、G-CSF、IL-6和GM-CSF的第二培养基中培养；和

(c)随后将所述细胞在包含CF、IL-15、IL-7、IL-2、G-CSF、IL-6和GM-CSF的第三培养基中培养。

139.权利要求138的方法，其中所述培养步骤(a)的持续时间为7-9天，其中所述培养步骤(b)的持续时间为5-7天，且其中所述培养步骤(c)的持续时间为5-9天。

140.权利要求138的方法，其中所述培养步骤(a)的持续时间为7-9天，其中所述培养步骤(b)的持续时间为5-7天，且其中所述培养步骤(c)的持续时间为21-35天。

141.权利要求138、139或140的方法，其中用于该方法的造血干细胞或祖细胞是$CD34^+$。

142.权利要求138、139或140的方法，其中所述造血干细胞或祖细胞包含来自人胎盘灌注液的造血干细胞或祖细胞和来自脐带的造血干细胞或祖细胞，其中所述胎盘灌注液和所述脐带血来自同一胎盘。

143.权利要求138-142中任一项的方法，其中在步骤(a)结束时CD34-细胞包含超过80％的TSPNK细胞。

144.权利要求138-143中任一项的方法，其中所述TSPNK细胞包含不超过40％CD3-CD56+细胞。

145.权利要求138-144中任一项的方法，其中所述TSPNK细胞包含是$CD52^+CD117^+$的细胞。

146.权利要求1-108中任一项的方法，其中所述NK细胞通过包括以下的步骤产生：

(a)将造血干细胞或祖细胞在包含干细胞动员剂和血小板生成素(Tpo)的第一培养基中培养以产生第一细胞群；

(b)将第一细胞群在包含干细胞动员剂和白介素-15(IL-15)却缺乏Tpo的第二培养基中培养以产生第二细胞群；和

(c)将第二细胞群在包含IL-2和IL-15却缺乏干细胞动员剂和LMWH的第三培养基中培养以产生第三细胞群；

其中第三细胞群包含是CD56+、CD3-、CD16-或CD16+和CD94+或CD94-的自然杀伤细胞，且其中至少80％的自然杀伤细胞是有活力的。

147.权利要求1-146中任一项的方法，其中所述对象是人。

148.一种用于治疗有需要的对象的癌症的药盒，其包含：

(a)分离的NK细胞群或其药物组合物；和

(b)第二药剂或其药物组合物，其中所述第二药剂可用于治疗所述癌症。

149.权利要求148的药盒，其中所述第二药剂是与肿瘤相关抗原(TAA)特异性结合的抗体或其抗原结合片段。

150.权利要求149的药盒，其中所述抗体是单克隆抗体。

151.权利要求149或150的药盒、其中所述TAA选自CD123、CLL-1、CD38、CS-1、CD138、

ROR1、FAP、MUC1、PSCA、EGFRvIII、EPHA2和GD2。

152.权利要求148的药盒，其中所述第二药剂是与肿瘤微环境相关抗原(TMAA)特异性结合的抗体或其抗原结合片段。

153.权利要求152的药盒，其中所述抗体是单克隆抗体。

154.权利要求152或153的药盒，其中所述TMAA选自VEGF-A、EGF、PDGF、IGF和bFGF。

155.权利要求148的药盒，其中所述第二药剂是与免疫检查点蛋白特异性结合并拮抗其活性的抗体或其抗原结合片段。

156.权利要求155的药盒，其中所述抗体是单克隆抗体。

157.权利要求155或156的药盒，其中所述免疫检查点蛋白选自CTLA-4、PD-1、PD-L1、PD-L2和LAG-3。

158.权利要求148的方法，其中所述第二药剂是双特异性杀伤细胞衔接器(BiKE)。

159.权利要求158的药盒，其中所述BiKE包含与TAA特异性结合的第一单链可变片段(scFv)。

160.权利要求159的药盒，其中所述TAA选自CD123、CLL-1、CD38、CS-1、CD138、ROR1、FAP、MUC1、PSCA、EGFRvIII、EPHA2和GD2。

161.权利要求158-160中任一项的药盒，其中所述BiKE包含与CD16特异性结合的第二scFv。

162.权利要求148的药盒，其中所述第二药剂是抗炎药。

163.权利要求148的药盒，其中所述第二药剂是免疫调节剂。

164.权利要求148的药盒，其中所述第二药剂是细胞毒性剂。

165.权利要求148的药盒，其中所述第二药剂是癌症疫苗。

166.权利要求148的药盒，其中所述第二药剂是化疗剂。

167.权利要求148的药盒，其中所述第二药剂是HDAC抑制剂。

168.权利要求148的药盒，其中所述第二药剂是siRNA。

169.权利要求148-168中任一项的药盒，其中所述癌症是血液癌症。

170.权利要求148-168中任一项的方法，其中所述癌症是实体瘤。

171.一种用于治疗有需要的对象的病毒感染的药盒，其包含：

(a)分离的自然杀伤(NK)细胞群或其药物组合物；和

(b)第二药剂或其药物组合物，其中所述第二药剂可用于治疗所述病毒感染。

172.权利要求171的药盒，其中所述第二药剂是与免疫检查点蛋白特异性结合并拮抗其活性的抗体或其抗原结合片段。

173.权利要求172的药盒，其中所述抗体是单克隆抗体。

174.权利要求171或172的药盒，其中所述免疫检查点蛋白选自CTLA-4、PD-1、PD-L1、PD-L2和LAG-3。

175.权利要求171的方法，其中所述第二药剂是双特异性杀伤细胞衔接器(BiKE)。

176.权利要求148-160和162-175中任一项的药盒，其中所述NK细胞是胎盘中间体自然杀伤(PiNK)细胞。

177.权利要求148-175中任一项的药盒，其中所述NK细胞是活化NK细胞。

178.权利要求148-175中任一项的药盒，其中所述NK细胞是TSPNK细胞。

179.权利要求178的药盒,其中所述TSPNK细胞是NK祖细胞。

180.权利要求176的药盒,其中所述PiNK细胞来源于胎盘细胞。

181.权利要求180的药盒,其中所述胎盘细胞获自胎盘灌注液。

182.权利要求181的药盒,其中所述胎盘细胞获自经机械和/或酶破坏的胎盘组织。

183.权利要求177的药盒,其中所述活化NK细胞通过包括以下的步骤产生:

(a)将造血干细胞群或祖细胞群接种在包含白介素-15(IL-15)和可选的干细胞因子(SCF)和白介素-7(IL-7)的一种或多种的第一培养基中,其中所述IL-15和可选的SCF和IL-7不包括在所述培养基的成分不确定的组分中,使得该群扩增,且所述造血干细胞群或祖细胞群内的大量造血干细胞或祖细胞在所述扩增期间分化成NK细胞;和

(b)使来自步骤(a)的细胞在包含白介素-2(IL-2)的第二培养基中扩增,以产生活化NK细胞群。

184.权利要求177的药盒,其中所述活化NK细胞通过包括以下的步骤产生:使造血干细胞群或祖细胞群在包含干细胞因子(SCF)、白介素-7(IL-7)和白介素-15(IL-15)的一种或多种的第一培养基中扩增,且其中所述SCF、IL-7和IL-15不包括在所述培养基的成分不确定的组分中,其中所述造血干细胞群或祖细胞群内的大量造血干细胞或祖细胞在所述扩增期间分化成NK细胞;且其中所述方法的第二步包括将来自第一步骤的细胞在包含白介素-2(IL-2)的第二培养基中培养,以产生活化NK细胞。

185.权利要求183的药盒,其中所述第一培养基另包含Fms样酪氨酸激酶3配体(Flt3-L)、血小板生成素(Tpo)、白介素-2(IL-2)或肝素的一种或多种。

186.权利要求185的药盒,其中所述第一培养基另包含胎牛血清或人血清。

187.权利要求185的药盒,其中所述SCF以约1-约150ng/mL的浓度存在于第一培养基中。

188.权利要求185的药盒,其中所述Flt3-L以约1-约150ng/mL的浓度存在于第一培养基中。

189.权利要求185的药盒,其中所述IL-2以约50-约1500IU/mL的浓度存在于第一培养基中。

190.权利要求185的药盒,其中所述IL-7以约1-约150ng/mL的浓度存在于第一培养基中。

191.权利要求185的药盒,其中所述IL-15以1-约150ng/mL的浓度存在于第一培养基中。

192.权利要求185的药盒,其中所述Tpo以约1-约150ng/mL的浓度存在于第一培养基中。

193.权利要求185的药盒,其中所述肝素以约0.1-约30U/mL的浓度存在于第一培养基中。

194.权利要求183的药盒,其中第二步中的所述IL-2以50-约1500IU/mL的浓度存在于第二培养基中。

195.权利要求183的药盒,其中所述第二培养基另外包含胎牛血清(FCS)、运铁蛋白、胰岛素、乙醇胺、油酸、亚油酸、棕榈酸、牛血清白蛋白(BSA)和植物凝集素的一种或多种。

196.权利要求183的药盒,其中所述造血干细胞或祖细胞是$CD34^+$。

197.权利要求183的药盒，其中所述造血干细胞或祖细胞包含来自人胎盘灌注液的造血干细胞或祖细胞和来自脐带的造血干细胞或祖细胞，其中所述胎盘灌注液和所述脐带血来自同一胎盘。

198.权利要求183的药盒，其中步骤(b)中的所述饲养细胞包含丝裂霉素C处理的外周血单核细胞(PBMC)、K562细胞或组织培养贴壁干细胞。

199.权利要求183的药盒，其中所述NK细胞是$CD3^{-}CD56^{+}CD16-$。

200.权利要求199的药盒，其中所述NK细胞另外是$CD94^{+}CD117^{+}$。

201.权利要求199的药盒，其中所述NK细胞另外是$CD161^{-}$。

202.权利要求199的药盒，其中所述NK细胞另外是$NKG2D^{+}$。

203.权利要求199的药盒，其中所述NK细胞另外是$NKp46^{+}$。

204.权利要求199的药盒，其中所述NK细胞另外是$CD226^{+}$。

205.权利要求178或179的药盒，其中所述TSPNK细胞通过包括以下的步骤产生：

(a)将造血干细胞或祖细胞在包含Flt3L、TPO、SCF、IL-7、G-CSF、IL-6和GM-CSF的第一培养基中培养；

(b)随后将所述细胞在包含Flt3L、SCF、IL-15和IL-7、IL-17和IL-15、G-CSF、IL-6和GM-CSF的第二培养基中培养；和

(c)随后将所述细胞在包含CF、IL-15、IL-7、IL-2、G-CSF、IL-6和GM-CSF的第三培养基中培养。

206.权利要求205的药盒，其中所述培养步骤(a)的持续时间为7-9天，其中所述培养步骤(b)的持续时间为5-7天，且其中所述培养步骤(c)的持续时间为5-9天。

207.权利要求205的药盒，其中所述培养步骤(a)的持续时间为7-9天，其中所述培养步骤(b)的持续时间为5-7天，且其中所述培养步骤(c)的持续时间为21-35天。

208.权利要求205、206、207的药盒，其中用于该方法的造血干细胞或祖细胞是$CD34^{+}$。

209.权利要求205、206、207的药盒，其中所述造血干细胞或祖细胞包含来自人胎盘灌注液的造血干细胞或祖细胞和来自脐带的造血干细胞或祖细胞，其中所述胎盘灌注液和所述脐带血来自同一胎盘。

210.权利要求205-209中任一项的药盒，其中在步骤(a)结束时CD34-细胞包含超过80％的TSPNK细胞。

211.权利要求205-210中任一项的药盒，其中所述TSPNK细胞包含不超过40％CD3-CD56+细胞。

212.权利要求205-211中任一项的药盒，其中所述TSPNK细胞包含是$CD52^{+}CD117^{+}$的细胞。

213.权利要求148-175中任一项的药盒，其中所述NK细胞通过包括以下的步骤产生：

(a)将造血干细胞或祖细胞在包含干细胞动员剂和血小板生成素(Tpo)的第一培养基中培养以产生第一细胞群；

(b)将第一细胞群在包含干细胞动员剂和白介素-15(IL-15)却缺乏Tpo的第二培养基中培养以产生第二细胞群；和

(c)将第二细胞群在包含IL-2和IL-15却缺乏干细胞动员剂和LMWH的第三培养基中培养以产生第三细胞群；

其中第三细胞群包含是CD56+、CD3-、CD16-或CD16+和CD94+或CD94-的自然杀伤细胞，且其中至少80％的自然杀伤细胞是有活力的。

214.权利要求147-213中任一项的药盒，其中所述对象是人。

使用自然杀伤细胞治疗血液病症、实体瘤或感染性疾病的方法

[0001] 本申请要求2014年12月31日提交的美国临时申请号62/098,547和2015年3月30日提交的美国临时申请号62/139,952的权益，其每一个的公开内容通过引用以其整体并入本文。

1.发明领域

[0002] 本文提供使用与第二药剂组合的自然杀伤细胞或使用具有针对靶特异性和/或归巢特异性的遗传修饰的自然杀伤细胞治疗有需要的对象的血液病症、实体瘤或感染性疾病的方法。

[0003] 2.发明背景

[0004] 自然杀伤(NK)细胞是构成先天免疫系统的主要组分的细胞毒性淋巴细胞。

[0005] NK细胞在响应干扰素或巨噬细胞衍生细胞因子时被激活。NK细胞具有控制细胞的细胞毒活性的两种类型的表面受体，标记为“激活性受体”和“抑制性受体”。

[0006] 在其它活性中，NK细胞在肿瘤的宿主排斥中起作用，并且显示能够杀伤病毒感染细胞。自然杀伤细胞可被缺乏主要组织相容性复合体(MHC)蛋白或显示其水平降低的细胞激活。来自外周血的激活或增殖的NK细胞和LAK细胞已被用于患有晚期癌症的患者的离体疗法和体内治疗两者中，其针对骨髓相关疾病(例如白血病)、乳腺癌和某些类型的淋巴瘤有某种成效。

[0007] 尽管NK细胞在杀伤肿瘤细胞和病毒感染细胞中的有利性质，但对于开发利用NK细胞的更有效的NK细胞和更有效的治疗方案仍有巨大需求。

[0008] 3.发明概述

[0009] 本发明提供使用自然杀伤(NK)细胞与可用于治疗该疾病的第二药剂组合治疗有需要的对象的疾病(例如血液病症、实体瘤或感染性疾病)的方法。本文还提供利用具有针对靶特异性和/或归巢特异性的遗传修饰的NK细胞(例如包含嵌合抗原受体(CAR)和/或归巢受体的NK细胞)治疗有需要的对象的疾病(例如血液病症、实体瘤或感染性疾病)的方法。

[0010] 一方面，本文提供治疗有需要的对象的癌症的方法，所述方法包括：(a)给予所述对象分离的自然杀伤(NK)细胞群或其药物组合物；和(b)给予所述对象第二药剂或其药物组合物，其中所述第二药剂可用于治疗所述癌症。在一个具体的实施方案中，所述癌症是多发性骨髓瘤。

[0011] 在某些实施方案中，第二药剂是与肿瘤相关抗原(TAA)特异性结合的抗体或其抗原结合片段。在具体的实施方案中，抗体是单克隆抗体。在具体的实施方案中，TAA选自CD123、CLL-1、CD38、CS-1(亦称为SLAM7、SLAMF7、CD319和CRACC)、CD138、ROR1、FAP、MUC1、PSCA、EGFRvIII、EPHA2和GD2。在一个更具体的实施方案中，第二药剂是与CS-1结合的抗体。在更具体的实施方案中，第二药剂是elotuzumab(HuLuc63，Bristol Myers-Squibb/AbbVie人源化抗CS-1单克隆抗体)。

[0012] 在某些实施方案中，第二药剂是与肿瘤微环境相关抗原(TMAA)特异性结合的抗体

或其抗原结合片段。在具体的实施方案中，抗体是单克隆抗体。在具体的实施方案中，TMAA选自VEGF-A、EGF、PDGF、IGF和bFGF。

[0013] 在某些实施方案中，第二药剂是与免疫检查点蛋白特异性结合并拮抗其活性的抗体或其抗原结合片段。在具体的实施方案中，抗体是单克隆抗体。在具体的实施方案中，免疫检查点蛋白选自CTLA-4、PD-1、PD-L1、PD-L2和LAG-3。

[0014] 在某些实施方案中，第二药剂是双特异性杀伤细胞衔接器(bispecific killer cell engager，BiKE)。在具体的实施方案中，BiKE包含与TAA特异性结合的第一单链可变片段(scFv)。在其它具体的实施方案中，TAA选自CD123、CLL-1、CD38、CS-1、CD138、ROR1、FAP、MUC1、PSCA、EGFRvIII、EPHA2和GD2。在具体的实施方案中，BiKE包含与CD16特异性结合的第二scFv。

[0015] 在某些实施方案中，第二药剂是抗炎药。

[0016] 在某些实施方案中，第二药剂是免疫调节剂。在具体的实施方案中，第二药剂是来那度胺(lenalidomide)或泊马度胺(pomalidomide)。

[0017] 在某些实施方案中，第二药剂是细胞毒性剂。

[0018] 在某些实施方案中，第二药剂是癌症疫苗。

[0019] 在某些实施方案中，第二药剂是化疗剂。

[0020] 在某些实施方案中，第二药剂是HDAC抑制剂。在其它具体的实施方案中，第二药剂是罗米地新(ISTODAX®，Celgene)。

[0021] 在某些实施方案中，第二药剂是siRNA。

[0022] 在一些实施方案中，分离的NK细胞群或其药物组合物在第二药剂或其药物组合物之前给予。在一些实施方案中，分离的NK细胞群或其药物组合物在第二药剂或其药物组合物之后给予。在其它实施方案中，分离的NK细胞群或其药物组合物与第二药剂或其药物组合物在同一时间给予。

[0023] 在具体的实施方案中，给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。在具体的实施方案中，给予所述对象分离的NK细胞群或其药物组合物的步骤用装置(devise)、基质或支架进行。在具体的实施方案中，给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射。在具体的实施方案中，NK细胞的注射是局部注射。在更具体的实施方案中，局部注射是直接进入实体瘤(例如肉瘤)中。在具体的实施方案中，NK细胞的给药经注射器注射。在具体的实施方案中，NK细胞经注射的给予借助于腹腔镜检查、内窥镜检查、超声、计算机体层摄影术、磁共振或放射检查。

[0024] 在具体的实施方案中，给予所述对象第二药剂或其药物组合物的步骤是通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。在具体的实施方案中，给予所述对象第二药剂或其药物组合物的步骤用装置、基质或支架进行。

[0025] 在不同的实施方案中，NK细胞在细胞表面上被岩藻糖基化。

[0026] 在一些实施方案中，分离的NK细胞群或其药物组合物以单剂量给予。在其它实施方案中，分离的NK细胞群或其药物组合物以多剂量给予。

[0027] 在一些实施方案中，第二药剂或其药物组合物以单剂量给予。在其它实施方案中，第二药剂或其药物组合物以多剂量给予。

[0028] 另一方面，本文提供治疗有需要的对象的癌症的方法，所述方法包括给予所述对象分离的NK细胞群或其药物组合物，其中NK细胞包含嵌合抗原受体(CAR)，其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和可选的共刺激结构域。本文还提供治疗有需要的对象的癌症的方法，所述方法包括给予所述对象分离的NK细胞群或其药物组合物，其中NK细胞包含归巢受体，且本文还提供治疗有需要的对象的癌症的方法，所述方法包括给予所述对象分离的自然杀伤(NK)细胞群或其药物组合物，其中NK细胞包含嵌合抗原受体(CAR)和归巢受体，其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和可选的共刺激结构域。在不同的实施方案中，CAR包含胞外域、跨膜结构域、胞内刺激结构域和共刺激结构域。

[0029] 在具体的实施方案中，包含CAR和/或归巢受体的NK细胞来源于经工程改造表达CAR和/或归巢受体的CD34+造血干细胞(HSC)。

[0030] 在不同的实施方案中，CAR的胞外域是抗原结合域。在具体的实施方案中，抗原结合域是scFv结构域。在某些实施方案中，抗原结合域与TAA特异性结合。在具体的实施方案中，TAA选自CD123、CLL-1、CD38、CD20和CS-1。在更具体的实施方案中，抗原结合域包含来源于结合CS-1的抗体的单链Fv(scFv)或抗原结合片段。在更具体的实施方案中，抗原结合域包含elotuzumab的单链形式和/或elotuzumab的抗原结合片段。在具体的实施方案中，抗原结合域包含来源于结合CD20的抗体的单链Fv(scFv)或抗原结合片段。

[0031] 在不同的实施方案中，CAR的胞内刺激结构域是CD3ζ信号转导结构域。

[0032] 在不同的实施方案中，CAR的共刺激结构域包含CD28、4-1BB、PD-1、OX40、CTLA-4、NKp46、NKp44、NKp30、DAP10或DAP12的胞内域。

[0033] 在不同的实施方案中，归巢受体是趋化性受体。在具体的实施方案中，趋化性受体选自CXCR4、VEGFR2和CCR7。

[0034] 在一个实施方案中，本文提供治疗患有多发性骨髓瘤的个体的方法，所述方法包括给予个体(1)来那度胺或泊马度胺和(2)包含CAR的NK细胞(“CAR NK细胞”)，其中所述CAR NK细胞在治疗所述个体的多发性骨髓瘤时是有效的。在治疗患有多发性骨髓瘤的个体的方法的具体实施方案中，所述CAR NK细胞包含CAR胞外域，所述胞外域是CS-1结合域。在具体的实施方案中，CS-1结合域包含结合CS-1的抗体的scFv或抗原结合片段。在某些具体实施方案中，CS-1结合域包含elotuzumab的单链形式和/或elotuzumab的抗原结合片段。

[0035] 在另一个实施方案中，本文提供治疗患有多发性骨髓瘤的个体的方法，所述方法包括给予个体(1)来那度胺或泊马度胺；(2)elotuzumab；和(3)CAR NK细胞，其中所述CAR NK细胞对治疗所述个体的多发性骨髓瘤是有效的。在治疗患有多发性骨髓瘤的个体的方法的某些具体实施方案中，所述CAR NK细胞包含CAR胞外域，所述胞外域是CS-1结合域。在具体的实施方案中，CS-1结合域包含结合CS-1的抗体的scFv或抗原结合片段。

[0036] 在另一个实施方案中，本文提供治疗患有血液癌症(例如伯基特淋巴瘤(Burkitt’s lymphoma))的个体的方法，所述方法包括给予个体(1)罗米地新和(2)CAR NK细胞，其中所述CAR NK细胞对治疗所述个体的血液癌症(例如伯基特淋巴瘤)是有效的。在治疗患有血液癌症(例如伯基特淋巴瘤)的个体的方法的某些具体实施方案中，所述CAR NK细胞包含CAR胞外域，所述胞外域是CD20结合域。在具体的实施方案中，CD20结合域包含结合CD20的抗体的scFv或抗原结合片段。

[0037] 在具体的实施方案中，给予所述对象分离的NK细胞群或其药物组合物的步骤是通

过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。在具体的实施方案中,给予所述对象分离的NK细胞群或其药物组合物的步骤用装置、基质或支架进行。在具体的实施方案中,给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射。在具体的实施方案中,NK细胞的注射是局部注射。在更具体的实施方案中,局部注射直接进入实体瘤(例如肉瘤)中。在具体的实施方案中,NK细胞的给药经注射器注射。在具体的实施方案中,NK细胞经注射给予借助于腹腔镜检查、内窥镜检查、超声、计算机体层摄影术、磁共振或放射检查。

[0038] 在不同的实施方案中,NK细胞在细胞表面上被岩藻糖基化。

[0039] 在一些实施方案中,分离的NK细胞群或其药物组合物以单剂量给予。在其它实施方案中,分离的NK细胞群或其药物组合物以多剂量给予。

[0040] 另一方面,本文提供治疗有需要的对象的病毒感染的方法,所述方法包括:(a)给予所述对象分离的自然杀伤(NK)细胞群或其药物组合物;和(b)给予所述对象第二药剂或其药物组合物,其中所述第二药剂可用于治疗所述病毒感染。

[0041] 在某些实施方案中,第二药剂是与免疫检查点蛋白特异性结合并拮抗其活性的抗体或其抗原结合片段。在具体的实施方案中,抗体是单克隆抗体。在具体的实施方案中,免疫检查点蛋白选自CTLA-4、PD-1、PD-L1、PD-L2和LAG-3。

[0042] 在某些实施方案中,第二药剂是双特异性杀伤细胞衔接器(BiKE)。

[0043] 在一些实施方案中,分离的NK细胞群或其药物组合物在第二药剂或其药物组合物之前给予。在一些实施方案中,分离的NK细胞群或其药物组合物在第二药剂或其药物组合物之后给予。在其它实施方案中,分离的NK细胞群或其药物组合物与第二药剂或其药物组合物在同一时间给予。

[0044] 在具体的实施方案中,给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。在具体的实施方案中,给予所述对象分离的NK细胞群或其药物组合物的步骤用装置、基质或支架进行。在具体的实施方案中,给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射。在具体的实施方案中,NK细胞的注射是局部注射。在更具体的实施方案中,局部注射直接进入实体瘤(例如肉瘤)中。在具体的实施方案中,NK细胞的给药经注射器注射。在具体的实施方案中,NK细胞经注射给予借助于腹腔镜检查、内窥镜检查、超声、计算机体层摄影术、磁共振或放射检查。

[0045] 在具体的实施方案中,给予所述对象第二药剂或其药物组合物的步骤是通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。在具体的实施方案中,给予所述对象第二药剂或其药物组合物的步骤用装置、基质或支架进行。

[0046] 在不同的实施方案中,NK细胞在细胞表面上被岩藻糖基化。

[0047] 在一些实施方案中,分离的NK细胞群或其药物组合物以单剂量给予。在其它实施方案中,分离的NK细胞群或其药物组合物以多剂量给予。

[0048] 在一些实施方案中,第二药剂或其药物组合物以单剂量给予。在其它实施方案中,第二药剂或其药物组合物以多剂量给予。

[0049] 另一方面,本文提供治疗有需要的对象的病毒感染的方法,所述方法包括给予所述对象分离的NK细胞群或其药物组合物,其中NK细胞包含嵌合抗原受体(CAR),其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和可选的共刺激结构域。本文还提供治疗有需要的对象的病毒感染的方法,所述方法包括给予所述对象分离的NK细胞群或其药物组合

物,其中NK细胞包含归巢受体,且本文还提供治疗有需要的对象的病毒感染的方法,所述方法包括给予所述对象分离的自然杀伤(NK)细胞群或其药物组合物,其中NK细胞包含嵌合抗原受体(CAR)和归巢受体,其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和可选的共刺激结构域。在不同的实施方案中,CAR包含胞外域、跨膜结构域、胞内刺激结构域和共刺激结构域。

[0050] 在具体的实施方案中,包含CAR和/或归巢受体的NK细胞来源于经工程改造表达CAR和/或归巢受体的CD34+造血干细胞(HSC)。

[0051] 在不同的实施方案中,CAR的胞外域是抗原结合域。在具体的实施方案中,抗原结合域是scFv结构域。

[0052] 在不同的实施方案中,CAR的胞内刺激结构域是CD3ζ信号转导结构域。

[0053] 在不同的实施方案中,CAR的共刺激结构域包含CD28、4-1BB、PD-1、OX40、CTLA-4、NKp46、NKp44、NKp30、DAP10或DAP12的胞内域。

[0054] 在不同的实施方案中,归巢受体是趋化性受体。在具体的实施方案中,趋化性受体选自CXCR4、VEGFR2和CCR7。

[0055] 在具体的实施方案中,给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药。在具体的实施方案中,给予所述对象分离的NK细胞群或其药物组合物的步骤用装置、基质或支架进行。在具体的实施方案中,给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射。在具体的实施方案中,NK细胞的注射是局部注射。在更具体的实施方案中,局部注射直接进入实体瘤(例如肉瘤)中。在具体的实施方案中,NK细胞的给药经注射器注射。在具体的实施方案中,NK细胞经注射给予借助于腹腔镜检查、内窥镜检查、超声、计算机体层摄影术、磁共振或放射检查。

[0056] 在不同的实施方案中,NK细胞在细胞表面上被岩藻糖基化。

[0057] 在一些实施方案中,分离的NK细胞群或其药物组合物以单剂量给予。在其它实施方案中,分离的NK细胞群或其药物组合物以多剂量给予。

[0058] 本发明还提供治疗有需要的对象的疾病(例如血液病症、实体瘤或感染性疾病)的药盒,其包含分离的NK细胞群和可用于治疗该疾病的第二药剂。

[0059] 一方面,本文提供治疗有需要的对象的癌症的药盒,其包含:(a)分离的NK细胞群或其药物组合物;和(b)第二药剂或其药物组合物,其中所述第二药剂可用于治疗所述癌症。第二药剂可为上文提供的可用于治疗癌症的方法的任何药剂。

[0060] 另一方面,本文提供治疗有需要的对象的病毒感染的药盒,其包含:(a)分离的NK细胞群或其药物组合物;和(b)第二药剂或其药物组合物,其中所述第二药剂可用于治疗所述病毒感染。第二药剂可以是上文提供的可用于治疗病毒感染的方法的任何药剂。

[0061] 在本文提供的方法或药盒的不同实施方案中,NK细胞是胎盘中间体自然杀伤(PiNK)细胞。在某些实施方案中,PiNK细胞来源于胎盘细胞。在具体的实施方案中,胎盘细胞获自胎盘灌注液。在具体的实施方案中,胎盘细胞获自经机械和/或酶破坏的胎盘组织。

[0062] 在本文提供的方法或药盒的不同实施方案中,NK细胞是活化NK细胞。在某些实施方案中,活化NK细胞通过包括以下的步骤产生:(a)将造血干细胞群或祖细胞群接种在包含白介素-15(IL-15)和可选的干细胞因子(SCF)和白介素-7(IL-7)的一种或多种的第一培养基中,其中所述IL-15和可选的SCF和IL-7不包括在所述培养基的成分不确定的组分中,使

得该群扩增，且所述造血干细胞群或祖细胞群内的大量造血干细胞或祖细胞在所述扩增期间分化成NK细胞；和(b)使来自步骤(a)的细胞在包含白介素-2(IL-2)的第二培养基中扩增，以产生活化NK细胞群。在某些实施方案中，活化NK细胞通过包括以下的步骤产生：使造血干细胞群或祖细胞群在包含干细胞因子(SCF)、白介素-7(IL-7)和白介素-15(IL-15)的一种或多种的第一培养基中扩增，其中所述SCF、IL-7和IL-15不包括在所述培养基的成分不确定的组分中，其中所述造血干细胞群或祖细胞群内的大量造血干细胞或祖细胞在所述扩增期间分化成NK细胞；且其中所述方法的第二步骤包括将来自第一步骤的细胞在包含白介素-2(IL-2)的第二培养基中培养，以产生活化NK细胞。

[0063] 在具体的实施方案中，第一培养基另包含Fms样酪氨酸激酶3配体(Flt3-L)、血小板生成素(Tpo)、白介素-2(IL-2)或肝素的一种或多种。在其它具体的实施方案中，第一培养基另包含胎牛血清或人血清。在其它具体的实施方案中，SCF以约1-约150ng/mL的浓度存在于第一培养基中。在其它具体的实施方案中，Flt3-L以约1-约150ng/mL的浓度存在于第一培养基中。在其它具体的实施方案中，IL-2以约50-约1500IU/mL的浓度存在于第一培养基中。在其它具体的实施方案中，IL-7以约1-约150ng/mL的浓度存在于第一培养基中。在其它具体的实施方案中，IL-15以1-约150ng/mL的浓度存在于第一培养基中。在其它具体的实施方案中，Tpo以约1-约150ng/mL的浓度存在于第一培养基中。在其它具体的实施方案中，肝素以约0.1-约30U/mL的浓度存在于第一培养基中。

[0064] 在具体的实施方案中，上文第二步骤中的所述IL-2以50-约1500IU/mL的浓度存在于第二培养基中。

[0065] 在具体的实施方案中，所述第二培养基另外包含胎牛血清(FCS)、运铁蛋白、胰岛素、乙醇胺、油酸、亚油酸、棕榈酸、牛血清白蛋白(BSA)和植物凝集素的一种或多种。

[0066] 在具体的实施方案中，造血干细胞或祖细胞是$CD34^+$。

[0067] 在具体的实施方案中，造血干细胞或祖细胞包含来自人胎盘灌注液的造血干细胞或祖细胞和来自脐带的造血干细胞或祖细胞，其中所述胎盘灌注液和所述脐带血来自同一胎盘。

[0068] 在具体的实施方案中，上述步骤(b)中的饲养细胞包含丝裂霉素C处理的外周血单核细胞(PBMC)、K562细胞或组织培养贴壁干细胞。

[0069] 在具体的实施方案中，NK细胞是$CD3^-CD56^+CD16^-$。在又一个具体的实施方案中，NK细胞另外是$CD94^+CD117^+$。在另外又一个具体的实施方案中，NK细胞另外是$CD161^-$。在另外又一个具体的实施方案中，NK细胞另外是$NKG2D^+$。在另外又一个具体的实施方案中，NK细胞另外是$NKp46^+$。在另外又一个具体的实施方案中，NK细胞另外是$CD226^+$。

[0070] 在本文提供的方法或药盒的不同实施方案中，NK细胞是三步法NK(TSPNK)细胞。在具体的实施方案中，TSPNK细胞是NK祖细胞。在某些实施方案中，TSPNK细胞通过包括以下的步骤产生：(a)将造血干细胞或祖细胞在包含Flt3L、TPO、SCF、IL-7、G-CSF、IL-6和GM-CSF的第一培养基中培养；(b)随后将所述细胞在包含Flt3L、SCF、IL-15和IL-7、IL-17和IL-15、G-CSF、IL-6和GM-CSF的第二培养基中培养；和(c)随后将所述细胞在包含SCF、IL-15、IL-7、IL-2、G-CSF、IL-6和GM-CSF的第三培养基中培养。

[0071] 在具体的实施方案中，培养步骤(a)的持续时间为7-9天，培养步骤(b)的持续时间为5-7天，而养步骤(c)的持续时间为5-9天。在具体的实施方案中，培养步骤(a)的持续时间

为7-9天，培养步骤(b)的持续时间为5-7天，而养步骤(c)的持续时间为21-35天。

[0072] 在具体的实施方案中，用于该方法的造血干细胞或祖细胞是$CD34^+$。

[0073] 在具体的实施方案中，造血干细胞或祖细胞包含来自人胎盘灌注液的造血干细胞或祖细胞和来自脐带的造血干细胞或祖细胞，其中所述胎盘灌注液和所述脐带血来自同一胎盘。

[0074] 在具体的实施方案中，CD34-细胞在上述产生TSPNK细胞的方法的步骤(a)结束时包含超过80％的TSPNK细胞。

[0075] 在具体的实施方案中，TSPNK细胞包含不超过40％CD3-CD56+细胞。

[0076] 在具体的实施方案中，TSPNK细胞包含是$CD52^+CD117^+$的细胞。

[0077] 在本文所述方法或药盒的不同实施方案中，NK细胞通过包括以下的步骤产生：(a)将造血干细胞或祖细胞在包含干细胞动员剂和血小板生成素(Tpo)的第一培养基中培养以产生第一细胞群；(b)将第一细胞群在包含干细胞动员剂和白介素-15(IL-15)却缺乏Tpo的第二培养基中培养以产生第二细胞群；和(c)将第二细胞群在包含IL-2和IL-15却缺乏干细胞动员剂和LMWH的第三培养基中培养以产生第三细胞群；其中第三细胞群包含是CD56+、CD3-、CD16-或CD16+和CD94+或CD94-的自然杀伤细胞，且其中至少80％的自然杀伤细胞是有活力的。

[0078] 本文提供的方法或药盒的任一个中的癌症可为血液癌症或实体瘤。

[0079] 在本文提供的方法或药盒的任一个的优选实施方案中，对象是人。

[0080] 3.1.术语

[0081] 如本文所用，“自然杀伤细胞”或“NK细胞”在没有更多修饰的情况下包括来源于任何组织来源的自然杀伤细胞，并包括成熟的自然杀伤细胞以及自然杀伤祖细胞。在一些实施方案中，NK细胞是第5.1.1节描述的胎盘中间体自然杀伤(PiNK)细胞。在一些实施方案中，NK细胞是第5.1.2节描述的活化NK细胞。在一些实施方案中，NK细胞是第5.1.3节描述的三步法NK(TSPNK)细胞。自然杀伤细胞可来源于任何组织来源，并包括成熟的自然杀伤细胞以及NK祖细胞。

[0082] 本文所用术语“NK祖细胞群”是指包含尚未发育成成熟的NK细胞的自然杀伤细胞谱系的细胞的细胞群，成熟NK细胞通过例如表达一个或多个表型标志物(例如CD56、CD16和KIR)的水平表示。在一个实施方案中，NK祖细胞群包含具有低CD16和高CD56的细胞。

[0083] 如本文所用，“PiNK”和“PiNK细胞”是指获自人胎盘例如人胎盘灌注液或经机械和/或酶破坏的胎盘组织的胎盘中间体自然杀伤细胞。该细胞是$CD56^+$和$CD16^-$，例如通过流式细胞术测定，例如使用抗CD56和CD16抗体的荧光激活细胞分选术测定。

[0084] 如本文所用，“胎盘灌注液”意指流经胎盘(例如人胎盘)的至少一部分，例如流经胎盘血管系统的灌注液，包括在流经胎盘期间通过灌注液收集的大量细胞。

[0085] 如本文所用，“胎盘灌注液细胞”意指分离自或可分离自胎盘灌注液的有核细胞，例如总有核细胞。

[0086] 如本文所用，“饲养细胞”是指与第二种类型的细胞共培养以提供其中第二种类型的细胞可维持并且可能增殖的环境的一种细胞类型。虽不受任何理论的束缚，但是饲养细胞可向靶细胞提供例如肽、多肽、电信号、有机分子(例如类固醇类)、核酸分子、生长因子(例如bFGF)、其它因子(例如细胞因子)和代谢营养物。在某些实施方案中，饲养细胞在单层

中生长。

[0087] 本文所用术语“造血细胞”包括造血干细胞和造血祖细胞。

[0088] 如本文所用，“成分未确定组分”是培养基领域的专业术语，是指其组成一般未提供或量化的组分。“成分未确定组分”的实例包括而不限于人血清(例如人血清AB)和胎儿血清(例如胎牛血清或胎牛犊血清)。

[0089] 如本文所用，“+”，当用来表明特殊细胞标志物的存在时，意指细胞标志物相对于同种型对照在荧光激活细胞分选术中可检出地存在；或在定量或半定量RT-PCR中高于背景地可检出。

[0090] 如本文所用，“-”，当用来表明特殊细胞标志物的存在时，意指细胞标志物相对于同种型对照在荧光激活细胞分选术中不可检出地存在；在定量或半定量RT-PCR中不高于背景地检出。

[0091] 如本文所用，“癌症”是指血液癌症或实体瘤。

[0092] 4.附图简述

[0093] 图1表示在利妥昔单抗的不同浓度下PiNK细胞针对Daudi细胞的依赖抗体的细胞毒性(ADCC)活性。

[0094] 图2表示MM细胞系MM285、MM293、RPMI8226和OPM2中PD-L1和CS-1的表达。按照生产商的方案，将细胞用抗PD-L1 APC(Biolegend，目录号329708)、抗CS1 PE-Cy7(Biolegend，目录号331816)和7-AAD(BD Bioscience，目录号559925)染色。数据在BD LSRFortessa(BD Biosciences)上获取，并应用FLOWJO®软件(Tree Star)分析。数据表示为根据7-AAD-单细胞选通的％阳性细胞。％阳性门控的设置使用未染色样品作为对照进行。图中最左边的峰表示对照，最右边的峰表示样品。对PD-L1是阳性的细胞的百分比如下：71.6％MM285、70.7％MM293、66.2％OPM-2和94.4％RPMI8226。对CS-1是阳性的细胞的百分比如下：31.8％MM285、58.8％MM293、93.4％OPM-2和29.5％RPMI8226。

[0095] 图3表示在3:1的效应物-靶标比率下三阶段NK细胞针对指定的MM细胞系和原代MM样品的24小时细胞毒性测定。按照生产商提供的方案，各样品中有活力的靶细胞($PKH26^+$ $TO\text{-}PRO\text{-}3^-$)的数目使用计数珠粒通过流式细胞术确定(Invitrogen，目录号C36950)。将计数珠粒引入该测定法中以计算在持续24小时培养期间肿瘤细胞的任何潜在增殖。在37℃和5％CO_2下孵育24小时后，收获细胞，接着用1μM TO-PRO-3染色以鉴定死细胞。结果表示为均值±均值的标准差。

[0096] 图4表示在3:1的效应物-靶标比率以及下列额外条件下三阶段NK细胞在48孔板中针对OPM2细胞的24小时细胞毒性测定：IL-15(5ng/mL)(Invitrogen，目录号PHC9153)；IL-2(200IU/mL)(Invitrogen，目录号PHC0023)；抗PD-L1(10ng/mL)(Affymetrix，目录号16-5983-82)；抗IgG(10ng/mL)(Affymetrix，目录号16-4714-82)；REVLIMID®(来那度胺；1uM)或DMSO(0.1％)。接种单独的靶细胞作为对照。在37℃和5％CO_2下孵育24小时后，收获细胞，接着用1μM TO-PRO-3染色以鉴定死细胞。结果表示为均值±均值的标准差。

[0097] 5.发明详述

[0098] 本文提供使用自然杀伤(NK)细胞与可用于治疗该疾病的第二药剂组合治疗有需要的对象的疾病(例如血液病症、实体瘤或感染性疾病)的方法。本文还提供使用具有针对靶特异性和/或归巢特异性的遗传修饰的NK细胞(例如包含嵌合抗原受体(CAR)和/或归巢

受体的NK细胞)治疗有需要的对象的疾病(例如血液病症、实体瘤或感染性疾病)的方法。本文还提供治疗有需要的对象的疾病(例如血液病症、实体瘤或感染性疾病)的药盒,其包含分离的NK细胞群和可用于治疗该疾病的第二药剂,或其包含具有遗传修饰的分离的NK细胞群(例如包含嵌合抗原受体(CAR)和/或归巢受体的NK细胞)。

[0099] 5.1.NK细胞

[0100] 本文描述了NK细胞,包括PiNK细胞、活化NK细胞、TSPNK细胞和通过三阶段方法产生的NK细胞。

[0101] 5.1.1.胎盘中间体自然杀伤(PiNK)细胞

[0102] 在一些实施方案中,自然杀伤细胞是胎盘中间体自然杀伤(PiNK)细胞(另参见美国专利号8,263,065,其公开内容通过引用以其整体并入本文)。在不同的实施方案中,PiNK细胞来源于胎盘细胞。在具体的实施方案中,胎盘细胞获自胎盘灌注液,例如人胎盘灌注液。在具体的实施方案中,胎盘细胞获自经机械和/或酶破坏的胎盘组织。

[0103] PiNK细胞的特征在于是$CD56^{+}CD16-$,即显示CD56细胞标志物并缺乏CD16细胞标志物,例如,通过流式细胞术测定,例如上文所述使用针对CD16和CD56的抗体荧光激活细胞分选术的测定。

[0104] 在某些实施方案中,PiNK细胞是$CD3^{-}$。

[0105] 在其它实施方案中,PiNK细胞不显示完全成熟的自然杀伤细胞所显示的一种或多种细胞标志物(例如CD16),或显示与完全成熟的自然杀伤细胞相比水平可检测地降低的所述一种或多种标志物,或显示与自然杀伤细胞前体有关但不与完全成熟的自然杀伤细胞有关的一种或多种细胞标志物。在一个具体的实施方案中,与完全成熟的NK细胞相比较,本文所述PiNK细胞以低的可检出水平表达NKG2D、CD94和/或NKp46。在另一个具体的实施方案中,与同等数目的完全成熟的NK细胞相比,本文所述的大量PiNK细胞共计以较低的可检出水平表达NKG2D、CD94和/或NKp46。

[0106] 在某些实施方案中,与外周血自然杀伤细胞相比,PiNK细胞以较高的可检出水平表达以下的一种或多种:microRNA hsa-miR-100、hsa-miR-127、hsa-miR-211、hsa-miR-302c、hsa-miR-326、hsa-miR-337、hsa-miR-497、hsa-miR-512-3p、hsa-miR-515-5p、hsa-miR-517b、hsa-miR-517c、hsa-miR-518a、hsa-miR-518e、hsa-miR-519d、hsa-miR-520g、hsa-miR-520h、hsa-miR-564、hsa-miR-566、hsa-miR-618和/或hsa-miR-99a。

[0107] 因为根据采集方法产后胎盘包含来自胎儿和来自母体胎盘灌注液的组织和细胞,所以PiNK细胞可只包含胎儿细胞,或基本上大部分的胎儿细胞(例如大于约90%、95%、98%或99%),或可包含胎儿和母体细胞的混合物(例如胎儿细胞包含小于灌注液总有核细胞的约90%、80%、70%、60%或50%)。在一个实施方案中,PiNK细胞只来源于胎儿胎盘细胞,例如,细胞获自胎盘闭路式灌注(见上),其中灌注产生包含基本上多数或仅胎儿胎盘细胞的灌注液。在另一个实施方案中,PiNK细胞来源于胎儿和母体细胞,例如,细胞通过借助盘法(pan method)的灌注获得(见上),其中灌注产生包含胎儿和母体胎盘细胞的混合物的灌注液。因此,在一个实施方案中,NK细胞是一群胎盘衍生中间体自然杀伤细胞,其基本上大部分具有胎儿基因型。在另一个实施方案中,NK细胞是一群胎盘衍生中间体自然杀伤细胞,包含具有胎儿基因型的自然杀伤细胞和具有母体表型的自然杀伤细胞。

[0108] 5.1.2.活化NK细胞

[0109] 在一些实施方案中，自然杀伤细胞是活化NK细胞(即两步NK细胞或TSNK细胞)(另参见美国专利申请公布号2012/0148553，其公开内容通过引用以其整体并入本文)，其是通过下文第5.2.4节所述任何方法/流程产生的NK细胞。

[0110] 在一个具体的实施方案中，活化NK细胞是$CD3^-CD56^+$。在一个具体的实施方案中，活化NK细胞是$CD3^-CD56^+$CD16-。在另一个具体的实施方案中，活化NK细胞另外是$CD94^+CD117^+$。在另一个具体的实施方案中，活化NK细胞另外是$CD161^-$。在另一个具体的实施方案中，活化NK细胞另外是$NKG2D^+$。在另一个具体的实施方案中，活化NK细胞另外是$NKp46^+$。在另一个具体的实施方案中，活化NK细胞另外是$CD226^+$。

[0111] 在某些实施方案中，大于50％、60％、70％、80％、90％、92％、94％、96％、98％的所述活化NK细胞是$CD56^+$和CD16-。在其它实施方案中，至少50％、60％、70％、80％、82％、84％、86％、88％或90％的所述活化NK细胞是$CD3^-$和$CD56^+$。在其它实施方案中，至少50％、52％、54％、56％、58％或60％的所述活化NK细胞是$NKG2D^+$。在其它实施方案中，少于30％、20％、10％、9％、8％、7％、6％、5％、4％或3％的所述细胞是$NKB1^+$。在某些其它实施方案中，少于30％、20％、10％、8％、6％、4％或2％的所述活化NK细胞是$NKAT2^+$。在某些其它实施方案中，少于30％、20％、10％、8％、6％、4％或2％的所述活化NK细胞是$CD56^+$和$CD16^+$。在更具体的实施方案中，至少10％、20％、25％、30％、35％、40％、50％、55％、60％、65％或70％的所述$CD3^-$，$CD56^+$活化NK细胞是$NKp46^+$。在其它更具体的实施方案中，至少10％、20％、25％、30％、35％、40％、50％、55％、60％、65％、70％、75％、80％或85％的所述$CD3^-$，$CD56^+$活化NK细胞是$CD117^+$。在其它更具体的实施方案中，至少10％、20％、25％、30％、35％、40％、45％或50％的所述$CD3^-$，$CD56^+$活化NK细胞是$CD94^+$。在其它更具体的实施方案中，至少10％、20％、25％、30％、35％、40％、45％或50％的所述$CD3^-$，$CD56^+$活化NK细胞是$CD161^-$。在其它更具体的实施方案中，至少10％、12％、14％、16％、18％或20％的所述$CD3^-$，$CD56^+$活化NK细胞是$CD226^+$。在更具体的实施方案中，至少20％、25％、30％、35％或40％的所述$CD3^-$，$CD56^+$活化NK细胞是$CD7^+$。在更具体的实施方案中，至少30％、35％、40％、45％、50％、55％或60％的所述$CD3^-$，$CD56^+$活化NK细胞是$CD5^+$。

[0112] 活化NK细胞可具有胎儿基因型或母体基因型。例如，由于作为适于产生活化NK细胞的造血细胞源的产后胎盘包含来自胎儿和来自母体的组织和细胞，因此胎盘灌注液可只包含胎儿细胞，或基本上大部分的胎儿细胞(例如大于约90％、95％、98％或99％)，或可包含胎儿和母体细胞的混合物(例如胎儿细胞包含小于灌注液总有核细胞的约90％、80％、70％、60％或50％)。在一个实施方案中，活化NK细胞只来源于胎儿胎盘造血细胞，例如，细胞获自胎盘闭路式灌注，其中灌注产生包含基本上多数或仅胎儿胎盘造血细胞的灌注液。在另一个实施方案中，活化NK细胞来源于胎儿和母体细胞，例如，细胞通过借助盘法的灌注获得(见上)，其中灌注产生包含胎儿和母体胎盘细胞的混合物的灌注液。因此，在一个实施方案中，活化NK细胞来源于一群胎盘衍生中间体自然杀伤细胞，其基本上大部分具有胎儿基因型。在另一个实施方案中，活化NK细胞来源于一群胎盘衍生中间体自然杀伤细胞，包含具有胎儿基因型的自然杀伤细胞和具有母体表型的自然杀伤细胞。

[0113] 在某些实施方案中，可通过检测一种或多种功能上相关的标志物，例如CD94、CD161、NKp44、DNAM-1、2B4、NKp46、CD94、KIR和激活性受体的NKG2家族(例如NKG2D)，来评价活化NK细胞或活化NK细胞富集群。

[0114] 任选例如可在细胞毒性测定法中使用肿瘤细胞，例如培养的K562、LN-18、U937、WERI-RB-1、U-118MG、HT-29、HCC2218、KG-1或U266肿瘤细胞等作为靶细胞，评价分离或富集的自然杀伤细胞的细胞毒活性。

[0115] 5.1.3.三步法NK(TSPNK)细胞

[0116] 在一些实施方案中，自然杀伤细胞是三步法NK(TSPNK)细胞，其是通过下文第5.2.5节描述的任何方法/流程产生的NK细胞。在具体的实施方案中，TSPNK细胞是NK祖细胞(另参见美国专利申请公布号2012/0148553，其公开内容通过引用以其整体并入本文)。

[0117] 5.1.3.1.TSPNK细胞

[0118] 在一个实施方案中，与通过本文所述三步法产生的NK祖细胞群相比，通过本文所述三步法产生的所述分离的TSPNK细胞群包含CD3-CD56+细胞的较大百分比，例如，与用于产生TSPNK细胞群的第三培养步骤相比，采用除用于产生NK祖细胞群的第三培养步骤以外均相同的三步法产生的NK祖细胞群具有较短的持续时间。在一个具体的实施方案中，所述TSPNK细胞群包含约65％、70％、75％、80％、85％、90％、95％、98％或99％CD3-CD56+细胞。在另一个具体的实施方案中，所述TSPNK细胞群包含不小于65％、70％、75％、80％、85％、90％、95％、98％或99％CD3-CD56+细胞。在另一个具体的实施方案中，所述TSPNK细胞群包含介于65％-70％、70％-75％、75％-80％、80％-85％、85％-90％、90％-95％或95％-99％之间的CD3-CD56+细胞。在另一个具体的实施方案中，使用包括长的第三培养步骤的三步法，例如18-20、19-21、20-22或21-23天的第三培养步骤，产生通过本文所述三步法产生的所述TSPNK细胞群。

[0119] 在某些实施方案中，所述TSPNK细胞群中的所述$CD3^-CD56^+$细胞包含另外是$CD117^+$的$CD3^-CD56^+$细胞，其中所述TSPNK细胞群包含与通过本文所述三步法产生的NK祖细胞群相比较小百分比的$CD3^-CD56^+CD117^+$细胞，例如，与用于产生TSPNK细胞群的第三培养步骤相比，采用除用于产生NK祖细胞群的第三培养步骤以外均相同的三步法产生的NK祖细胞群具有较短的持续时间。

[0120] 在某些实施方案中，所述TSPNK细胞群中的所述$CD3^-CD56^+$细胞包含另外是$CD161^+$的$CD3^-CD56^+$细胞，其中所述TSPNK细胞群包含与通过本文所述三步法产生的NK祖细胞群相比较小百分比的$CD3^-CD56^+CD161^+$细胞，例如，与用于产生TSPNK细胞群的第三培养步骤相比，采用除用于产生NK祖细胞群的第三培养步骤以外均相同的三步法产生的NK祖细胞群具有较短的持续时间。

[0121] 在某些实施方案中，所述TSPNK细胞群中的所述$CD3^-CD56^+$细胞包含另外是$NKp46^+$的$CD3^-CD56^+$的细胞，其中所述TSPNK细胞群包含与通过本文所述三步法产生的NK祖细胞群相比较大百分比的$CD3^-CD56^+NKp46^+$细胞，例如，与用于产生TSPNK细胞群的第三培养步骤相比，采用除用于产生NK祖细胞群的第三培养步骤以外均相同的三步法产生的NK祖细胞群具有较短的持续时间。

[0122] 在某些实施方案中，所述TSPNK细胞群中的所述$CD3^-CD56^+$细胞包含另外是CD16-的$CD3^-CD56^+$的细胞，其中所述TSPNK细胞群包含与通过本文所述三步法产生的NK祖细胞群相比较大百分比的$CD3^-CD56^+CD16^-$细胞，例如，与用于产生TSPNK细胞群的第三培养步骤相比，采用除用于产生NK祖细胞群的第三培养步骤以外均相同的三步法产生的NK祖细胞群具有较短的持续时间。在另一个实施方案中，与外周血(PB)衍生NK细胞相比，使用本文所述三步

法产生的TSPNK细胞具有较长的端粒。

[0123] 在一个实施方案中，通过本文所述三步法产生的TSPNK细胞群包含是CD117+的细胞。在一个具体的实施方案中，所述TSPNK细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％$CD117^+$细胞。在一个实施方案中，通过本文所述三步法产生的TSPNK细胞群包含是NKG2D+的细胞。在一个具体的实施方案中，所述TSPNK细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％$NKG2D^+$细胞。在一个实施方案中，通过本文所述三步法产生的TSPNK细胞群包含是NKp44+的细胞。在一个具体的实施方案中，所述TSPNK细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％$NKp44^+$细胞。在一个实施方案中，通过本文所述三步法产生的TSPNK细胞群包含是CD52+的细胞。在一个具体的实施方案中，所述TSPNK细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％$CD52^+$细胞。在一个具体的实施方案中，通过本文所述三步法产生的所述TSPNK细胞群包含是$CD52^+CD117^+$的细胞。在一个实施方案中，通过本文所述三步法产生的TSPNK细胞群包含是CD244+的细胞。在一个具体的实施方案中，所述TSPNK细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％$CD244^+$细胞。在一个具体的实施方案中，通过本文所述三步法产生的所述TSPNK细胞群包含是CD244+CD117+的细胞。在一个实施方案中，通过本文所述三步法产生的TSPNK细胞群包含是LFA-1+的细胞。在一个具体的实施方案中，所述TSPNK细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％LFA-1+细胞。在一个实施方案中，通过本文所述三步法产生的TSPNK细胞群包含是CD94+的细胞。在一个具体的实施方案中，所述TSPNK细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％CD94+细胞。

[0124] 5.1.3.2.NK祖细胞

[0125] 在一个实施方案中，所述分离的NK祖细胞群包含与非祖代NK细胞群(例按本文所述三步方法产生的非祖代NK细胞群)有关的CD3-CD56+细胞的百分比相比低百分比的CD3-CD56+细胞，例如，NK祖细胞群包含约5％、10％、15％、20％、25％、30％、35％、40％、45％或50％CD3-CD56+细胞。在另一个具体的实施方案中，所述NK祖细胞群包含不超过5％、10％、15％、20％、25％、30％、35％、40％、45％或50％CD3-CD56+细胞。在另一个具体的实施方案中，所述NK祖细胞群包含介于0％-5％、5％-10％、10％-15％、15％-20％、20％-25％、25％-30％、30％-35％、35％-40％、40％-45％或45％-50％之间的CD3-CD56+细胞。在一些实施方案中，所述NK祖细胞群，例如包含与非祖代NK细胞群有关的CD3-CD56+细胞的百分比相比低百分比的CD3-CD56+细胞的NK祖细胞群，包含不超过1％、不超过2％、不超过3％、不超过4％、不超过5％、不超过10％或不超过15％CD3-CD56+细胞。在另一个具体的实施方案中，通过本文所述三步法产生的所述NK祖细胞群使用包括短的第三培养步骤(例如4-6、5-7、6-8或7-9天的第三培养步骤)的三步法产生。

[0126] 在某些实施方案中，所述NK祖细胞群中的所述$CD3^-CD56^+$细胞另外是$CD117^+$。在一个具体的实施方案中，所述NK祖细胞群中约65％、70％、75％、80％、85％、90％、95％、98％

或99％的所述$CD3^-CD56^+$细胞是$CD117^+$。在另一个具体的实施方案中，所述NK祖细胞群中不小于65％、70％、75％、80％、85％、90％、95％、98％或99％的所述$CD3^-CD56^+$细胞是$CD117^+$。在另一个具体的实施方案中，所述NK祖细胞群中介于65％-70％、70％-75％、75％-80％、80％-85％、85％-90％、90％-95％或95％-99％之间的所述$CD3^-CD56^+$细胞是$CD117^+$。

[0127] 在某些实施方案中，所述NK祖细胞群中的所述$CD3^-CD56^+$细胞另外是CD161+。在一个具体的实施方案中，所述NK祖细胞群中约40％、45％、50％、55％、60％、65％、70％或75％的所述$CD3^-CD56^+$细胞是CD161+。在另一个具体的实施方案中，所述NK祖细胞群中不小于40％、45％、50％、55％、60％、65％、70％或75％的所述$CD3^-CD56^+$细胞是CD161+。在另一个具体的实施方案中，所述NK祖细胞群中介于40％-45％、45％-50％、50％-55％、55％-60％、60％-65％、65％-70％或70％-75％之间的所述$CD3^-CD56^+$细胞是CD161+。

[0128] 在某些实施方案中，所述NK祖细胞群中的所述$CD3^-CD56^+$细胞另外是NKp46+。在一个具体的实施方案中，所述NK祖细胞群中约25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％或更多的所述$CD3^-CD56^+$细胞是NKp46+。在一个更具体的实施方案中，所述NK祖细胞群中约25％、30％、35％、40％、45％、50％或55％的所述$CD3^-CD56^+$细胞是NKp46+。在另一个具体的实施方案中，所述NK祖细胞群中不超过25％、30％、35％、40％、45％、50％或55％的所述$CD3^-CD56^+$细胞是NKp46+。在另一个具体的实施方案中，所述NK祖细胞群中介于25％-30％、30％-35％、35％-40％、40％-45％、45％-50％、50％-55％、55％-60％、60％-65％、65％-70％、70％-75％、75％-80％、80％-85％、85％-90％或更多的所述$CD3^-CD56^+$细胞是NKp46+。在一个更具体的实施方案中，所述NK祖细胞群中介于25％-30％、30％-35％、35％-40％、40％-45％、45％-50％或50％-55％的所述$CD3^-CD56^+$细胞是$NKp46^+$。

[0129] 在某些实施方案中，所述NK祖细胞群含有是$CD56^+CD16^-$的细胞。在某些实施方案中，所述NK祖细胞群中的$CD3^-CD56^+$细胞是CD16-。在某些实施方案中，所述NK祖细胞群中的$CD3^-CD56^+$细胞是$CD16^+$。在一个具体的实施方案中，所述NK祖细胞群包含不超过5％、10％、15％、20％、25％、30％、35％、40％、45％或50％$CD16^+$细胞。在另一个具体的实施方案中，所述NK祖细胞群包含介于0％-5％、5％-10％、10％-15％、15％-20％或20％-25％之间的$CD16^+$细胞。在一些实施方案中，所述NK祖细胞群包含不超过1％、不超过2％、不超过3％、不超过4％、不超过5％、不超过10％或不超过15％$CD16^+$细胞。

[0130] 在某些实施方案中，所述NK祖细胞群中的所述$CD3^-CD56^+$细胞另外是CD16-。在某些实施方案中，所述NK祖细胞群中的所述$CD3^-CD56^+$细胞另外是CD117+和CD161+。在某些实施方案中，所述NK祖细胞群中的所述$CD3^-CD56^+$细胞另外是CD16-、CD117+和CD161+。在某些实施方案中，所述NK祖细胞群中的所述$CD3^-CD56^+$细胞另外是CD16-、CD117+、CD161+和$NKp46^+$。

[0131] 在一个实施方案中，由本文所述三步法产生的NK祖细胞群包含不超过约40％CD3-CD56+细胞。在一个实施方案中，由本文所述三步法产生的NK祖细胞群包含是CD117+的细胞。在一个具体的实施方案中，所述NK祖细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％CD117+细胞。在一个实施方案中，由本文所述三步法产生的NK祖细胞群包含是CD52+的细胞。在一个具体的实施方案中，所述NK祖细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％CD52+细胞。在一个具体的

实施方案中，由本文所述三步法产生的所述NK祖细胞群包含是$CD52^+CD117^+$的细胞。在一个实施方案中，由本文所述三步法产生的NK祖细胞群包含是CD244+的细胞。在一个具体的实施方案中，所述NK祖细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％CD244+细胞。在一个具体的实施方案中，由本文所述三步法产生的所述NK祖细胞群包含是CD244+CD117+的细胞。在一个实施方案中，由本文所述三步法产生的NK祖细胞群包含是LFA-1+的细胞。在一个具体的实施方案中，所述NK祖细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％LFA-1+细胞。在一个实施方案中，由本文所述三步法产生的NK祖细胞群包含是CD94+的细胞。在一个具体的实施方案中，所述NK祖细胞群包含不超过约5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或90％CD94+细胞。

[0132] 在具体的实施方案中，与CD56+细胞相比，通过本文所述三步法产生的NK祖细胞群包含较大比例的CD56-细胞。在具体的实施方案中，由本文所述三步法产生的NK祖细胞群体内或离体分化成CD56+细胞比例提高的群。

[0133] 在一个具体的实施方案中，通过本文所述三步法产生的NK祖细胞群包含与非祖代NK细胞群有关的$CD34^-CD117^+$细胞的百分比相比低百分比的$CD34^-CD117^+$细胞，例如NK祖细胞群包含约5％、10％、15％、20％、25％、30％、35％、40％、45％或50％$CD34^-CD117^+$细胞。在另一个具体的实施方案中，所述NK祖细胞群包含不超过5％、10％、15％、20％、25％、30％、35％、40％、45％或50％$CD34^-CD117^+$细胞。在另一个具体的实施方案中，所述NK祖细胞群包含介于0％-5％、5％-10％、10％-15％、15％-20％、20％-25％、25％-30％、30％-35％、35％-40％、40％-45％或45％-50％之间的$CD34^-CD117^+$细胞。在一些实施方案中，所述NK祖细胞群包含不超过1％、不超过2％、不超过3％、不超过4％、不超过5％、不超过10％或不超过15％$CD34^-CD117^+$细胞。在另一个具体的实施方案中，由本文所述三步法产生的所述NK祖细胞群采用包括短的第三培养步骤(例如4-6、5-7、6-8或7-9天的第三培养步骤)的三步法产生。

[0134] 在一个具体的实施方案中，通过本文所述三步法产生的NK祖细胞群包含与非祖代NK细胞群有关的$CD161^+$细胞的百分比相比低百分比的CD161+细胞，例如，NK祖细胞群包含约5％、10％、15％、20％、25％、30％、35％、40％、45％或50％$CD161^+$细胞。在另一个具体的实施方案中，所述NK祖细胞群包含不超过5％、10％、15％、20％、25％、30％、35％、40％、45％或50％$CD161^+$细胞。在另一个具体的实施方案中，所述NK祖细胞群包含介于0％-5％、5％-10％、10％-15％、15％-20％、20％-25％、25％-30％、30％-35％、35％-40％、40％-45％或45％-50％之间的$CD161^+$细胞。在一些实施方案中，所述NK祖细胞群包含不超过1％、不超过2％、不超过3％、不超过4％、不超过5％、不超过10％或不超过15％$CD161^+$细胞。在另一个具体的实施方案中，由本文所述三步法产生的所述NK祖细胞群采用包括短的第三培养步骤(例如4-6、5-7、6-8或7-9天的第三培养步骤)的三步法产生。

[0135] 在一个具体的实施方案中，通过本文所述三步法产生的NK祖细胞群包含与非祖代NK细胞群有关的$NKp46^+$细胞的百分比相比低百分比的$NKp46^+$细胞，例如，NK祖细胞群包含约1％、5％、10％、15％、20％、25％、30％、35％、40％、45％或50％$NKp46^+$细胞。在另一个具体的实施方案中，所述NK祖细胞群包含不超过1％、5％、10％、15％、20％、25％、30％、35％、

40％、45％或50％NKp46$^+$细胞。在另一个具体的实施方案中，所述NK祖细胞群包含介于0％-5％、5％-10％、10％-15％、15％-20％、20％-25％、25％-30％、30％-35％、35％-40％、40％-45％或45％-50％之间的NKp46$^+$细胞。在一些实施方案中，所述NK祖细胞群包含不超过1％、不超过2％、不超过3％、不超过4％、不超过5％、不超过10％或不超过15％NKp46$^+$细胞。在另一个具体的实施方案中，由本文所述三步法产生的所述NK祖细胞群采用包括短的第三培养步骤(例如4-6、5-7、6-8或7-9天的第三培养步骤)的三步法产生。

[0136] 在一个具体的实施方案中，通过本文所述三步法产生的NK祖细胞群包含与非祖代NK细胞群有关的CD56$^+$CD16-细胞的百分比相比低百分比的CD56$^+$CD16-细胞，例如，NK祖细胞群包含约1％、5％、10％、15％、20％、25％、30％、35％、40％、45％或50％CD56$^+$CD16-细胞。在另一个具体的实施方案中，所述NK祖细胞群包含不超过1％、5％、10％、15％、20％、25％、30％、35％、40％、45％或50％CD56$^+$CD16-细胞。在另一个具体的实施方案中，所述NK祖细胞群包含介于0％-5％、5％-10％、10％-15％、15％-20％、20％-25％、25％-30％、30％-35％、35％-40％、40％-45％或45％-50％之间的CD56$^+$CD16-细胞。在一些实施方案中，所述NK祖细胞群包含不超过1％、不超过2％、不超过3％、不超过4％、不超过5％、不超过10％或不超过15％CD56$^+$CD16-细胞。在另一个具体的实施方案中，由本文所述三步法产生的所述NK祖细胞群采用包括短的第三培养步骤(例如4-6、5-7、6-8或7-9天的第三培养步骤)的三步法产生。

[0137] 在一个实施方案中，由本文所述三步法产生的NK祖细胞群包含是CD52+CD117+的细胞。在一个具体的实施方案中，通过本文所述三步法产生的NK祖细胞群包含与造血祖细胞群有关的CD52$^+$CD117+细胞的百分比相比较高百分比的CD52$^+$CD117+细胞。在一个具体的实施方案中，通过本文所述三步法产生的NK祖细胞群包含与非祖代NK细胞群有关的CD52$^+$CD117+细胞的百分比相比较高百分比的CD52$^+$CD117+细胞，例如，NK祖细胞群包含约50％、55％、60％、65％、70％、75％、80％、85％、90％或更多的CD52$^+$CD117+细胞。在另一个具体的实施方案中，所述NK祖细胞群包含不小于50％、55％、60％、65％、70％、75％、80％、85％或90％CD52$^+$CD117+细胞。在另一个具体的实施方案中，所述NK祖细胞群包含介于50％-55％、55％-60％、60％-65％、65％-70％、70％-75％、75％-80％、80％-85％、85％-90％、90％-95％之间或更多的CD52$^+$CD117+细胞。在另一个具体的实施方案中，包含通过本文所述三步法产生的CD52$^+$CD117+细胞的所述NK祖细胞群采用包括短的第三培养步骤(例如4-6、5-7、6-8或7-9天的第三培养步骤)的三步法产生。在一个具体的实施方案中，包含CD52$^+$CD117+细胞的所述NK祖细胞群采用包括培养共12天或更多天、13天或更多天、14天或更多天、15天或更多天、16天或更多天、17天或更多天、18天或更多天、19天或更多天、20天或更多天或21天或更多天的三步法产生。在一个具体的实施方案中，包含CD52$^+$CD117+细胞的所述NK祖细胞群采用包括培养共至少12天、13天或14天但不超过21-25天、25-30天或30-35天的三步法产生。在一个具体的实施方案中，包含CD52$^+$CD117+细胞的所述NK祖细胞群采用包括培养共21天的三步法产生。

[0138] 在一个具体的实施方案中，本文所述NK祖细胞具有比非祖代NK细胞(例如采用同类方法产生的非祖代NK细胞)大的植入骨髓(例如体内)的能力。例如，在某些实施方案中，采用包括短的第三培养步骤(例如4-6、5-7、6-8或7-9天的第三培养步骤)的三步法产生的NK祖细胞以比采用包括较长的第三培养步骤(例如18-20、19-21、20-22或21-23天的第三培

养步骤)的三步法产生的非祖代NK细胞高的效率植入骨髓(例如体内)。在另一个实施方案中,本文所述NK祖细胞具有比外周血(PB)衍生NK细胞长的端粒。

[0139] 5.1.4.通过三阶段方法产生的NK细胞

[0140] 在一个实施方案中,本文提供分离的NK细胞群,其中所述NK细胞按照下文所述三阶段方法产生。

[0141] 在一个实施方案中,本文提供通过本文所述三阶段方法产生的分离的NK细胞群,其中所述NK细胞群包含与通过本文所述三阶段方法产生的NK祖细胞群相比较大百分比的CD3-CD56+细胞,例如,与用于产生NK细胞群的第三培养步骤相比,通过除用于产生NK祖细胞群的第三培养步骤以外均相同的三阶段方法产生的NK祖细胞群具有较短的持续时间。在一个具体的实施方案中,所述NK细胞群包含约70%或更多,在一些实施方案中75%、80%、85%、90%、95%、98%或99%CD3-CD56+细胞。在另一个具体的实施方案中,所述NK细胞群包含不小于80%、85%、90%、95%、98%或99%CD3-CD56+细胞。在另一个具体的实施方案中,所述NK细胞群包含介于70%-75%、75%-80%、80%-85%、85%-90%、90%-95%或95%-99%之间的CD3-CD56+细胞。

[0142] 在某些实施方案中,所述NK细胞群中的所述CD3-CD56+细胞包含另外是NKp46+的CD3-CD56+细胞。在某些实施方案中,所述NK细胞群中的所述CD3-CD56+细胞包含另外是CD16-的CD3-CD56+细胞。在某些实施方案中,所述NK细胞群中的所述CD3-CD56+细胞包含另外是CD16+的CD3-CD56+细胞。在某些实施方案中,所述NK细胞群中的所述CD3-CD56+细胞包含另外是CD94-的CD3-CD56+细胞。在某些实施方案中,所述NK细胞群中的所述CD3-CD56+细胞包含另外是CD94+的CD3-CD56+细胞。

[0143] 在一个实施方案中,通过本文所述三阶段方法产生的NK细胞群包含是CD117+的细胞。在一个实施方案中,通过本文所述三阶段方法产生的NK细胞群包含是NKG2D+的细胞。在一个实施方案中,通过本文所述三阶段方法产生的NK细胞群包含是NKp44+的细胞。在一个实施方案中,通过本文所述三阶段方法产生的NK细胞群包含是CD244+的细胞。

[0144] 5.1.5.细胞组合和细胞/灌注液组合

[0145] NK细胞,例如活化NK细胞和/或TSPNK细胞在本发明中可与胎盘灌注液、胎盘灌注液细胞和/或贴壁胎盘细胞进一步组合。

[0146] 5.1.5.1.NK细胞和灌注液或灌注液细胞的组合

[0147] 在具体的实施方案中,自然杀伤细胞包含与$CD56^+CD16^+$自然杀伤细胞组合的$CD56^+$CD16-PiNK细胞。在更具体的实施方案中,$CD56^+CD16^+$自然杀伤细胞可自胎盘或自另一来源例如外周血、脐带血、骨髓等分离。因此,在不同的其它实施方案中,PiNK细胞例如可以例如以下比率与$CD56^+CD16^+$自然杀伤细胞组合:约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或约9:1。当用于本文时,"分离的"意指细胞已从其正常环境(例如胎盘)中取出。

[0148] 在不同的具体实施方案中,分离的NK细胞群包含至少约50%、55%、60%、65%、70%、75%、80%、85%、90%、95%、98%或至少约99%PiNK细胞。在另一个实施方案中,大量的PiNK细胞包含未经扩增的、例如与从胎盘灌注液收集一样的PiNK细胞或由其组成。在另一个实施方案中,大量的PiNK细胞包含经扩增的PiNK细胞或由其组成。扩增自然杀伤细胞的方法描述于本文的其它部分,例如描述于Ohno等,美国专利申请公布号2003/0157713;

另参见Yssel等,J.Immunol.Methods 72(1):219-227(1984)和Litwin等,J.Exp.Med.178(4):1321-1326(1993)。

[0149] 在具体的实施方案中,分离的NK细胞群包含PiNK细胞的胎盘细胞群。在一个具体的实施方案中,分离的NK细胞群是来自包含自体的分离PiNK细胞的胎盘灌注液(例如胎盘灌注液细胞)的总有核细胞。在不同的其它实施方案中,活化NK细胞可以例如以下比率与例如其中所述NK细胞自组织来源分离且未经扩增的NK细胞、自组织来源分离并扩增的NK细胞或通过不同方法产生的NK细胞(例如$CD56^+CD16^+$自然杀伤细胞)组合:约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或约9:1。当用于本文时,"分离的"意指细胞已从其正常组织环境中取出。

[0150] 在具体的实施方案中,活化NK细胞还可以例如以下比率与例如其中所述NK细胞自组织来源分离且未经扩增的NK细胞、自组织来源分离并扩增的NK细胞或通过不同方法产生的NK细胞(例如$CD56^+CD16^+$自然杀伤细胞)组合:约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或约9:1。当用于本文时,"分离的"意指细胞已从其正常组织环境中取出。

[0151] 在一个实施方案中,例如,使用补充采用本文所述方法产生的NK细胞,例如活化NK细胞或TSPNK细胞(例如NK祖细胞)的一定体积的胎盘灌注液。在具体的实施方案中,例如,每毫升胎盘灌注液补充约$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$或更多个采用本文所述方法产生的NK细胞,例如活化NK细胞或TSPNK细胞(例如NK祖细胞)。在另一个实施方案中,胎盘灌注液细胞补充采用本文所述方法产生的NK细胞,例如活化NK细胞或TSPNK细胞(例如NK祖细胞)。在某些其它实施方案中,当胎盘灌注液细胞与采用本文所述方法产生的NK细胞例如活化NK细胞或TSPNK细胞(例如NK祖细胞)组合时,胎盘灌注液细胞一般包含细胞总数的约、大于约或少于约50%、45%、40%、35%、30%、25%、20%、15%、10%、8%、6%、4%、2%或1%。在某些其它实施方案中,当采用本文所述方法产生的NK细胞例如活化NK细胞或TSPNK细胞(例如NK祖细胞)与大量胎盘灌注液细胞和/或混合自然杀伤细胞组合时,NK细胞一般包含细胞总数的约、大于约或少于约50%、45%、40%、35%、30%、25%、20%、15%、10%、8%、6%、4%、2%或1%。在某些其它实施方案中,当使用采用本文所述方法产生的NK细胞例如活化NK细胞或TSPNK细胞(例如NK祖细胞)补充胎盘灌注液时,细胞悬浮于其中的溶液(例如盐溶液,培养基等)的体积包含灌注液加细胞的总体积的约、大于约或小于约50%、45%、40%、35%、30%、25%、20%、15%、10%、8%、6%、4%、2%或1%,其中在补充前将NK细胞悬浮至约$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$或多个细胞/毫升。

[0152] 在其它实施方案中,细胞的上述组合的任一个进而与来自脐带血的脐带血细胞或有核细胞组合。

[0153] 获自两个或更多个来源(例如两个或更多个胎盘)且混合(例如合并)的合并的胎盘灌注液可进一步用于本发明中。所述合并的灌注液可包含来自各个来源的大致等体积的灌注液,或可包含来自各个来源的不同体积。来自各个来源的相对体积可随机选择,或可基于例如一种或多种细胞因子(例如细胞因子、生长因子、激素等)的浓度或量;来自各个来源的灌注液中的胎盘细胞数;或来自各个来源的灌注液的其它特征。同样地可合并同一胎盘的多次灌注的灌注液。

[0154] 同样地，获自两个或更多个来源(例如两个或更多个胎盘)并合并的胎盘灌注液细胞和胎盘衍生中间体自然杀伤细胞也可用于本发明中。所述合并的细胞可包含来自两个或更多个来源的大致相同数目的细胞，或来自合并来源的一个或多个的不同数目的细胞。来自各个来源的细胞的相对数可基于例如待合并的细胞中一个或多个具体细胞类型的数目，例如$CD34^+$细胞等的数目。

[0155] 可测定采用本文所述方法产生的NK细胞例如活化NK细胞或TSPNK细胞(例如NK祖细胞)和所述细胞与胎盘灌注液和/或胎盘灌注液细胞的组合以确定预期来自例如指定数目的NK细胞或指定体积的灌注液的肿瘤/感染抑制(即效能)的程度或量。例如，使细胞的等分样或样品数在其中肿瘤/感染细胞另可增殖的条件下与已知数目的肿瘤/感染细胞接触或与之接近，并且将在胎盘灌注液、灌注液细胞、胎盘自然杀伤细胞或其组合存在时随时间推移(例如1、2、3、4、5、6、7、8、9或10周或更久)的肿瘤/感染细胞的增殖速率与在灌注液、灌注液细胞、胎盘自然杀伤细胞或其组合不存在时相同数目的肿瘤/感染细胞的增殖进行比较。细胞的效能可表示为例如抑制肿瘤细胞生长/感染扩散例如达约10％、15％、20％、25％、30％、35％、40％、45％、50％等所需要的细胞的数目或溶液的体积。

[0156] 在某些实施方案中，采用本文所述方法产生的NK细胞例如活化NK细胞或TSPNK细胞(例如NK祖细胞)作为药品级可给予单位提供。所述单位可以分立的体积提供，例如15mL、20mL、25mL、30nL。35mL、40mL、45mL、50mL、55mL、60mL、65mL、70mL、75mL、80mL、85mL、90mL、95mL、100mL、150mL、200mL、250mL、300mL、350mL、400mL、450mL、500mL等。可提供所述单位以含有规定数目的细胞，例如NK细胞或NK细胞群或与其它NK细胞或灌注液细胞组合的NK祖细胞群，例如$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$或更多个细胞/毫升，或$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$、$1x10^9$、$5x10^9$、$1x10^{10}$、$5x10^{10}$、$1x10^{11}$或更多个细胞/单位。在具体的实施方案中，单位可包含约、至少约或至多约$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$或更多个NK细胞/毫升，或$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$、$1x10^9$、$5x10^9$、$1x10^{10}$、$5x10^{10}$、$1x10^{11}$或更多个细胞/单位。可提供所述单位以含有规定数目的NK细胞和/或其它细胞的任一种。

[0157] 在上述实施方案中，NK细胞或NK细胞与灌注液细胞或灌注液的组合对于接受者可为自体的(即获自接受者)，或对于接受者可以为同种异体的(即获自所述接受者以外的至少一个其它个体)。

[0158] 在某些实施方案中，对每单位的细胞进行标记以指明体积、细胞数、细胞类型、该单位是否已富集了特定类型的细胞和/或该单位中给定细胞数或给定毫升数的单位效能(即，该单位中的细胞是否引起一种或多种特定类型的肿瘤细胞增殖的可测量抑制)的一种或多种。

[0159] 5.1.5.2.来自匹配的灌注液和脐带血的NK细胞的组合

[0160] 自然杀伤细胞在本发明中可进一步获自胎盘灌注液和脐带血的匹配单位的组合，在本文称为混合自然杀伤细胞。本文所用“匹配单位”表示NK细胞获自胎盘灌注液细胞和脐带血细胞，其中脐带血细胞获自从中获得胎盘灌注液的胎盘的脐带血，即胎盘灌注液细胞和脐带血细胞且因此来自各个的自然杀伤细胞来自同一个体。

[0161] 在某些实施方案中，组合的胎盘杀伤细胞只包含或基本只包含是$CD56^+$和CD16-的

自然杀伤细胞。在某些其它实施方案中，组合的胎盘杀伤细胞包含是$CD56^+$和CD16-的NK细胞和是$CD56^+$和$CD16^+$的NK细胞。在某些具体实施方案中，组合的胎盘杀伤细胞包含至少50％、60％、70％、80％、90％、95％、96％、97％、98％、99％或99.5％$CD56^+$CD16-自然杀伤细胞(PiNK细胞)。

[0162] 在一个实施方案中，混合自然杀伤细胞不经过培养。在一个具体的实施方案中，与同等数目的来自外周血的自然杀伤细胞相比，混合自然杀伤细胞包含可检测的较高数目的$CD3^-CD56^+$CD16-自然杀伤细胞。在另一个具体的实施方案中，与同等数目的来自外周血的自然杀伤细胞相比，混合自然杀伤细胞包含可检测的较低数目的$CD3^-CD56^+$CD16-自然杀伤细胞。在另一个具体的实施方案中，与同等数目的来自外周血的自然杀伤细胞相比，混合自然杀伤细胞包含可检测的较高数目的$CD3^-CD56^+$KIR2DL2/L3$^+$自然杀伤细胞。在另一个具体的实施方案中，与同等数目的来自外周血的自然杀伤细胞相比，混合自然杀伤细胞包含可检测的较低数目的$CD3^-CD56^+$NKp46$^+$自然杀伤细胞。在另一个具体的实施方案中，与同等数目的来自外周血的自然杀伤细胞相比，混合自然杀伤细胞包含可检测的较低数目的$CD3^-CD56^+$NKp30$^+$自然杀伤细胞。在另一个具体的实施方案中，与同等数目的来自外周血的自然杀伤细胞相比，混合自然杀伤细胞包含可检测的较低数目的$CD3^-CD56^+$2B4$^+$自然杀伤细胞。在另一个具体的实施方案中，与同等数目的来自外周血的自然杀伤细胞相比，混合自然杀伤细胞包含可检测的较低数目的$CD3^-CD56^+CD94^+$自然杀伤细胞。

[0163] 在另一个实施方案中，混合自然杀伤细胞经培养例如21天。在一个具体的实施方案中，与同等数目的来自外周血的自然杀伤细胞相比，混合自然杀伤细胞包含可检测的较低数目的$CD3^-CD56^+$KIR2DL2/L3$^+$自然杀伤细胞。在另一个具体的实施方案中，混合自然杀伤细胞不经过培养。在另一个具体的实施方案中，与同等数目的来自外周血的自然杀伤细胞相比，混合自然杀伤细胞包含可检测的较高数目的$CD3^-CD56^+$NKp44$^+$自然杀伤细胞。在一个具体的实施方案中，与同等数目的来自外周血的自然杀伤细胞相比，混合自然杀伤细胞包含可检测的较高数目的$CD3^-CD56^+$NKp30$^+$自然杀伤细胞。

[0164] 在另一个实施方案中，与同等数目的外周血自然杀伤细胞相比，混合自然杀伤细胞表达可检测的较高量的粒酶B。

[0165] 混合自然杀伤细胞可进一步与脐带血组合。在不同的实施方案中，脐带血以约$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$个混合自然杀伤细胞/毫升脐带血与混合自然杀伤细胞组合。

[0166] 5.1.5.3.NK细胞与贴壁胎盘干细胞的组合

[0167] 在其它实施方案中，采用本文所述方法产生的NK细胞，例如采用本文所述三步法产生的活化NK细胞或TSPNK细胞(例如NK祖细胞)，单独或与胎盘灌注液或胎盘灌注液细胞组合地补充了分离的贴壁胎盘细胞，例如描述于例如Hariri的美国专利号7,045,148和7,255,879及美国专利申请公布号2007/0275362(其公开内容通过引用以其整体并入本文)的胎盘干细胞和胎盘多能细胞。“贴壁胎盘细胞”意指附着在组织培养表面(例如组织培养塑料)的细胞。可用于本文公开的组合物和方法的贴壁胎盘细胞不是滋养层、胚胎生殖细胞或胚干细胞。在某些实施方案中，贴壁胎盘干细胞在上述方法(例如两步方法)期间用作饲养细胞。

[0168] 采用本文所述方法产生的NK细胞例如活化NK细胞或TSPNK细胞(例如NK祖细胞)，

单独或与胎盘灌注液或胎盘灌注液细胞组合地可补充例如$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$或更多个贴壁胎盘细胞/毫升，或$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$、$1x10^9$、$5x10^9$、$1x10^{10}$、$5x10^{10}$、$1x10^{11}$更多个贴壁胎盘细胞。组合中的贴壁胎盘细胞可以是例如已培养例如1、2、3、4、5、6、7、8、9、10、12、14、16、18、20、22、24、26、28、30、32、34、36、38或40个群体倍增或更多的贴壁胎盘细胞。

[0169] 分离的贴壁胎盘细胞，当在原代培养中培养或在细胞培养中扩增时，附着在组织培养基质例如组织培养容器表面(例如组织培养塑料)上。培养中的贴壁胎盘细胞一般呈成纤维样星状外形，其多个胞突从中心细胞体延伸出来。然而，贴壁胎盘细胞在形态上可与培养在相同条件下的成纤维细胞区分开来，因为贴壁胎盘细胞显示比成纤维细胞较多数目的这类胞突。在形态上，贴壁胎盘细胞还可与造血干细胞区分开来，后者在培养时一般呈更圆形的或鹅卵石的形态。

[0170] 可用于本文提供的组合物和方法的分离的贴壁胎盘细胞和贴壁胎盘细胞群表达可用来鉴定和/或分离细胞或包含贴壁胎盘细胞的细胞群的多种标志物。可用于本文提供的组合物和方法的贴壁胎盘细胞和贴壁胎盘细胞群包括直接获自胎盘的贴壁胎盘细胞和含贴壁胎盘细胞的细胞群或其任何部分(例如羊膜、绒毛膜、羊膜-绒毛膜板、胎盘绒毛叶、脐带等)。在一个实施方案中，贴壁胎盘干细胞群是培养中的一群(即两个或更多个)贴壁胎盘干细胞，例如容器(例如袋)中的一群。

[0171] 贴壁胎盘细胞一般表达标志物CD73、CD105和CD200和/或OCT-4，而不表达CD34、CD38或CD45。贴壁胎盘干细胞还可表达HLA-ABC(MHC-1)和HLA-DR。这些标志物可用来鉴定贴壁胎盘细胞，并将贴壁胎盘细胞与其它细胞类型区分开来。因为贴壁胎盘细胞可表达CD73和CD105，所以它们具有间充质干细胞样性质。CD34、CD38和/或CD45表达的缺乏鉴定贴壁胎盘干细胞为非造血干细胞。

[0172] 在某些实施方案中，本文描述的分离的贴壁胎盘细胞可检测地抑制癌细胞增殖或肿瘤生长。

[0173] 在某些实施方案中，分离的贴壁胎盘细胞是分离的胎盘干细胞。在某些其它实施方案中，分离的贴壁胎盘细胞是分离的胎盘多能细胞。在一个具体的实施方案中，分离的贴壁胎盘细胞是通过流式细胞术检出的$CD34^-$、$CD10^+$和$CD105^+$。在一个更具体的实施方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$贴壁胎盘细胞是胎盘干细胞。在另一个更具体的实施方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$胎盘细胞是多能贴壁胎盘细胞。在另一个具体的实施方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$胎盘细胞具有分化成神经表型的细胞、成骨表型的细胞或软骨形成表型的细胞的潜力。在一个更具体的实施方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$贴壁胎盘细胞另外是$CD200^+$。在另一个更具体的实施方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$贴壁胎盘细胞另外是通过流式细胞术检出的$CD90^+$或$CD45^-$。在另一个更具体的实施方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$贴壁胎盘细胞另外是通过流式细胞术检出的$CD90^+$或$CD45^-$。在一个更具体的实施方案中，$CD34^-$、$CD10^+$、$CD105^+$，$CD200^+$贴壁胎盘细胞另外是通过流式细胞术检出的$CD90^+$或$CD45^-$。在另一个更具体的实施方案中，$CD34^-$、$CD10^+$、$CD105^+$，$CD200^+$贴壁胎盘细胞另外是通过流式细胞术检出的$CD90^+$和$CD45^-$。在另一个更具体的实施方案中，$CD34^-$、$CD10^+$、$CD105^+$、$CD200^+$、$CD90^+$、$CD45^-$贴壁胎盘细胞另外是通过流式细胞术检出的$CD80^-$和$CD86^-$。

[0174] 在一个实施方案中，分离的贴壁胎盘细胞是$CD200^+$、$HLA\text{-}G^+$。在一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD73^+$和$CD105^+$。在另一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$或$CD45^-$。在一个更具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$、$CD45^-$、$CD73^+$和$CD105^+$。在另一个实施方案中，所述分离的贴壁胎盘细胞当在允许胚状体样体(embryoid-like bodies)形成的条件下培养时产生一个或多个胚状体样体。

[0175] 在另一个实施方案中，分离的贴壁胎盘细胞是CD73+、$CD105^+$、$CD200^+$。在一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$HLA\text{-}G^+$。在另一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$或$CD45^-$。在另一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$和$CD45^-$。在一个更具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$、$CD45^-$和$HLA\text{-}G^+$。在另一个具体的实施方案中，所述分离的贴壁胎盘细胞当在允许胚状体样体形成的条件下培养时产生一个或多个胚状体样体。

[0176] 在另一个实施方案中，分离的贴壁胎盘细胞是$CD200^+$、$OCT\text{-}4^+$。在一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD73^+$和$CD105^+$。在另一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$HLA\text{-}G^+$。在另一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$和$CD45^-$。在一个更具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$、$CD45^-$、$CD73^+$、$CD105^+$和$HLA\text{-}G^+$。在另一个具体的实施方案中，分离的贴壁胎盘细胞当在允许胚状体样体形成的条件下培养时还产生一个或多个胚状体样体。

[0177] 在另一个实施方案中，分离的贴壁胎盘细胞是$CD73^+$、$CD105^+$和$HLA\text{-}G^+$。在一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$或$CD45^-$。在另一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$和$CD45^-$。在另一个具体的实施方案中，所述贴壁干细胞还是$OCT\text{-}4^+$。在另一个具体的实施方案中，所述贴壁干细胞还是$CD200^+$。在一个更具体的实施方案中，所述贴壁干细胞还是$CD34^-$、$CD38^-$、$CD45^-$、$OCT\text{-}4^+$和$CD200^+$。

[0178] 在另一个实施方案中，分离的贴壁胎盘细胞是$CD73^+$、$CD105^+$干细胞，其中所述细胞在允许胚状体样体形成的条件下产生一个或多个胚状体样体。在一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$或$CD45^-$。在另一个具体的实施方案中，分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$和$CD45^-$。在另一个具体的实施方案中，分离的贴壁胎盘细胞还是$OCT\text{-}4^+$。在一个更具体的实施方案中，所述分离的贴壁胎盘细胞还是$OCT\text{-}4^+$、$CD34^-$、$CD38^-$和$CD45^-$。

[0179] 在另一个实施方案中，贴壁胎盘干细胞是$OCT\text{-}4^+$干细胞，其中所述贴壁胎盘干细胞当在允许胚状体样体形成的条件下培养时产生一个或多个胚状体样体，且其中所述干细胞被鉴定为可检测地抑制癌细胞增殖或肿瘤生长。

[0180] 在不同的实施方案中，至少10％、至少20％、至少30％、至少40％、至少50％至少60％、至少70％、至少80％、至少90％或至少95％的所述分离的贴壁胎盘细胞是$OCT\text{-}4^+$。在一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD73^+$和$CD105^+$。在另一个具体的实施方案中，所述分离的贴壁胎盘细胞还是$CD34^-$、$CD38^-$或$CD45^-$。在另一个具体的实施方案中，所述干细胞是$CD200^+$。在一个更具体的实施方案中，所述分离的贴壁胎盘细胞还是$CCD73^+$、$CD105^+$、$CD200^+$、$CD34^-$、$CD38^-$和$CD45^-$。在另一个具体的实施方案中，所述分离的贴壁胎盘细胞已扩增，例如传代至少一次、至少3次、至少5次、至少10次、至少15次或至少20次。

[0181] 在上述实施方案的一个更具体的实施方案中，分离的贴壁胎盘细胞表达ABC-p(一种胎盘特异性ABC转运蛋白；参见例如Allikmets等，Cancer Res.58(23):5337-9(1998))。

[0182] 在另一个实施方案中，分离的贴壁胎盘细胞$CD29^+$、$CD44^+$、$CD73^+$、$CD90^+$、$CD105^+$、$CD200^+$、$CD34^-$和$CD133^-$。在另一个实施方案中，分离的贴壁胎盘细胞组成型地分泌IL-6、IL-8和单核细胞化学引诱物蛋白(MCP-1)。

[0183] 上文提及的分离的贴壁胎盘细胞的每一种可包含直接自哺乳动物获得和分离的细胞或已培养和传代至少1、2、3、4、5、6、7、8、9、10、12、14、16、18、20、25、30次或更多次的细胞或其组合。肿瘤细胞抑制量的上述分离的贴壁胎盘细胞可包含约、至少或不超过$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$、$1x10^9$、$5x10^9$、$1x10^{10}$、$5x10^{10}$、$1x10^{11}$或更多个分离的贴壁胎盘细胞。

[0184] 5.1.5.4.包含贴壁胎盘细胞条件培养基的组合物

[0185] 还可用于本发明的是包含采用本文所述方法产生的NK细胞，例如采用本文所述三步法产生的活化NK细胞或TSPNK细胞(例如NK祖细胞)以及额外的条件培养基的组合物，其中所述组合物是肿瘤抑制性的，或在癌症或病毒感染的治疗中是有效的。本文所述的贴壁胎盘细胞可用来产生肿瘤细胞抑制性的抗癌或抗病毒的条件培养基，即包括由具有可检测的肿瘤细胞抑制作用、抗癌作用或抗病毒作用的细胞分泌或排出的一种或多种生物分子的培养基。在不同的实施方案中，条件培养基包括细胞在其中增殖(即已培养)至少1、2、3、4、5、6、7、8、9、10、11、12、13、14或更多天的培养基。在其它实施方案中，条件培养基包括这类细胞在其中生长至至少30％、40％、50％、60％、70％、80％、90％汇合或直到100％汇合的培养基。所述条件培养基可用来支持各个细胞群(例如胎盘细胞或任何类型的细胞)的培养。在另一个实施方案中，本文提供的条件培养基包括其中已培养了分离的贴壁胎盘细胞(例如分离的贴壁胎盘干细胞)或分离的贴壁胎盘多能细胞和分离的贴壁胎盘细胞以外的细胞(例如非胎盘干细胞或多能细胞)的培养基。

[0186] 这类条件培养基可与采用本文所述方法产生的NK细胞例如活化NK细胞或TSPNK细胞(例如NK祖细胞)、胎盘灌注液、胎盘灌注液细胞的任一种或其任何组合进行组合以形成是肿瘤细胞抑制性的抗癌或抗病毒的组合物。在某些实施方案中，组合物包含以体积计小于一半的条件培养基，例如约或小于约50％、45％、40％、35％、30％、25％、20％、15％、10％、5％、4％、3％、2％或1％(体积)。

[0187] 因此，在一个实施方案中，用于本发明的是包含采用本文所述方法产生的NK细胞例如活化NK细胞或TSPNK细胞(例如NK祖细胞)和来自分离的贴壁胎盘细胞培养的培养基的组合物，其中所述分离的贴壁胎盘细胞(a)附着在基质上；和(b)是$CD34^-$、$CD10^+$和$CD105^+$；其中所述组合物可检测地抑制肿瘤细胞的生长或增殖，或是抗癌或抗病毒的。在一个具体的实施方案中，分离的贴壁胎盘细胞是通过流式细胞术检出的$CD34^-$、$CD10^+$和$CD105^+$。在一个更具体的实施方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$贴壁胎盘细胞是胎盘干细胞。在另一个更具体的实施方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$胎盘细胞是多能贴壁胎盘细胞。在另一个具体的实施方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$胎盘细胞具有分化成神经表型的细胞、成骨表型的细胞或软骨形成表型的细胞的潜力。在一个更具体的实施方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$贴壁胎盘细胞另外是$CD200^+$。在另一个更具体的实施方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$贴壁胎盘细胞另外是通过流式细胞术检出的$CD90^+$或$CD45^-$。在另一个更具体的实施

方案中，分离的$CD34^-$、$CD10^+$、$CD105^+$贴壁胎盘细胞另外是通过流式细胞术检出的$CD90^+$或$CD45^-$。在一个更具体的实施方案中，$CD34^-$、$CD10^+$、$CD105^+$、$CD200^+$贴壁胎盘细胞另外是通过流式细胞术检出的$CD90^+$或$CD45^-$。在另一个更具体的实施方案中，$CD34^-$、$CD10^+$、$CD105^+$、$CD200^+$贴壁胎盘细胞另外是通过流式细胞术检出的$CD90^+$和$CD45^-$。在另一个更具体的实施方案中，$CD34^-$、$CD10^+$、$CD105^+$、$CD200^+$、$CD90^+$、$CD45^-$贴壁胎盘细胞另外是通过流式细胞术检出的$CD80^-$和$CD86^-$。

[0188] 在另一个实施方案中，用于本发明的是包含采用本文所述方法产生的NK细胞例如活化NK细胞或TSPNK细胞(例如NK祖细胞)和来自分离的贴壁胎盘细胞培养的培养基的组合物，其中所述分离的贴壁胎盘细胞(a)附着在基质上；和(b)表达CD200和HLA-G，或表达CD73、CD105和CD200，或表达CD200和OCT-4，或表达CD73、CD105和HLA-G，或表达CD73和CD105，并且当包含胎盘干细胞的胎盘细胞群在允许胚状体样体形成的条件下培养时，促进所述群中的一个或多个胚状体样体的形成，或表达OCT-4并且当包含胎盘干细胞的胎盘细胞群在允许胚状体样体形成的条件下培养时，促进所述群中的一个或多个胚状体样体的形成；其中所述组合物可检测地抑制肿瘤细胞的生长或增殖，或是抗癌或抗病毒的。在一个具体的实施方案中，组合物还包含大量所述分离的胎盘贴壁细胞。在另一个具体的实施方案中，组合物包含大量的非胎盘细胞。在一个更具体的实施方案中，所述非胎盘细胞包含$CD34^+$细胞，例如造血祖细胞，例如外周血造血祖细胞、脐带血造血祖细胞或胎盘血造血祖细胞。非胎盘细胞还可包含干细胞，例如间充质干细胞，例如骨髓衍生间充质干细胞。非胎盘细胞还可以是成体细胞或细胞系的一个或多个类型。在另一个具体的实施方案中，组合物包含抗增殖剂，例如，抗MIP-1α或抗MIP-1β抗体。

[0189] 在一个具体的实施方案中，通过上述细胞或细胞组合调节的培养基获自以分离的贴壁胎盘细胞与肿瘤细胞约1:1、约2:1、约3:1、约4:1或约5:1的比率、与大量肿瘤细胞共培养的大量分离的贴壁胎盘细胞。例如，条件培养基或上清液可获自包含约$1x10^5$个分离的贴壁胎盘细胞、约$1x10^6$个分离的贴壁胎盘细胞、约$1x10^7$个分离的贴壁胎盘细胞或约$1x10^8$个分离的贴壁胎盘细胞或更多的培养物。在另一个具体的实施方案中，条件培养基或上清液获自包含约$1x10^5$-约$5x10^5$个分离贴壁胎盘细胞和约$1x10^5$个肿瘤细胞、约$1x10^6$-约$5x10^6$个分离的贴壁胎盘细胞和约$1x10^6$个肿瘤细胞、约$1x10^7$-约$5x10^7$个分离的贴壁胎盘细胞和约$1x10^7$个肿瘤细胞或约$1x10^8$-约$5x10^8$个分离的贴壁胎盘细胞和约$1x10^8$个肿瘤细胞的共培养物。

[0190] 5.2.产生NK细胞的方法

[0191] NK细胞可产生自例如胎盘组织、胎盘灌注液、脐带血、胎盘血、外周血、脾、肝等任何来源的造血细胞，例如造血干细胞和祖细胞。

[0192] 自然杀伤细胞和如上所述可用于衍生自然杀伤细胞的细胞的一个重要来源是胎盘，例如足月胎盘，例如足月人胎盘。包含胎盘灌注液细胞的胎盘灌注液可通过例如美国专利号7,045,148和7,468,276和美国专利申请公布号2009/0104164公开的方法获得，其每一个的公开内容通过引用以其整体并入本文。

[0193] 5.2.1.细胞收集组合物

[0194] 可从中分离造血干细胞和祖细胞，或例如可与NK细胞，例如按照本文提供的三阶段方法产生的NK细胞群组合用于肿瘤抑制或带有肿瘤细胞、患有癌症或病毒感染的个体的

治疗的胎盘灌注液和灌注液细胞可使用胎盘细胞收集组合物通过哺乳动物(例如人)产后胎盘灌注来收集。灌注液可通过用任何生理上可接受的溶液,例如盐溶液、培养基或更复杂的细胞收集组合物灌注胎盘自胎盘收集。适于灌注胎盘和适于收集和保存灌注液细胞的细胞收集组合物详细描述于相关的美国申请公布号2007/0190042,其通过引用以其整体并入本文。

[0195] 细胞收集组合物可包含适于干细胞收集和/或培养的任何生理上可接受的溶液,例如盐溶液(例如磷酸缓冲盐水、Kreb氏溶液、改良的Kreb氏溶液、Eagle氏溶液、0.9%NaCl等)、培养基(例如DMEM、H.DMEM等)等。

[0196] 细胞收集组合物可包含有助于从收集时间起到培养时间保存胎盘细胞的一种或多种组分,即防止胎盘细胞干燥,或延迟胎盘细胞死亡、降低死亡细胞群中胎盘细胞的数目等。这类组分可以是例如细胞凋亡抑制剂(例如胱天蛋白酶抑制剂或JNK抑制剂);血管扩张药(例如硫酸镁、抗高血压药、心房钠尿肽(ANP)、促肾上腺皮质激素、促肾上腺皮质素释放激素、硝普盐、肼屈嗪、腺苷三磷酸、腺苷、吲哚美辛或硫酸镁、磷酸二酯酶抑制剂等);坏死抑制剂(例如2-(1H-吲哚-3-基)-3-戊基氨基-马来酰亚胺、吡咯烷二硫代氨基甲酸酯或氯硝西泮);TNF-α抑制剂和/或携氧全氟化碳(例如全氟辛基溴化物、全氟癸基溴化物等)。

[0197] 细胞收集组合物可包含一种或多种组织降解酶,例如金属蛋白酶、丝氨酸蛋白酶、中性蛋白酶、透明质酸酶、RNA酶或DNA酶等。这类酶包括但不限于胶原酶(例如胶原酶I、II、III或IV、来自溶组织梭状芽胞杆菌(Clostridium histolyticum)的胶原酶等);分散酶、嗜热菌蛋白酶、弹性蛋白酶、胰蛋白酶、LIBERASE、透明质酸酶等。

[0198] 细胞收集组合物可包含杀细菌或抑细菌有效量的抗生素。在某些非限制性的实施方案中,抗生素是大环内酯(例如妥布霉素)、头孢菌素(例如头孢氨苄、头孢拉定、头孢呋辛、头孢丙烯、头孢克洛、头孢克肟或头孢羟氨苄)、克拉霉素、红霉素、青霉素(例如青霉素V)或喹诺酮(例如氧氟沙星、环丙沙星或诺氟沙星)、四环素、链霉素等。在一个具体的实施方案中,抗生素对革兰氏(+)和/或革兰氏(-)细菌,例如绿脓假单胞菌(Pseudomonas aeruginosa)、金黄色葡萄球菌(Staphylococcus aureus)等有活性。

[0199] 细胞收集组合物还可包含一种或多种下列化合物:腺苷(约1mM-约50mM);D-葡萄糖(约20mM-约100mM);镁离子(约1mM-约50mM);分子量大于20,000道尔顿的大分子,在一个实施方案中,以足以维持内皮完整性和细胞活力的量存在(例如合成的或天然存在的胶体、多糖例如葡聚糖或聚乙二醇,以约25g/l-约100g/l或约40g/l-约60g/l存在);抗氧化剂(例如丁羟茴醚、丁羟甲苯、谷胱甘肽、维生素C或维生素E,以约25μM-约100μM存在);还原剂(例如N-乙酰半胱氨酸,以约0.1mM-约5mM存在);防止钙进入细胞的药剂(例如维拉帕米,以约2μM-约25μM存在);硝酸甘油(例如约0.05g/L-约0.2g/L);抗凝血药,在一个实施方案中,足以有助于防止残余血液凝结的量存在(例如肝素或蛭素,以约1000单位/l-约100,000单位/l的浓度存在);或含阿米洛利的化合物(例如阿米洛利、乙基异丙基阿米洛利、亚己基阿米洛利、二甲基阿米洛利或异丁基阿米洛利,以约1.0μM-约5μM存在)。

[0200] 5.2.2.胎盘的收集和处理

[0201] 一般而言,人胎盘在出生后在其娩出后不久回收。在一个实施方案中,在知情同意后、在取得患者的完整病历并且与胎盘关联后,向患者回收胎盘。在一个实施方案中,病历在分娩后继续。

[0202] 在回收灌注液之前，除去脐带血和胎盘血。在某些实施方案中，在分娩后，回收胎盘中的脐带血。胎盘可经过常规的脐带血回收过程。借助重力，通常使用针或插管以使胎盘放血(参见例如Anderson，美国专利号5,372,581;Hessel等，美国专利号5,415,665)。通常将针或插管置于脐静脉中，可轻轻按摩胎盘以助于将脐带血从胎盘中排出。这类脐带血回收可商业化地进行，例如LifeBank Inc.、Cedar Knolls,N.J.、ViaCord、Cord Blood Registry和CryoCell。在一个实施方案中，胎盘经重力放血而无需进一步操作以使脐带血回收期间的组织破坏减到最小。

[0203] 通常，将胎盘从分娩或生产室转移到另一位置(例如实验室)，用于脐带血回收和灌注液收集。可通过例如将近端脐带夹紧的胎盘置于无菌自封(zip-lock)塑料袋，然后将塑料袋置于绝热容器中，将胎盘在无菌的隔热运输装置(保持在20-28℃之间的胎盘温度)中运输。在另一个实施方案中，基本按美国专利号7,147,626所述，在脐带血收集试剂盒中运输胎盘。在一个实施方案中，在分娩后4-24小时，把胎盘送到实验室。在某些实施方案中，在脐带血回收之前，例如在插入胎盘的4-5cm(厘米)内夹紧近端脐带。在其它实施方案中，在脐带血回收之后但在胎盘进一步处理之前将近端脐带夹紧。

[0204] 在收集灌注液之前，可以将胎盘保存在无菌条件下和在室温下或在5-25℃(摄氏)的温度下。在灌注胎盘以除去任何残留脐带血之前，可将胎盘保存超过48小时的一段时间，或保存4-24小时的一段时间。可将胎盘保存在5℃-25℃(摄氏)温度下的抗凝血剂溶液中。合适的抗凝血剂溶液是本领域众所周知的。例如，可以使用肝素或华法林钠溶液。在一个实施方案中，抗凝血剂溶液包含肝素溶液(例如1％w/w的1:1000溶液中)。在一些实施方案中，在收集胎盘灌注液前，经放血的胎盘保存不超过36小时。

[0205] 5.2.3.胎盘灌注

[0206] 灌注哺乳动物胎盘和获取胎盘灌注液的方法公开于例如Hariri，美国专利号7,045,148和7,255,879及美国申请公布号2009/0104164、2007/0190042和20070275362(以美国专利号8,057,788授予专利权)，其每一个的公开内容本文通过引用以其整体并入本文。

[0207] 灌注液可通过使例如盐溶液、培养基或上述细胞收集组合物等灌注溶液流经胎盘血管系统来获得。在一个实施方案中，通过使灌注液流经脐动脉和脐静脉的任一个或两个来灌注哺乳动物胎盘。灌注液经胎盘的流动可利用例如流向胎盘的重力实现。例如，使用泵(例如蠕动泵)迫使灌注液通过胎盘。例如，可用与无菌连接装置(例如无菌管)连接的插管(例如TEFLON®或塑料插管)插入脐静脉。使无菌连接装置与灌注歧管连接。

[0208] 在灌注的准备中，以使脐动脉和脐静脉位于胎盘最高点的方式使胎盘定位。可通过使灌注液流经胎盘血管系统，或流经胎盘血管系统和周围组织，来灌注胎盘。在一个实施方案中，同时将脐动脉和脐静脉连接至例如通过挠性连接器连接至灌注液贮器的移液管。灌注液流进脐静脉和动脉。灌注液从血管壁渗出和/或流经血管壁进入胎盘的周围组织，并在来自妊娠期间连接至母体子宫的胎盘表面的适合的开放血管中采集。还可以将灌流液引入通过脐带开口，并允许从与母体子宫壁连接的胎盘壁中的开口中流出或渗出。在另一个实施方案中，使灌注液流经脐静脉，并从脐动脉收集，或使灌注液流经脐动脉，并从脐静脉收集，即只流经胎盘血管系统(胎儿组织)。

[0209] 在一个实施方案中，例如，同时将脐动脉和脐静脉连接至例如通过挠性连接器连接至灌注液贮器的移液管。灌注液流进脐静脉和动脉。灌注液从血管壁渗出和/或流经血管

壁进入胎盘的周围组织，并在来自妊娠期间连接至母体子宫的胎盘表面的适合的开放血管中采集。还可以将灌流液引入通过脐带开口，并允许从与母体子宫壁连接的胎盘壁中的开口中流出或渗出。通过该方法(可称为“盘”法)收集的胎盘细胞通常是胎儿和母体细胞的混合物。

[0210] 在另一个实施方案中，使灌注液流经脐静脉，并从脐动脉收集，或使灌注液流经脐动脉，并从脐静脉收集。通过该方法(可称为“闭路”方法)收集的胎盘细胞，通常几乎不包括胎儿的细胞。

[0211] 在一个实施方案中，闭路灌注方法可如下进行。在出生后约48小时内获取产后胎盘。把脐带夹住，并在夹钳之上剪断。脐带可弃去，或可加工以回收例如脐带干细胞，和/或处理脐带膜用于产生生物材料。灌注期间可以保留羊膜，或使用例如用手使用钝器解剖，使羊膜与绒毛膜分离。如果在灌注前将羊膜与绒毛膜分离，则羊膜例如可弃去，或例如通过酶消化处理获得干细胞，或产生例如羊膜生物材料，例如描述于美国申请公布号2004/0048796的生物材料。在例如使用消毒纱布清洗胎盘的所有可见血凝块和残留血液后，例如通过部分切割脐带膜以暴露脐带横截面，来露出脐带血管。暴露血管，并例如通过使闭合的鳄鱼夹向前通过每根血管的切口打开血管。然后将装置(例如与灌注装置或蠕动泵连接的塑料管)插入每根胎盘动脉。泵可以是适于所述目的的任何泵，例如蠕动泵。然后将与无菌收集贮器(例如血液袋，例如250mL收集袋)连接的塑料管插入胎盘静脉。或者，将与泵连接的管插入胎盘静脉，并将连至收集贮器的管插入胎盘动脉的一个或两个。然后用一定体积的灌注溶液(例如约750ml灌注溶液)灌注胎盘。然后，例如通过离心收集灌注液中的细胞。

[0212] 在一个实施方案中，在灌注期间夹住近端脐带，更具体地说，可在插入胎盘脐带的4-5cm(厘米)内夹住近端脐带。

[0213] 在放血过程中最早从哺乳动物胎盘收集的灌注液一般被脐带血和/或胎盘血的残留红细胞染色。当灌注继续进行并且从胎盘中洗出残留的脐带血细胞时，灌注液变得更浅。一般而言，30-100mL灌注液最初适于从胎盘中冲洗血液，但根据观察到的结果，可使用或多或少的灌注液。

[0214] 在某些实施方案中，在灌注前从胎盘取出脐带血(例如通过重力引流)，但是不用溶液冲洗(例如灌注)胎盘以除去残留血液。在某些实施方案中，在灌注前从胎盘中取出脐带血(例如通过重力引流)，并用溶液冲洗(例如灌注)胎盘以除去残留血液。

[0215] 用来灌注胎盘的灌注液的体积可随待收集胎盘细胞的数目、胎盘的大小、从单个胎盘进行收集的次数等而变化。在不同的实施方案中，灌注液的体积可为50mL-5000mL、50mL-4000mL、50mL-3000mL、100mL-2000mL、250mL-2000mL、500mL-2000mL或750mL-2000mL。通常，胎盘在放血后用700-800mL灌注液灌注。

[0216] 可在几小时或几天进程内将胎盘灌注多次。当将胎盘灌注多次时，可将其在无菌条件下在容器或其它合适的容器中维持或培养，并用在含或不含抗凝血剂(例如肝素、华法林钠、香豆素、双羟香豆素)时，和/或在含或不含抗微生物剂(例如β-巯基乙醇(0.1mM)；抗生素例如链霉素(例如40-100μg/ml)、青霉素(例如40U/ml)、两性霉素B(例如0.5μg/ml)时用细胞收集组合物或标准灌注液(例如平常的盐溶液例如磷酸缓冲盐水(“PBS”)灌注。在一个实施方案中，将分离的胎盘维持或培养一段时间而不收集灌注液，从而在灌注液灌注和收集前，将胎盘维持或培养1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、

22、23或24小时，或2或3天或更多天。可使灌注胎盘再维持一段或多段额外的时间，例如1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24小时或更多个小时，用例如700-800mL灌注液灌注第二次。可将胎盘灌注1、2、3、4、5次或更多次，例如每1、2、3、4、5或6小时一次。在一个实施方案中，重复胎盘的灌注和灌注溶液(例如胎盘细胞收集组合物)的收集，直到回收的有核细胞的数目降至低于100细胞/ml。可对不同时间点的灌注液分别进一步处理以回收时间相关细胞群，例如总有核细胞。还可以合并不同时间点的灌注液。

[0217] 5.2.4.胎盘灌注液和胎盘灌注液细胞

[0218] 通常，来自单次胎盘灌注的胎盘灌注液包含约$100x10^6$-约$500x10^6$个有核细胞，包括NK细胞(例如按照本文所述三阶段方法产生的NK细胞)来自其中的造血细胞可通过本文公开的方法产生。在某些实施方案中，胎盘灌注液或灌注液细胞包含$CD34^+$细胞，例如，造血干细胞或祖细胞。在一个更具体的实施方案中，这类细胞可包含$CD34^+CD45^-$干细胞或祖细胞、$CD34^+CD45^+$干细胞或祖细胞等。在某些实施方案中，在造血细胞从中分离之前对灌注液或灌注液细胞冷藏保存。在某些其它实施方案中，胎盘灌注液包含或灌注液细胞包含仅胎儿细胞或胎儿细胞和母体细胞的组合。

[0219] 5.2.5.造血细胞

[0220] 在不同的实施方案中，NK细胞从造血细胞(例如造血干细胞或祖细胞)中产生。

[0221] 本文所用的造血细胞可以是能够分化成NK细胞的任何造血细胞，例如，祖细胞、造血祖细胞、造血干细胞等。造血细胞可获自组织来源，例如骨髓、脐带血、胎盘血、外周血、肝等或其组合。造血细胞可获自胎盘。在一个具体的实施方案中，造血细胞获自胎盘灌注液。来自胎盘灌注液的造血细胞可包含胎儿和母体造血细胞的混合物，例如，其中母体细胞包含大于造血细胞总数5％的混合物。在一个实施方案中，来自胎盘灌注液的造血细胞包含至少约90％、95％、98％、99％或99.5％胎儿细胞。

[0222] 在另一个具体的实施方案中，造血细胞(例如造血干细胞或祖细胞)获自胎盘灌注液、脐带血或外周血。在另一个具体的实施方案中，造血细胞(例如造血干细胞或祖细胞)是来自胎盘灌注液和脐带血的混合细胞，例如来自与灌注液同一胎盘的脐带血。在另一个具体的实施方案中，所述脐带血获自从中获得所述胎盘灌注液的胎盘以外的胎盘。在某些实施方案中，混合细胞可通过将脐带血和胎盘灌注液合并或进行混合获得。在某些实施方案中，按体积计将脐带血和胎盘灌注液以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等的比率进行混合以得到混合细胞。在一个具体的实施方案中，将脐带血和胎盘灌注液以10:1-1:10、5:1-1:5或3:1-1:3的比率进行混合。在另一个具体的实施方案中，将脐带血和胎盘灌注液以10:1、5:1、3:1、1:1、1:3、1:5或1:10的比率进行混合。在一个更具体的实施方案中，将脐带血和胎盘灌注液以8.5:1.5(85％:15％)的比率进行混合。

[0223] 在某些实施方案中，将脐带血和胎盘灌注液按总有核细胞(TNC)含量计以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等的比率进行混合得到混合细胞。在一个具体的实施方案中，将脐带血和胎盘灌注液以10:1-10:1、5:1-1:5或3:1-1:3的比率进行混合。在另一个具体的实施方案中，将脐带血和胎盘灌注液以10:1、5:1、3:1、1:1、1:3、1:5或1:10的比率进行混合。

[0224] 在另一个具体的实施方案中，造血细胞(例如造血干细胞或祖细胞)来自脐带血和胎盘灌注液两者，但其中所述脐带血获自从中获得所述胎盘灌注液的胎盘以外的胎盘。

[0225] 在某些实施方案中，造血细胞是$CD34^+$细胞。在具体的实施方案中，可用于本文公开的方法的造血细胞是$CD34^+CD38^+$或$CD34^+CD38^-$。在一个更具体的实施方案中，造血细胞是$CD34^+CD38^-Lin^-$。在另一个具体的实施方案中，造血细胞是$CD2^-$、$CD3^-$、$CD11b^-$、$CD11c^-$、$CD14^-$、CD16-、$CD19^-$、$CD24^-$、$CD56^-$、$CD66b^-$和/或血型糖蛋白A^-的一种或多种。在另一个具体的实施方案中，造血细胞是$CD2^-$、$CD3^-$、$CD11b^-$、$CD11c^-$、$CD14^-$、CD16-、$CD19^-$、$CD24^-$、$CD56^-$、$CD66b^-$和血型糖蛋白A^-。在另一个更具体的实施方案中，造血细胞是$CD34^+CD38^-CD33^-CD117^-$。在另一个更具体的实施方案中，造血细胞是$CD34^+CD38^-CD33^-CD117^-CD235^-CD36^-$。

[0226] 在另一个实施方案中，造血细胞是$CD45^+$。在另一个具体的实施方案中，造血细胞是$CD34^+CD45^+$。在另一个实施方案中，造血细胞是$Thy\text{-}1^+$。在一个具体的实施方案中，造血细胞是$CD34^+Thy\text{-}1^+$。在另一个实施方案中，造血细胞是$CD133^+$。在具体的实施方案中，造血细胞是$CD34^+CD133^+$或$CD133^+Thy\text{-}1^+$。在另一个具体的实施方案中，$CD34^+$造血细胞是$CXCR4^+$。在另一个具体的实施方案中，$CD34^+$造血细胞是$CXCR4^-$。在另一个实施方案中，造血细胞对KDR(血管生长因子受体2)为阳性。在具体的实施方案中，造血细胞是$CD34^+KDR^+$、$CD133^+KDR^+$或$Thy\text{-}1^+KDR^+$。在某些其它实施方案中，造血细胞对为阳性醛脱氢酶($ALDH^+$)，例如，细胞是$CD34^+ALDH^+$。

[0227] 在某些其它实施方案中，$CD34^+$细胞是$CD45^-$。在具体的实施方案中，$CD34^+$细胞，例如$CD34^+$，$CD45^-$细胞表达miRNA hsa-miR-380、hsa-miR-512、hsa-miR-517、hsa-miR-518c、hsa-miR-519b和/或hsa-miR-520a的一种或多种或全部。

[0228] 在某些实施方案中，造血细胞是$CD34^-$。

[0229] 造血细胞还可缺乏表明谱系定型或发育naiveté缺乏的某些标志物。例如，在另一个实施方案中，造血细胞是$HLA\text{-}DR^-$。在具体的实施方案中，造血细胞是$CD34^+HLA\text{-}DR^-$、$CD133^+HLA\text{-}DR^-$、$Thy\text{-}1^+HLA\text{-}DR^-$或$ALDH^+HLA\text{-}DR^-$。在另一个实施方案中，造血细胞对谱系标志物CD2、CD3、CD11b、CD11c、CD14、CD16、CD19、CD24、CD56、CD66b和血型糖蛋白A的一个或多个、优选全部呈阴性。

[0230] 因此，可根据表明未分化状态的标志物的存在，或根据表明发生至少某种谱系分化的谱系标志物的缺乏，选择造血细胞用于本文公开的方法。下文详细论述了根据特异性标志物存在与否分离细胞(包括造血细胞)的方法。

[0231] 本文所用的造血细胞可以是基本同质群体，例如包含至少约95％、至少约98％或至少约99％来自单个组织来源的造血细胞的群体，或包含显示相同的造血细胞有关细胞标志物的造血细胞的群体。例如，在不同的实施方案中，造血细胞可包含至少约95％、98％或99％来自骨髓、脐带血、胎盘血、外周血或胎盘(例如胎盘灌注液)的造血细胞。

[0232] 本文所用的造血细胞可获自单一个体(例如来自单个胎盘)，或例如可以合并来自

多个个体的造血细胞。当造血细胞获自多个个体并合并时，造血细胞可获自相同的组织来源。因此，在不同的实施方案中，合并的造血细胞全部来自胎盘(例如胎盘灌注液)、全部来自胎盘血、全部来自脐带血，全部来自外周血等。

[0233] 在某些实施方案中，本文所用的造血细胞可包含来自两个或更多个组织来源的造血细胞。例如，在某些实施方案中，当来自两个或更多个来源的造血细胞进行混合用于本文方法时，用来产生NK细胞的大量造血细胞包含来自胎盘(例如胎盘灌注液)的造血细胞。在不同的实施方案中，用来产生NK细胞的造血细胞包含来自胎盘和来自脐带血、来自胎盘和外周血、来自胎盘和胎盘血或胎盘和骨髓的造血细胞。在一个优选的实施方案中，造血细胞包含来自胎盘灌注液的造血细胞与脐带血的造血细胞的组合，其中脐带血和胎盘来自同一个体，即，其中灌注液和脐带血是匹配的。在其中造血细胞包含来自2个组织来源的造血细胞的实施方案中，可将来自所述来源的造血细胞以例如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或9:1的比率进行混合。

[0234] 5.2.5.1.胎盘造血干细胞

[0235] 在某些实施方案中，造血细胞是胎盘造血细胞。如本文所用，“胎盘造血细胞”意指造血细胞获自胎盘本身，并非获自胎盘血或脐带血。在一个实施方案中，胎盘造血细胞是$CD34^+$。在一个具体的实施方案中，胎盘造血细胞主要是(例如至少约50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或98％)$CD34^+CD38^-$细胞。在另一个具体的实施方案中，胎盘造血细胞主要是(例如至少约50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或98％)$CD34^+CD38^+$细胞。可通过通过本领域技术人员已知的任何方法，例如通过灌注，从产后哺乳动物(例如人)胎盘获得胎盘造血细胞。

[0236] 在另一个实施方案中，胎盘造血细胞是$CD45^-$。在一个具体的实施方案中，造血细胞是$CD34^+CD45^-$。在另一个具体的实施方案中，胎盘造血细胞是$CD34^+CD45^+$。

[0237] 5.2.6.产生PiNK细胞的方法

[0238] 在不同的实施方案中，PiNK细胞来源于胎盘细胞。在具体的实施方案中，胎盘细胞获自胎盘灌注液，例如人胎盘灌注液。在具体的实施方案中，胎盘细胞获自经机械和/或酶破坏的胎盘组织。

[0239] 5.2.6.1.从胎盘灌注液获得PiNK细胞

[0240] 在一个实施方案中，通过获得胎盘灌注液，然后使胎盘灌注液与$CD56^+$细胞特异性结合的组分(例如针对CD56的抗体)接触，接着根据形成$CD56^+$细胞群的所述结合分离$CD56^+$细胞，来收集PiNK细胞。$CD56^+$细胞群包含分离的自然杀伤细胞群。在一个具体的实施方案中，使$CD56^+$细胞与$CD16^+$细胞特异性结合的组分(例如针对CD16的抗体)接触，并将$CD16^+$细胞从$CD56^+$细胞群中除去。在另一个具体的实施方案中，另将$CD3^+$细胞从$CD56^+$细胞群中除去。

[0241] 在一个实施方案中，PiNK细胞如下获自胎盘灌注液。给产后人胎盘放血，并用例如约200-800mL灌注溶液只通过胎盘血管系统灌注。在一个具体的实施方案中，使胎盘的脐带血排出，并在所述灌注之前用例如经过胎盘血管系统的灌注溶液冲洗以除去残余血。灌注液经收集并处理以除去任何残留的红细胞。可根据CD56和CD16的表达，分离灌注液中总有核细胞中的自然杀伤细胞。在某些实施方案中，PiNK细胞的分离包括使用抗CD56抗体的分

离，其中分离的细胞是$CD56^+$。在另一个实施方案中，PiNK细胞的分离包括使用抗CD16抗体的分离，其中分离的细胞是CD16-。在另一个实施方案中，PiNK细胞的分离包括使用抗CD56抗体的分离，并使用抗CD16抗体除去大量的非PiNK细胞，其中分离的细胞包含$CD56^+$，CD16-细胞。

[0242] 可通过本领域已知的任何方法，例如荧光激活细胞分选术(FACS)，或者优选使用与特异性抗体缀合的微珠的磁性细胞分选，来实现细胞分离。例如，可采用AUTOMACS™分离器(Miltenyi)，进行磁性细胞分离并使之自动化。

[0243] 另一方面，分离胎盘自然杀伤细胞(例如PiNK细胞)的方法包括获得大量胎盘细胞，并从所述大量胎盘细胞中分离自然杀伤细胞。在一个具体的实施方案中，胎盘细胞是或包含胎盘灌注液细胞，例如来自胎盘灌注液的总有核细胞。在另一个具体的实施方案中，所述大量胎盘细胞是或包含胎盘组织的机械和/或酶消化获得的胎盘细胞。在另一个实施方案中，所述分离使用一种或多种抗体进行。在一个更具体的实施方案中，所述一种或多种抗体包含抗CD3、CD16或CD56的一种或多种抗体。在一个更具体的实施方案中，所述分离包括从所述大量胎盘细胞中的$CD56^-$细胞中分离$CD56^+$细胞。在一个更具体的实施方案中，所述分离包括从是$CD56^-$或$CD16^+$的胎盘细胞中分离$CD56^+$、CD16-胎盘细胞，例如胎盘自然杀伤细胞，例如PiNK细胞。在一个更具体的实施方案中，所述分离包括是$CD56^-$、$CD16^+$或$CD3^+$的细胞胎盘中分离$CD56^+$、CD16-、$CD3^-$胎盘细胞。在另一个实施方案中，分离胎盘自然杀伤细胞的所述方法产生是至少50％、55％、60％、65％、70％、75％、80％、85％、90％、95％、98％或至少99％$CD56^+$，CD16-自然杀伤细胞的胎盘细胞群。

[0244] 在某些实施方案中，胎盘自然杀伤细胞(例如PiNK细胞)在培养物中扩增。在某些其它实施方案中，胎盘灌注液细胞在培养物中扩增。在一个具体的实施方案中，所述胎盘灌注液细胞在饲养层存在下和/或在至少一种细胞因子存在下扩增。在一个更具体的实施方案中，所述饲养层包含K562细胞或外周血单核细胞。在另一个更具体的实施方案中，所述至少一种细胞因子是白介素-2。在具体的实施方案中，PiNK细胞在培养物中培养，例如扩增至少约或至多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或28天。在一个具体的实施方案中，培养PiNK细胞约21天。

[0245] 5.2.6.2.破坏和消化胎盘组织以获得PiNK细胞

[0246] 胎盘自然杀伤细胞(例如PiNK细胞)还可获自经机械和/或酶破坏的胎盘组织。

[0247] 胎盘组织可使用一种或多种组织降解酶，例如金属蛋白酶、丝氨酸蛋白酶、中性蛋白酶、RNA酶或DNA酶等进行破坏。这类酶包括但不限于胶原酶(例如胶原酶I、II、III或IV、来自溶组织梭状芽胞杆菌的胶原酶等)；分散酶、嗜热菌蛋白酶、弹性蛋白酶、胰蛋白酶、LIBERASE、透明质酸酶等。通常在消化后，使消化的组织流经滤器或过滤器，除去部分消化的细胞块，留下基本上是单细胞的悬液。

[0248] 在获得胎盘细胞的悬液后，自然杀伤细胞可使用例如抗CD3和CD56的抗体分离。在一个具体的实施方案中，胎盘自然杀伤细胞如下分离：通过选择是$CD56^+$的细胞以产生第一细胞群；使所述第一细胞群与对CD3和/或CD16有特异性的抗体接触；从是$CD3^+$或$CD56^+$的所述第一细胞群中取出细胞，从而产生基本上是$CD56^+$和$CD3^-$，$CD56^+$和CD16-或$CD56^+$，$CD3^-$和CD16-的第二细胞群。

[0249] 在一个实施方案中，使用磁珠从胎盘细胞悬液中分离胎盘自然杀伤细胞。例如，可

采用磁性激活细胞分选(MACS)技术,一种用于根据其结合包含一种或多种特异性抗体(例如抗CD56抗体)的磁珠(例如约0.5-100μm直径)的能力分离粒子的方法,来分离细胞。可对磁性微球进行各种有用的修饰,包括共价添加特异性识别具体的细胞表面分子或半抗原的抗体。然后将珠粒与细胞混合以使之结合。使细胞通过磁场以分离出具有特异性细胞表面标志物的细胞。在一个实施方案中,然随后可将这些细胞分离,并且和与抗另外的细胞表面标志物的抗体偶联的磁珠再混合。使这些细胞再次通过磁场,从而分离出结合两种抗体的细胞。然后可将这类细胞稀释至单独的培养皿(例如微量滴定培养皿)中供克隆分离。

[0250] 5.2.7.产生活化NK细胞的方法

[0251] 活化NK细胞可产生自上文描述的造血细胞。在某实施方案中,活化NK细胞自扩增的造血细胞(例如造血干细胞和/或造血祖细胞)产生。在一个具体的实施方案中,造血细胞在不使用饲养细胞的第一培养基中连续扩增并分化。然后将该细胞在存在饲养细胞的第二培养基中培养。这类分离、扩增和分化可在中央设施中进行,所述中央设施提供扩增的造血细胞用于运输以在使用点(例如医院、军事基地、军事前线等)进行分散扩增和分化。

[0252] 在一些实施方案中,活化NK细胞的产生包括扩增造血细胞群。在细胞扩增期间,造血细胞群内的大量造血细胞分化成NK细胞。

[0253] 在一个实施方案中,产生活化自然杀伤(NK)细胞群的方法包括:(a)将造血干细胞群或祖细胞群接种在包含白介素-15(IL-15)和可选的干细胞因子(SCF)和白介素-7(IL-7)的一种或多种的第一培养基中,其中所述IL-15和可选的SCF和IL-7不包括在所述培养基的成分不确定的组分中,使得该群扩增,且所述造血干细胞群或祖细胞群内的大量造血干细胞或祖细胞在所述扩增期间分化成NK细胞;和(b)使来自步骤(a)的细胞在包含白介素-2(IL-2)的第二培养基中扩增,以产生活化NK细胞群。

[0254] 在另一个实施方案中,本文所述活化NK细胞通过NK细胞扩增/分化和成熟的两步方法产生。第一步和第二步包括将细胞在含独特的细胞因子组合的培养基中培养。在某些实施方案中,所述方法包括(a)将造血细胞群在第一培养基中培养和扩增,其中造血细胞群内的大量造血干细胞或祖细胞分化成NK细胞;和(b)使来自步骤(a)的NK细胞在第二培养基扩增,其中NK细胞进一步扩增和分化,且其中NK细胞成熟(例如被激活或另具有细胞毒活性)。在某些实施方案中,所述方法不包括步骤(a)和(b)间的中间步骤、步骤(a)前的其它培养步骤和/或步骤(b)后的其它步骤(例如成熟步骤)。

[0255] 5.2.7.1.第一步

[0256] 在某些实施方案中,产生活化NK细胞的步骤包括将造血细胞群在第一培养基中培养和扩增的第一步,其中造血细胞群内的大量造血干细胞或祖细胞分化成NK细胞。

[0257] 虽不希望受任何参数、机制或理论的束缚,但如上文所述造血细胞的培养导致造血细胞的持续扩增和来自所述细胞的NK细胞的分化。在某些实施方案中,使用于本文所述方法的造血细胞(例如干细胞或祖细胞)在使用饲养层的第一步中扩增和分化。在其它实施方案中,使造血细胞(例如干细胞或祖细胞)在不使用饲养层的第一步中扩增和分化。

[0258] 造血细胞不依赖饲养细胞的扩增和分化可发生在与细胞培养和扩增相容的任何容器中,例如培养瓶、管、烧杯、培养皿、多孔板、袋等。在一个具体的实施方案中,造血细胞的不依赖饲养细胞的扩增发生在袋中,例如柔性透气碳氟化合物培养袋(例如来自American Fluoroseal)。在一个具体的实施方案中,造血细胞在其中扩增的容器适于运输

至例如医院或军事区等场所，其中扩增的NK细胞进一步扩增和分化。

[0259] 在某些实施方案中，造血细胞例如以连续方式在第一培养基中扩增并分化。在一个实施方案中，第一培养基是无动物组分培养基。可用于本文所述方法的示例性无动物组分培养基包括但不限于Eagle基础培养基(BME)、Dulbecco改良Eagle培养基(DMEM)、Glasgow极限必需培养基(GMEM)、Dulbecco改良Eagle培养基/营养物混合物F-12Ham(DMEM/F-12)、极限必需培养基(MEM)、Iscove改良Dulbecco培养基(IMDM)、营养物混合物F-10Ham(Ham's F-10)、营养物混合物F-12Ham(Ham's F-12)、RPMI-1640培养基、Williams养基E、STEMSPAN®(目录号Stem Cell Technologies,Vancouver,Canada)、Glycostem基础生长培养基(GBGM®)、AIM-V®培养基(Invitrogen)、X-VIVO™ 10(Lonza)、X-VIVO™ 15(Lonza)、OPTMIZER(Invitrogen)、STEMSPAN®H3000(Stem Cell Technologies)、CELLGRO COMPLETE™(Mediatech)或其任何改良变体或组合。在本文任一个实施方案的具体实施方案中，培养基不是GBGM®。

[0260] 在优选的实施方案中，第一培养基包含一种或多种培养基补充剂(例如营养物、细胞因子和/或因子)。适用于本文所述方法的培养基补充剂包括例如而不限于、血清例如人血清AB、胎牛血清(FBS)或胎牛血清(FCS)、维生素、牛血清白蛋白(BSA)、氨基酸(例如L-谷氨酰胺)、脂肪酸(例如油酸、亚油酸或棕榈酸)、胰岛素(例如重组人胰岛素)、运铁蛋白(铁饱和人运铁蛋白)、β-巯基乙醇、干细胞因子(SCF)、Fms样酪氨酸激酶3配体(Flt3-L)、细胞因子例如白介素-2(IL-2)、白介素-7(IL-7)、白介素-15(IL-15)、血小板生成素(Tpo)、肝素或O-乙酰-肉碱(亦称为乙酰肉碱、O-乙酰-L-肉碱或OAC)。在一个具体的实施方案中，本文所用培养基包含人血清AB。在另一个具体的实施方案中，本文所用培养基包含FBS。在另一个具体的实施方案中，本文所用培养基包含OAC。

[0261] 在某些实施方案中，第一培养基不含以下的一种或多种：粒细胞集落刺激因子(G-CSF)、粒细胞/巨噬细胞集落刺激因子(GM-CSF)、白介素-6(IL-6)、巨噬细胞炎性蛋白1α(MIP1α)或白血病抑制因子(LIF)。

[0262] 因此，一方面，本文描述了产生NK细胞的两步方法，其中所述第一步包括将造血细胞群在饲养细胞不存在时在第一培养基中扩增和分化，其中所述造血细胞群内的大量造血细胞在所述扩增期间分化成NK细胞，且其中培养基包含浓度为约1-约150ng/mL的CF、浓度为约50-约1500IU/mL的IL-2、浓度为约1-约150ng/mL的IL-7、浓度为1-约150ng/mL的IL-15和浓度为约0.1-约30IU/mL的肝素，且其中所述SCF、IL-2、IL-7、IL-15和肝素不包含在所述培养基的成分不确定的组分(例如血清)内。在某些实施方案中，所述培养基包含以下的一种或多种：O-乙酰-肉碱(亦称为乙酰肉碱、O-乙酰-L-肉碱或OAC)或影响线粒体中的乙酰-CoA循环的化合物、thiazovivin、Y-27632、pyintegrin、Rho激酶(ROCK)抑制剂、胱天蛋白酶抑制剂或其它抗凋亡化合物/肽、NOVA-RS(Sheffield Bio-Science)或其它小分子生长促进剂。在某些实施方案中，所述培养基包含烟酰胺。在某些实施方案中，所述培养基包含约0.5mM-10mM OAC。在一个实施方案中，所述培养基包含temspan®H3000和/或DMEM:F12和约0.5、1、2、3、4、5、6、7、8、9或10mM OAC。在一个具体的实施方案中，所述培养基是GBGM®。在另一个具体的实施方案中，培养基不是GBGM®。在另一个具体的实施方案中，所述培养基包含temspan®H3000和约5mM的OAC。在另一个具体的实施方案中，所述培

养基包含DMEM:F12和约5mM的OAC。OAC可在本文所述培养过程期间的任何时间添加。在某些实施方案中,将所述OAC加入第一培养基中和/或在第一培养步骤期间加入。在一些实施方案中,在培养的第0、1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21天将所述OAC加入第一培养基中。在一个具体的实施方案中,在第一培养步骤的第7天将所述OAC加入第一培养基中。在一个更具体的实施方案中,在培养的第7天将所述OAC加入第一培养基中,且所述OAC在整个第一和第二培养步骤中存在。在某些实施方案中,将所述OAC加入第二培养基中和/或在第二培养步骤期间加入。在一些实施方案中,在培养的第22、23、24、25、26、27、28、29、30、31、32、33、34、35天将所述OAC加入第二培养基中。

[0263] 在另一个具体的实施方案中,所述培养基是补充约5-20%BSA、约1-10μg/mL重组人胰岛素、约10-50μg/mL铁饱和人运铁蛋白和约10-50μMβ-巯基乙醇的IMDM。在另一个具体的实施方案中,所述培养基不含IL-11、IL-3、同源框-B4(HoxB4)和/或甲基纤维素的一种或多种或任一种。

[0264] 在其它具体的实施方案中,所述培养基包含浓度为约0.1-约500ng/mL、约5-约100ng/mL或约20ng/mL的CF。在其它具体的实施方案中,所述培养基包含浓度为约10-约2000IU/mL或约100-约500IU/mL或约200IU/mL的IL-2。在其它具体的实施方案中,所述培养基包含浓度为约0.1-约500ng/mL、约5-约100ng/mL或约20ng/mL的IL-7。在其它具体的实施方案中,所述培养基包含浓度为约0.1-约500ng/mL、约5-约100ng/mL或约10ng/mL的IL-15。在其它具体的实施方案中,所述培养基包含浓度为约0.05-约100U/mL或约0.5-约20U/mL或约1.5U/mL的肝素。

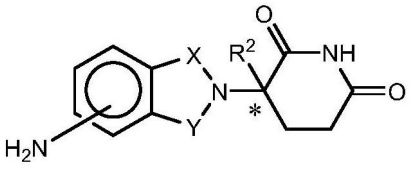
[0265] 在另外其它的具体实施方案中,所述培养基另包含浓度为约1-约150ng/mL的Fms样酪氨酸激酶3配体(Flt-3L)、浓度为约1-约150ng/mL的血小板生成素(Tpo)或两者的组合。在其它具体的实施方案中,所述培养基包含浓度为约0.1-约500ng/mL、约5-约100ng/mL或约20ng/mL的Flt-3L。在其它具体的实施方案中,所述培养基包含浓度为约0.1-约500ng/mL、约5-约100ng/mL或约20ng/mL的Tpo。

[0266] 在一个更具体的实施方案中,第一培养基是GBGM®,其包含约20ng/mL SCF、约20ng/mL IL-7、约10ng/mL IL-15。在另一个更具体的实施方案中,第一培养基是GBGM®,其包含约20ng/mL SCF、约20ng/mL Flt3-L、约200IU/mL IL-2、约20ng/mL IL-7、约10ng/mL IL-15、约20ng/mL Tpo和约1.5U/mL肝素。在另一个具体的实施方案中,所述第一培养基另包含10%人血清(例如人血清AB)或胎儿血清(例如FBS)。在本文任一个实施方案的具体实施方案中,培养基不是GBGM®。

[0267] 在另一个实施方案中,造血细胞是扩增通过将所述细胞培养在例如所述第一培养基中,与未与免疫调节化合物接触的相等数目的造血细胞相比,以足以在指定时间内引起造血细胞增殖可检出增加的时间和量与免疫调节化合物(例如TNF-α抑制化合物)接触。参见例如美国专利申请公布号2003/0235909,其公开内容通过引用以其整体并入本文。在某些实施方案中,免疫调节化合物是氨基取代的二氢异吲哚。在一个优选的实施方案中,免疫调节化合物是3-(4-氨基-1-氧代-1,3-二氢异吲哚-2-基)-哌啶-2,6-二酮、3-(4'氨基二氢异吲哚-1'-酮)-1-哌啶-2,6-二酮、4-(氨基)-2-(2,6-二氧代(3-哌啶基))-二氢异吲哚-1,3-二酮或4-氨基-2-(2,6-二氧代哌啶-3-基)异吲哚-1,3-二酮。在另一个优选的实施方案中,免疫调节化合物是泊马度胺或来那度胺。

[0268] 免疫调节化合物的具体实例包括但不限于取代的苯乙烯的氰基和羧基衍生物，例如美国专利号5,929,117中公开的那些；1-氧代-2-(2,6-二氧代-3-氟哌啶-3-基)二氢异吲哚和1,3-二氧代-2-(2,6-二氧代-3-氟哌啶-3-基)二氢异吲哚，例如美国专利号5,874,448中公开的那些；美国专利号5,798,368中公开的四取代的2-(2,6-二氧代哌啶-3-基)-1-氧代二氢异吲哚；沙利度胺和EM-12的1-氧代和1,3-二氧代-2-(2,6-二氧代哌啶-3-基)二氢异吲哚(例如4-甲基衍生物)，包括但不限于美国专利号5,635,517中公开的那些；以及美国专利号5,698,579和5,877,200中公开的非多肽环酰胺类；沙利度胺的类似物和衍生物，包括沙利度胺的水解产物、代谢物、衍生物和前体，例如公开于D'Amato的美国专利号5,593,990、5,629,327和6,071,948中的那些；氨基沙利度胺以及氨基沙利度胺的类似物、水解产物、代谢物、衍生物和前体，及取代的2-(2,6-二氧代哌啶-3-基)邻苯二甲酰亚胺和取代的2-(2,6-二氧代哌啶-3-基)-1-氧代异吲哚，例如公开于美国专利号6,281,230和6,316,471中的那些；异吲哚-酰亚胺化合物例如公开于2001年10月5日提交的美国专利申请号09/972,487、2001年12月21日提交的美国专利申请号10/032,286和国际申请号PCT/US01/50401(国际公布号WO 02/059106)中的那些。本文标出的专利和专利申请每一个的整体内容通过引用并入本文。免疫调节化合物不包括沙利度胺。

[0269] 在另一个实施方案中，免疫调节化合物包括但不限于描述于美国专利号5,635,517(其通过引用并入本文)的在苯并环中被氨基取代的1-氧代-和1,3-二氧代-2-(2,6-二氧代哌啶-3-基)二氢异吲哚。这些化合物具有以下结构

[0270] 

，

[0271] 其中X和Y之一为C＝O，X和Y的另一个为C＝O或CH_2，且R^2为氢或低级烷基，或其药学上可接受的盐、水合物、溶剂合物、包合物、对映异构体、非对映异构体、外消旋体或立体异构体的混合物。

[0272] 在另一个实施方案中，具体的免疫调节化合物包括但不限于：

[0273] 1-氧代-2-(2,6-二氧代哌啶-3-基)-4-氨基二氢异吲哚；

[0274] 1-氧代-2-(2,6-二氧代哌啶-3-基)-5-氨基二氢异吲哚；

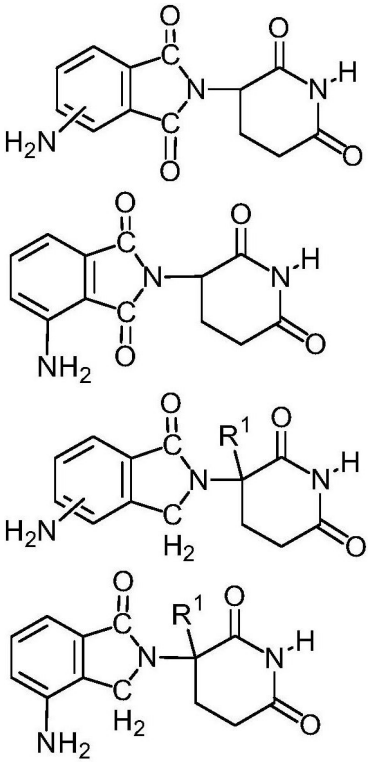
[0275] 1-氧代-2-(2,6-二氧代哌啶-3-基)-6-氨基二氢异吲哚；

[0276] 1-氧代-2-(2,6-二氧代哌啶-3-基)-7-氨基二氢异吲哚；

[0277] 1,3-二氧代-2-(2,6-二氧代哌啶-3-基)-4-氨基二氢异吲哚；和

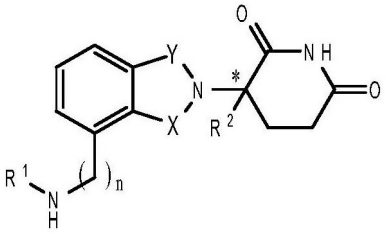
[0278] 1,3-二氧代-2-(2,6-二氧代哌啶-3-基)-5-氨基二氢异吲哚。

[0279] 其它具体的免疫调节化合物属于取代的2-(2,6-二氧代哌啶-3-基)邻苯二甲酰亚胺和取代的2-(2,6-二氧代哌啶-3-基)-1-氧代异吲哚的类别，例如公开于美国专利号6,281,230、6,316,471、6,335,349和6,476,052及国际专利申请号PCT/US97/13375(国际公布号WO 98/03502)中的那些，其每一个通过引用并入本文。这个类别的代表性化合物具有下式：

[0280] 

[0281] 其中R^1为氢或甲基。在个别的实施方案中，本发明包括使用这些化合物的对映异构体纯的形式(例如旋光纯(R)或(S)对映异构体)。

[0282] 还有具体的免疫调节化合物属于公开于美国专利申请号10/032,286和09/972,487及国际申请号PCT/US01/50401(国际公布号WO 02/059106)的异吲哚-酰亚胺类别，其每一个通过引用并入本文。在一个代表性实施方案中，所述免疫调节化合物是化合物具有以下结构

[0283]  ，

[0284] 其中X和Y之一为C＝O，另一个为CH_2或C＝O；

[0285] R^1为H、(C_1-C_8)烷基、(C_3-C_7)环烷基、(C_2-C_8)烯基、(C_2-C_8)炔基、苄基、芳基、(C_0-C_4)烷基-(C_1-C_6)杂环烷基、(C_0-C_4)烷基-(C_2-C_5)杂芳基、C(O)R^3、C(S)R^3、C(O)OR^4、(C_1-C_8)烷基-$N(R^6)_2$、(C_1-C_8)烷基-OR^5、(C_1-C_8)烷基-C(O)OR^5、C(O)NHR^3、C(S)NHR^3、C(O)$NR^3R^{3'}$、C(S)$NR^3R^{3'}$或(C_1-C_8)烷基-O(CO)R^5；

[0286] R^2为H、F、苄基、(C_1-C_8)烷基、(C_2-C_8)烯基或(C_2-C_8)炔基；

[0287] R^3和R^3独立地为(C_1-C_8)烷基、(C_3-C_7)环烷基、(C_2-C_8)烯基、(C_2-C_8)炔基、苄基、芳基、(C_0-C_4)烷基-(C_1-C_6)杂环烷基、(C_0-C_4)烷基-(C_2-C_5)杂芳基、(C_0-C_8)烷基-$N(R^6)_2$、(C_1-C_8)烷基-OR^5、(C_1-C_8)烷基-C(O)OR^5、(C_1-C_8)烷基-O(CO)R^5或C(O)OR^5；

[0288] R^4为(C_1-C_8)烷基、(C_2-C_8)烯基、(C_2-C_8)炔基、(C_1-C_4)烷基-OR^5、苄基、芳基、(C_0-C_4)烷基-(C_1-C_6)杂环烷基或(C_0-C_4)烷基-(C_2-C_5)杂芳基；

[0289] R^5为(C_1-C_8)烷基、(C_2-C_8)烯基、(C_2-C_8)炔基、苄基、芳基或(C_2-C_5)杂芳基；

[0290] R^6每次出现独立地为H、(C_1-C_8)烷基、(C_2-C_8)烯基、(C_2-C_8)炔基、苄基、芳基、(C_2-C_5)杂芳基或(C_0-C_8)烷基-C(O)O-R^5或R^6基团可连接形成杂环烷基；

[0291] n为0或1；和

[0292] *表示手性碳中心；

[0293] 或其药学上可接受的盐、水合物、溶剂合物、包合物、对映异构体、非对映异构体、外消旋体或立体异构体的混合物。

[0294] 在上式的具体化合物中，当n为0时，则R^1为(C_3-C_7)环烷基、(C_2-C_8)烯基、(C_2-C_8)炔基、苄基、芳基、(C_0-C_4)烷基-(C_1-C_6)杂环烷基、(C_0-C_4)烷基-(C_2-C_5)杂芳基、C(O)R^3、C(O)OR^4、(C_1-C_8)烷基-N$(R^6)_2$、(C_1-C_8)烷基-OR^5、(C_1-C_8)烷基-C(O)OR^5、C(S)NHR^3或(C_1-C_8)烷基-O(CO)R^5；

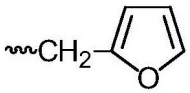
[0295] R^2为H或(C_1-C_8)烷基；和

[0296] R^3为(C_1-C_8)烷基、(C_3-C_7)环烷基、(C_2-C_8)烯基、(C_2-C_8)炔基、苄基、芳基、(C_0-C_4)烷基-(C_1-C_6)杂环烷基、(C_0-C_4)烷基-(C_2-C_5)杂芳基、(C_5-C_8)烷基-N$(R^6)_2$、(C_0-C_8)烷基-NH-C(O)O-R^5、(C_1-C_8)烷基-OR^5、(C_1-C_8)烷基-C(O)OR^5、(C_1-C_8)烷基-O(CO)R^5或C(O)OR^5；且其它变量具有相同的定义。

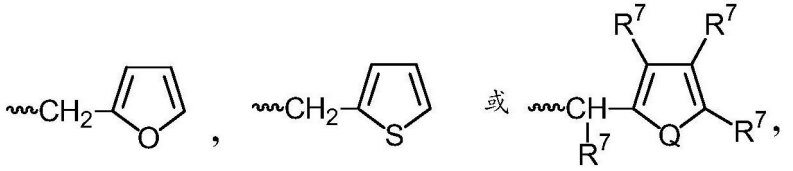
[0297] 在上式的其它具体化合物中，R^2为H或(C_1-C_4)烷基。

[0298] 在上式的其它具体化合物中，R^1为(C_1-C_8)烷基或苄基。

[0299] 在上式的其它具体化合物中，R^1为H、(C_1-C_8)烷基、苄基、CH_2OCH_3、$CH_2CH_2OCH_3$或

[0300] 

[0301] 在上式的化合物的另一个实施方案中，R^1为

[0302] 

[0303] 其中Q为O或S，且每次出现R^7独立地为H、(C_1-C_8)烷基、苄基、CH_2OCH_3或$CH_2CH_2OCH_3$。

[0304] 在上式的其它具体化合物中，R^1为C(O)R^3。

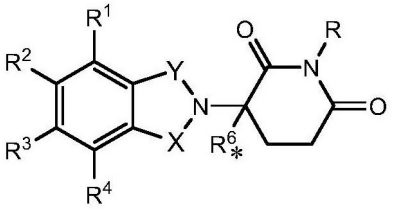
[0305] 在上式的其它具体化合物中，R^3为(C0-C4)烷基-(C2-C5)杂芳基、(C1-C8)烷基、芳基或(C_0-C_4)烷基-OR^5。

[0306] 在上式的其它具体化合物中，杂芳基为吡啶基、呋喃基或噻吩基。

[0307] 在上式的其它具体化合物中，R^1为C(O)OR^4。

[0308] 在上式的其它具体化合物中，C(O)NHC(O)的H可被(C_1-C_4)烷基、芳基或苄基取代。

[0309] 在另一个实施方案中，所述免疫调节化合物是具有以下结构的化合物

[0310] 

[0311] 其中：

[0312] X和Y之一为C＝O，另一个为CH_2或C＝O；

[0313] R为H或CH_2OCOR'；

[0314] (i)R^1、R^2、R^3或R^4的每一个彼此独立地为卤素、1-4个碳原子的烷基或1-4个碳原子的烷氧基，或(ii)R^1、R^2、R^3或R^4之一为硝基或-NHR^5，而剩余的R^1、R^2、R^3或R^4为氢；

[0315] R^5为氢或1-8个碳的烷基；

[0316] R^6为氢、1-8个碳原子的烷基、苯并、氯或氟；

[0317] R'为R^7-CHR^{10}-N(R^8R^9)；

[0318] R^7为间亚苯基或对亚苯基或-(C_nH_{2n})-，其中n的值为0-4；

[0319] R^8和R^9的每一个彼此独立地为氢或1-8个碳原子的烷基，或R^8和R^9合在一起为四亚甲基、五亚甲基、六亚甲基或-$CH_2CH_2X_1CH_2CH_2$-，其中X_1为-O-、-S-或-NH-；

[0320] R^{10}为氢、1-8个碳原子的烷基或苯基；和

[0321] *表示手性碳中心；

[0322] 或其药学上可接受的盐、水合物、溶剂合物、包合物、对映异构体、非对映异构体、外消旋体或立体异构体的混合物。

[0323] 在一个具体的实施方案中，造血细胞的扩增在补充20％BITS(牛血清白蛋白、重组人胰岛素和运铁蛋白)、SCF、Flt-3配体、IL-3和4-(氨基)-2-(2,6-二氧代(3-哌啶基))-二氢异吲哚-1,3-二酮(10μM，在0.05％DMSO中)的IMDM中进行。在一个更具体的实施方案中，使约5x10^7个造血细胞(例如$CD34^+$细胞)在培养基中扩增形成约5x10^{10}个细胞-约5x10^{12}个细胞，将其重新悬浮于100mL IMDM中以产生扩增的造血细胞群。优选将扩增的造血细胞群冷冻保存以利于运输。

[0324] 在不同的具体实施方案中，至少50％、55％、60％、65％、70％。75％、80％、85％、90％、95％、97％、98％或99％的造血细胞分化成NK细胞。

[0325] 在某些实施方案中，按本文所述造血细胞扩增和分化的方法包括将包含所述造血细胞的细胞群在扩增和分化期间维持在约2x10^4和约2x10^5个细胞/毫升之间。在某些其它实施方案中，按本文所述造血细胞扩增和分化的方法包括将包含所述造血细胞的细胞群维持在不超过约1x10^5个细胞/毫升。

[0326] 造血细胞扩增和分化成NK细胞的时间可为例如约3天-约120天。在一个实施方案中，分化时间为约7天-约75天。在另一个实施方案中，分化时间为约14天-约50天。在一个具体的实施方案中，分化时间为约21天-约28天。

[0327] 5.2.7.2.第二步

[0328] 造血细胞(例如干细胞或祖细胞)和产生于第一步的自然杀伤细胞例如在不使用饲养层时或在饲养细胞存在下在第二步中进一步增和分化。如上所述细胞的培养导致来自第一步的NK细胞的持续扩增、分化以及成熟。在第二步中，NK细胞在例如包含与所述第一培养基相比不同的细胞因子和/或生物活性分子的第二培养基中以连续方式扩增、分化和成熟。在某些实施方案中，第二培养基是无动物组分培养基。本公开内容中描述了示例性无动物组分细胞培养基。

[0329] 因此，一方面，本文描述了产生活化NK细胞的方法，所述方法包括在存在饲养细胞的第二培养基中并与白介素-2(IL-2)接触时扩增上文所述来自第一步的NK细胞。在具体的

实施方案中，所述第二培养基含有包含IL-2(例如10IU/mL-1000IU/mL)和以下的一种或多种的细胞生长培养基：人血清(例如人血清AB)、胎牛血清(FBS)或胎牛血清(FCS)，例如5%-15%FCS v/v；运铁蛋白，例如10μg/mL-50μg/mL；胰岛素，例如5μg/mL-20μg/mL；乙醇胺，例如$5x10^{-4}$to $5x10^{-5}$M；油酸，例如0.1μg/mL-5μg/mL；亚油酸，例如0.1μg/mL-5μg/mL；棕榈酸，例如0.05μg/mL-2μg/mL；牛血清白蛋白(BSA)，例如1μg/mL-5μg/mL和/或植物凝集素，例如0.01μg/mL-1μg/mL。在一个更具体的实施方案中，所述第二培养基含有包含FBS或FCS(例如10%FCS v/v)、IL-2、运铁蛋白、胰岛素、乙醇胺、油酸、亚油酸、棕榈酸、牛血清白蛋白(BSA)和植物凝集素的细胞生长培养基。在一个更具体的实施方案中，所述第二培养基包含Iscove改良Dulbecco培养基(IMDM)、10%FBS或FCS、400IU IL-2、35μg/mL运铁蛋白、5μg/mL胰岛素、$2x10^{-5}$M乙醇胺、1μg/mL油酸、1μg/mL亚油酸(Sigma-Aldrich)、0.2μg/mL棕榈酸(Sigma-Aldrich)、2.5μg/mL BSA(Sigma-Aldrich)和0.1μg/mL植物凝集素。

[0330] 在某些实施方案中，第二培养基不含粒细胞集落刺激因子(G-CSF)、粒细胞/巨噬细胞集落刺激因子(GM-CSF)、白介素-6(IL-6)、巨噬细胞炎性蛋白1α(MIP1α)或白血病抑制因子(LIF)的一种或多种。

[0331] 饲养细胞，当使用时可自不同的细胞类型建立。这些细胞类型的实例包括而不限于成纤维细胞、干细胞(例如组织培养贴壁胎盘干细胞)、血细胞(例如外周血单核细胞(PBMC))和癌性细胞(例如慢性髓细胞性白血病(CML)细胞例如K562)。在一个具体的实施方案中，在所述第二培养基中的所述培养包括使用饲养细胞(例如K562细胞和/或外周血单核细胞(PBMC))，例如在之后1、2、3、4、5、6、7、8、9或10天细胞在所述第二培养基中开始时的培养。在某些实施方案中，饲养细胞任选来自与它们支持的细胞不同的物种。例如，人NK细胞可受小鼠胚胎成纤维细胞(来自原代培养或telomerized line)支持。

[0332] 在某些实施方案中，饲养细胞任选被辐射(例如γ-辐射)灭活或用抗有丝分裂剂例如丝裂霉素C处理以防止它们的生长超过其支持的细胞，却允许支持NK细胞的重要因子的合成。例如，细胞可以抑制增殖但允许支持人胚胎干(hES)细胞的重要因子合成的剂量(约4000拉德γ辐射)照射。

[0333] 用于第二步的NK细胞的培养可发生在与细胞培养和扩增相容的任何容器中，例如培养瓶、管、烧杯、培养皿、多孔板、袋等。在一个具体的实施方案中，NK细胞的依赖饲养细胞的培养发生在袋，例如柔性透气碳氟化合物培养袋(例如来自American Fluoroseal)中。在一个具体的实施方案中，其中NK细胞培养的容器适于运输至医院或军事区的场所，例如扩增的NK细胞在其中进一步扩增、分化和成熟。

[0334] 可通过例如流式细胞术，通过检测NK细胞特异性标志物，来评价来自步骤1的细胞向活化NK细胞的分化。NK细胞特异性标志物包括但不限于CD56、CD94、CD117和NKp46。还可通过NK细胞的形态性质，例如大的尺寸、大量内质网中的高蛋白质合成活性和/或预成型颗粒来评价分化。

[0335] 来自步骤1的细胞向活化NK细胞的扩增和分化的时间可为例如约3天-约120天。在一个实施方案中，分化时间为约7天-约75天。在另一个实施方案中，分化时间为约14天-约50天。在一个具体的实施方案中，分化时间为约10天-约21天。

[0336] 可通过例如流式细胞术，通过检测例如CD56、CD94、CD117、NKG2D、DNAM-1和NKp46等标志物，来评价造血细胞向NK细胞的分化。还可通过NK细胞的形态性质，例如大的尺寸、

大量内质网中的高蛋白质合成活性和/或预成型颗粒，来评价分化。可通过检测一种或多种功能相关的标志物(例如CD94、CD161、NKp44、DNAM-1、2B4、NKp46、CD94、KIR)和激活性受体的NKG2家族(例如NKG2D)，评价NK细胞(例如活化NK细胞)的成熟。还可通过在不同发育阶段检测特异性标志物评价NK细胞(例如活化NK细胞)的成熟。例如，在一个实施方案中，NK细胞前体(pro-NK cell)是$CD34^+$、$CD45RA^+$、$CD10^+$、$CD117^-$和/或$CD161^-$。在另一个实施方案中，前NK细胞(pre-NK cell)是$CD34^+$、$CD45RA^+$、$CD10^-$、$CD117^+$和/或$CD161^-$。在另一个实施方案中，未成熟NK细胞是$CD34^-$、$CD117^+$、$CD161^+$、$NKp46^-$和/或$CD94/NKG2A^-$。在另一个实施方案中，$CD56^{bright}$ NK细胞是$CD117^+$、$NKp46^+$、$CD94/NKG2A^+$、CD16-和/或$KIR^{+/-}$。在另一个实施方案中，$CD56^{dim}$ NK细胞是$CD117^-$、$NKp46^+$、$CD94/NKG2A^{+/-}$、CD16+和/或KIR^+。在一个具体的实施方案中，NK细胞(例如活化NK细胞)的成熟通过是$CD161^-$、$CD94^+$和/或$NKp46^+$的NK细胞(例如活化NK细胞)的百分比确定。在一个更具体的实施方案中，至少10％、20％、25％、30％、35％、40％、50％、55％、60％、65％或70％成熟的NK细胞(例如活化NK细胞)是$NKp46^+$。在另一个更具体的实施方案中，至少10％、20％、25％、30％、35％、40％、45％或50％成熟的NK细胞(例如活化NK细胞)是$CD94^+$。在另一个更具体的实施方案中，至少10％、20％、25％、30％、35％、40％、45％或50％成熟的NK细胞(例如活化NK细胞)是$CD161^-$。

[0337] 在某些实施方案中，使用例如抗这些细胞标志物的一种或多种的抗体，通过检测例如CD3、CD7或CD127、CD10、CD14、CD15、CD16、CD33、CD34、CD56、CD94、CD117、CD161、NKp44、NKp46、NKG2D、DNAM-1、2B4或TO-PRO-3的表达水平，评价造血细胞向NK细胞的分化。这类抗体可与可检测标记，例如FITC、R-PE、PerCP、PerCP-Cy5.5、APC、APC-Cy7或APC-H7等荧光标记缀合。

[0338] 5.2.8.产生TSPNK细胞的方法

[0339] TSPNK细胞可产生自上文描述的造血细胞。在某实施方案中，TSPNK细胞自扩增的造血细胞(例如造血干细胞和/或造血祖细胞)产生。

[0340] 在一个实施方案中，TSPNK细胞通过三步法产生。在某些实施方案中，按本文所述使造血细胞扩增和分化以产生本文所述三步法的NK祖细胞群或NK细胞群的方法包括在扩增和分化期间将包含所述造血细胞的细胞群维持在约$2x10^4$和约$6x10^6$个细胞/毫升之间，例如约$2x10^4$和约$2x10^5$个细胞/毫升之间。在某些其它实施方案中，如本文所述造血细胞扩增和分化的方法包括将包含所述造血细胞的细胞群维持在不超过约$1x10^5$个细胞/毫升。在某些其它实施方案中，如本文所述造血细胞扩增和分化的方法包括将包含所述造血细胞的细胞群维持在不超过约$1x10^5$个细胞/毫升、$2x10^5$个细胞/毫升、$3x10^5$个细胞/毫升、$4x10^5$个细胞/毫升、$5x10^5$个细胞/毫升、$6x10^5$个细胞/毫升、$7x10^5$个细胞/毫升、$8x10^5$个细胞/毫升、$9x10^5$个细胞/毫升、$1x10^6$个细胞/毫升、$2x10^6$个细胞/毫升、$3x10^6$个细胞/毫升、$4x10^6$个细胞/毫升、$5x10^6$个细胞/毫升、$6x10^6$个细胞/毫升、$7x10^6$个细胞/毫升、$8x10^6$个细胞/毫升或$9x10^6$个细胞/毫升。

[0341] 在某个实施方案中，包括第一步(“步骤1”)的三步法包括例如如本文所述将造血干细胞或祖细胞(例如$CD34^+$干细胞或祖细胞)培养在第一培养基中培养规定的一段时间。在某些实施方案中，第一培养基含有促进造血祖细胞扩增的一种或多种因子、开启扩增性造血祖细胞群内淋巴分化的一种或多种因子和/或模拟基质饲养细胞支持的一种或多种因子。在某些实施方案中，第一培养基包含一种或多种细胞因子(例如Flt3L、TPO、SCF)。在某

些实施方案中，第一培养基包含IL-7。在某些实施方案中，第一培养基包含亚ng/mL浓度的G-CSF、IL-6和/或GM-CSF。在一个具体的实施方案中，第一培养基包含细胞因子Flt3L、TPO和SCF、IL-7和亚ng/mL浓度的G-CSF、IL-6和GM-CSF。在具体的实施方案中，在第一培养基中，CD34+细胞进行向谱系特异性祖细胞的扩增，所述祖细胞然后变成CD34-。在某些实施方案中，这种扩增快速发生。在某些实施方案中，在步骤1结束时CD34-细胞包含总群体的超过50％、超过55％、超过60％、超过65％、超过70％、超过75％、超过80％或更多。在一个更具体的实施方案中，在步骤1结束时CD34-细胞包含总群体的超过80％。

[0342] 在某些实施方案中，随后在“步骤2”中，例如如上所述将所述细胞在第二培养基中培养规定的一段时间。在某些实施方案中，第二培养基含有促进淋巴祖细胞进一步扩增的因子、可有利于沿NK谱系发育的因子和/或模拟基质饲养细胞支持的因子。在某些实施方案中，第二培养基包含一种或多种细胞因子(例如Flt3L、SCF、IL-15和/或IL-7)。在某些实施方案中，第二培养基包含IL-17和/或IL-15。在某些实施方案中，第二培养基包含亚ng/mL浓度的G-CSF、IL-6和/或GM-CSF。在一个具体的实施方案中，第二培养基包含细胞因子Flt3L、SCF、IL-15，和IL-7、IL-17和IL-15，以及亚ng/mL浓度的G-CSF、IL-6和GM-CSF。

[0343] 在某些实施方案中，随后在“步骤3”中，例如按本文所述将所述细胞在第三培养基中培养规定的一段时间。在某些实施方案中，第三培养基包含促进CD56+CD3-CD16-细胞(其可以是NK祖细胞)的分化和功能活化的因子。在一个实施方案中，这类因子包含IL2和IL12和IL18、IL12和IL15、IL12和IL18、IL2和IL12和IL15和IL18或IL2和IL15和IL18。在某些实施方案中，第三培养基包含模拟基质饲养细胞支持的因子。在某些实施方案中，第三培养基包含一种或多种细胞因子(例如SCF、IL-15、IL-7、IL-2)。在某些实施方案中，第三培养基包含亚ng/mL浓度的G-CSF、IL-6和/或GM-CSF。在一个具体的实施方案中，第三培养基包含细胞因子SCF、IL-15、IL-7、IL-2和亚ng/mL浓度的G-CSF、IL-6和GM-CSF。

[0344] 在具体的实施方案中，采用三步法产生NK细胞(例如成熟的NK细胞)群。在具体的实施方案中，采用三步法产生NK祖细胞群。在某些实施方案中，三步法在基质饲养细胞支持不存在下进行。在某些实施方案中，三步法在外源添加的类固醇类(例如可的松、氢化可的松或其衍生物)不存在下进行。

[0345] 在某些实施方案中，用于本文所述三步法的第一培养基可含有第5.2.4节中描述的与两步方法有关的第一或第二培养基的任何组分。在某些实施方案中，用于三步法的所述第一培养基包括包含以下的一种或多种的培养基：动物血清，例如人血清(例如人血清AB)、胎牛血清(FBS)或胎牛血清(FCS)，例如1％-20％v/v血清，例如5％-20％v/v血清；干细胞因子(SCF)，例如1ng/mL-50ng/mL SCF；FMS样酪氨酸激酶-3配体(Flt-3配体)，例如1ng/mL-30ng/mL Flt-3配体；白介素-7(IL-7)，例如1ng/mL-50ng/mL IL-7；血小板生成素(TPO)，例如1ng/mL-100ng/mL，例如1ng/mL-50ng/mLTPO；白介素-2(IL-2)，例如直到2000IU/mL，例如50IU/mL-500IU/mL；和/或肝素，例如低分子量肝素(LWH)，例如0.1IU/mL-10IU/mL肝素。在某些实施方案中，所述第一培养基另外包含下列的一种或多种：抗生素例如庆大霉素；抗氧化剂例如运铁蛋白、胰岛素和/或β-巯基乙醇；亚硒酸钠；抗坏血酸；乙醇胺和谷胱甘肽。在某些实施方案中，所述第一培养基另外包含OAC。在某些实施方案中，所述第一培养基另外包含白介素-6(IL-6)、白血病抑制因子(LIF)、G-CSF、GM-CSF和/或MIP-1α。在某些实施方案中，所述第一培养基另外包含一种或多种抗氧化剂，例如holo-

transferrin、胰岛素溶液、还原型谷胱甘肽、亚硒酸钠、乙醇胺、抗坏血酸、β-巯基乙醇、O-乙酰-L-肉碱、N-乙酰半胱氨酸、(+/-)硫辛酸、烟酰胺或白藜芦醇。在某些实施方案中，为第一培养基提供基料的培养基是本领域技术人员已知的细胞/组织培养基，例如市购可获得的细胞/组织培养基例如GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN®H3000、CELLGRO COMPLETE™、DMEM:Ham's F12("F12")(例如2:1比率，或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Advanced DMEM,Gibco)、EL08-1D2、Myelocult™H5100、IMDM和/或RPMI-1640；或是包含一般包括在已知细胞/组织培养基中的组分，例如包括在GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN®H3000、CELLGRO COMPLETE™、DMEM:Ham's F12("F12")(例如2:1比率，或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Gibco)、EL08-1D2、Myelocult™H5100、IMDM和/或RPMI-1640中的组分的培养基。在本文任一个实施方案的具体实施方案中，培养基不是GBGM®。

[0346] 在某些实施方案中，用于本文所述三步法的第二培养基可含有第5.2.4节中描述的与两步方法有关的第一或第二培养基的任何组分。在某些实施方案中，用于三步法的所述第二培养基含有包含以下一种或多种的培养基：动物血清，例如人血清(例如人血清AB)、FBS或FCS，例如5%-20%v/v血清；SCF，例如1ng/mL-50ng/mL SCF；Flt-3配体，例如1ng/mL-30ng/mL Flt-3配体；IL-7，例如1ng/mL-50ng/mL IL-7；白介素-15(IL-15)，例如1ng/mL-50ng/mL IL-15；和/或肝素，例如LWH，例如0.1IU/mL-10IU/mL肝素。在某些实施方案中，所述第二培养基另外包含下列的一种或多种：抗生素例如庆大霉素；抗氧化剂例如运铁蛋白、胰岛素和/或β-巯基乙醇；亚硒酸钠；抗坏血酸；乙醇胺和谷胱甘肽。在某些实施方案中，所述第二培养基另外包含OAC。在某些实施方案中，所述第二培养基另外包含白介素-6(IL-6)、白血病抑制因子(LIF)、G-CSF、GM-CSF和/或MIP-1α。在某些实施方案中，所述第二培养基另外包含以下的一种或多种：抗氧化剂，例如holo-transferrin、胰岛素溶液、还原型谷胱甘肽、亚硒酸钠、乙醇胺、抗坏血酸、β-巯基乙醇、O-乙酰-L-肉碱、N-乙酰半胱氨酸、(+/-)硫辛酸、烟酰胺或白藜芦醇。在某些实施方案中，为第二培养基提供基料的培养基是本领域技术人员已知的细胞/组织培养基，例如市购可获得的细胞/组织培养基例如GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN®H3000、CELLGRO COMPLETE™、DMEM:Ham's F12("F12")(例如2:1比率，或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Gibco)、EL08-1D2、Myelocult™ H5100、IMDM和/或RPMI-1640；或是包含一般包括在已知细胞/组织培养基中的组分，例如包括在GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN®H3000、CELLGRO COMPLETE™、DMEM:Ham's F12("F12")(例如2:1比率、或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Gibco)、EL08-1D2、Myelocult™ H5100、IMDM和/或RPMI-1640中的组分的培养基。在本文任一个实施方案的具体实施方案中，培养基不是GBGM®。

[0347] 在某些实施方案中，用于本文所述三步法的第三培养基可含有第5.2.4节中描述的与两步方法有关的第一或第二培养基的任何组分。在某些实施方案中，用于三步法的所述第三培养基包括包含以下一种或多种的培养基：动物血清，例如人血清(例如人血清AB)、FBS或FCS，例如5%-20%v/v血清；SCF，例如1ng/mL-50ng/mL SCF；Flt-3配体，例如1ng/mL-30ng/mL Flt-3配体；IL-7，例如1ng/mL-50ng/mL IL-7；IL-15，例如1ng/mL-50ng/mL IL-15；和白介素-2(IL-2)，例如0-2000IU/mL的范围，例如50IU/mL-1000IU/mL IL-2。在某些实

施方案中，所述第三培养基另外包含下列的一种或多种：抗生素例如庆大霉素；抗氧化剂例如运铁蛋白、胰岛素和/或β-巯基乙醇；亚硒酸钠；抗坏血酸；乙醇胺和谷胱甘肽。在某些实施方案中，所述第三培养基另外包含OAC。在某些实施方案中，所述第三培养基另外包含白介素-6(IL-6)、白血病抑制因子(LIF)、G-CSF、GM-CSF和/或MIP-1α。在某些实施方案中，所述第三培养基另外包含一种或多种抗氧化剂，例如holo-transferrin、胰岛素溶液、还原型谷胱甘肽、亚硒酸钠、乙醇胺、抗坏血酸、β-巯基乙醇、O-乙酰-L-肉碱、N-乙酰半胱氨酸、(+/-)硫辛酸、烟酰胺或白藜芦醇。在某些实施方案中，为第三培养基提供基料的培养基是本领域技术人员已知的细胞/组织培养基，例如市购可获得的细胞/组织培养基例如GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN®H3000、CELLGRO COMPLETE™、DMEM:Ham's F12("F12")(例如2:1比率，或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Gibco)、EL08-1D2、Myelocult™H5100、IMDM和/或RPMI-1640；或是包含一般包括在已知细胞/组织培养基中的组分，例如包括在GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN®H3000、CELLGRO COMPLETE™、DMEM:Ham's F12("F12")(例如2:1比率，或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Gibco)、EL08-1D2、Myelocult™H5100、IMDM和/或RPMI-1640中的组分的培养基。在本文任一个实施方案的具体实施方案中，培养基不是GBGM®。

[0348] 在某些实施方案中，在本文所述三步法中，在所述第二培养基中的所述培养之前，将所述造血干细胞或祖细胞在所述第一培养基中培养1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19或20天。在某些实施方案中，在所述第三培养基中的所述培养之前，将在所述第一培养基中培养的细胞在所述第二培养基中培养1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19或20天。在某些实施方案中，将在所述第一培养基和所述第二培养基中培养的细胞在所述第三培养基中培养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或30天或超过30天。

[0349] 在某些实施方案中，在本文所述三步法中，在所述第二培养基中的所述培养之前，将所述造血干细胞或祖细胞在所述第一培养基中培养2-12天、3-11天，例如3-5、4-6、5-7、6-8、7-9、8-10或9-11天。在某些实施方案中，在所述第三培养基中的所述培养之前，将在所述第一培养基中培养的细胞在所述第二培养基中培养1-10天，例如1-3、2-4、3-5、4-6、5-7、6-8或7-9天。在某些实施方案中，将在所述第一培养基和所述第二培养基中培养的细胞在所述第三培养基中培养2-27天，例如3-25天、例如、for 3-5、4-6、5-7、6-8、7-9、8-10、9-11、10-12、11-13、12-14、13-15、14-16、15-17、16-18、17-19、18-20、19-21、20-22、21-23、22-24或23-25天。

[0350] 在一个具体的实施方案中，在本文所述三步法中，在所述第二培养基中的所述培养之前，将所述造血干细胞或祖细胞在所述第一培养基中培养9天；在所述第三培养基中的所述培养之前在所述第二培养基中培养5天；并在所述第三培养基中培养7天，即，将细胞培养共21天。

[0351] 在一个具体的实施方案中，在本文所述三步法中，在所述第二培养基中的所述培养之前将所述造血干细胞或祖细胞在所述第一培养基中培养7-9天；在所述第三培养基中的所述培养之前在所述第二培养基中培养5-7天；并在所述第三培养基中培养21-35天，即，将细胞培养共35天。在一个更具体的实施方案中，在本文所述三步法中，在所述第二培养基

中的所述培养之前将所述造血干细胞或祖细胞在所述第一培养基中培养9天;在所述第三培养基中的所述培养之前在所述第二培养基中培养5天;并在所述第三培养基中培养21天,即,将细胞培养共35天。

[0352] 5.2.9.产生三阶段NK细胞的方法

[0353] 通过三阶段方法产生NK细胞和NK细胞群包括扩增造血细胞群。在细胞扩增期间,造血细胞群内的大量造血细胞分化成NK细胞。一方面,本文提供产生NK细胞的方法,所述方法包括将造血干细胞或祖细胞,例如$CD34^+$干细胞或祖细胞在包含干细胞动员剂和血小板生成素(Tpo)的第一培养基中培养以产生第一细胞群,随后将所述第一细胞群在包含干细胞动员剂和白介素-15(IL-15)却缺乏Tpo的第二培养基中培养以产生第二细胞群,随后将所述第二细胞群在包含IL-2和IL-15却缺乏干细胞动员剂和LMWH的第三培养基中培养以产生第三细胞群,其中第三细胞群包含是CD56+,CD3-的自然杀伤细胞,且其中至少70%、例如80%自然杀伤细胞对于某些实施方案是有活力的,这类自然杀伤细胞包含是CD16-的自然杀伤细胞。在某些实施方案中,这类自然杀伤细胞包含是CD94-的自然杀伤细胞。

[0354] 在一个实施方案中,本文提供产生NK细胞群的三阶段方法。在某些实施方案中,按本文所述使造血细胞扩增和分化以产生本文所述三阶段方法的NK细胞群的方法包括将包含所述造血细胞的细胞群维持在约$2x10^4$和约$6x10^6$个细胞/毫升之间。在某些方面,最初以$1x10^4$-$1x10^5$个细胞/mL将所述造血干细胞或祖细胞接种在所述第一培养基中。在一个具体方面,最初以约$3x10^4$个细胞/mL将所述造血干细胞或祖细胞接种在所述第一培养基中。

[0355] 在某些实施方案中,所述造血干细胞或祖细胞是哺乳动物细胞。在具体的实施方案中,所述造血干细胞或祖细胞是人细胞。在具体的实施方案中,所述造血干细胞或祖细胞是灵长类动物细胞。在具体的实施方案中,所述造血干细胞或祖细胞是犬科动物细胞。在具体的实施方案中,所述造血干细胞或祖细胞是啮齿动物细胞。

[0356] 在某些方面,最初以$5x10^4$-$5x10^5$个细胞/mL将所述第一细胞群接种在所述第二培养基中。在一个具体方面,最初以约$1x10^5$个细胞/mL将所述第一细胞群接种在所述第二培养基中。

[0357] 在某些方面,最初以$1x10^5$-$5x10^6$个细胞/mL将所述第二细胞群接种在所述第三培养基中。在某些方面,最初以$1x10^5$-$1x10^6$个细胞/mL将所述第二细胞群接种在所述第三培养基中。在一个具体方面,最初以约$5x10^5$个细胞/mL将所述第二细胞群接种在所述第三培养基中。在一个更具体的方面,最初以约$5x10^5$个细胞/mL将所述第二细胞群接种在转瓶中的所述第三培养基中。在一个具体方面,最初以约$3x10^5$个细胞/mL将所述第二细胞群接种在所述第三培养基中。在一个更具体的方面,最初以约$3x10^5$个细胞/mL将所述第二细胞群接种在所述第三培养基中呈静置培养。

[0358] 在某一实施方案中,三阶段方法包括:包括例如按本文所述将造血干细胞或祖细胞例如$CD34^+$干细胞或祖细胞在第一培养基中培养规定的一段时间以产生第一细胞群的第一阶段(“阶段1”)。在某些实施方案中,第一培养基包含干细胞动员剂和血小板生成素(Tpo)。在某些实施方案中,第一培养基除干细胞动员剂和Tpo以外还包含LMWH、Flt-3L、SCF、IL-6、IL-7、G-CSF和GM-CSF的一种或多种。在一个具体的实施方案中,第一培养基除干细胞动员剂和Tpo以外,第一培养基各自还包含LMWH、Flt-3L、SCF、IL-6、IL-7、G-CSF和GM-CSF的每一种。

[0359] 在某些实施方案中，随后在“阶段2”中，例如按本文所述将所述细胞在第二培养基中培养规定的一段时间以产生第二细胞群。在某些实施方案中，第二培养基包含干细胞动员剂和白介素-15(IL-15)，并缺乏Tpo。在某些实施方案中，第二培养基除干细胞动员剂和IL-15以外还包含LMWH、Flt-3、SCF、IL-6、IL-7、G-CSF和GM-CSF的一种或多种。在某些实施方案中，第二培养基除干细胞动员剂和IL-15以外还包含LMWH、Flt-3、SCF、IL-6、IL-7、G-CSF和GM-CSF的每一个。

[0360] 在某些实施方案中，随后在“阶段3”中，例如按本文所述，将所述细胞在第三培养基中培养规定的一段时间以产生第三细胞(例如自然杀伤细胞)群。在某些实施方案中，第三培养基包含IL-2和IL-15，并缺乏干细胞动员剂和LMWH。在某些实施方案中，第三培养基除IL-2和IL-15以外还包含SCF、IL-6、IL-7、G-CSF和GM-CSF的一种或多种。在某些实施方案中，第三培养基除IL-2和IL-15以外还包含SCF、IL-6、IL-7、G-CSF和GM-CSF的每一种。

[0361] 在一个具体的实施方案中，采用三阶段方法以产生NK细胞群。在某些实施方案中，三阶段方法在基质饲养细胞支持不存在下进行。在某些实施方案中，三阶段方法在外源添加的类固醇类(例如可的松、氢化可的松或其衍生物)不存在下进行。

[0362] 在某些方面，用于三阶段方法的所述第一培养基包含干细胞动员剂和血小板生成素(Tpo)。在某些方面，用于三阶段方法的第一培养基除干细胞动员剂和Tpo以外还包含低分子量肝素(LMWH)、Flt-3配体(Flt-3L)、干细胞因子(SCF)、IL-6、IL-7、粒细胞集落刺激因子(G-CSF)或粒细胞-巨噬细胞刺激因子(GM-CSF)的一种或多种。在某些方面，用于三阶段方法的第一培养基除干细胞动员剂和Tpo以外还包含LMWH、Flt-3L、SCF、IL-6、IL-7、G-CSF和GM-CSF的每一种。在某些方面，所述Tpo以1ng/mL-100ng/mL、1ng/mL-50ng/mL、20ng/mL-30ng/mL或约25ng/mL的浓度存在于第一培养基中。在某些方面，在第一培养基中，LMWH以1U/mL-10U/mL的浓度存在；Flt-3L以1ng/mL-50ng/mL的浓度存在；SCF以1ng/mL-50ng/mL的浓度存在；IL-6以0.01ng/mL-0.1ng/mL的浓度存在；IL-7以1ng/mL-50ng/mL的浓度存在；G-CSF以0.01ng/mL-0.50ng/mL的浓度存在；且GM-CSF以0.005ng/mL-0.1ng/mL的浓度存在。在某些方面，在第一培养基中，LMWH以4U/mL-5U/mL的浓度存在；Flt-3L以20ng/mL-30ng/mL的浓度存在；SCF以20ng/mL-30ng/mL的浓度存在；IL-6以0.04ng/mL-0.06ng/mL的浓度存在；IL-7以20ng/mL-30ng/mL的浓度存在；G-CSF以0.20ng/mL-0.30ng/mL的浓度存在；且GM-CSF以0.005ng/mL-0.5ng/mL的浓度存在。在某些方面，在第一培养基中，LMWH以约4.5U/mL的浓度存在；Flt-3L以约25ng/mL的浓度存在；SCF以约27ng/mL的浓度存在；IL-6以约0.05ng/mL的浓度存在；IL-7以约25ng/mL的浓度存在；G-CSF以约.25ng/mL的浓度存在；且GM-CSF以约0.01ng/mL的浓度存在。在某些实施方案中，所述第一培养基另外包含下列的一种或多种：抗生素例如庆大霉素；抗氧化剂例如运铁蛋白、胰岛素和/或β-巯基乙醇；亚硒酸钠；抗坏血酸；乙醇胺和谷胱甘肽。在某些实施方案中，为第一培养基提供基料的培养基是本领域技术人员已知的细胞/组织培养基，例如市购可获得的细胞/组织培养基例如SCGM™、STEMMACS™、GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN® H3000、CELLGRO COMPLETE™、DMEM:Ham's F12(“F12”)(例如2:1比率，或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Gibco)、EL08-1D2、Myelocult™H5100、IMDM和/或RPMI-1640；或是包含一般包括在已知细胞/组织培养基中的组分，例如包括在GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN®H3000、CELLGRO COMPLETE™、DMEM:Ham's F12

("F12")(例如2:1比率,或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Gibco)、EL08-1D2、Myelocult™H5100、IMDM和/或RPMI-1640中的组分的培养基。在本文任一个实施方案的具体实施方案中,培养基不是GBGM®。

[0363] 在某些方面,用于三阶段方法的所述第二培养基包含干细胞动员剂和白介素-15(IL-15),并缺乏Tpo。在某些方面,用于三阶段方法的第二培养基除干细胞动员剂和IL-15以外还包含LMWH、Flt-3、SCF、IL-6、IL-7、G-CSF和GM-CSF的一种或多种。在某些方面,用于三阶段方法的第二培养基除干细胞动员剂和IL-15以外还包含LMWH、Flt-3、SCF、IL-6、IL-7、G-CSF和GM-CSF的每一种。在某些方面,所述IL-15以1ng/mL-50ng/mL、10ng/mL-30ng/mL或约20ng/mL的浓度存在于所述第二培养基中。在某些方面,在所述第二培养基中,LMWH以1U/mL-10U/mL的浓度存在;Flt-3L以1ng/mL-50ng/mL的浓度存在;SCF以1ng/mL-50ng/mL的浓度存在;IL-6以0.01ng/mL-0.1ng/mL的浓度存在;IL-7以1ng/mL-50ng/mL的浓度存在;G-CSF以0.01ng/mL-0.50ng/mL的浓度存在;且GM-CSF以0.005ng/mL-0.1ng/mL的浓度存在。在某些方面,在第二培养基中,LMWH以4U/mL-5U/mL的浓度存在于第二培养基中;Flt-3L以20ng/mL-30ng/mL的浓度存在;SCF以20ng/mL-30ng/mL的浓度存在;IL-6以0.04ng/mL-0.06ng/mL的浓度存在;IL-7以20ng/mL-30ng/mL的浓度存在;G-CSF以0.20ng/mL-0.30ng/mL的浓度存在;且GM-CSF以0.005ng/mL-0.5ng/mL的浓度存在。在某些方面,在第二培养基中,LMWH以4U/mL-5U/mL的浓度存在于第二培养基中;Flt-3L以20ng/mL-30ng/mL的浓度存在;SCF以20ng/mL-30ng/mL的浓度存在;IL-6以0.04ng/mL-0.06ng/mL的浓度存在;IL-7以20ng/mL-30ng/mL的浓度存在;G-CSF以0.20ng/mL-0.30ng/mL的浓度存在;且GM-CSF以0.005ng/mL-0.5ng/mL的浓度存在。在某些方面,在第二培养基中,LMWH以约4.5U/mL的浓度存在于第二培养基中;Flt-3L以约25ng/mL的浓度存在;SCF以约27ng/mL的浓度存在;IL-6以约0.05ng/mL的浓度存在;IL-7以约25ng/mL的浓度存在;G-CSF以约0.25ng/mL的浓度存在;且GM-CSF以约0.01ng/mL的浓度存在。在某些实施方案中,所述第二培养基另外包含下列的一种或多种:抗生素例如庆大霉素;抗氧化剂例如运铁蛋白、胰岛素和/或β-巯基乙醇;亚硒酸钠;抗坏血酸;乙醇胺和谷胱甘肽。在某些实施方案中,为第二培养基提供基料的培养基是本领域技术人员已知的细胞/组织培养基,例如市购可获得的细胞/组织培养基例如SCGM™、STEMMACS™、GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN®H3000、CELLGRO COMPLETE™、DMEM:Ham's F12("F12")(例如2:1比率,或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Gibco)、EL08-1D2、Myelocult™H5100、IMDM和/或RPMI-1640;或是包含一般包括在已知细胞/组织培养基中的组分,例如包括在GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN®H3000、CELLGRO COMPLETE™、DMEM:Ham's F12("F12")(例如2:1比率,或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Gibco)、EL08-1D2、Myelocult™H5100、IMDM和/或RPMI-1640中的组分的培养基。在本文任一个实施方案的具体实施方案中,培养基不是GBGM®。

[0364] 在某些实施方案中,用于三阶段方法的所述第三培养基包含IL-2和IL-15,并缺乏干细胞动员剂和LMWH。在某些方面,用于三阶段方法的第三培养基除IL-2和IL-15以外还包含SCF、IL-6、IL-7、G-CSF或GM-CSF。在某些方面,用于三阶段方法的第三培养基除IL-2和IL-15以外还包含SCF、IL-6、IL-7、G-CSF和GM-CSF的每一种。在某些方面,所述IL-2以10U/mL-10,000U/mL的浓度存在于所述第三培养基中,所述IL-15以1ng/mL-50ng/mL的浓度存在

于所述第三培养基中。在某些方面，所述IL-2以100U/mL-10,000U/mL的浓度存在于所述第三培养基中，所述IL-15以1ng/mL-50ng/mL的浓度存在于所述第三培养基中。在某些方面，所述IL-2以300U/mL-3,000U/mL的浓度存在于所述第三培养基中，所述IL-15以10ng/mL-30ng/mL的浓度存在于所述第三培养基中。在某些方面，所述IL-2以约1,000U/mL的浓度存在于所述第三培养基中，所述IL-15以约20ng/mL的浓度存在于所述第三培养基中。在某些方面，在所述第三培养基中，SCF以1ng/mL-50ng/mL的浓度存在；IL-6以0.01ng/mL-0.1ng/mL的浓度存在；IL-7以1ng/mL-50ng/mL的浓度存在；G-CSF以0.01ng/mL-0.50ng/mL的浓度存在；且GM-CSF以0.005ng/mL-0.1ng/mL的浓度存在。在某些方面，在所述第三培养基中，SCF以20ng/mL-30ng/mL的浓度存在；IL-6以0.04ng/mL-0.06ng/mL的浓度存在；IL-7以20ng/mL-30ng/mL的浓度存在；G-CSF以0.20ng/mL-0.30ng/mL的浓度存在；且GM-CSF以0.005ng/mL-0.5ng/mL的浓度存在。在某些方面，在所述第三培养基中，SCF以约22ng/mL的浓度存在；IL-6以约0.05ng/mL的浓度存在；IL-7以约20ng/mL的浓度存在；G-CSF以约0.25ng/mL的浓度存在；且GM-CSF以约0.01ng/mL的浓度存在。在某些实施方案中，所述第三培养基另外包含下列的一种或多种：抗生素例如庆大霉素；抗氧化剂例如运铁蛋白、胰岛素和/或β-巯基乙醇；亚硒酸钠；抗坏血酸；乙醇胺和谷胱甘肽。在某些实施方案中，为第三培养基提供基料的培养基是本领域技术人员已知的细胞/组织培养基，例如市购可获得的细胞/组织培养基例如SCGM™、STEMMACS™、GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN®H3000、CELLGRO COMPLETE™、DMEM:Ham's F12("F12")(例如2:1比率，或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Gibco)、EL08-1D2、Myelocult™ H5100、IMDM和/或RPMI-1640；或是包含一般包括在已知细胞/组织培养基中的组分，例如包括在GBGM®、AIM-V®、X-VIVO™ 10、X-VIVO™ 15、OPTMIZER、STEMSPAN®H3000、CELLGRO COMPLETE™、DMEM:Ham's F12("F12")(例如2:1比率，或高葡萄糖或低葡萄糖DMEM)、高级DMEM(Gibco)、EL08-1D2、Myelocult™ H5100、IMDM和/或RPMI-1640中的组分的培养基。在本文任一个实施方案的具体实施方案中，培养基不是GBGM®。

[0365] 一般而言，具体列举的培养基组分不是指所述培养基的成分未确定组分中的可能成分。例如，所述Tpo、IL-2和IL-15不包含在第一培养基、第二培养基或第三培养基的成分未确定组分中，例如，所述Tpo、IL-2和IL-15不包含在血清中。此外，所述LMWH、Flt-3、SCF、IL-6、IL-7、G-CSF和/或GM-CSF不包含在第一培养基、第二培养基或第三培养基的成分未确定组分内，例如，所述LMWH、Flt-3、SCF、IL-6、IL-7、G-CSF和/或GM-CSF不包含在血清中。

[0366] 在某些方面，所述第一培养基、第二培养基或第三培养基包含人血清-AB。在某些方面，所述第一培养基、第二培养基或第三培养基的任一种包含1％-20％人血清-AB、5％-15％人血清-AB或约2％、5％或10％人血清-AB。

[0367] 在某些实施方案中，在本文所述三阶段方法中，将所述造血干细胞或祖细胞在所述第一培养基中培养1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19或20天。在某些实施方案中，在本文所述三阶段方法中，将细胞在所述第二培养基中培养1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19或20天。在某些实施方案中，在本文所述三阶段方法中，将细胞在所述第三培养基中培养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或30天或超过30天。

[0368] 在一个具体的实施方案中，在本文所述三阶段方法中，在所述第二培养基中的所

述培养之前，将所述造血干细胞或祖细胞在所述第一培养基中培养7-13天以产生第一细胞群；在所述第三培养基中的所述培养之前，将所述第一细胞群在所述第二培养基中培养2-6天以产生第二细胞群；将所述第二细胞群在所述第三培养基中培养10-30天，即，将细胞培养共19-49天。

[0369] 在一个具体的实施方案中，在本文所述三阶段方法中，在所述第二培养基中的所述培养之前，将所述造血干细胞或祖细胞在所述第一培养基中培养8-12天以产生第一细胞群；在所述第三培养基中的所述培养之前，将所述第一细胞群在所述第二培养基中培养3-5天以产生第二细胞群；将所述第二细胞群在所述第三培养基中培养15-25天，即，将细胞培养共26-42天。

[0370] 在一个具体的实施方案中，在本文所述三阶段方法中，在所述第二培养基中的所述培养之前，将所述造血干细胞或祖细胞在所述第一培养基中培养约10天以产生第一细胞群；在所述第三培养基中的所述培养之前，将所述第一细胞群在所述第二培养基中培养约4天以产生第二细胞群；将所述第二细胞群在所述第三培养基中培养约21天，即，将细胞培养共约35天。

[0371] 在某些方面，在所述第一培养基、第二培养基和第三培养基中的所述培养全都在静置培养条件下(例如在培养皿或培养瓶中)进行。在某些方面，在所述第一培养基、第二培养基或第三培养基的至少一种中的所述培养在转瓶中进行。在某些方面，在所述第一培养基和所述第二培养基中的所述培养在静置培养条件下进行，且在所述第三培养基中的所述培养在转瓶中进行。

[0372] 在某些方面，所述培养在转瓶中进行。在其它方面，所述培养在G-Rex装置中进行。在另外其它的方面，所述培养在WAVE生物反应器中进行。

[0373] 在某些方面，最初以$1x10^4$-$1x10^5$个细胞/mL将所述造血干细胞或祖细胞接种在所述第一培养基中。在一个具体方面，最初以约$3x10^4$个细胞/mL将所述造血干细胞或祖细胞接种在所述第一培养基中。

[0374] 在某些方面，最初以$5x10^4$-$5x10^5$个细胞/mL将所述第一细胞群接种在所述第二培养基中。在一个具体方面，最初以约$1x10^5$个细胞/mL将所述第一细胞群接种在所述第二培养基中。

[0375] 在某些方面，最初以$1x10^5$-$5x10^6$个细胞/mL将所述第二细胞群接种在所述第三培养基中。在某些方面，最初以$1x10^5$-$1x10^6$个细胞/mL将所述第二细胞群接种在所述第三培养基中。在一个具体方面，最初以约$5x10^5$个细胞/mL将所述第二细胞群接种在所述第三培养基中。在一个更具体的方面，最初以约$5x10^5$个细胞/mL将所述第二细胞群接种在转瓶中的所述第三培养基中。在一个具体方面，最初以约$3x10^5$个细胞/mL将所述第二细胞群接种在所述第三培养基中。在一个更具体的方面，最初以约$3x10^5$个细胞/mL将所述第二细胞群接种在所述第三培养基中呈静置培养。

[0376] 5.2.10.细胞的分离

[0377] 分离自然杀伤细胞的方法是本领域已知的，可用于分离采用本文所述三步法产生的自然杀伤细胞，例如活化NK细胞或TSPNK细胞(例如NK祖细胞)。NK细胞可通过将来自组织来源(例如外周血)的细胞用抗CD56和CD3的抗体染色来分离或富集，并选出$CD56^+CD3^-$细胞。NK细胞(例如活化NK细胞或TSPNK细胞)可使用市购可获得的试剂盒(例如NK细胞分离试剂

盒(Miltenyi Biotec))分离。NK细胞(例如活化NK细胞或TSPNK细胞)还可通过包含NK细胞(例如活化NK细胞或TSPNK细胞)的细胞群中除去NK细胞以外的细胞来分离或富集。例如NK细胞(例如活化NK细胞或TSPNK细胞)可通过使用例如抗CD3、CD4、CD14、CD19、CD20、CD36、CD66b、CD123、HLA DR和/或CD235a(血型糖蛋白A)的一种或多种的抗体,消耗显示非NK细胞标志物的细胞来分离或富集。阴性分离可使用市购可获得的试剂盒,例如NK细胞阴性分离试剂盒(Dynal Biotech)进行。通过这些方法分离的细胞可再分选以例如分离$CD16^+$和CD16-细胞。

[0378] 细胞分离可通过例如流式细胞术、荧光激活细胞分选术(FACS)或者优选使用与特异性抗体缀合的微珠的磁性细胞分选实现。可采用例如磁性激活细胞分选(MACS)技术,一种根据其结合包含一种或多种特异性抗体(例如抗CD56抗体)的磁珠(例如约0.5-100μm直径)的能力分离粒子的方法,来分离细胞。磁性细胞分离可采用例如AUTOMACS™分离器(Miltenyi)进行并使之自动化。可对磁性微球进行各种有用的修饰,包括共价添加特异性识别具体的细胞表面分子或半抗原的抗体。然后将珠粒与细胞混合以使之结合。使细胞通过磁场以分离出具有特异性细胞表面标志物的细胞。在一个实施方案中,随后可将这些细胞分离并且和与抗另外的细胞表面标志物的抗体偶联的磁珠再混合。使这些细胞再次通过磁场,从而分离出结合两种抗体的细胞。然后可将这类细胞稀释至单独的培养皿(例如微量滴定培养皿)中供克隆分离。

[0379] 在一些实施方案中,分离或富集的自然杀伤细胞的纯度可通过检测CD56、CD3和CD16的一种或多种来证实。

[0380] 5.2.11.细胞/灌注液的保存

[0381] 可保存采用本文所述方法产生的细胞(例如NK细胞),例如采用本文所述三步法产生的活化NK细胞或TSPNK细胞(例如NK祖细胞)、包含造血干细胞或祖细胞的或胎盘灌注液细胞或胎盘灌注液,即置于允许长期储存的条件下,或在抑制由例如细胞凋亡或坏死造成的细胞死亡的条件下。

[0382] 可通过使细胞收集组合物通过至少一部分胎盘(例如通过胎盘血管系统)来产生胎盘灌注液。细胞收集组合物包含起保存包含在灌注液内的细胞的作用的一种或多种化合物。这类胎盘细胞收集组合物可包含细胞凋亡抑制剂、坏死抑制剂和/或携氧全氟化碳,如相关美国申请公布号20070190042中所述,其公开内容通过引用以其整体并入本文。

[0383] 在一个实施方案中,通过使灌注液或细胞群与包含细胞凋亡抑制剂和携氧全氟化碳的细胞收集组合物接近,从哺乳动物(例如人)产后胎盘收集灌注液或胎盘细胞群,其中所述细胞凋亡抑制剂以与未接触或接近细胞凋亡抑制剂的细胞群相比足以降低或防止胎盘细胞群(例如贴壁胎盘细胞,例如胎盘干细胞或胎盘多能细胞)中的细胞凋亡的量和时间存在。例如,可用细胞收集组合物灌注胎盘,并从中分离胎盘细胞,例如总有核胎盘细胞。在一个具体的实施方案中,细胞凋亡抑制剂是胱天蛋白酶抑制剂。在另一个具体的实施方案中,所述细胞凋亡抑制剂是JNK抑制剂。在一个更具体的实施方案中,所述JNK抑制剂不调节贴壁胎盘细胞(例如贴壁胎盘干细胞或贴壁胎盘多能细胞)的分化或增殖。在另一个实施方案中,细胞收集组合物包含呈不同相的所述细胞凋亡抑制剂和所述携氧全氟化碳。在另一个实施方案中,细胞收集组合物包含呈乳液的所述细胞凋亡抑制剂和所述携氧全氟化碳。在另一个实施方案中,细胞收集组合物另外包含乳化剂,例如卵磷脂。在另一个实施方案

中，在将胎盘细胞与细胞收集组合物接近时，所述细胞凋亡抑制剂和所述全氟化碳介于约0℃和约25℃之间。在另一个更具体的实施方案中，在使胎盘细胞与细胞收集组合物接近时，所述细胞凋亡抑制剂和所述全氟化碳介于约2℃和10℃之间，或介于约2℃和约5℃之间。在另一个更具体的实施方案中，所述使之接近在所述细胞群运输期间进行。在另一个更具体的实施方案中，所述使之接近在所述细胞群的冻融期间进行。

[0384] 在另一个实施方案中，胎盘灌注液和/或胎盘细胞可通过使灌注液和/或细胞与细胞凋亡抑制剂和器官保存化合物接近来收集和保存，其中所述细胞凋亡抑制剂以与未接触或接近细胞凋亡抑制剂的灌注液或胎盘细胞相比足以降低或防止细胞凋亡的量和时间存在。在一个具体的实施方案中，器官保存化合物是UW溶液(描述于美国专利号4,798,824；亦称为VIASPAN™；另参见Southard等，Transplantation 49(2):251-257(1990)或描述于Stern等的美国专利号5,552,267的溶液，所述文献的公开内容通过引用以其整体并入本文。在另一个实施方案中，所述器官保存组合物是羟乙基淀粉、乳糖酸、棉子糖或其组合。在另一个实施方案中，胎盘细胞收集组合物另外包含呈两相或作为乳液的携氧全氟化碳。

[0385] 在另一个实施方案中，在灌注期间，使胎盘细胞与包含细胞凋亡抑制剂和携氧全氟化碳的细胞收集组合物、器官保存化合物或其组合接近。在另一个实施方案中，在通过灌注收集后，使胎盘细胞与所述细胞收集化合物接近。

[0386] 通常，在胎盘细胞收集、富集和分离期间，优选能够使因缺氧和机械应力所致的细胞胁迫降到最小或消除。因此，在所述方法的另一个实施方案中，在收集、富集或分离期间，胎盘灌注液或胎盘细胞群在所述保存期间暴露于缺氧条件小于6小时，其中缺氧条件是小于正常血液氧浓度的氧浓度。在一个更具体的实施方案中，在所述保存期间所述灌注液或胎盘细胞群暴露于所述缺氧条件小于2小时。在另一个更具体的实施方案中，在收集、富集或分离期间，所述胎盘细胞群暴露于所述缺氧条件小于1小时或小于30分钟，或不暴露于缺氧条件。在另一个具体的实施方案中，在收集、富集或分离期间，所述胎盘细胞群不暴露于剪切应力。

[0387] 细胞例如胎盘灌注液细胞，造血细胞例如$CD34^+$造血干细胞；采用本文所述方法产生的NK细胞(例如活化NK细胞或TSPNK细胞)(例如NK祖细胞)；本文提供的分离的贴壁胎盘细胞可被冷冻保存在例如小的容器(例如安瓿或隔膜小瓶)中的冷冻保存培养基中。在具体的实施方案中，细胞以或已以约$1x10^4$-$5x10^8$个细胞/mL的浓度冰冻保存。在具体的实施方案中，细胞以或已以约$1x10^6$-$1.5x10^7$个细胞/mL的浓度冰冻保存。在更具体的实施方案中，本文提供的细胞以或已以约$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$1.5x10^7$个细胞/mL的浓度冰冻保存。在某些实施方案中，NK细胞在给予前已被冷冻保存。在某些实施方案中，NK细胞在给予前未曾被冷冻保存。

[0388] 合适的冷冻保存培养基包括但不限于生理盐水、培养基，包括例如生长培养基，或细胞冰冻培养基，例如市购可获得的细胞冰冻培养基，例如C2695、C2639或C6039(Sigma)；CryoStor®CS2、CryoStor®CS5或CryoStor®CS10(BioLife溶液)。在一个实施方案中，冷冻保存培养基包含浓度为例如约1、2、3、4、5、6、7、8、9或10％(v/v)的DMSO(二甲亚砜)。冷冻保存培养基可包含其它作用剂，例如甲基纤维素、葡聚糖、白蛋白(例如人血清白蛋白)、海藻糖和/或甘油。在某些实施方案中，冷冻保存培养基包含约1％-10％DMSO、约25％-75％葡聚糖和/或约20-60％人血清白蛋白(HSA)。在某些实施方案中，冷冻保存培养基包含约

1%-10%DMSO、约25%-75%海藻糖和/或约20-60%人HSA。在一个具体的实施方案中，冷冻保存培养基包含5%DMSO、55%葡聚糖和40%HSA。在一个更具体的实施方案中，冷冻保存培养基包含5%DMSO、55%葡聚糖(10%w/v在生理盐水中)和40%HSA。在另一个具体的实施方案中，冷冻保存培养基包含5%DMSO、55%海藻糖和40%HSA。在一个更具体的实施方案中，冷冻保存培养基包含5%DMSO、55%海藻糖(10%w/v在生理盐水中)和40%HSA。在另一个具体的实施方案中，冷冻保存培养基包含CryoStor®CS5。在另一个具体的实施方案中，冷冻保存培养基包含CryoStor®CS10。

[0389] 可通过本领域已知不同方法的任一种和在细胞培养、扩增或分化的任何阶段将细胞冷冻保存。例如，可以恰好在从来源组织或器官分离之后(例如胎盘灌注液或脐带血)或在上述方法的第一或第二步期间或之后，将本文提供的细胞冷冻保存。在某些实施方案中，在从来源组织或器官分离后约1、5、10、15、20、30、45分钟内或约1、2、4、6、10、12、18、20或24小时内，将造血细胞(例如造血干细胞或祖细胞)冷冻保存。在某些实施方案中，在从来源组织或器官分离后1、2或3天内将所述细胞冷冻保存。在某些实施方案中，如上所述在第一培养基中培养后将所述细胞冷冻保存约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或28天。在一些实施方案中，如上所述在第一培养基中培养后将所述细胞冷冻保存约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或28天，并如上所述在第二培养基中冷冻保存约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或28天。在一些实施方案中，当TSPNK细胞(例如NK祖细胞)采用本文所述三步法制备时，在第一培养基中培养约1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25天后；和/或在第二培养基中培养约1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25天后；和/或在第三培养基中培养约1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25天后，将所述细胞冷冻保存。在一个具体的实施方案中，NK祖细胞采用本文所述三步法制备，且在第一培养基中培养9天后；在第二培养基中培养5天后；并在第三培养基中培养7天后，将所述细胞冷冻保存。

[0390] 一方面，NK细胞(例如活化NK细胞)群通过包括以下方法产生：(a)将造血干细胞群或祖细胞群接种在包含白介素-15(IL-15)和可选的干细胞因子(SCF)和白介素-7(IL-7)的一种或多种的第一培养基中，其中所述IL-15和可选的SCF和IL-7不包括在所述培养基的成分不确定的组分中，使得该群扩增，且所述造血干细胞群或祖细胞群内的大量造血干细胞或祖细胞在所述扩增期间分化成NK细胞；(b)使来自步骤(a)的细胞在包含白介素-2(IL-2)的第二培养基中扩增，以产生活化NK细胞群，(c)将来自步骤(b)的NK细胞在冷冻保存培养基中冷冻保存。在一个具体的实施方案中，所述步骤(c)另包括(1)制备细胞悬浮溶液；(2)将冷冻保存培养基加入来自步骤(1)的细胞悬浮溶液中以获得冷冻保存的细胞悬液；(3)使来自步骤(3)的冷冻保存细胞悬液冷却以获得冷冻保存的样品；和(4)将冷冻保存样品保存在-80℃下。在某些实施方案中，所述方法不包括步骤(a)和(b)之间及步骤(b)和(c)之间的中间步骤和/或步骤(a)前的其它培养步骤。

[0391] 在另一个实施方案中，NK细胞(例如活化NK细胞或TSPNK细胞)(例如NK祖细胞)群的冷冻保存包括：(a)在包含干细胞因子(SCF)、IL-2、白介素-7(IL-7)、白介素-15(IL-15)和肝素的一种或多种的第一培养基中扩增造血干细胞群或祖细胞群，且其中所述SCF、IL-

2、IL-7和IL-15不包括在所述培养基的成分不确定的组分中，且其中所述造血干细胞群或祖细胞群内的大量造血干细胞或祖细胞在所述扩增期间分化成NK细胞；(b)使来自步骤(a)的细胞在包含白介素-2(IL-2)的第二培养基中扩增，以产生活化NK细胞；和(c)将来自步骤(b)的NK细胞在冷冻保存培养基中冷冻保存。在一个具体的实施方案中，所述步骤(c)另包括(1)制备细胞悬浮溶液；(2)将冷冻保存培养基加入来自步骤(1)的细胞悬浮溶液中以获得冷冻保存的细胞悬液；(3)使来自步骤(3)的冷冻保存细胞悬液冷却以获得冷冻保存的样品；和(4)将冷冻保存样品保存在-80℃下。在某些实施方案中，所述方法不包括步骤(a)和(b)之间及步骤(b)和(c)之间的中间步骤。

[0392] 在冷冻保存期间，优选使细胞在控速冰箱中以例如约0.1、0.3、0.5、1或2℃/分钟冷却。优选的冷冻保存温度为约-80℃-约-180℃，优选约-125℃-约-140℃。在用前融化之前，可将冷冻保存细胞转移到液氮中。在一些实施方案中，例如，一旦安瓿达到约-90℃，便将它们转移到液氮储存区。冷冻保存细胞优选以约25℃-约40℃的温度融化，优选至约37℃的温度。在某些实施方案中，在冷冻保存约1、2、4、6、10、12、18、20或24小时，或约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或28天后，使冷冻保存细胞融化。在某些实施方案中，在冷冻保存约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或28个月后，使冷冻保存细胞融化。在某些实施方案中，在冷冻保存约1、2、3、4、5、6、7、8、9或10年后，使冷冻保存细胞融化。

[0393] 合适的融化培养基包括但不限于生理盐水、plasmalyte培养基包括例如生长培养基，例如RPMI培养基。在优选的实施方案中，融化培养基包含一种或多种培养基补充剂(例如营养物、细胞因子和/或因子)。适于本文提供的融化细胞的培养基补充剂包括例如而不限于血清例如人血清AB、胎牛血清(FBS)或胎牛血清(FCS)、维生素、人血清白蛋白(HSA)、牛血清白蛋白(BSA)、氨基酸(例如L-谷氨酰胺)、脂肪酸(例如油酸、亚油酸或棕榈酸)、胰岛素(例如重组人胰岛素)、运铁蛋白(铁饱和人运铁蛋白)、β-巯基乙醇、干细胞因子(SCF)、Fms样酪氨酸激酶3配体(Flt3-L)、细胞因子例如白介素-2(IL-2)、白介素-7(IL-7)、白介素-15(IL-15)、血小板生成素(Tpo)或肝素。在一个具体的实施方案中，可用于本文提供的方法的融化培养基包含RPMI。在另一个具体的实施方案中，所述融化培养基包含plasmalyte。在另一个具体的实施方案中，所述融化培养基包含约0.5-20％FBS。在另一个具体的实施方案中，所述融化培养基包含约1、2、5、10、15或20％FBS。在另一个具体的实施方案中，所述融化培养基包含约0.5％-20％HSA。在另一个具体的实施方案中，所述融化培养基包含约1、2.5、5、10、15、或20％HSA。在一个更具体的实施方案中，所述融化培养基包含RPMI和约10％FBS。在另一个更具体的实施方案中，所述融化培养基包含plasmalyte和约5％HSA。

[0394] 本文提供的冷冻保存方法可优化以允许长期储存，或置于抑制由例如细胞凋亡或坏死造成的细胞死亡的条件下。在一个实施方案中，融化后细胞包含大于60％、65％、70％、75％、80％、85％、90％、95％或98％活细胞，如通过例如自动细胞计数器或锥虫蓝方法测定。在另一个实施方案中，融化后细胞包含约0.5、1、5、10、15、20或25％死细胞。在另一个实施方案中，融化后细胞包含约0.5、1、5、10、15、20或25％早凋亡细胞。在另一个实施方案中，约0.5、1、5、10、15或20％的融化后细胞在融化后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或28天后进行细胞凋亡，例如，如通过细胞凋亡测定法(例如TO-PRO3或AnnV/PI细胞凋亡测定法试剂盒)测定。在某些实施方案中，在采用本

文提供的方法培养、扩增或分化后，将融化后细胞重新冷冻保存。

[0395] 5.3.遗传修饰的NK细胞

[0396] 另一方面，NK细胞可经遗传修饰以提高靶特异性和/或归巢特异性。

[0397] 在一些实施方案中，遗传修饰的NK细胞是包含嵌合抗原受体(CAR)的NK细胞。CAR是人工膜结合蛋白，它引导免疫细胞(例如T淋巴细胞)至抗原，并刺激免疫细胞杀死显示抗原的细胞。参见例如Eshhar，美国专利号7,741,465；美国专利申请公布号2012/0093842；国际申请公布号WO 2014/100385和国际申请公布号WO 2014/124143。至少，CAR包含与抗原(例如细胞上的抗原)结合的胞外域、跨膜结构域和将最初的激活信号传送至免疫细胞的胞内(胞质)信号转导结构域(即胞内刺激结构域)。所有其它条件都满足时，当CAR在例如T淋巴细胞例如原代T淋巴细胞的表面表达，且CAR的胞外域与抗原结合时，胞内信号转导结构域传送信号至T淋巴细胞以激活和/或增殖，并且，如果抗原在细胞表面呈现，则杀死表达抗原的细胞。因为一些免疫细胞，例如T淋巴细胞和NK细胞，需要2种信号，初级激活信号和共刺激信号从而最大地激活，CAR还可任选包含共刺激结构域，使得抗原与胞外域的结合导致初级激活信号和共刺激信号两者的传送。

[0398] 适应性免疫应答始于周围淋巴样器官，包括淋巴结。B细胞和T细胞被隔离在淋巴结的不同区域，分别称为“B细胞带”(其位于淋巴结外皮质区或滤泡中)和“T细胞带”，后者更广泛地分布在滤泡周围的区域(亦称为副皮质)。B细胞和T细胞表达允许它们归巢至这些各自的带的受体，使得它们可暴露于抗原。完整抗原呈现于B细胞带中，而在T细胞带中，抗原通过抗原呈递细胞(例如树突细胞)呈递。完整抗原(例如肿瘤抗原)还在肿瘤部位呈现。

[0399] 在一些实施方案中，遗传修饰的NK细胞是包含归巢受体的NK细胞，所述受体使包含所述归巢受体的细胞归巢至具体的解剖带、具体的组织或具体的细胞类型，例如淋巴结、胃肠道或皮肤的B细胞带。

[0400] 在某些实施方案中，遗传修饰的NK细胞是按本文所述包含CAR和归巢受体两者的NK细胞。

[0401] 虽不受任何特殊机制或理论的束缚，但认为当本文的遗传修饰的细胞表达使表达所述归巢受体的细胞归巢至特定区带的归巢受体时，它们更可能被暴露于天然抗原，其中细胞(例如表达CAR的细胞)能够被激活。

[0402] 包含CAR和/或归巢受体的NK细胞可通过本领域已知的任何方法产生。在一些实施方案中，包含CAR和/或归巢受体的NK细胞先按第5.2节中所述(例如通过两步方法或通过三步方法)产生，然后通过将包含编码CAR和/或归巢受体的核酸序列的一个或多个载体导入(例如通过转染)NK细胞，进行工程改造以表达CAR和/或归巢受体。在一些实施方案中，通过将包含编码CAR和/或归巢受体的核酸序列的一个或多个载体导入(例如通过转染)细胞，先对NK细胞可从中产生的细胞(例如CD34+造血干细胞)进行工程改造以表达CAR和/或归巢受体，然后通过第5.2节中所述的任何方法(例如两步方法或三步法)，将其用来衍生包含CAR和/或归巢受体的NK细胞。

[0403] 5.3.1.通用CAR结构和胞内域

[0404] 在某些实施方案中，CAR的胞内域是或包含在免疫细胞表面表达的蛋白质的胞内域或基序，并引发所述NK细胞的激活和/或增殖。这类结构域或基序能够传送在响应抗原与CAR胞外部分结合时对激活NK细胞是必需的主要抗原结合信号。通常，该结构域或基序包含

或是ITAM(基于免疫受体酪氨酸的激活基序)。适于CAR的含ITAM的多肽包括例如ζCD3链(CD3ζ)或其含ITAM的部分。在一个具体的实施方案中,胞内域是CD3ζ胞内信号转导结构域。在其它具体的实施方案中,胞内域来自淋巴细胞受体链、TCR/CD3复合蛋白、Fc受体亚基或IL-2受体亚基。

[0405] 在某些实施方案中,CAR另外包含一个或多个共刺激结构域或基序,例如作为多肽胞内域的部分。一个或多个共刺激结构域或基序可以是或包含共刺激CD27多肽序列、共刺激CD28多肽序列、共刺激OX40(CD134)多肽序列、共刺激4-1BB(CD137)多肽序列、共刺激诱导型T细胞共刺激(ICOS)多肽序列、共刺激PD-1多肽序列、共刺激CTLA-4多肽序列、共刺激NKp46多肽序列、共刺激NKp44多肽序列、共刺激NKp30多肽序列、共刺激NKG2D多肽序列、共刺激DAP10多肽序列、共刺激DAP12多肽序列或其它共刺激结构域或基序的一个或多个。

[0406] 跨膜区可以是可掺入功能性CAR的任何跨膜区,通常为来自CD4或CD8分子的跨膜区。

[0407] 5.3.2.CAR胞外域

[0408] 多肽的胞外域与目标抗原结合。在某些实施方案中,胞外域包含与所述抗原结合的受体或受体的部分。胞外域可以是例如与所述抗原结合的受体或受体的部分。在某些实施方案中,胞外域包含或是抗体或其抗原结合部分。在具体的实施方案中,胞外域包含或是单链Fv结构域。单链Fv结构域可包含例如通过柔性接头与V_H连接结合的V_L,其中所述V_L和V_H来自结合所述抗原的抗体。

[0409] 多肽的胞外域与之结合的抗原可以是任何目标抗原,例如可以是肿瘤细胞上的抗原或感染细胞上的抗原。肿瘤细胞可以是例如实体瘤中的细胞或血液癌症的细胞。抗原可以是在以下任何肿瘤或癌症类型的细胞上表达的任何抗原:例如淋巴瘤、肺癌、乳腺癌、前列腺癌、肾上腺皮质癌、甲状腺癌、鼻咽癌、黑素瘤(例如恶性黑素瘤)、皮肤癌、结肠直肠癌、带状瘤、促结缔组织增生性小圆细胞肿瘤、内分泌肿瘤、尤因肉瘤(Ewing sarcoma)、外周原始神经外胚层瘤、实体生殖细胞肿瘤、肝胚细胞瘤、成神经细胞瘤、非横纹肌肉瘤软组织肉瘤、骨肉瘤、成视网膜细胞瘤、横纹肌肉瘤、维尔姆斯瘤、成胶质细胞瘤、粘液瘤、纤维瘤、脂肪瘤等的细胞。在更具体的实施方案中,所述淋巴瘤可以是慢性淋巴细胞白血病(小淋巴细胞淋巴瘤)、B细胞幼淋巴细胞白血病、淋巴浆细胞淋巴瘤、瓦尔登斯特伦巨球蛋白血症(Waldenström macroglobulinemia)、脾边缘区淋巴瘤、浆细胞骨髓瘤、浆细胞瘤、结外边缘区B细胞淋巴瘤、MALT淋巴瘤、节边缘区B细胞淋巴瘤、滤泡性淋巴瘤、套细胞淋巴瘤、弥散性大B细胞淋巴瘤、纵隔(胸腺)大B细胞淋巴瘤、血管内大B细胞淋巴瘤、原发性渗出性淋巴瘤、伯基特淋巴瘤、T淋巴细胞幼淋巴细胞白血病、大颗粒T淋巴细胞白血病、侵袭性NK细胞白血病、成人T淋巴细胞白血病/淋巴瘤、鼻型结外NK/T淋巴细胞淋巴瘤、肠病型T淋巴细胞淋巴瘤、肝脾T淋巴细胞淋巴瘤、母细胞性NK细胞淋巴瘤、蕈样肉芽肿病、塞扎里综合征(Sezary syndrome)、原发性皮肤间变性大细胞淋巴瘤、淋巴瘤样丘疹病、血管免疫母细胞性T淋巴细胞淋巴瘤、外周T淋巴细胞淋巴瘤(未分类的)、间变性大细胞淋巴瘤、霍奇金淋巴瘤(Hodgkin lymphoma)、非霍奇金淋巴瘤或多发性骨髓瘤。

[0410] 在某些实施方案中,抗原是肿瘤相关抗原(TAA)或肿瘤特异性抗原(TSA)。在不同的具体实施方案中,无限制地,肿瘤相关抗原或肿瘤特异性抗原为Her2、前列腺干细胞抗原(PSCA)、α-甲胎蛋白(AFP)、癌胚抗原(CEA)、癌抗原-125(CA-125)、CA19-9、钙视网膜蛋白、

MUC-1、上皮膜蛋白(EMA)、上皮肿瘤抗原(ETA)、酪氨酸酶、黑素瘤相关抗原(MAGE)、CD19、CD20、CD34、CD45、CD99、CD117、嗜铬粒蛋白、细胞角蛋白、结蛋白、胶质细胞原纤维酸性蛋白(GFAP)、巨囊性病液体蛋白(GCDFP-15)、HMB-45抗原、高分子量黑素瘤相关抗原(HMW-MAA)、蛋白质melan-A(MART-1)、myo-D1、肌肉特异性肌动蛋白(MSA)、神经丝、神经元特异性烯醇化酶(NSE)、胎盘碱性磷酸酶、突触小泡蛋白(synaptophysis)、甲状腺球蛋白、甲状腺转录因子-1、丙酮酸激酶同功酶M2型二聚形式(肿瘤M2-PK)、异常ras蛋白或异常p53蛋白。

[0411] 在某些实施方案中,TAA或TSA是癌症/睾丸(CT)抗原,例如BAGE、CAGE、CTAGE、FATE、GAGE、HCA661、HOM-TES-85、MAGEA、MAGEB、MAGEC、NA88、NY-ESO-1、NY-SAR-35、OY-TES-1、SPANXB1、SPA17、SSX、SYCP1或TPTE。

[0412] 在某些其它实施方案中,TAA或TSA是糖或神经节苷脂,例如fuc-GM1、GM2(肿瘤胚胎抗原-免疫原性-1;OFA-I-1);GD2(OFA-I-2)、GM3、GD3等。

[0413] 在某些其它实施方案中,TAA或TSA是α-辅肌动蛋白-4、Bage-1、BCR-ABL、Bcr-Abl融合蛋白、β-联蛋白、CA 125、CA 15-3(CA 27.29\BCAA)、CA 195、CA 242、CA-50、CAM43、Casp-8、cdc27、cdk4、cdkn2a、CEA、coa-1、dek-can融合蛋白、EBNA、EF2、EB病毒抗原、ETV6-AML1融合蛋白、HLA-A2、HLA-A11、hsp70-2、KIAA0205、Mart2、Mum-1、2和3、neo-PAP、肌球蛋白I类、OS-9、pml-RARα融合蛋白、PTPRK、K-ras、N-ras、磷酸丙糖异构酶、Gage 3,4,5,6,7、GnTV、Herv-K-mel、Lage-1、NA-88、NY-Eso-1/Lage-2、SP17、SSX-2、TRP2-Int2、gp100(Pmel 17)、酪氨酸酶、TRP-1、TRP-2、MAGE-1、MAGE-3、RAGE、GAGE-1、GAGE-2、p15(58)、RAGE、、SCP-1、Hom/Mel-40、PRAME、p53、H-Ras、HER-2/neu、E2A-PRL、H4-RET、IGH-IGK、MYL-RAR、人乳头状瘤病毒(HPV)抗原E6和E7、TSP-180、MAGE-4、MAGE-5、MAGE-6、p185erbB2、p180erbB-3、c-met、nm-23H1、PSA、TAG-72-4、CA 19-9、CA 72-4、CAM 17.1、NuMa、K-ras、13-联蛋白、Mum-1、p16、TAGE、PSMA、CT7、端粒酶、43-9F、5T4、791Tgp72、13HCG、BCA225、BTAA、CD68\KP1、CO-029、FGF-5、G250、Ga733(EpCAM)、HTgp-175、M344、MA-50、MG7-Ag、MOV18、NB\70K、NY-CO-1、RCAS1、SDCCAG16、TA-90、TAAL6、TAG72、TLP、TPS、CD19、CD22、CD27、CD30、CD70、GD2(神经节苷脂G2)、EGFRvIII(表皮生长因子变体III)、精子蛋白17(Sp17)、间皮素、PAP(前列腺酸性磷酸酶)、prostein、TARP(T细胞受体γ交替读框蛋白)、Trp-p8、STEAP1(前列腺六跨膜上皮抗原1)、异常ras蛋白或异常p53蛋白。在另一个具体的实施方案中,所述肿瘤相关抗原或肿瘤特异性抗原是整联蛋白αvβ3(CD61)、泌乳激素、K-Ras(V-Ki-ras2 Kirsten大鼠肉瘤病毒癌基因)或Ral-B。

[0414] 在具体的实施方案中,TAA或TSA是CD20、CD123、CLL-1、CD38、CS-1、CD138、ROR1、FAP、MUC1、PSCA、EGFRvIII、EPHA2或GD2。在其它具体的实施方案中,TAA或TSA是CD123、CLL-1、CD38或CS-1。在一个具体的实施方案中,CAR的胞外域结合CS-1。在又一个具体的实施方案中,胞外域包含elotuzumab的单链形式和/或elotuzumab的抗原结合片段。在一个具体的实施方案中,CAR的胞外域与CD20结合。在一个更具体的实施方案中,CAR的胞外域是与CD20结合的scFv或其抗原结合片段。

[0415] 其它肿瘤相关抗原和肿瘤特异性抗原为本领域人员所知。

[0416] 与TSA和TAA结合的抗体和scFv是本领域已知的,像编码它们的核苷酸序列一样。

[0417] 在某些具体实施方案中,抗原是不被视为TSA或TAA的抗原,但是尽管如此与肿瘤细胞或被肿瘤造成的损害有关。在具体的实施方案中,抗原是肿瘤微环境相关抗原(TMAA)。

在某些实施方案中，例如，TMAA是例如生长因子、细胞因子或白介素，例如与血管生成或血管发生相关的生长因子、细胞因子或白介素。这类生长因子、细胞因子或白介素可包括例如血管内皮生长因子(VEGF)、碱性成纤维细胞生长因子(bFGF)、血小板衍生生长因子(PDGF)、肝细胞生长因子(HGF)、胰岛素样生长因子(IGF)或白介素-8(IL-8)。肿瘤还可在肿瘤局部造成低氧环境。因此，在其它具体的实施方案中，TMAA是缺氧相关因子，例如HIF-1α、HIF-1β、HIF-2α、HIF-2β、HIF-3α或HIF-3β。肿瘤还可对正常组织造成局部损害，引起称为损伤相关分子模式分子(DAMP；亦称为alarmins)的分子释放。在某些其它具体的实施方案中，TMAA因此是DAMP，例如热激蛋白、染色质相关蛋白高迁移率族框1(HMGB1)、S100A8(MRP8、钙粒蛋白A)、S100A9(MRP14、钙粒蛋白B)、血清淀粉样蛋白A(SAA)，或可以是脱氧核糖核酸、腺苷三磷酸、尿酸或硫酸肝素。在具体的实施方案中，TMAA是VEGF-A、EGF、PDGF、IGF或bFGF。

[0418] 在一个具体的实施方案中，其中癌症是胃肠癌，例如肝癌、胃癌、食管癌、胆囊癌、结肠直肠癌、肛门癌或胰腺癌，抗原是对胃肠癌特异性或与之有关的抗原。在一个具体的实施方案中，NK细胞包含胃肠归巢受体且还包含具有与胃肠癌相关抗原结合的胞外域的CAR。在一个具体的实施方案中，CAR的胞外域结合CEA。在其它具体的实施方案中，CAR的胞外域结合Her2、CA242、MUC1、CA125或CA19-9。

[0419] 在一个具体的实施方案中，其中癌症是皮肤癌，例如黑素瘤、鳞状细胞癌或基细胞癌，抗原是对皮肤癌有特异性或与之有关的抗原。在一个具体的实施方案中，NK细胞包含皮肤归巢受体且还包含具有与皮肤癌相关抗原结合的胞外域的CAR。在一个具体的实施方案中，CAR的胞外域结合HMW-MAA。在其它具体的实施方案中，CAR的胞外域结合Her2、GD2、GD3、CEA或SPAG9。

[0420] 在某些实施方案中，胞外域通过接头、间隔基或铰链多肽序列(例如来自CD28的序列)与所述跨膜结构域连接。

[0421] 5.3.3.循环系统归巢受体

[0422] 在某些实施方案中，归巢受体引起包含所述归巢受体的细胞归巢至循环系统。这类受体在本文称为“循环系统归巢受体”。在不同的实施方案中，循环系统归巢受体是趋化性受体。在具体的实施方案中，趋化性受体是CXCR4、VEGFR2或CCR7。

[0423] 在一个实施方案中，归巢受体引起包含所述归巢受体的细胞归巢至骨髓。这类受体在本文称为“骨髓归巢受体”。在具体的实施方案中，骨髓归巢受体是CXCR4，例如人CXCR4。GenBank™登记号NM_001008540.1和NM_003467.2提供人CXCR4的示例性核苷酸序列。GenBank™登记号NP_001008540.1和NP_003458.1提供人CXCR4的示例性氨基酸序列。人归巢受体的示例性核苷酸和氨基酸序列可见表1。

[0424] 在另一个实施方案中，归巢受体引起包含所述归巢受体的细胞归巢至周围淋巴样器官，例如淋巴结。这类受体在本文称为“周围淋巴器官归巢受体”。在具体的实施方案中，周围淋巴器官归巢受体是CCR7，例如人CCR7。GenBank™登记号NM_001301714.1、NM_001301716.1、NM_001301717.1、NM_001301718.1和NM_001838.3提供人CCR7的示例性核苷酸序列。GenBank™登记号NP_001288643.1、NP_001288645.1NP_001288646.1、NP_001288647.1和NP_001829.1提供人CCR7的示例性氨基酸序列。人归巢受体的示例性核苷酸和氨基酸序列可见表1。

[0425] 在另一个实施方案中，归巢受体引起包含所述归巢受体的细胞归巢至血管内皮。

这类受体在本文称为“血管内皮归巢受体”。在具体的实施方案中，血管内皮归巢受体是VEGFR2，例如人VEGFR2。GenBank™登记号NM_002253.2提供人VEGFR2的示例性核苷酸序列。GenBank™登记号NP_002244.1提供人VEGFR2的示例性氨基酸序列。人归巢受体的示例性核苷酸和氨基酸序列可见表1。

[0426] 在另一个实施方案中，归巢受体引起包含所述归巢受体的细胞归巢至淋巴结的B细胞带，例如淋巴结的滤泡。这类受体在本文称为“B细胞带归巢受体”。在具体的实施方案中，B细胞带归巢受体是CXCR5，例如人CXCR5。GenBank™登记号NM_001716.4和NM_032966.2提供人CXCR5的示例性核苷酸序列。GenBank™登记号NP_116743.1和NP_001707.1提供人CXCR5的示例性氨基酸序列。人归巢受体的示例性核苷酸和氨基酸序列可见表1。

[0427] 在一些实施方案中，对NK细胞进行工程改造以包含循环系统归巢受体的步骤包括将包含受体核酸序列(即编码受体的核酸序列)的一个或多个载体导入细胞的步骤。在具体的实施方案中，载体包含人CXCR4、CCR7、VEGFR2或CXCR5的核酸序列。在某一实施方案中，对NK细胞进行工程改造以包含循环系统归巢受体的步骤通过本领域技术人员已知的任何方法进行。

[0428] 本文还描述了产生归巢至循环系统的遗传改造的NK细胞的方法，所述方法包括对NK细胞进行工程改造以包含循环系统归巢受体(例如CXCR4、CCR7、VEGFR2或CXCR5)的步骤，其中所述循环系统归巢受体由细胞以引起细胞归巢至循环系统的足够水平或足够量表达。在一些实施方案中，对NK细胞进行工程改造以包含循环系统归巢受体的步骤包括将包含受体核酸序列(即编码受体的核酸序列)的一个或多个载体导入细胞的步骤。在具体的实施方案中，载体包含人CXCR4、CCR7、VEGFR2或CXCR5的核酸序列。在某一实施方案中，对NK细胞进行工程改造以包含循环系统归巢受体的步骤通过本领域技术人员已知的任何方法进行。

[0429] 5.3.4.胃肠归巢受体

[0430] 在一个实施方案中，归巢受体引起包含所述归巢受体的细胞归巢至胃肠道，例如胃肠器官、组织或细胞。引起细胞归巢至胃肠道的这类受体在本文称为“胃肠归巢受体”。在某些实施方案中，胃肠归巢受体是CCR9或整联蛋白α4β7，例如人CCR9或人整联蛋白α4β7。GenBank™登记号NM_031200.2和NM001256369.1提供人CCR9的示例性核苷酸序列。GenBank™登记号NP_112477.1和NP_001243298.1提供人CCR9的示例性氨基酸序列。GenBank™登记号NM_000885.4和NM_000889.2分别提供人α4和人β7的示例性核苷酸序列。GenBank™登记号NP_000876.3和NP_000880.1分别提供人α4和人β7的示例性氨基酸序列。人归巢受体的示例性核苷酸和氨基酸序列可见表1。在一些实施方案中，NK细胞另包含第二胃肠归巢受体。在一些实施方案中，NK细胞包含第一胃肠归巢受体，其中第一胃肠归巢受体是CCR9，并另包含第二胃肠归巢受体，其中第二胃肠归巢受体是整联蛋白α4β7。在其它具体的实施方案中，NK细胞包含胃肠归巢受体CXCR3。

[0431] 在某些实施方案中，在维生素A代谢物存在下扩增、激活或既扩增又激活包含一种或多种胃肠归巢受体的NK细胞。在具体的实施方案中，扩增、激活或既扩增又激活在体内、体外或离体发生。在具体的实施方案中，维生素A代谢物是视黄酸。在某些实施方案中，包含一种或多种胃肠归巢受体的NK细胞还包含B细胞带归巢受体。在具体的实施方案中，B细胞带归巢受体是CXCR5。

[0432] 本文还描述了产生归巢至胃肠道(例如胃肠器官、皮肤或组织)的遗传修饰的NK细

胞的方法。在某些实施方案中，包含引起包含一种或多种受体(例如CCR9或整联蛋白α4β7)的细胞归巢至胃肠道的一种或多种归巢受体的NK细胞，通过包括对NK细胞进行工程改造以表达一种或多种胃肠归巢受体的步骤的方法产生。在一些实施方案中，对NK细胞进行工程改造以包含一种或多种胃肠归巢受体的步骤包括将包含编码归巢受体的核酸序列的一个或多个载体导入细胞。在具体的实施方案中，载体包含人CCR9的核酸序列、人整联蛋白α4β7的核酸序列或两者。

[0433] 在某些实施方案中，归巢至胃肠道的NK细胞通过包括用诱导一种或多种胃肠归巢受体(例如CCR9或α4β7)表达的分子处理细胞的步骤的方法产生。在具体的实施方案中，所述分子是维生素A。

[0434] 在某些实施方案中，用于产生包含引起包含一种或多种受体的细胞归巢至胃肠道一种或多种受体的遗传修饰的NK细胞的方法包括扩增细胞的步骤，该步骤在维生素A代谢物存在下进行。在某些实施方案中，用于包含归巢胃肠道的一种或多种受体的遗传修饰的NK细胞的方法包括激活细胞的步骤，该步骤在维生素A代谢物存在下进行。在某些实施方案中，扩增和激活两个步骤在维生素A代谢物存在下进行。在某些实施方案中，维生素A代谢物是视黄酸。在某一实施方案中，对NK细胞进行工程改造以包含胃肠归巢受体的步骤通过本领域技术人员已知的任何方法进行。

[0435] 5.3.5.皮肤归巢受体

[0436] 在一个实施方案中，归巢受体引起包含所述归巢受体的细胞归巢至皮肤，例如皮肤组织或皮肤细胞。在某些实施方案中，皮肤归巢受体是CCR10、CCR8、CCR4或CLA，例如人CCR10、人CCR8、人CCR4或人CLA。GenBank™登记号NM_016602.2和AF215981.1提供人CCR10的示例性核苷酸序列。GenBank™登记号NP_057686.2和P46092.3提供人CCR10的示例性氨基酸序列。GenBank™登记号NM_005201.3和BC107159.1提供人CCR8的示例性核苷酸序列。GenBank™登记号NP_005192.1和AAI07160.1提供人CCR8的示例性氨基酸序列。GenBank™登记号NM_005508.4提供人CCR4的示例性核苷酸序列。GenBank™登记号P51679.1提供人CCR4的示例性氨基酸序列。GenBank™登记号NM_001206609.1和NM_003006.4提供人CLA的示例性核苷酸序列。GenBank™登记号NP_001193538.1和NP_002997.2提供人CLA的示例性氨基酸序列。人归巢受体的示例性核苷酸和氨基酸序列可见表1。在一些实施方案中，NK细胞另包含第二皮肤归巢受体。在一些实施方案中，NK细胞包含第一皮肤归巢受体，其中第一皮肤归巢受体是CCR10，并另包含第二皮肤归巢受体，其中第二皮肤归巢受体是CLA。在一些实施方案中，NK细胞包含第一皮肤归巢受体，其中第一皮肤归巢受体是CCR10，并另包含第二皮肤归巢受体，其中第二皮肤归巢受体是CCR4。在一些实施方案中，NK细胞包含第一皮肤归巢受体，其中第一皮肤归巢受体是CCR4，并另包含第二皮肤归巢受体，其中第二皮肤归巢受体是CLA。在一些实施方案中，NK细胞另包含第三皮肤归巢受体。在一些实施方案中，NK细胞包含第一皮肤归巢受体，其中第一皮肤归巢受体是CCR10，另包含第二皮肤归巢受体，其中第二皮肤归巢受体是CCR4，并另包含第三皮肤归巢受体，其中第三皮肤归巢受体是CLA。在一些实施方案中，NK细胞包含第一皮肤归巢受体，其中第一皮肤归巢受体是CCR8，并另包含第二皮肤归巢受体，其中第二皮肤归巢受体是CLA、CCR4或CCR10。在一些实施方案中，NK细胞包含第一皮肤归巢受体，其中第一皮肤归巢受体是CCR8，另包含第二皮肤归巢受体，其中第二皮肤归巢受体是CLA、CCR4或CCR10，并另包含第三皮肤归巢受体，其中第三皮肤归巢受体不

同于第二皮肤归巢受体，并选自CLA、CCR4和CCR10。在一些实施方案中，NK细胞另包含第三皮肤归巢受体。在一些实施方案中，NK细胞包含第一皮肤归巢受体，其中第一皮肤归巢受体是CCR10，另包含第二皮肤归巢受体，其中第二皮肤归巢受体是CCR4，另包含第三皮肤归巢受体，其中第三皮肤归巢受体是CLA，且另包含第四皮肤归巢受体，其中第四皮肤归巢受体是CCR8。在某些实施方案中，NK细胞包含一种或多种皮肤归巢受体。在其它具体的实施方案中，NK细胞包含皮肤归巢受体CCR6。

[0437] 在某些实施方案中，在维生素D代谢物存在下扩增、激活或既扩增又激活包含一种或多种皮肤归巢受体的NK细胞。在具体的实施方案中，扩增、激活或既扩增又激活在体内、体外或离体发生。在具体的实施方案中，维生素D代谢物是1,25-二羟胆钙化醇(1,25(OH)$_2$D$_3$)。在某些实施方案中，在IL-12存在下扩增、激活或既扩增又激活包含一种或多种皮肤归巢受体的NK细胞。在具体的实施方案中，扩增、激活或既扩增又激活在体内、体外或离体发生。在更具体的实施方案中，在维生素D代谢物和IL-12存在下扩增、激活或既扩增又激活包含一种或多种皮肤归巢受体的NK细胞。在具体的实施方案中，扩增、激活或既扩增又激活在体内、体外或离体发生。在某些实施方案中，包含一种或多种皮肤归巢受体的NK细胞还包含B细胞带归巢受体。在具体的实施方案中，B细胞带归巢受体是CXCR5。

[0438] 本文还描述了产生归巢至皮肤(例如皮肤组织或细胞)的遗传修饰的NK细胞的方法。在某些实施方案中，归巢至皮肤的NK细胞通过包括对NK细胞进行改造以包含皮肤归巢受体(例如CCR4、CCR8、CCR10或CLA)的步骤的方法产生。在一些实施方案中，对NK细胞进行改造以包含皮肤归巢受体的步骤包括将包含受体核酸序列(即编码受体的核酸序列)的一个或多个载体导入细胞。在具体的实施方案中，载体包含人CCR10的核酸序列、人CLA的核酸序列或两者。在具体的实施方案中，载体包含人CCR4的核酸序列和可选的人CLA的核酸序列。在具体的实施方案中，载体包含人CCR4的核酸序列和人CCR10的核酸序列。在具体的实施方案中，载体包含人CCR10的核酸序列、人CCR4的核酸序列和人CLA的核酸序列。在具体的实施方案中，载体包含人CCR8的核酸序列。在具体的实施方案中，载体包含人CCR8的核酸序列和可选的人CLA的核酸序列。在具体的实施方案中，载体包含人CCR8的核酸序列和人CCR10的核酸序列。在具体的实施方案中，载体包含人CCR8的核酸序列、人CCR4的核酸序列和人CLA的核酸序列。在具体的实施方案中，载体包含人CCR8的核酸序列、人CCR10的核酸序列和人CLA的核酸序列。在具体的实施方案中，载体包含人CCR8的核酸序列、人CCR4的核酸序列和人CCR10的核酸序列。在具体的实施方案中，载体包含人CCR8的核酸序列、人CCR4的核酸序列、CCR10的核酸和人CLA的核酸序列。

[0439] 在某些实施方案中，归巢至皮肤的细胞(例如NK细胞)通过包括诱导(例如增加)一种或多种皮肤归巢受体(例如CCR4、CCR10、CCR8或CLA)表达的分子处理细胞(例如NK细胞)的步骤的方法产生。在具体的实施方案中，所述分子是维生素D。在某些实施方案中，皮肤归巢受体表达的诱导辅以用IL-12处理细胞(例如NK细胞)，例如，以足以通过所述细胞增加CCR4、CCR8、CCR10或CLA的一种或多种的表达的量和一段时间使细胞与IL-12接触。

[0440] 在某些实施方案中，用于产生包含引起包含一种或多种受体的细胞归巢至皮肤的一种或多种归巢受体的NK细胞的方法包括扩增细胞的步骤，该步骤在维生素D代谢物和可选的IL-12存在下进行。在某些实施方案中，用于产生包含引起包含一种或多种受体的细胞归巢至胃肠道的一种或多种受体的NK细胞的方法包括激活细胞的步骤，该步骤在维生素D

代谢物和可选的IL-12存在下进行。在某些实施方案中，扩增和激活两个步骤在维生素D代谢物和可选的IL-12存在下进行。在某些实施方案中，维生素D代谢物是1,25(OH)$_2$D$_3$。在某一实施方案中，产生包含皮肤归巢受体的NK细胞的步骤通过本领域技术人员已知的任何方法进行。

[0441] 表1.人归巢受体的示例性核苷酸和氨基酸序列。

[0442]

SEQ ID NO:	GenBank 登记号和描述	序列
1	NM_001008540.1 编码人CXCR4同种型a的示例性核酸序列	1 tttttttttct tccctctagt gggcggggca gaggagttag ccaagatgtg actttgaaac 61 cctcagcgtc tcagtgccct tttgttctaa acaaagaatt ttgtaattgg ttctaccaaa 121 gaaggatata atgaagtcac tatgggaaaa gatggggagg agagttgtag gattctacat 181 taattctctt gtgcccttag cccactactt cagaatttcc tgaagaaagc aagcctgaat 241 tggtttttta aattgcttta aaaatttttt ttaactgggt taatgcttgc tgaattggaa 301 gtgaatgtcc attcctttgc ctcttttgca gatatacact tcagataact acaccgagga 361 aatgggctca ggggactatg actccatgaa ggaaccctgt ttccgtgaag aaaatgctaa 421 tttcaataaa atcttcctgc ccaccatcta ctccatcatc ttcttaactg gcattgtggg 481 caatggattg gtcatcctgg tcatgggtta ccagaagaaa ctgagaagca tgacggacaa 541 gtacaggctg cacctgtcag tggccgacct cctctttgtc atcacgcttc ccttctgggc 601 agttgatgcc gtggcaaact ggtactttgg gaacttccta tgcaaggcag tccatgtcat 661 ctacacagtc aacctctaca gcagtgtcct catcctggcc ttcatcagtc tggaccgcta 721 cctggccatc gtccacgcca ccaacagtca gaggccaagg aagctgttgg ctgaaaaggt 781 ggtctatgtt ggcgtctgga tccctgccct cctgctgact attcccgact tcatctttgc

[0443]

SEQ ID NO:	GenBank 登记号和描述	序列
		841 caacgtcagt gaggcagatg acagatatat ctgtgaccgc ttctacccca atgacttgtg 901 ggtggttgtg ttccagtttc agcacatcat ggttggcctt atcctgcctg gtattgtcat 961 cctgtcctgc tattgcatta tcatctccaa gctgtcacac tccaagggcc accagaagcg 1021 caaggccctc aagaccacag tcatcctcat cctggctttc ttcgcctgtt ggctgcctta 1081 ctacattggg atcagcatcg actccttcat cctcctggaa atcatcaagc aagggtgtga 1141 gtttgagaac actgtgcaca agtggatttc catcaccgag gccctagctt tcttccactg 1201 ttgtctgaac cccatcctct atgctttcct tggagccaaa tttaaaacct ctgcccagca 1261 cgcactcacc tctgtgagca gagggtccag cctcaagatc ctctccaaag gaaagcgagg 1321 tggacattca tctgtttcca ctgagtctga gtcttcaagt tttcactcca gctaacacag 1381 atgtaaaaga ctttttttta tacgataaat aacttttttt taagttacac atttttcaga 1441 tataaaagac tgaccaatat tgtacagttt ttattgcttg ttggattttt gtcttgtgtt 1501 tctttagttt ttgtgaagtt taattgactt atttatataa atttttttg tttcatattg 1561 atgtgtgtct aggcaggacc tgtggccaag ttcttagttg ctgtatgtct cgtggtagga 1621 ctgtagaaaa gggaactgaa cattccagag cgtgtagtga atcacgtaaa gctagaaatg 1681 atccccagct gtttatgcat agataatctc tccattcccg tggaacgttt ttcctgttct 1741 taagacgtga ttttgctgta gaagatggca cttataacca aagcccaaag tggtatagaa 1801 atgctggttt ttcagttttc aggagtgggt tgatttcagc acctacagtg tacagtcttg 1861 tattaagttg ttaataaaag tacatgttaa acttaaaaaa aaaaaaaaaa aa
2	NM_003467.2 编码人 CXCR4 同种型 b 的示例性核酸序列	1 aacttcagtt tgttggctgc ggcagcaggt agcaaagtga cgccgagggc ctgagtgctc 61 cagtagccac cgcatctgga gaaccagcgg ttaccatgga ggggatcagt atatacactt 121 cagataacta caccgaggaa atgggctcag gggactatga ctccatgaag gaaccctgtt 181 tccgtgaaga aaatgctaat ttcaataaaa tcttcctgcc caccatctac tccatcatct 241 tcttaactgg cattgtgggc aatggattgg tcatcctggt catgggttac cagaagaaac 301 tgagaagcat gacggacaag tacaggctgc acctgtcagt ggccgacctc ctctttgtca 361 tcacgcttcc cttctgggca gttgatgccg tggcaaactg gtactttggg aacttcctat 421 gcaaggcagt ccatgtcatc tacacagtca acctctacag cagtgtcctc atcctggcct 481 tcatcagtct ggaccgctac ctggccatcg tccacgccac caacagtcag aggccaagga 541 agctgttggc tgaaaaggtg gtctatgttg gcgtctggat ccctgccctc ctgctgacta 601 ttcccgactt catctttgcc aacgtcagtg aggcagatga cagatatatc tgtgaccgct 661 tctaccccaa tgacttgtgg gtggttgtgt tccagtttca gcacatcatg gttggcctta 721 tcctgcctgg tattgtcatc ctgtcctgct attgcattat catctccaag ctgtcacact 781 ccaagggcca ccagaagcgc aaggccctca agaccacagt catcctcatc ctggctttct 841 tcgcctgttg gctgccttac tacattggga tcagcatcga ctccttcatc ctcctggaaa 901 tcatcaagca agggtgtgag tttgagaaca ctgtgcacaa gtggatttcc atcaccgagg 961 ccctagcttt cttccactgt tgtctgaacc ccatcctcta tgctttcctt ggagccaaat 1021 ttaaaacctc tgcccagcac gcactcacct ctgtgagcag agggtccagc ctcaagatcc 1081 tctccaaagg aaagcgaggt ggacattcat ctgtttccac tgagtctgag tcttcaagtt 1141 ttcactccag ctaacacaga tgtaaaagac tttttttat acgataaata actttttttt 1201 aagttacaca tttttcagat ataaaagact gaccaatatt gtacagtttt tattgcttgt 1261 tggatttttg tcttgtgttt ctttagtttt tgtgaagttt aattgactta tttatataaa 1321 tttttttgt ttcatattga tgtgtgtcta ggcaggacct gtggccaagt tcttagttgc 1381 tgtatgtctc gtggtaggac tgtagaaaag ggaactgaac attccagagc gtgtagtgaa 1441 tcacgtaaag ctagaaatga tccccagctg tttatgcata gataatctct ccattcccgt 1501 ggaacgtttt tcctgttctt aagacgtgat tttgctgtag aagatggcac ttataaccaa 1561 agcccaaagt ggtatagaaa tgctggtttt tcagttttca ggagtgggtt gatttcagca 1621 cctacagtgt acagtcttgt attaagttgt taataaaagt acatgttaaa cttaaaaaaa 1681 aaaaaaaaaa a
3	NP_001008540	1 msiplpllqi ytsdnyteem gsgdydsmke pcfreenanf nkiflptiys iifltgivgn

[0444]

SEQ ID NO:	GenBank 登记号和描述	序列
	.1 人 CXCR4 同种型 a 的示例性氨基酸序列	61 glvilvmgyq kklrsmtdky rlhlsvadll fvitlpfwav davanwyfgn flckavhviy 121 tvnlyssvli lafisldryl aivhatnsqr prkllaekvv yvgvwipall ltipdfifan 181 vseaddryic drfypndlwv vvfqfqhimv glilpgivil scyciiiskl shskghqkrk 241 alkttvilil affacwlpyy igisidsfil leiikqgcef entvhkwisi tealaffhcc 301 lnpilyaflg akfktsaqha ltsvsrgssl kilskgkrgg hssvsteses ssfhss
4	NP_003458.1 人 CXCR4 同种型 b 的示例性氨基酸序列	1 megisiytsd nyteemgsgd ydsmkepcfr eenanfnkif lptiysiifl tgivgnglvi 61 lvmgyqkklr smtdkyrlhl svadllfvit lpfwavdava nwyfgnflck avhviytvnl 121 yssvlilafi sldrylaivh atnsqrprkl laekvvyvgv wipallltip dfifanvsea 181 ddryicdrfy pndlwvvvfq fqhimvglil pgivilscyc iiisklshsk ghqkrkalkt 241 tvililaffa cwlpyyigis idsfilleii kqgcefentv hkwisiteal affhcclnpi 301 lyaflgakfk tsaqhaltsv srgsslkils kgkrgghssv stsesesssfh ss
5	NM_001301714.1 编码人 CCR7 同种型 b 的示例性核酸序列	1 cacttcctcc ccagacaggg gtagtgcgag gccgggcaca gccttcctgt gtggttttac 61 cgcccagaga gcgtcatgga cctgggtatg cctgtgtcaa gatgaggtca cggacgatta 121 catcggagac aacaccacag tggactacac tttgttcgag tctttgtgct ccaagaagga 181 cgtgcggaac tttaaagcct ggttcctccc tatcatgtac tccatcattt gtttcgtggg 241 cctactgggc aatgggctgg tcgtgttgac ctatatctat ttcaagaggc tcaagaccat 301 gaccgatacc tacctgctca acctggcggt ggcagacatc ctcttcctcc tgacccttcc 361 cttctgggcc tacagcgcgg ccaagtcctg ggtcttcggt gtccactttt gcaagctcat 421 ctttgccatc tacaagatga gcttcttcag tggcatgctc ctacttcttt gcatcagcat 481 tgaccgctac gtggccatcg tccaggctgt ctcagctcac cgccaccgtg cccgcgtcct 541 tctcatcagc aagctgtcct gtgtgggcat ctggatacta gccacagtgc tctccatccc 601 agagctcctg tacagtgacc tccagaggag cagcagtgag caagcgatgc gatgctctct 661 catcacagag catgtggagg cctttatcac catccaggtg gcccagatgg tgatcggctt 721 tctggtcccc ctgctggcca tgagcttctg ttaccttgtc atcatccgca ccctgctcca 781 ggcacgcaac tttgagcgca acaaggccat caaggtgatc atcgctgtgg tcgtggtctt 841 catagtcttc cagctgccct acaatggggt ggtcctggcc cagacggtgg ccaacttcaa 901 catcaccagt agcacctgtg agctcagtaa gcaactcaac atcgcctacg acgtcaccta 961 cagcctggcc tgcgtccgct gctgcgtcaa ccctttcttg tacgccttca tcggcgtcaa 1021 gttccgcaac gatctcttca agctcttcaa ggacctgggc tgcctcagcc aggagcagct 1081 ccggcagtgg tcttcctgtc ggcacatccg gcgctcctcc atgagtgtgg aggccgagac 1141 caccaccacc ttctccccat aggcgactct tctgcctgga ctagagggac ctctcccagg 1201 gtccctgggg tggggatagg gagcagatgc aatgactcag gacatccccc cgccaaaagc 1261 tgctcaggga aaagcagctc tcccctcaga gtgcaagccc ctgctccaga agatagcttc 1321 accccaatcc cagctacctc aaccaatgcc aaaaaaagac agggctgata agctaacacc 1381 agacagacaa cactgggaaa cagaggctat tgtcccctaa accaaaaact gaaagtgaaa 1441 gtccagaaac tgttcccacc tgctggagtg aaggggccaa ggagggtgag tgcaaggggc 1501 gtgggagtgg cctgaagagt cctctgaatg aaccttctgg cctcccacag actcaaatgc 1561 tcagaccagc tcttccgaaa accaggcctt atctccaaga ccagagatag tggggagact 1621 tcttggcttg gtgaggaaaa gcggacatca gctggtcaaa caaactctct gaacccctcc 1681 ctccatcgtt ttcttcactg tcctccaagc cagcgggaat ggcagctgcc acgccgccct 1741 aaaagcacac tcatcccctc acttgccgcg tcgccctccc aggctctcaa caggggagag 1801 tgtggtgttt cctgcaggcc aggccagctg cctccgcgtg atcaaagcca cactctgggc 1861 tccagagtgg ggatgacatg cactcagctc ttggctccac tgggatggga

[0445]

SEQ ID NO:	GenBank 登记号和描述	序列
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6	NM_001301716.1 编码人 CCR7 同种型 c 前体的示例性核酸序列	1 ctctagatga gtcagtggag ggcgggtgga gcgttgaacc gtgaagagtg tggttgggcg 61 taaacgtgga cttaaactca ggagctaagg ggtaattcag tgaaaaaggg gaatgagcgg 121 tggggagctc tgttgcaaca gggtccaatc gcagcaggac tacaaatgcc cgagcgcagg 181 ctgggaacga ggggacagcg gctgcctgtc cccagaatag aaaatgcagc taggaagccc 241 tctttgagtg gacagcggag gactggactg ccaggccaag catcaggggc ttcatcctca 301 gggccggtta gagcccctga ggatttagga ggaagggaaa ccaatgaaaa gcgtgctggt 361 ggtggctctc cttgtcattt tccaggtatg cctgtgtcaa gatgaggtca cggacgatta 421 catcggagac aacaccacag tggactacac tttgttcgag tctttgtgct ccaagaagga 481 cgtgcggaac tttaaagcct ggttcctccc tatcatgtac tccatcattt gtttcgtggg 541 cctactgggc aatgggctgg tcgtgttgac ctatatctat ttcaagaggc tcaagaccat 601 gaccgatacc tacctgctca acctggcggt ggcagacatc ctcttcctcc tgacccttcc 661 cttctgggcc tacagcgcgg ccaagtcctg ggtcttcggt gtccactttt gcaagctcat 721 ctttgccatc tacaagatga gcttcttcag tggcatgctc ctacttcttt gcatcagcat 781 tgaccgctac gtggccatcg tccaggctgt ctcagctcac cgccaccgtg cccgcgtcct 841 tctcatcagc aagctgtcct gtgtgggcat ctggatacta gccacagtgc tctccatccc 901 agagctcctg tacagtgacc tccagaggag cagcagtgag caagcgatgc gatgctctct 961 catcacagag catgtggagg cctttatcac catccaggtg gcccagatgg tgatcggctt 1021 tctggtcccc ctgctggcca tgagcttctg ttaccttgtc atcatccgca ccctgctcca 1081 ggcacgcaac tttgagcgca acaaggccat caaggtgatc atcgctgtgg tcgtggtctt 1141 catagtcttc cagctgccct acaatggggt ggtcctggcc cagacggtgg ccaacttcaa 1201 catcaccagt agcacctgtg agctcagtaa gcaactcaac atcgcctacg acgtcaccta 1261 cagcctggcc tgcgtccgct gctgcgtcaa ccctttcttg tacgccttca tcggcgtcaa 1321 gttccgcaac gatctcttca agctcttcaa ggacctgggc tgcctcagcc aggagcagct 1381 ccggcagtgg tcttcctgtc ggcacatccg gcgctcctcc atgagtgtgg aggccgagac 1441 caccaccacc ttctccccat aggcgactct tctgcctgga ctagagggac ctctcccagg 1501 gtccctgggg tggggatagg gagcagatgc aatgactcag gacatccccc cgccaaaagc 1561 tgctcaggga aaagcagctc tcccctcaga gtgcaagccc ctgctccaga agatagcttc 1621 accccaatcc cagctacctc aaccaatgcc aaaaaaagac agggctgata agctaacacc 1681 agacagacaa cactgggaaa cagaggctat tgtcccctaa accaaaaact gaaagtgaaa 1741 gtccagaaac tgttcccacc tgctggagtg aaggggccaa ggagggtgag tgcaaggggc 1801 gtgggagtgg cctgaagagt cctctgaatg aaccttctgg cctcccacag actcaaatgc 1861 tcagaccagc tcttccgaaa accaggcctt atctccaaga ccagagatag tggggagact 1921 tcttggcttg gtgaggaaaa gcggacatca gctggtcaaa caaactctct gaacccctcc 1981 ctccatcgtt ttcttcactg tcctccaagc cagcgggaat ggcagctgcc acgccgccct 2041 aaaagcacac tcatcccctc acttgccgcg tcgccctccc aggctctcaa caggggagag 2101 tgtggtgttt cctgcaggcc aggccagctg cctccgcgtg atcaaagcca cactctgggc 2161 tccagagtgg ggatgacatg cactcagctc ttggctccac tgggatggga ggagaggaca

[0446]

SEQ ID NO:	GenBank 登记号和描述	序列
		2221 agggaaatgt caggggcggg gagggtgaca gtggccgccc aaggcccacg agcttgttct 2281 ttgttctttg tcacagggac tgaaaacctc tcctcatgtt ctgctttcga ttcgttaaga 2341 gagcaacatt ttacccacac acagataaag ttttcccttg aggaaacaac agctttaaaa 2401 gaaaaagaaa aaaaaagtct ttggtaaatg gcaaaaaaaa aaaaaaaaaa aaaaaaa
7	NM_001301717.1 编码人 CCR7 同种型 c 前体的示例性核酸序列	1 ctctagatga gtcagtggag ggcgggtgga gcgttgaacc gtgaagagtg tggttgggcg 61 taaacgtgga cttaaactca ggagctaagg gggaaaccaa tgaaaagcgt gctggtggtg 121 gctctccttg tcattttcca ggtatgcctg tgtcaagatg aggtcacgga cgattacatc 181 ggagacaaca ccacagtgga ctacactttg ttcgagtctt tgtgctccaa gaaggacgtg 241 cggaacttta aagcctggtt cctccctatc atgtactcca tcatttgttt cgtgggccta 301 ctgggcaatg ggctggtcgt gttgacctat atctatttca agaggctcaa gaccatgacc 361 gatacctacc tgctcaacct ggcggtggca gacatcctct tcctcctgac ccttcccttc 421 tgggcctaca gcgcggccaa gtcctgggtc ttcggtgtcc acttttgcaa gctcatcttt 481 gccatctaca agatgagctt cttcagtggc atgctcctac ttctttgcat cagcattgac 541 cgctacgtgg ccatcgtcca ggctgtctca gctcaccgcc accgtgcccg cgtccttctc 601 atcagcaagc tgtcctgtgt gggcatctgg atactagcca cagtgctctc catcccagag 661 ctcctgtaca gtgacctcca gaggagcagc agtgagcaag cgatgcgatg ctctctcatc 721 acagagcatg tggaggcctt tatcaccatc caggtggccc agatggtgat cggctttctg 781 gtccccctgc tggccatgag cttctgttac cttgtcatca tccgcaccct gctccaggca 841 cgcaactttg agcgcaacaa ggccatcaag gtgatcatcg ctgtggtcgt ggtcttcata 901 gtcttccagc tgccctacaa tggggtggtc ctggcccaga cggtggccaa cttcaacatc 961 accagtagca cctgtgagct cagtaagcaa ctcaacatcg cctacgacgt cacctacagc 1021 ctggcctgcg tccgctgctg cgtcaaccct ttcttgtacg ccttcatcgg cgtcaagttc 1081 cgcaacgatc tcttcaagct cttcaaggac ctgggctgcc tcagccagga gcagctccgg 1141 cagtggtctt cctgtcggca catccggcgc tcctccatga gtgtggaggc cgagaccacc 1201 accaccttct ccccataggc gactcttctg cctggactag agggacctct cccagggtcc 1261 ctggggtggg gatagggagc agatgcaatg actcaggaca tccccccgcc aaaagctgct 1321 cagggaaaag cagctctccc ctcagagtgc aagcccctgc tccagaagat agcttcaccc 1381 caatcccagc tacctcaacc aatgccaaaa aaagacaggg ctgataagct aacaccagac 1441 agacaacact gggaaacaga ggctattgtc ccctaaacca aaaactgaaa gtgaaagtcc 1501 agaaactgtt cccacctgct ggagtgaagg ggccaaggag ggtgagtgca aggggcgtgg 1561 gagtggcctg aagagtcctc tgaatgaacc ttctggcctc ccacagactc aaatgctcag 1621 accagctctt ccgaaaacca ggccttatct ccaagaccag agatagtggg gagacttctt 1681 ggcttggtga ggaaaagcgg acatcagctg gtcaaacaaa ctctctgaac ccctccctcc 1741 atcgttttct tcactgtcct ccaagccagc gggaatggca gctgccacgc cgccctaaaa 1801 gcacactcat cccctcactt gccgcgtcgc cctcccaggc tctcaacagg ggagagtgtg 1861 gtgtttcctg caggccaggc cagctgcctc cgcgtgatca aagccacact ctgggctcca 1921 gagtggggat gacatgcact cagctcttgg ctccactggg atgggaggag aggacaaggg 1981 aaatgtcagg ggcggggagg gtgacagtgg ccgcccaagg cccacgagct tgttctttgt 2041 tctttgtcac agggactgaa aacctctcct catgttctgc tttcgattcg ttaagagagc 2101 aacattttac ccacacacag ataaagtttt cccttgagga aacaacagct ttaaaagaaa 2161 aagaaaaaaa aagtctttgg taaatggcaa aaaaaaaaaa aaaaaaaaaa aaa
8	NM_00130171	1 aggagaaggt gccttaaaca ggttcccacg catttcctgg cgctattgag cttggagctg

[0447]

SEQ ID NO:	GenBank 登记号和描述	序列
	8.1 编码人 CCR7 同种型 c 前体的示例性核酸序列	61 ccaagggcct gccttcactt gtggcatcgc agttactgac tctccagtgg gccaggccct 121 acctagctgg gacctgaggg tcaggatacg ggaagagggc tactgccgcc ctgacttgta 181 gggaaaccaa tgaaaagcgt gctggtggtg gctctccttg tcattttcca ggtatgcctg 241 tgtcaagatg aggtcacgga cgattacatc ggagacaaca ccacagtgga ctacactttg 301 ttcgagtctt tgtgctccaa gaaggacgtg cggaacttta aagcctggtt cctccctatc 361 atgtactcca tcatttgttt cgtgggccta ctgggcaatg ggctggtcgt gttgacctat 421 atctatttca agaggctcaa gaccatgacc gatacctacc tgctcaacct ggcggtggca 481 gacatcctct tcctcctgac ccttcccttc tgggcctaca gcgcggccaa gtcctgggtc 541 ttcggtgtcc acttttgcaa gctcatcttt gccatctaca agatgagctt cttcagtggc 601 atgctcctac ttctttgcat cagcattgac cgctacgtgg ccatcgtcca ggctgtctca 661 gctcaccgcc accgtgcccg cgtccttctc atcagcaagc tgtcctgtgt gggcatctgg 721 atactagcca cagtgctctc catcccagag ctcctgtaca gtgacctcca gaggagcagc 781 agtgagcaag cgatgcgatg ctctctcatc acagagcatg tggaggcctt tatcaccatc 841 caggtggccc agatggtgat cggctttctg gtccccctgc tggccatgag cttctgttac 901 cttgtcatca tccgcaccct gctccaggca cgcaactttg agcgcaacaa ggccatcaag 961 gtgatcatcg ctgtggtcgt ggtcttcata gtcttccagc tgccctacaa tggggtggtc 1021 ctggcccaga cggtggccaa cttcaacatc accagtagca cctgtgagct cagtaagcaa 1081 ctcaacatcg cctacgacgt cacctacagc ctggcctgcg tccgctgctg cgtcaaccct 1141 ttcttgtacg ccttcatcgg cgtcaagttc cgcaacgatc tcttcaagct cttcaaggac 1201 ctgggctgcc tcagccagga gcagctccgg cagtggtctt cctgtcggca catccggcgc 1261 tcctccatga gtgtggaggc cgagaccacc accaccttct ccccataggc gactcttctg 1321 cctggactag agggacctct cccagggtcc ctggggtggg gatagggagc agatgcaatg 1381 actcaggaca tccccccgcc aaaagctgct cagggaaaag cagctctccc ctcagagtgc 1441 aagcccctgc tccagaagat agcttcaccc caatcccagc tacctcaacc aatgccaaaa 1501 aaagacaggg ctgataagct aacaccagac agacaacact gggaaacaga ggctattgtc 1561 ccctaaacca aaaactgaaa gtgaaagtcc agaaactgtt cccacctgct ggagtgaagg 1621 ggccaaggag ggtgagtgca aggggcgtgg gagtggcctg aagagtcctc tgaatgaacc 1681 ttctggcctc ccacagactc aaatgctcag accagctctt ccgaaaacca ggccttatct 1741 ccaagaccag agatagtggg gagacttctt ggcttggtga ggaaaagcgg acatcagctg 1801 gtcaaacaaa ctctctgaac ccctccctcc atcgttttct tcactgtcct ccaagccagc 1861 gggaatggca gctgccacgc cgccctaaaa gcacactcat cccctcactt gccgcgtcgc 1921 cctcccaggc tctcaacagg ggagagtgtg gtgtttcctg caggccaggc cagctgcctc 1981 cgcgtgatca aagccacact ctgggctcca gagtggggat gacatgcact cagctcttgg 2041 ctccactggg atgggaggag aggacaaggg aaatgtcagg ggcggggagg gtgacagtgg 2101 ccgcccaagg cccacgagct tgttctttgt tctttgtcac agggactgaa aacctctcct 2161 catgttctgc tttcgattcg ttaagagagc aacattttac ccacacacag ataaagtttt 2221 cccttgagga aacaacagct ttaaaagaaa aagaaaaaaa aagtctttgg taaatggcaa 2281 aaaaaaaaaa aaaaaaaaaa aaa
9	NM_001838.3 编码人 CCR7	1 cacttcctcc ccagacaggg gtagtgcgag gccgggcaca gccttcctgt gtggttttac 61 cgcccagaga gcgtcatgga cctggggaaa ccaatgaaaa gcgtgctggt ggtggctctc 121 cttgtcattt tccaggtatg cctgtgtcaa gatgaggtca cggacgatta catcggagac 181 aacaccacag tggactacac tttgttcgag tctttgtgct ccaagaagga cgtgcggaac

[0448]

SEQ ID NO:	GenBank 登记号和描述	序列
	同种型 a 前体的示例性核酸序列	241 tttaaagcct ggttcctccc tatcatgtac tccatcattt gtttcgtggg cctactgggc 301 aatgggctgg tcgtgttgac ctatatctat ttcaagaggc tcaagaccat gaccgatacc 361 tacctgctca acctggcggt ggcagacatc ctcttcctcc tgacccttcc cttctgggcc 421 tacagcgcgg ccaagtcctg ggtcttcggt gtccactttt gcaagctcat ctttgccatc 481 tacaagatga gcttcttcag tggcatgctc ctacttcttt gcatcagcat tgaccgctac 541 gtggccatcg tccaggctgt ctcagctcac cgccaccgtg cccgcgtcct tctcatcagc 601 aagctgtcct gtgtgggcat ctggatacta gccacagtgc tctccatccc agagctcctg 661 tacagtgacc tccagaggag cagcagtgag caagcgatgc gatgctctct catcacagag 721 catgtggagg cctttatcac catccaggtg gcccagatgg tgatcggctt tctggtcccc 781 ctgctggcca tgagcttctg ttaccttgtc atcatccgca ccctgctcca ggcacgcaac 841 tttgagcgca acaaggccat caaggtgatc atcgctgtgg tcgtggtctt catagtcttc 901 cagctgccct acaatggggt ggtcctggcc cagacggtgg ccaacttcaa catcaccagt 961 agcacctgtg agctcagtaa gcaactcaac atcgcctacg acgtcaccta cagcctggcc 1021 tgcgtccgct gctgcgtcaa ccctttcttg tacgccttca tcggcgtcaa gttccgcaac 1081 gatctcttca agctcttcaa ggacctgggc tgcctcagcc aggagcagct ccggcagtgg 1141 tcttcctgtc ggcacatccg gcgctcctcc atgagtgtgg aggccgagac caccaccacc 1201 ttctccccat aggcgactct tctgcctgga ctagagggac ctctcccagg gtccctgggg 1261 tggggatagg gagcagatgc aatgactcag gacatccccc cgccaaaagc tgctcaggga 1321 aaagcagctc tcccctcaga gtgcaagccc ctgctccaga agatagcttc accccaatcc 1381 cagctacctc aaccaatgcc aaaaaaagac agggctgata agctaacacc agacagacaa 1441 cactgggaaa cagaggctat tgtcccctaa accaaaaact gaaagtgaaa gtccagaaac 1501 tgttcccacc tgctggagtg aaggggccaa ggagggtgag tgcaaggggc gtgggagtgg 1561 cctgaagagt cctctgaatg aaccttctgg cctcccacag actcaaatgc tcagaccagc 1621 tcttccgaaa accaggcctt atctccaaga ccagagatag tggggagact tcttggcttg 1681 gtgaggaaaa gcggacatca gctggtcaaa caaactctct gaacccctcc ctccatcgtt 1741 ttcttcactg tcctccaagc cagcgggaat ggcagctgcc acgccgccct aaaagcacac 1801 tcatcccctc acttgccgcg tcgccctccc aggctctcaa caggggagag tgtggtgttt 1861 cctgcaggcc aggccagctg cctccgcgtg atcaaagcca cactctgggc tccagagtgg 1921 ggatgacatg cactcagctc ttggctccac tgggatggga ggagaggaca agggaaatgt 1981 caggggcggg gagggtgaca gtggccgccc aaggcccacg agcttgttct ttgttctttg 2041 tcacagggac tgaaaacctc tcctcatgtt ctgctttcga ttcgttaaga gagcaacatt 2101 ttacccacac acagataaag ttttcccttg aggaaacaac agctttaaaa gaaaaagaaa 2161 aaaaaagtct ttggtaaatg gcaaaaaaaa aaaaaaaaaa aaaaaaa
10	NP_001288643.1 人 CCR7 同种型 b 的示例性氨基酸序列	1 mysiicfvgl lgnglvvlty iyfkrlktmt dtyllnlava dilflltlpf waysaakswv 61 fgvhfcklif aiykmsffsg mllllcisid ryvaivqavs ahrhrarvll isklscvgiw 121 ilatvlsipe llysdlqrss seqamrcsli tehveafiti qvaqmvigfl vpllamsfcy 181 lviirtllqa rnfernkaik viiavvvvfi vfqlpyngvv laqtvanfni tsstcelskq 241 lniaydvtys lacvrccvnp flyafigvkf rndlfklfkd lgclsqeqlr qwsscrhirr 301 ssmsveaett ttfsp
11	NP_001288645.1 人 CCR7 同种	1 mksvlvvall vifqvclcqd evtddyigdn ttvdytlfes lcskkdvrnf kawflpimys 61 iicfvgllgn glvvltyiyf krlktmtdty llnlavadil flltlpfway saakswvfgv 121 hfcklifaiy kmsffsgmll llcisidryv aivqavsahr hrarvllisk lscvgiwila 181 tvlsipelly sdlqrssseq amrcsliteh veafitiqva qmvigflvpl lamsfcylvi

[0449]

SEQ ID NO:	GenBank 登记号和描述	序列
	型 c 前体的示例性氨基酸序列	241 irtllqarnf ernkaikvii avvvvfivfq lpyngvvlaq tvanfnitss tcelskqlni 301 aydvtyslac vrccvnpfly afigvkfrnd lfklfkdlgc lsqeqlrqws scrhirrssm 361 sveaettttf sp
12	NP_001288646.1 人 CCR7 同种型 c 前体的示例性氨基酸序列	1 mksvlvvall vifqvclcqd evtddyigdn ttvdytlfes lcskkdvrnf kawflpimys 61 iicfvgllgn glvvltyiyf krlktmtdty llnlavadil flltlpfway saakswvfgv 121 hfcklifaiy kmsffsgmll llcisidryv aivqavsahr hrarvllisk lscvgiwila 181 tvlsipelly sdlqrssseq amrcsliteh veafitiqva qmvigflvpl lamsfcylvi 241 irtllqarnf ernkaikvii avvvvfivfq lpyngvvlaq tvanfnitss tcelskqlni 301 aydvtyslac vrccvnpfly afigvkfrnd lfklfkdlgc lsqeqlrqws scrhirrssm 361 sveaettttf sp
13	NP_001288647.1 人 CCR7 同种型 c 前体的示例性氨基酸序列	1 mksvlvvall vifqvclcqd evtddyigdn ttvdytlfes lcskkdvrnf kawflpimys 61 iicfvgllgn glvvltyiyf krlktmtdty llnlavadil flltlpfway saakswvfgv 121 hfcklifaiy kmsffsgmll llcisidryv aivqavsahr hrarvllisk lscvgiwila 181 tvlsipelly sdlqrssseq amrcsliteh veafitiqva qmvigflvpl lamsfcylvi 241 irtllqarnf ernkaikvii avvvvfivfq lpyngvvlaq tvanfnitss tcelskqlni 301 aydvtyslac vrccvnpfly afigvkfrnd lfklfkdlgc lsqeqlrqws scrhirrssm 361 sveaettttf sp
14	NP_001829.1 人 CCR7 同种型 a 前体的示例性氨基酸序列	1 mdlgkpmksv lvvallvifq vclcqdevtd dyigdnttvd ytlfeslcsk kdvrnfkawf 61 lpimysiicf vgllgnglvv ltyiyfkrlk tmtdtyllnl avadilfllt lpfwaysaak 121 swvfgvhfck lifaiykmsf fsgmllllci sidryvaivq avsahrhrar vllisklscv 181 giwilatvls ipellysdlq rssseqamrc slitehveaf itiqvaqmvi gflvpllams 241 fcylviirtl lqarnfernk aikviiavvv vfivfqlpyn gvvlaqtvan fnitsstcel 301 skqlniaydv tyslacvrcc vnpflyafig vkfrndlfkl fkdlgclsqe qlrqwsscrh 361 irrssmsvea ettttfsp
15	NM_002253.2 编码人 VEGFR2 前体的示例性核酸序列	1 actgagtccc gggaccccgg gagagcggtc aatgtgtggt cgctgcgttt cctctgcctg 61 cgccgggcat cacttgcgcg ccgcagaaag tccgtctggc agcctggata tcctctccta 121 ccggcacccg cagacgcccc tgcagccgcg gtcggcgccc gggctcccta gccctgtgcg 181 ctcaactgtc ctgcgctgcg gggtgccgcg agttccacct ccgcgcctcc ttctctagac 241 aggcgctggg agaaagaacc ggctcccgag ttctgggcat ttcgcccggc tcgaggtgca 301 ggatgcagag caaggtgctg ctggccgtcg ccctgtggct ctgcgtggag acccgggccg 361 cctctgtggg tttgcctagt gtttctcttg atctgcccag gctcagcata caaaaagaca 421 tacttacaat taaggctaat acaactcttc aaattacttg caggggacag agggacttgg 481 actggctttg gcccaataat cagagtggca gtgagcaaag ggtggaggtg actgagtgca 541 gcgatggcct cttctgtaag acactcacaa ttccaaaagt gatcggaaat gacactggag 601 cctacaagtg cttctaccgg gaaactgact tggcctcggt catttatgtc tatgttcaag 661 attacagatc tccatttatt gcttctgtta gtgaccaaca tggagtcgtg tacattactg 721 agaacaaaaa caaaactgtg gtgattccat gtctcgggtc catttcaaat ctcaacgtgt 781 cactttgtgc aagataccca gaaaagagat ttgttcctga tggtaacaga atttcctggg 841 acagcaagaa gggctttact attcccagct acatgatcag ctatgctggc atggtcttct 901 gtgaagcaaa aattaatgat gaaagttacc agtctattat gtacatagtt gtcgttgtag 961 ggtataggat ttatgatgtg gttctgagtc cgtctcatgg aattgaacta tctgttggag

[0450]

SEQ ID NO:	GenBank 登记号和描述	序列
		1021 aaaagcttgt cttaaattgt acagcaagaa ctgaactaaa tgtggggatt gacttcaact 1081 gggaataccc ttcttcgaag catcagcata agaaacttgt aaaccgagac ctaaaaaccc 1141 agtctgggag tgagatgaag aaatttttga gcaccttaac tatagatggt gtaacccgga 1201 gtgaccaagg attgtacacc tgtgcagcat ccagtgggct gatgaccaag aagaacagca 1261 catttgtcag ggtccatgaa aaacctttg ttgcttttgg aagtggcatg gaatctctgg 1321 tggaagccac ggtgggggag cgtgtcagaa tccctgcgaa gtaccttggt tacccacccc 1381 cagaaataaa atggtataaa aatggaatac cccttgagtc caatcacaca attaaagcgg 1441 ggcatgtact gacgattatg gaagtgagtg aaagagacac aggaaattac actgtcatcc 1501 ttaccaatcc catttcaaag gagaagcaga gccatgtggt ctctctggtt gtgtatgtcc 1561 caccccagat tggtgagaaa tctctaatct ctcctgtgga ttcctaccag tacggcacca 1621 ctcaaacgct gacatgtacg gtctatgcca ttcctccccc gcatcacatc cactggtatt 1681 ggcagttgga ggaagagtgc gccaacgagc ccagccaagc tgtctcagtg acaaacccat 1741 acccttgtga agaatggaga agtgtggagg acttccaggg aggaaataaa attgaagtta 1801 ataaaaatca atttgctcta attgaaggaa aaaacaaaac tgtaagtacc cttgttatcc 1861 aagcggcaaa tgtgtcagct ttgtacaaat gtgaagcggt caacaaagtc gggagaggag 1921 agagggtgat ctccttccac gtgaccaggg gtcctgaaat tactttgcaa cctgacatgc 1981 agcccactga gcaggagagc gtgtctttgt ggtgcactgc agacagatct acgtttgaga 2041 acctcacatg gtacaagctt ggcccacagc ctctgccaat ccatgtggga gagttgccca 2101 cacctgtttg caagaacttg gatactcttt ggaaattgaa tgccaccatg ttctctaata 2161 gcacaaatga cattttgatc atggagctta agaatgcatc cttgcaggac caaggagact 2221 atgtctgcct tgctcaagac aggaagacca agaaaagaca ttgcgtggtc aggcagctca 2281 cagtcctaga gcgtgtggca cccacgatca caggaaacct ggagaatcag acgacaagta 2341 ttggggaaag catcgaagtc tcatgcacgg catctgggaa tccccctcca cagatcatgt 2401 ggtttaaaga taatgagacc cttgtagaag actcaggcat tgtattgaag gatgggaacc 2461 ggaacctcac tatccgcaga gtgaggaagg aggacgaagg cctctacacc tgccaggcat 2521 gcagtgttct tggctgtgca aaagtggagg catttttcat aatagaaggt gcccaggaaa 2581 agacgaactt ggaaatcatt attctagtag gcacggcggt gattgccatg ttcttctggc 2641 tacttcttgt catcatccta cggaccgtta agcgggccaa tggaggggaa ctgaagacag 2701 gctacttgtc catcgtcatg gatccagatg aactcccatt ggatgaacat tgtgaacgac 2761 tgccttatga tgccagcaaa tgggaattcc ccagagaccg gctgaagcta ggtaagcctc 2821 ttggccgtgg tgcctttggc caagtgattg aagcagatgc ctttggaatt gacaagacag 2881 caacttgcag gacagtagca gtcaaaatgt tgaaagaagg agcaacacac agtgagcatc 2941 gagctctcat gtctgaactc aagatcctca ttcatattgg tcaccatctc aatgtggtca 3001 accttctagg tgcctgtacc aagccaggag ggccactcat ggtgattgtg gaattctgca 3061 aatttggaaa cctgtccact tacctgagga gcaagagaaa tgaatttgtc ccctacaaga 3121 ccaaaggggc acgattccgt caagggaaag actacgttgg agcaatccct gtggatctga 3181 aacggcgctt ggacagcatc accagtagcc agagctcagc cagctctgga tttgtggagg 3241 agaagtccct cagtgatgta gaagaagagg aagctcctga agatctgtat aaggacttcc 3301 tgaccttgga gcatctcatc tgttacagct tccaagtggc taagggcatg gagttcttgg 3361 catcgcgaaa gtgtatccac agggacctgg cggcacgaaa tatcctctta tcggagaaga 3421 acgtggttaa aatctgtgac tttggcttgg cccgggatat ttataaagat ccagattatg 3481 tcagaaaagg agatgctcgc ctccctttga aatggatggc cccagaaaca atttttgaca 3541 gagtgtacac aatccagagt gacgtctggt cttttggtgt tttgctgtgg gaaatatttt

[0451]

SEQ ID NO:	GenBank 登记号和描述	序列
		3601 ccttaggtgc ttctccatat cctggggtaa agattgatga agaattttgt aggcgattga 3661 aagaaggaac tagaatgagg gcccctgatt atactacacc agaaatgtac cagaccatgc 3721 tggactgctg gcacggggag cccagtcaga gacccacgtt ttcagagttg gtggaacatt 3781 tgggaaatct cttgcaagct aatgctcagc aggatggcaa agactacatt gttcttccga 3841 tatcagagac tttgagcatg gaagaggatt ctggactctc tctgcctacc tcacctgttt 3901 cctgtatgga ggaggaggaa gtatgtgacc ccaaattcca ttatgacaac acagcaggaa 3961 tcagtcagta tctgcagaac agtaagcgaa agagccggcc tgtgagtgta aaaacatttg 4021 aagatatccc gttagaagaa ccagaagtaa aagtaatccc agatgacaac cagacggaca 4081 gtggtatggt tcttgcctca gaagagctga aaactttgga agacagaacc aaattatctc 4141 catcttttgg tggaatggtg cccagcaaaa gcagggagtc tgtggcatct gaaggctcaa 4201 accagacaag cggctaccag tccggatatc actccgatga cacagacacc accgtgtact 4261 ccagtgagga agcagaactt ttaaagctga tagagattgg agtgcaaacc ggtagcacag 4321 cccagattct ccagcctgac tcggggacca cactgagctc tcctcctgtt taaaaggaag 4381 catccacacc cccaactcct ggacatcaca tgagaggtgc tgctcagatt ttcaagtgtt 4441 gttctttcca ccagcaggaa gtagccgcat ttgattttca tttcgacaac agaaaaagga 4501 cctcggactg cagggagcca gtcttctagg catatcctgg aagaggcttg tgacccaaga 4561 atgtgtctgt gtcttctccc agtgttgacc tgatcctctt tttcattcat ttaaaaagca 4621 tttatcatgc cccctgctgc gggtctcacc atgggtttag aacaaagacg ttcaagaaat 4681 ggccccatcc tcaaagaagt agcagtacct ggggagctga cacttctgta aaactagaag 4741 ataaaccagg caatgtaagt gttcgaggtg ttgaagatgg gaaggatttg cagggctgag 4801 tctatccaag aggctttgtt taggacgtgg gtcccaagcc aagccttaag tgtggaattc 4861 ggattgatag aaaggaagac taacgttacc ttgctttgga gagtactgga gcctgcaaat 4921 gcattgtgtt tgctctggtg gaggtgggca tggggtctgt tctgaaatgt aaagggttca 4981 gacggggttt ctggttttag aaggttgcgt gttcttcgag ttgggctaaa gtagagttcg 5041 ttgtgctgtt tctgactcct aatgagagtt ccttccagac cgttacgtgt ctcctggcca 5101 agccccagga aggaaatgat gcagctctgg ctccttgtct cccaggctga tcctttattc 5161 agaataccac aaagaaagga cattcagctc aaggctccct gccgtgttga agagttctga 5221 ctgcacaaac cagcttctgg tttcttctgg aatgaatacc ctcatatctg tcctgatgtg 5281 atatgtctga gactgaatgc gggaggttca atgtgaagct gtgtgtggtg tcaaagtttc 5341 aggaaggatt ttacccttttt gttcttcccc ctgtccccaa cccactctca ccccgcaacc 5401 catcagtatt ttagttattt ggcctctact ccagtaaacc tgattgggtt tgttcactct 5461 ctgaatgatt attagccaga cttcaaaatt attttatagc ccaaattata acatctattg 5521 tattatttag acttttaaca tatagagcta tttctactga ttttttgccct tgttctgtcc 5581 tttttttcaa aaaagaaaat gtgtttttttg tttggtacca tagtgtgaaa tgctgggaac 5641 aatgactata agacatgcta tggcacatat atttatagtc tgtttatgta gaaacaaatg 5701 taatatatta aagccttata tataatgaac tttgtactat tcacattttg tatcagtatt 5761 atgtagcata acaaaggtca taatgctttc agcaattgat gtcattttat taaagaacat 5821 tgaaaaactt gaaggaatcc ctttgcaagg ttgcattact gtacccatca tttctaaaat 5881 ggaagagggg gtggctgggc acagtggccg acacctaaaa acccagcact ttgggggggcc 5941 aaggtgggag gatcgcttga gcccaggagt tcaagaccag tctggccaac atggtcagat 6001 tccatctcaa agaaaaaagg taaaaataaa ataaaatgga gaagaaggaa tcaga
16	NP_002244.1 编码人VEGFR2前体的示例性核酸	1 mqskvllava lwlcvetraa svglpsvsld lprlsiqkdi ltikanttlq itcrgqrdld 61 wlwpnnqsgs eqrvevtecs dglfcktlti pkvigndtga ykcfyretdl asviyvyvqd 121 yrspfiasvs dqhgvvyite nknktvvipc lgsisnlnvs lcarypekrf vpdgnriswd 181 skkgftipsy misyagmvfc eakindesyq simyivvvvg yriydvvlsp shgielsvge 241 klvlnctart elnvgidfnw eypsskhqhk klvnrdlktq sgsemkkfls tltidgvtrs

[0452]

SEQ ID NO:	GenBank 登记号和描述	序列
	序列	301 dqglytcaas sglmtkknst fvrvhekpfv afgsgmeslv eatvgervri pakylgyppp 361 eikwykngip lesnhtikag hvltimevse rdtgnytvil tnpiskekqs hvvslvvyvp 421 pqigekslis pvdsyqygtt qtltctvyai ppphhihwyw qleeecanep sqavsvtnpy 481 pceewrsved fqggnkievn knqfaliegk nktvstlviq aanvsalykc eavnkvgrge 541 rvisfhvtrg peitlqpdmq pteqesvslw ctadrstfen ltwyklgpqp lpihvgelpt 601 pvcknldtlw klnatmfsns tndilimelk naslqdqgdy vclaqdrktk krhcvvrqlt 661 vlervaptit gnlenqttsi gesievscta sgnpppqimw fkdnetlved sgivlkdgnr 721 nltirrvrke deglytcqac svlgcakvea ffiiegaqek tnleiiilvg taviamffwl 781 llviilrtvk ranggelktg ylsivmdpde lpldehcerl pydaskwefp rdrlklgkpl 841 grgafgqvie adafgidkta tcrtvavkml kegathsehr almselkili highhlnvvn 901 llgactkpgg plmvivefck fgnlstylrs krnefvpykt kgarfrqgkd yvgaipvdlk 961 rrldsitssq ssassgfvee kslsdveeee apedlykdfl tlehlicysf qvakgmefla 1021 srkcihrdla arnillsekn vvkicdfgla rdiykdpdyv rkgdarlplk wmapetifdr 1081 vytiqsdvws fgvllweifs lgaspypgvk ideefcrrlk egtrmrapdy ttpemyqtml 1141 dcwhgepsqr ptfselvehl gnllqanaqq dgkdyivlpi setlsmeeds glslptspvs 1201 cmeeeevcdp kfhydntagi sqylqnskrk srpvsvktfe dipleepevk vipddnqtds 1261 gmvlaseelk tledrtklsp sfggmvpsks resvasegsn qtsgyqsgyh sddtdttvys 1321 seeaellkli eigvqtgsta qilqpdsgtt lssppv
17	NM_001716.4 编码人CXCR5的示例性核酸序列	1 aaaaaaaaaa agtgatgagt tgtgaggcag gtcgcggccc tactgcctca ggagacgatg 61 cgcagctcat ttgcttaaat ttgcagctga cggctgccac ctctctagag gcacctggcg 121 gggagcctct caacataaga cagtgaccag tctggtgact cacagccggc acagccatga 181 actacccgct aacgctggaa atggacctcg agaacctgga ggacctgttc tgggaactgg 241 acagattgga caactataac gacacctccc tggtggaaaa tcatctctgc cctgccacag 301 aggggcccct catggcctcc ttcaaggccg tgttcgtgcc cgtggcctac agcctcatct 361 tcctcctggg cgtgatcggc aacgtcctgg tgctggtgat cctggagcgg caccggcaga 421 cacgcagttc cacggagacc ttcctgttcc acctggccgt ggccgacctc ctgctggtct 481 tcatcttgcc ctttgccgtg gccgagggct ctgtgggctg ggtcctgggg accttcctct 541 gcaaaactgt gattgccctg cacaaagtca acttctactg cagcagcctg ctcctggcct 601 gcatcgccgt ggaccgctac ctggccattg tccacgccgt ccatgcctac cgccaccgcc 661 gcctcctctc catccacatc acctgtggga ccatctggct ggtgggcttc ctccttgcct 721 tgccagagat tctcttcgcc aaagtcagcc aaggccatca caacaactcc ctgccacgtt 781 gcaccttctc ccaagagaac caagcagaaa cgcatgcctg gttcacctcc cgattcctct 841 accatgtggc gggattcctg ctgcccatgc tggtgatggg ctggtgctac gtgggggtag 901 tgcacaggtt gcgccaggcc cagcggcgcc ctcagcggca gaaggcagtc agggtggcca 961 tcctggtgac aagcatcttc ttcctctgct ggtcacccta ccacatcgtc atcttcctgg 1021 acaccctggc gaggctgaag gccgtggaca atacctgcaa gctgaatggc tctctccccg 1081 tggccatcac catgtgtgag ttcctgggcc tggcccactg ctgcctcaac cccatgctct 1141 acactttcgc cggcgtgaag ttccgcagtg acctgtcgcg gctcctgacg aagctgggct 1201 gtaccggccc tgcctccctg tgccagctct tccctagctg gcgcaggagc agtctctctg 1261 agtcagagaa tgccacctct ctcaccacgt tctaggtccc agtgtccccc tttattgctg 1321 cttttccttg gggcaggcag tgatgctgga tgctccttcc aacaggagct gggatcctaa

[0453]

SEQ ID NO:	GenBank 登记号和描述	序列
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18	NM_032966.2 编码人CXCR5的示例性核酸序列	1 ccactctaag gaatgcggtc cctttgacag gcgaaaaact gaagttggaa aagacaaagt 61 gatttgttca aaattgaaat ttgaaacttg acatttggtc agtgggccct atgtaggaaa 121 aaacctccaa gagagctagg gttcctctca gagaggaaag acaggtcctt aggtcctcac 181 cctcccgtct ccttgccctt gcagttctgg gaactggaca gattggacaa ctataacgac 241 acctccctgg tggaaaatca tctctgccct gccacagagg ggcccctcat ggcctccttc 301 aaggccgtgt tcgtgcccgt ggcctacagc ctcatcttcc tcctgggcgt gatcggcaac 361 gtcctggtgc tggtgatcct ggagcggcac cggcagacac gcagttccac ggagaccttc 421 ctgttccacc tggccgtggc cgacctcctg ctggtcttca tcttgccctt tgccgtggcc 481 gagggctctg tgggctgggt cctggggacc ttcctctgca aaactgtgat tgccctgcac 541 aaagtcaact tctactgcag cagcctgctc ctggcctgca tcgccgtgga ccgctacctg 601 gccattgtcc acgccgtcca tgcctaccgc caccgccgcc tcctctccat ccacatcacc 661 tgtgggacca tctggctggt gggcttcctc cttgccttgc cagagattct cttcgccaaa 721 gtcagccaag gccatcacaa caactccctg ccacgttgca ccttctccca agagaaccaa

[0454]

SEQ ID NO:	GenBank 登记号和描述	序列
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19	NP_116743.1 人 CXCR5 前	1 masfkavfvp vaysliflig vignvlvlvi lerhrqtrss tetflfhlav adlllvfilp 61 favaegsvgw vlgtflcktv ialhkvnfyc ssllaciav drylaivhav hayrhrrlls 121 ihitcgtiwl vgfllalpei lfakvsqghh nnslprctfs qenqaethaw ftsrflyhva 181 gfllpmlvmg wcyvgvvhrl rqaqrrpqrq kavrvailvt sifflcwspy hivifldtla

[0455]

SEQ ID NO:	GenBank 登记号和描述	序列
	体的示例性氨基酸序列	241 rlkavdntck lngslpvait mceflglahc clnpmlytfa gvkfrsdlsr lltklgctgp 301 aslcqlfpsw rrsslsesen atslttf
20	NP_001707.1 人 CXCR5 前体的示例性氨基酸序列	1 mnypltlemd lenledlfwe ldrldnyndt slvenhlcpa tegplmasfk avfvpvaysl 61 ifllgvignv lvlvilerhr qtrsstetfl fhlavadlll vfilpfavae gsvgwvlgtf 121 lcktvialhk vnfyctsslll aciavdryla ivhavhayrh rrllsihitc gtiwlvgfll 181 alpeilfakv sqghhnnslp rctfsqenqa ethawftsrf lyhvagfllp mlvmgwcyvg 241 vvhrlrqaqr rpqrqkavrv ailvtsiffl cwspyhivif ldtlarlkav dntcklngsl 301 pvaitmcefl glahcclnpm lytfagvkfr sdlsrlltkl gctgpaslcq lfpswrrssl 361 sesenatslt tf
21	NM_031200.2 编码人 CCR9 的示例性核酸序列	1 gcttcctttc tcgtgttgtt atcgggtagc tgcctgctca gaacccacaa agcctgcccc 61 tcatcccagg cagagagcaa cccagctctt tccccagaca ctgagagctg gtggtgcctg 121 ctgtcccagg gagagttgca tcgccctcca cagagcaggc ttgcatctga ctgacccacc 181 atgacaccca cagacttcac aagccctatt cctaacatgg ctgatgacta tggctctgaa 241 tccacatctt ccatggaaga ctacgttaac ttcaacttca ctgacttcta ctgtgagaaa 301 aacaatgtca ggcagtttgc gagccatttc ctcccaccct tgtactggct cgtgttcatc 361 gtgggtgcct tgggcaacag tcttgttatc cttgtctact ggtactgcac aagagtgaag 421 accatgaccg acatgttcct tttgaatttg gcaattgctg acctcctctt tcttgtcact 481 cttcccttct gggccattgc tgctgctgac cagtggaagt tccagacctt catgtgcaag 541 gtggtcaaca gcatgtacaa gatgaacttc tacagctgtg tgttgctgat catgtgcatc 601 agcgtggaca ggtacattgc cattgcccag gccatgagag cacatacttg gagggagaaa 661 aggcttttgt acagcaaaat ggtttgcttt accatctggg tattggcagc tgctctctgc 721 atcccagaaa tcttatacag ccaaatcaag gaggaatccg gcattgctat ctgcaccatg 781 gtttacccta gcgatgagag caccaaactg aagtcagctg tcttgaccct gaaggtcatt 841 ctggggttct tccttccctt cgtggtcatg gcttgctgct ataccatcat cattcacacc 901 ctgatacaag ccaagaagtc ttccaagcac aaagccctaa aagtgaccat cactgtcctg 961 accgtctttg tcttgtctca gtttccctac aactgcattt tgttggtgca gaccattgac 1021 gcctatgcca tgttcatctc caactgtgcc gtttccacca acattgacat ctgcttccag 1081 gtcacccaga ccatcgcctt cttccacagt tgcctgaacc ctgttctcta tgtttttgtg 1141 ggtgagagat tccgccggga tctcgtgaaa accctgaaga acttgggttg catcagccag 1201 gcccagtggg tttcatttac aaggagagag ggaagcttga agctgtcgtc tatgttgctg 1261 gagacaacct caggagcact ctccctctga ggggtcttct ctgaggtgca tggttctttt 1321 ggaagaaatg agaaatacag aaacagtttc cccactgatg ggaccagaga gagtgaaaga 1381 gaaaagaaaa ctcagaaagg gatgaatctg aactatatga ttacttgtag tcagaatttg 1441 ccaaagcaaa tatttcaaaa tcaactgact agtgcaggag gctgttgatt ggctcttgac 1501 tgtgatgccc gcaattctca aaggaggact aaggaccggc actgtggagc accctggctt 1561 tgccactcgc cggagcatca atgccgctgc ctctggagga gcccttggat tttctccatg 1621 cactgtgaac ttctgtggct tcagttctca tgctgcctct tccaaaaggg gacacagaag 1681 cactggctgc tgctacagac cgcaaaagca gaaagtttcg tgaaaatgtc catctttggg 1741 aaattttcta ccctgctctt gagcctgata acccatgcca ggtcttatag attcctgatc 1801 tagaaccttt ccaggcaatc tcagacctaa tttccttctg ttctccttgt tctgttctgg 1861 gccagtgaag gtccttgttc tgattttgaa acgatctgca ggtcttgcca gtgaacccct 1921 ggacaactga ccacacccac aaggcatcca aagtctgttg gcttccaatc catttctgtg 1981 tcctgctgga ggttttaacc tagacaagga ttccgcttat tccttggtat ggtgacagtg 2041 tctctccatg gcctgagcag ggagattata acagctgggt tcgcaggagc cagccttggc 2101 cctgttgtag gcttgttctg ttgagtggca cttgctttgg gtccaccgtc tgtctgctcc 2161 ctagaaaatg ggctggttct tttggccctc ttctttctga ggcccacttt attctgagga

[0456]

SEQ ID NO:	GenBank 登记号和描述	序列
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22	NM001256369.1 编码人 CCR9 的示例性核酸序列	1 gcttcctttc tcgtgttgtt atcgggtagc tgcctgctca gaacccacaa agcctgcccc 61 tcatcccagg cagagagcaa cccagctctt tccccagaca ctgagagctg gtggtgcctg 121 ctgtcccagg gagagttgca tcgccctcca cagagcaggc ttgcatctga ctgacccacc 181 atgacaccca cagacttcac atctcctcca ggccccgctc cagatcacct tccctcgctg 241 gcccaggaat ccatctcctt ccaggacctt agcccaggac taacacaagc cctattccta 301 acatggctga tgactatggc tctgaatcca catcttccat ggaagactac gttaacttca 361 acttcactga cttctactgt gagaaaaaca atgtcaggca gtttgcgagc catttcctcc 421 caccettgta ctggctcgtg ttcatcgtgg gtgccttggg caacagtctt gttatccttg 481 tctactggta ctgcacaaga gtgaagacca tgaccgacat gttccttttg aatttggcaa 541 ttgctgacct cctctttctt gtcactcttc ccttctgggc cattgctgct gctgaccagt 601 ggaagttcca gaccttcatg tgcaaggtgg tcaacagcat gtacaagatg aacttctaca 661 gctgtgtgtt gctgatcatg tgcatcagcg tggacaggta cattgccatt gcccaggcca 721 tgagagcaca tacttggagg gagaaaaggc ttttgtacag caaaatggtt tgctttacca 781 tctgggtatt ggcagctgct ctctgcatcc cagaaatctt atacagccaa atcaaggagg 841 aatccggcat tgctatctgc accatggttt accctagcga tgagagcacc aaactgaagt 901 cagctgtctt gaccctgaag gtcattctgg ggttcttcct tcccttcgtg gtcatggctt 961 gctgctatac catcatcatt cacaccctga tacaagccaa gaagtcttcc aagcacaaag 1021 ccctaaaagt gaccatcact gtcctgaccg tctttgtctt gtctcagttt ccctacaact 1081 gcattttgtt ggtgcagacc attgacgcct atgccatgtt catctccaac tgtgccgttt 1141 ccaccaacat tgacatctgc ttccaggtca cccagaccat cgccttcttc cacagttgcc 1201 tgaaccctgt tctctatgtt tttgtgggtg agagattccg ccgggatctc gtgaaaaccc 1261 tgaagaactt gggttgcatc agccaggccc agtgggtttc atttacaagg agagagggaa 1321 gcttgaagct gtcgtctatg ttgctggaga caacctcagg agcactctcc ctctgagggg 1381 tcttctctga ggtgcatggt tcttttggaa gaaatgagaa atacagaaac agtttcccca 1441 ctgatgggac cagagagagt gaaagagaaa agaaaactca gaaagggatg aatctgaact 1501 atatgattac ttgtagtcag aatttgccaa agcaaatatt tcaaaatcaa ctgactagtg 1561 caggaggctg ttgattggct cttgactgtg atgcccgcaa ttctcaaagg aggactaagg 1621 accggcactg tggagcaccc tggctttgcc actcgccgga gcatcaatgc cgctgcctct 1681 ggaggagccc ttggattttc tccatgcact gtgaacttct gtggcttcag ttctcatgct 1741 gcctcttcca aaaggggaca cagaagcact ggctgctgct acagaccgca aaagcagaaa 1801 gtttcgtgaa aatgtccatc tttgggaaat ttctaccct gctcttgagc ctgataaccc 1861 atgccaggtc ttatagattc ctgatctaga acctttccag gcaatctcag acctaatttc 1921 cttctgttct ccttgttctg ttctgggcca gtgaaggtcc ttgttctgat tttgaaacga 1981 tctgcaggtc ttgccagtga acccctggac aactgaccac acccacaagg catccaaagt 2041 ctgttggctt ccaatccatt tctgtgtcct gctggaggtt ttaacctaga caaggattcc 2101 gcttattcct tggtatggtg acagtgtctc tccatggcct gagcagggag attataacag 2161 ctgggttcgc aggagccagc cttggccctg ttgtaggctt gttctgttga gtggcacttg 2221 ctttgggtcc accgtctgtc tgctccctag aaaatgggct ggttcttttg gccctcttct 2281 ttctgaggcc cactttattc tgaggaatac agtgagcaga tatgggcagc agccaggtag 2341 ggcaaagggg tgaagcgcag gccttgctgg aaggctattt acttccatgc ttctcctttt 2401 cttactctat agtggcaaca ttttaaaagc ttttaactta gagattaggc tgaaaaaaat

SEQ ID NO:	GenBank 登记号和描述	序列
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23	NP_112477.1 人 CCR9 前体的示例性氨基酸序列	1 mtptdftspi pnmaddygse stssmedyvn fnftdfycek nnvrqfashf lpplywlvfi 61 vgalgnslvi lvywyctrvk tmtdmfllnl aiadllflvt lpfwaiaaad qwkfqtfmck 121 vvnsmykmnf yscvllimci svdryiaiaq amrahtwrek rllyskmvcf tiwvlaaalc 181 ipeilysqik eesgiaictm vypsdestkl ksavltlkvi lgfflpfvvm accytiiiht 241 liqakksskh kalkvtitvl tvfvlsqfpy ncillvqtid ayamfisnca vstnidicfq 301 vtqtiaffhs clnpvlyvfv gerfrrdlvk tlknlgcisq aqwvsftrre gslklssmll 361 ettsgalsl
24	NP_001243298.1 人 CCR9 前体的示例性氨基酸序列	1 maddygsest ssmedyvnfn ftdfyceknn vrqfashflp plywlvfivg algnslvilv 61 ywyctrvktm tdmfllnlai adllflvtlp fwaiaaadqw kfqtfmckvv nsmykmnfys 121 cvllimcisv dryiaiaqam rahtwrekrl lyskmvcfti wvlaaalcip eilysqikee 181 sgiaictmvy psdestklks avltlkvilg fflpfvvmac cytiiihtli qakksskhka 241 lkvtitvltv fvlsqfpync illvqtiday amfisncavs tnidicfqvt qtiaffhscl 301 npvlyvfvge rfrrdlvktl knlgcisqaq wvsftrregs lklssmllet tsgalsl
[0457] 25	NM_000885.4 编码人α4的示例性核酸序列	1 ataacgtctt tgtcactaaa atgttcccca ggggccttcg gcgagtcttt ttgtttggtt 61 ttttgttttt aatctgtggc tcttgataat ttatctagtg gttgcctaca cctgaaaaac 121 aagacacagt gtttaactat caacgaaaga actggacggc tccccgccgc agtcccactc 181 cccgagtttg tggctggcat ttgggccacg ccgggctggg cggtcacagc gaggggcgcg 241 cagtttgggg tcacacagct ccgcttctag gccccaacca ccgttaaaag gggaagcccg 301 tgccccatca ggtccgctct tgctgagccc agagccatcc cgcgctctgc gggctgggag 361 gcccgggcca ggacgcgagt cctgcgcagc cgaggttccc cagcgccccc tgcagccgcg 421 cgtaggcaga gacggagccc ggccctgcgc ctccgcacca cgcccgggac cccacccagc 481 ggcccgtacc cggagaagca gcgcgagcac ccgaagctcc cggctggcgg cagaaaccgg 541 gagtggggcc gggcgagtgc gcggcatccc aggccggccc gaacgctccg cccgcggtgg 601 gccgacttcc cctcctcttc cctctctcct tcctttagcc cgctggcgcc ggacacgctg 661 cgcctcatct cttggggcgt tcttccccgt tggccaaccg tcgcatcccg tgcaactttg 721 gggtagtggc cgtttagtgt tgaatgttcc ccaccgagag cgcatggctt gggaagcgag 781 gcgcgaaccc ggcccccgaa gggccgccgt ccgggagacg gtgatgctgt tgctgtgcct 841 gggggtcccg accggccgcc cctacaacgt ggacactgag agcgcgctgc tttaccaggg 901 cccccacaac acgctgttcg gctactcggt cgtgctgcac agccacgggg cgaaccgatg 961 gctcctagtg ggtgcgccca ctgccaactg gctcgccaac gcttcagtga tcaatcccgg 1021 ggcgatttac agatgcagga tcggaaagaa tcccggccag acgtgcgaac agctccagct 1081 gggtagccct aatggagaac cttgtggaaa gacttgtttg gaagagagag acaatcagtg 1141 gttgggggtc acactttcca gacagccagg agaaaatgga tccatcgtga cttgtgggca 1201 tagatggaaa aatatatttt acataaagaa tgaaaataag ctccccactg gtggttgcta

[0458]

SEQ ID NO:	GenBank 登记号和描述	序列
		1261 tggagtgccc cctgatttac gaacagaact gagtaaaaga atagctccgt gttatcaaga 1321 ttatgtgaaa aaatttggag aaaattttgc atcatgtcaa gctggaatat ccagttttta 1381 cacaaaggat ttaattgtga tgggggcccc aggatcatct tactggactg gctctctttt 1441 tgtctacaat ataactacaa ataaatacaa ggctttttta gacaaacaaa atcaagtaaa 1501 atttggaagt tatttaggat attcagtcgg agctggtcat tttcggagcc agcatactac 1561 cgaagtagtc ggaggagctc ctcaacatga gcagattggt aaggcatata tattcagcat 1621 tgatgaaaaa gaactaaata tcttacatga aatgaaaggt aaaaagcttg gatcgtactt 1681 tggagcttct gtctgtgctg tggacctcaa tgcagatggc ttctcagatc tgctcgtggg 1741 agcacccatg cagagcacca tcagagagga aggaagagtg tttgtgtaca tcaactctgg 1801 ctcgggagca gtaatgaatg caatggaaac aaacctcgtt ggaagtgaca aatatgctgc 1861 aagatttggg gaatctatag ttaatcttgg cgacattgac aatgatggct ttgaagatgt 1921 tgctatcgga gctccacaag aagatgactt gcaaggtgct atttatattt acaatggccg 1981 tgcagatggg atctcgtcaa ccttctcaca gagaattgaa ggacttcaga tcagcaaatc 2041 gttaagtatg tttggacagt ctatatcagg acaaattgat gcagataata atggctatgt 2101 agatgtagca gttggtgctt ttcggtctga ttctgctgtc ttgctaagga caagacctgt 2161 agtaattgtt gacgcttctt taagccaccc tgagtcagta aatagaacga aatttgactg 2221 tgttgaaaat ggatggcctt ctgtgtgcat agatctaaca ctttgtttct catataaggg 2281 caaggaagtt ccaggttaca ttgttttgtt ttataacatg agtttggatg tgaacagaaa 2341 ggcagagtct ccaccaagat tctatttctc ttctaatgga acttctgacg tgattacagg 2401 aagcatacag gtgtccagca gagaagctaa ctgtagaaca catcaagcat ttatgcggaa 2461 agatgtgcgg gacatcctca ccccaattca gattgaagct gcttaccacc ttggtcctca 2521 tgtcatcagt aaacgaagta cagaggaatt cccaccactt cagccaattc ttcagcagaa 2581 gaaagaaaaa gacataatga aaaaaacaat aaactttgca aggttttgtg cccatgaaaa 2641 ttgttctgct gatttacagg tttctgcaaa gattgggttt ttgaagcccc atgaaaataa 2701 aacatatctt gctgttggga gtatgaagac attgatgttg aatgtgtcct tgtttaatgc 2761 tggagatgat gcatatgaaa cgactctaca tgtcaaacta cccgtgggtc tttatttcat 2821 taagatttta gagctggaag agaagcaaat aaactgtgaa gtcacagata actctggcgt 2881 ggtacaactt gactgcagta ttggctatat atatgtagat catctctcaa ggatagatat 2941 tagctttctc ctggatgtga gctcactcag cagagcggaa gaggacctca gtatcacagt 3001 gcatgctacc tgtgaaaatg aagaggaaat ggacaatcta aagcacagca gagtgactgt 3061 agcaatacct ttaaaatatg aggttaagct gactgttcat gggtttgtaa acccaacttc 3121 atttgtgtat ggatcaaatg atgaaaatga gcctgaaacg tgcatggtgg agaaaatgaa 3181 cttaactttc catgttatca acactggcaa tagtatggct cccaatgtta gtgtggaaat 3241 aatggtacca aattctttta gcccccaaac tgataagctg ttcaacattt tggatgtcca 3301 gactactact ggagaatgcc actttgaaaa ttatcaaaga gtgtgtgcat tagagcagca 3361 aaagagtgca atgcagacct tgaaaggcat agtccggttc ttgtccaaga ctgataagag 3421 gctattgtac tgcataaaag ctgatccaca ttgtttaaat ttcttgtgta attttgggaa 3481 aatggaaagt ggaaaagaag ccagtgttca tatccaactg gaaggccggc catccatttt 3541 agaaatggat gagacttcag cactcaagtt tgaaataaga gcaacaggtt ttccagagcc 3601 aaatccaaga gtaattgaac taaacaagga tgagaatgtt gcgcatgttc tactggaagg 3661 actacatcat caaagaccca aacgttattt caccatagtg attatttcaa gtagcttgct 3721 acttggactt attgtacttc tgttgatctc atatgttatg tggaaggctg gcttctttaa 3781 aagacaatac aaatctatcc tacaagaaga aaacagaaga gacagttgga gttatatcaa 3841 cagtaaaagc aatgatgatt aaggacttct ttcaaattga gagaatggaa aacagactca 3901 ggttgtagta aagaaattta aaagacactg tttacaagaa aaaatgaatt ttgtttggac 3961 ttcttttact catgatcttg tgacatatta tgtcttcatg caaggggaaa atctcagcaa 4021 tgattactct ttgagataga agaactgcaa aggtaataat acagccaaag ataatctctc 4081 agcttttaaa tgggtagaga aacactaaag cattcaattt attcaagaaa agtaagccct 4141 tgaagatatc ttgaaatgaa agtataactg agttaaatta tactggagaa gtcttagact 4201 tgaaatacta cttaccatat gtgcttgcct cagtaaaatg aaccccactg ggtgggcaga 4261 ggttcatttc aaatacatct ttgatacttg ttcaaaatat gttctttaaa aatataattt

[0459]

SEQ ID NO:	GenBank 登记号和描述	序列
		4321 tttagagagc tgttcccaaa ttttctaacg agtggaccat tatcacttta aagcccttta 4381 tttataatac atttcctacg ggctgtgttc caacaaccat ttttttttcag cagactatga 4441 atattatagt attataggcc aaactggcaa acttcagact gaacatgtac actggtttga 4501 gcttagtgaa attacttctg gataattatt tttttataat tatggatttc accatctttc 4561 tttctgtata tatacatgtg tttttatgta ggtatatatt taccattctt cctatctatt 4621 cttcctataa cacaccttta tcaagcatac ccaggagtaa tcttcaaatc ttttgttata 4681 ttctgaaaca aaagattgtg agtgttgcac tttacctgat acacgctgat ttagaaaata 4741 cagaaaccat acctcactaa taactttaaa atcaaagctg tgcaaagact agggggccta 4801 tacttcatat gtattatgta ctatgtaaaa tattgactat cacacaacta tttccttgga 4861 tgtaattctt tgttaccctt tacaagtata agtgttacct tacatggaaa cgaagaaaca 4921 aaattcataa atttaaattc ataaatttag ctgaaagata ctgattcaat ttgtatacag 4981 tgaatataaa tgagacgaca gcaaaatttt catgaaatgt aaaatatttt tatagtttgt 5041 tcatactata tgaggttcta ttttaaatga ctttctggat tttaaaaaat ttctttaaat 5101 acaatcattt ttgtaatatt tattttatgc ttatgatcta gataattgca gaatatcatt 5161 ttatctgact ctgccttcat aagagagctg tggccgaatt ttgaacatct gttataggga 5221 gtgatcaaat tagaaggcaa tgtggaaaaa caattctggg aaagatttct ttatatgaag 5281 tccctgccac tagccagcca tcctaattga tgaaagttat ctgttcacag gcctgcagtg 5341 atggtgagga atgttctgag atttgcgaag gcatttgagt agtgaaatgt aagcacaaaa 5401 cctcctgaac ccagagtgtg tatacacagg aataaacttt atgacattta tgtatttttа 5461 aaaaactttg tatcgttata aaaaggctag tcattctttc aggagaacat ctaggatcat 5521 agatgaaaaa tcaagccccg atttagaact gtcttctcca ggatggtctc taaggaaatt 5581 tacatttggt tctttcctac tcagaactac tcagaaacaa ctatatattt caggttatct 5641 gagcacagtg aaagcagagt actatggttg tccaacacag gcctctcaga tacaagggga 5701 acacaattac atattgggct agattttgcc cagttcaaaa tagtatttgt tatcaactta 5761 ctttgttact tgtatcatga attttaaaac cctaccactt taagaagaca gggatgggtt 5821 attctttttt ggcaggtagg ctatataact atgtgatttt gaaatttaac tgctctggat 5881 tagggagcag tgaatcaagg cagacttatg aaatctgtat tatatttgta acagaatata 5941 ggaaatttaa cataattgat gagctcaaat cctgaaaaat gaaagaatcc aaattatttc 6001 agaattatct aggttaaata ttgatgtatt atgatggttg caaagttttt ttgtgtgtcc 6061 aataaacaca ttgtaaaaaa aa
26	NM_000889.2 编码人β7的示例性核酸序列	1 aaatcttccc caccctgggg agtgtcactt cctcctctgc cgtctcccag atcagtacac 61 aaaggctgct gctgccgcca gaggaaggac tgctctgcac gcacctatgt ggaaactaaa 121 gcccagagag aaagtctgac ttgccccaca gccagtgagt gactgcagca gcaccagaat 181 ctggtctgtt tcctgtttgg ctcttctacc actacggctt gggatctcgg gcatggtggc 241 tttgccaatg gtccttgttt tgctgctggt cctgagcaga ggtgagagtg aattggacgc 301 caagatccca tccacagggg atgccacaga atggcggaat cctcacctgt ccatgctggg 361 gtcctgccag ccagcccccc cctgccagaa gtgcatcctc tcacacccca gctgtgcatg 421 gtgcaagcaa ctgaacttca ccgcgtcggg agaggcggag gcgcggcgct gcgcccgacg 481 agaggagctg ctggctcgag gctgcccgct ggaggagctg gaggagcccc gcggccagca 541 ggaggtgctg caggaccagc cgctcagcca gggcgcccgc ggagagggtg ccacccagct 601 ggcgccgcag cgggtccggg tcacgctgcg gcctggggag ccccagcagc tccaggtccg 661 cttccttcgt gctgagggat acccggtgga cctgtactac cttatggacc tgagctactc 721 catgaaggac gacctggaac gcgtgcgcca gctcgggcac gctctgctgg tccggctgca 781 ggaagtcacc cattctgtgc gcattggttt tggttccttt gtggacaaaa cggtgctgcc 841 ctttgtgagc acagtaccct ccaaactgcg ccacccctgc cccacccggc tggagcgctg

[0460]

SEQ ID NO:	GenBank 登记号和描述	序列
		901 ccagtcacca ttcagctttc accatgtgct gtccctgacg ggggacgcac aagccttcga 961 gcgggaggtg gggcgccaga gtgtgtccgg caatctggac tcgcctgaag gtggcttcga 1021 tgccattctg caggctgcac tctgccagga gcagattggc tggagaaatg tgtcccggct 1081 gctggtgttc acttcagacg acacattcca tacagctggg gacgggaagt tgggcggcat 1141 tttcatgccc agtgatgggc actgccactt ggacagcaat ggcctctaca gtcgcagcac 1201 agagtttgac taccttctg tgggtcaggt agcccaggcc ctctctgcag caaatatcca 1261 gcccatcttt gctgtcacca gtgccgcact gcctgtctac caggagctga gtaaactgat 1321 tcctaagtct gcagttgggg agctgagtga ggactccagc aacgtggtac agctcatcat 1381 ggatgcttat aatagcctgt cttccaccgt gacccttgaa cactcttcac tccctcctgg 1441 ggtccacatt tcttacgaat cccagtgtga gggtcctgag aagagggagg gtaaggctga 1501 ggatcgagga cagtgcaacc acgtccgaat caaccagacg gtgactttct gggtttctct 1561 ccaagccacc cactgcctcc cagagcccca tctcctgagg ctccgggccc ttggcttctc 1621 agaggagctg attgtggagt tgcacacgct gtgtgactgt aattgcagtg acacccagcc 1681 ccaggctccc cactgcagtg atggccaggg acacctacaa tgtggtgtat gcagctgtgc 1741 ccctggccgc ctaggtcggc tctgtgagtg ctctgtggca gagctgtcct ccccagacct 1801 ggaatctggg tgccgggctc ccaatggcac agggcccctg tgcagtggaa agggtcactg 1861 tcaatgtgga cgctgcagct gcagtggaca gagctctggg catctgtgcg agtgtgacga 1921 tgccagctgt gagcgacatg agggcatcct ctgcggaggc tttggtcgct gccaatgtgg 1981 agtatgtcac tgtcatgcca accgcacggg cagagcatgc gaatgcagtg gggacatgga 2041 cagttgcatc agtcccgagg gagggctctg cagtgggcat ggacgctgca aatgcaaccg 2101 ctgccagtgc ttggacggct actatggtgc tctatgcgac caatgcccag gctgcaagac 2161 accatgcgag agacaccggg actgtgcaga gtgtggggcc ttcaggactg gcccactggc 2221 caccaactgc agtacagctt gtgcccatac caatgtgacc ctggccttgg cccctatctt 2281 ggatgatggc tggtgcaaag agcggaccct ggacaaccag ctgttcttct tcttggtgga 2341 ggatgacgcc agaggcacgg tcgtgctcag agtgagaccc caagaaaagg gagcagacca 2401 cacgcaggcc attgtgctgg gctgcgtagg gggcatcgtg gcagtggggc tggggctggt 2461 cctggcttac cggctctcgg tggaaatcta tgaccgccgg gaatacagtc gctttgagaa 2521 ggagcagcaa caactcaact ggaagcagga cagtaatcct ctctacaaaa gtgccatcac 2581 gaccaccatc aatcctcgct ttcaagaggc agacagtccc actctctgaa ggagggaggg 2641 acacttaccc aaggctcttc tccttggagg acagtgggaa ctggagggtg agaggaaggg 2701 tgggtctgta agaccttggt aggggactaa ttcactggcg aggtgcggcc accaccctac 2761 ttcattttca gagtgacacc caagagggct gcttcccatg cctgcaacct tgcatccatc 2821 tgggctaccc cacccaagta tacaataaag tcttacctca gaccacaaaa aaaaaaaa
27	NP_000876.3 人α4前体的示例性氨基酸序列	1 mawearrepg prraavretv mllclgvpt grpynvdtes allyqgphnt lfgysvvlhs 61 hganrwllvg aptanwlana svinpgaiyr crigknpgqt ceqlqlgspn gepcgktcle 121 erdnqwlgvt lsrqpgengs ivtcghrwkn ifyiknenkl ptggcygvpp dlrtelskri 181 apcyqdyvkk fgenfascqa gissfytkdl ivmgapgssy wtgslfvyni ttnkykafld 241 kqnqvkfgsy lgysvgaghf rsqhttevvg gapqheqigk ayifsideke lnilhemkgk 301 klgsyfgasv cavdlnadgf sdllvgapmq stireegrvf vyinsgsgav mnametnlvg 361 sdkyaarfge sivnlgdidn dgfedvaiga pqeddlqgai yiyngradgi sstfsqrieg

[0461]

SEQ ID NO:	GenBank 登记号和描述	序列
		421 lqiskslsmf gqsisgqida dnngyvdvav gafrsdsavl lrtrpvvivd aslshpesvn 481 rtkfdcveng wpsvcidltl cfsykgkevp gyivlfynms ldvnrkaesp prfyfssngt 541 sdvitgsiqv ssreancrth qafmrkdvrd iltpiqieaa yhlgphvisk rsteefpplq 601 pilqqkkekd imkktinfar fcahencsad lqvsakigfl kphenktyla vgsmktlmln 661 vslfnagdda yettlhvklp vglyfikile leekqincev tdnsgvvqld csigyiyvdh 721 lsridisfll dvsslsraee dlsitvhatc eneeemdnlk hsrvtvaipl kyevkltvhg 781 fvnptsfvyg sndenepetc mvekmnltfh vintgnsmap nvsveimvpn sfspqtdklf 841 nildvqtttg echfenyqrv caleqqksam qtlkgivrfl sktdkrllyc ikadphclnf 901 lcnfgkmesg keasvhiqle grpsilemde tsalkfeira tgfpepnprv ielnkdenva 961 hvlleglhhq rpkryftivi issslllgli vlllisyvmw kagffkrqyk silqeenrrd 1021 swsyinsksn dd
28	NP_000880.1 人β7前体的示例性氨基酸序列	1 mvalpmvlvl llvlsrgese ldakipstgd atewrnphls mlgscqpaps cqkcilshps 61 cawckqlnft asgeaearrc arreellarg cpleeleepr gqqevlqdqp lsqgargega 121 tqlapqrvrv tlrpgepqql qvrflraegy pvdlyylmdl sysmkddler vrqlghallv 181 rlqevthsvr igfgsfvdkt vlpfvstvps klrhpcptrl ercqspfsfh hvlsltgdaq 241 aferevgrqs vsgnldspeg gfdailqaal cqeqigwrnv srllvftsdd tfhtagdgkl 301 ggifmpsdgh chldsnglys rstefdypsv gqvaqalsaa niqpifavts aalpvyqels 361 klipksavge lsedssnvvq limdaynsls stvtlehssl ppgvhisyes qcegpekreg 421 kaedrgqcnh vrinqtvtfw vslqathclp ephllrlral gfseelivel htlcdcncsd 481 tqpqaphcsd gqghlqcgvc scapgrlgrl cecsvaelss pdlesgcrap ngtgplcsgk 541 ghcqcgrcsc sgqssghlce cddascerhe gilcggfgrc qcgvchchan rtgracecsg 601 dmdscispeg glcsghgrck cnrcqcldgy ygalcdqcpg cktpcerhrd caecgafrtg 661 platncstac ahtnvtlala pilddgwcke rtldnqlfff lveddargtv vlrvrpqekg 721 adhtqaivlg cvggivavgl glvlayrlsv eiydrreysr fekeqqqlnw kqdsnplyks 781 aitttinprf qeadsptl
29	NM_016602.2 编码人CCR10的示例性核酸序列	1 agagatgggg acggaggcca cagagcaggt ttcctggggc cattactctg gggatgaaga 61 ggacgcatac tcggctgagc cactgccgga gctttgctac aaggccgatg tccaggcctt 121 cagccgggcc ttccaaccca gtgtctccct gaccgtggct gcgctgggtc tggccggcaa 181 tggcctggtc ctggccaccc acctggcagc ccgacgcgca gcgcgctcgc ccacctctgc 241 ccacctgctc cagctggccc tggccgacct cttgctggcc ctgactctgc ccttcgcggc 301 agcaggggct cttcagggct ggagtctggg aagtgccacc tgccgcacca tctctggcct 361 ctactcggcc tccttccacg ccggcttcct cttcctggcc tgtatcagcg ccgaccgcta 421 cgtggccatc gcgcgagcgc tcccagccgg gccgcggccc tccactcccg gccgcgcaca 481 cttggtctcc gtcatcgtgt ggctgctgtc actgctcctg gcgctgcctg cgctgctctt 541 cagccaggat gggcagcggg aaggccaacg acgctgtcgc ctcatcttcc ccgagggcct 601 cacgcagacg gtgaaggggg cgagcgccgt ggcgcaggtg gccctgggct tcgcgctgcc 661 gctgggcgtc atggtagcct gctacgcgct tctgggccgc acgctgctgg ccgccagggg 721 gcccgagcgc cggcgtgcgc tgcgcgtcgt ggtggctctg gtggcggcct tcgtggtgct 781 gcagctgccc tacagcctcg ccctgctgct ggatactgcc gatctactgg ctgcgcgcga 841 gcggagctgc cctgccagca aacgcaagga tgtcgcactg ctggtgacca gcggcttggc 901 cctcgcccgc tgtggcctca atcccgttct ctacgccttc ctgggcctgc gcttccgcca 961 ggacctgcgg aggctgctac ggggtgggag ctgcccctca gggcctcaac cccgccgcgg

[0462]

SEQ ID NO:	GenBank 登记号和描述	序列
		1021 ctgcccccgc cggccccgcc tttcttcctg ctcagctccc acggagaccc acagtctctc 1081 ctgggacaac tagggctgcg aatctagagg agggggcagg ctgagggtcg tgggaaaggg 1141 gagtaggtgg gggaacactg agaaagaggc agggacctaa agggactacc tctgtgcctt 1201 gccacattaa attgataaca tggaaatgag atgcaaccca acaa
30	AF215981.1 编码人 CCR10 的示例性核酸序列	1 agagatgggg acggaggcca cagagcaggt ttcctggggc cattactctg gggatgaaga 61 ggacgcatac tcggctgagc cactgccgga gctttgctac aaggccgatg tccaggcctt 121 cagccgggcc ttccaaccca gtgtctccct gaccgtggct gcgctgggtc tggccggcaa 181 tggcctggtc ctggccaccc acctggcagc ccgacgcgca gcgcgctcgc ccacctctgc 241 ccacctgctc cagctggccc tggccgacct cttgctggcc ctgactctgc ccttcgcggc 301 agcaggggct cttcagggct ggagtctggg aagtgccacc tgccgcacca tctctggcct 361 ctactcggcc tccttccacg ccggcttcct cttcctggcc tgtatcagcg ccgaccgcta 421 cgtggccatc gcgcgagcgc tcccagccgg gccgcggccc tccactcccg gccgcgcaca 481 cttggtctcc gtcatcgtgt ggctgctgtc actgctcctg gcgctgcctg cgctgctctt 541 cagccaggat gggcagcggg aaggccaacg acgctgtcgc ctcatcttcc ccgagggcct 601 cacgcagacg gtgaaggggg cgagcgccgt ggcgcaggtg gccctgggct tcgcgctgcc 661 gctgggcgtc atggtagcct gctacgcgct tctgggccgc acgctgctgg ccgccagggg 721 gcccgagcgc cggcgtgcgc tgcgcgtcgt ggtggctctg gtggcggcct tcgtggtgct 781 gcagctgccc tacagcctcg ccctgctgct ggatactgcc gatctactgg ctgcgcgcga 841 gcggagctgc cctgccagca aacgcaagga tgtcgcactg ctggtgacca gcggcttggc 901 cctcgcccgc tgtggcctca atcccgttct ctacgccttc ctgggcctgc gcttccgcca 961 ggacctgcgg aggctgctac ggggtgggag ctcgccctca gggcctcaac cccgccgcgg 1021 ctgcccccgc cggccccgcc tttcttcctg ctcagctccc acggagaccc acagtctctc 1081 ctgggacaac tagggctgcg aatctagagg agggggcagg ctgagggtcg tgggaaaggg 1141 gagtaggtgg gggaacactg agaaagaggc agggacctaa agggactacc tctgtgcctt 1201 gccacattaa attgataaca tggaaatgaa aaaaaaaaaa aaaa
31	NP_057686.2 人 CCR10 前体的示例性氨基酸序列	1 mgteateqvs wghysgdeed aysaeplpel cykadvqafs rafqpsvslt vaalglagng 61 lvlathlaar raarsptsah llqlaladll laltlpfaaa galqgwslgs atcrtisgly 121 sasfhagflf lacisadryv aiaralpagp rpstpgrahl vsvivwllsl llalpallfs 181 qdgqregqrr crlifpeglt qtvkgasava qvalgfalpl gvmvacyall grtllaargp 241 errralrvvv alvaafvvlq lpyslalld tadllaarer scpaskrkdv allvtsglal 301 arcglnpvly aflglrfrqd lrrllrggsc psgpqprrgc prrprlsscs aptethslsw 361 dn
32	P46092.3 人 CCR10 前体的示例性氨基酸序列	1 mgteateqvs wghysgdeed aysaeplpel cykadvqafs rafqpsvslt vaalglagng 61 lvlathlaar raarsptsah llqlaladll laltlpfaaa galqgwslgs atcrtisgly 121 sasfhagflf lacisadryv aiaralpagp rpstpgrahl vsvivwllsl llalpallfs 181 qdgqregqrr crlifpeglt qtvkgasava qvalgfalpl gvmvacyall grtllaargp 241 errralrvvv alvaafvvlq lpyslalld tadllaarer scpaskrkdv allvtsglal 301 arcglnpvly aflglrfrqd lrrllrggsc psgpqprrgc prrprlsscs aptethslsw 361 dn

[0463]

SEQ ID NO:	GenBank 登记号和描述	序列
33	NM_005201.3 编码人 CCR8 的示例性核酸序列	1 tttgtagtgg gaggatacct ccagagaggc tgctgctcat tgagctgcac tcacatgagg 61 atacagactt tgtgaagaag gaattggcaa cactgaaacc tccagaacaa aggctgtcac 121 taaggtcccg ctgccttgat ggattataca cttgacctca gtgtgacaac agtgaccgac 181 tactactacc ctgatatctt ctcaagcccc tgtgatgcgg aacttattca gacaaatggc 241 aagttgctcc ttgctgtctt ttattgcctc ctgtttgtat tcagtcttct gggaaacagc 301 ctggtcatcc tggtccttgt ggtctgcaag aagctgagga gcatcacaga tgtatacctc 361 ttgaacctgg ccctgtctga cctgcttttt gtcttctcct tcccctttca gacctactat 421 ctgctggacc agtgggtgtt tgggactgta atgtgcaaag tggtgtctgg cttttattac 481 attggcttct acagcagcat gtttttcatc accctcatga gtgtggacag gtacctggct 541 gttgtccatg ccgtgtatgc cctaaaggtg aggacgatca ggatgggcac aacgctgtgc 601 ctggcagtat ggctaaccgc cattatggct accatcccat tgctagtgtt ttaccaagtg 661 gcctctgaag atggtgttct acagtgttat tcattttaca atcaacagac tttgaagtgg 721 aagatcttca ccaacttcaa aatgaacatt ttaggcttgt tgatcccatt caccatcttt 781 atgttctgct acattaaaat cctgcaccag ctgaagaggt gtcaaaacca caacaagacc 841 aaggccatca ggttggtgct cattgtggtc attgcatctt tacttttctg ggtcccattc 901 aacgtggttc ttttcctcac ttccttgcac agtatgcaca tcttggatgg atgtagcata 961 agccaacagc tgacttatgc cacccatgtc acagaaatca tttcctttac tcactgctgt 1021 gtgaaccctg ttatctatgc ttttgttggg gagaagttca agaaacacct ctcagaaata 1081 tttcagaaaa gttgcagcca aatcttcaac tacctaggaa gacaaatgcc tagggagagc 1141 tgtgaaaagt catcatcctg ccagcagcac tcctcccgtt cctccagcgt agactacatt 1201 ttgtgaggat caatgaagac taaatataaa aaacattttc ttgaatggca tgctagtagc 1261 agtgagcaaa ggtgtgggtg tgaaaggttt ccaaaaaaag ttcagcatga aggatgccat 1321 atatgttgtt gccaacactt ggaacacaat gactaaagac atagttgtgc atgcctggca 1381 caacatcaag cctgtgattg tgtttattga tgatgttgaa caagtggtaa ctttaaagga 1441 ttctgtatgc caagtgaaaa aaaaagatgt ctgacctcct tacatat
34	BC107159.1 编码人 CCR8 的示例性核酸序列	1 ctttgtgaag aaggaattgg caacactgaa acctccagaa caaaggctgt cactaaggtc 61 ccgctgcctt gatggattat acacttgacc tcagtgtgac aacagtgacc gactactact 121 accctgatat cttctcaagc ccctgtgatg cggaacttat tcagacaaat ggcaagttgc 181 tccttgctgt cttttattgc ctcctgtttg tattcagtct tctgggaaac agcctggtca 241 tcctggtcct tgtggtctgc aagaagctga ggagcatcac agatgtatac ctcttgaacc 301 tggccctgtc tgacctgctt tttgtcttct ccttcccctt tcagacctac tatctgctgg 361 accagtgggt gtttgggact gtaatgtgca aagtggtgtc tggcttttat tacattggct 421 tctacagcag catgtttttc atcaccctca tgagtgtgga caggtacctg gctgttgtcc 481 atgccgtgta tgccctaaag gtgaggacga tcaggatggg cacaacgctg tgcctggcag 541 tatggctaac cgccattatg gctaccatcc cattgctagt gttttaccaa gtggcctctg 601 aagatggtgt tctacagtgt tattcatttt acaatcaaca gactttgaag tggaagatct 661 tcaccaactt caaaatgaac attttaggct tgttgatccc attcaccatc tttatgttct 721 gctacattaa aatcctgcac cagctgaaga ggtgtcaaaa ccacaacaag accaaggcca 781 tcaggttggt gctcattgtg gtcattgcat ctttactttt ctgggtccca ttcaacgtgg 841 ttcttttcct cacttccttg cacagtatgc acatcttgga tggatgtagc ataagccaac 901 agctgactta tgccacccat gtcacagaaa tcatttcctt tactcactgc tgtgtgaacc 961 ctgttatcta tgcttttgtt ggggagaagt tcaagaaaca cctctcagaa atatttcaga 1021 aaagttgcag ccaaatcttc aactacctag gaagacaaat gcctagggag agctgtgaaa 1081 agtcatcatc ctgccagcag cactcctccc gttcctccag cgtagactac attttgtgag 1141 gatcaatgaa gactaaatat aaaaaacatt ttcttgaatg gcatgctagt agcagtgagc 1201 aaaggtgtgg gtgtgaaagg tttccaaaaa aagttcagca tgaaggatgc cgtgtgtgtt 1261 gttgccaaca cttggaacac gatgactggg gacgtggttg tgcatgcctg gcacaacatc 1321 aagcctgtga ttgtgtttat tgatgatgtt gaacaagtgg tggctttgga ggattctgta 1381 tgccaagtga aaggggagat gtctgacctc cttcatatag

[0464]

SEQ ID NO:	GenBank 登记号和描述	序列
35	NP_005192.1 人 CCR8 前体的示例性氨基酸序列	1 mdytldlsvt tvtdyyypdi fsspcdaeli qtngklllav fycllfvfsl lgnslvilvl 61 vvckklrsit dvyllnlals dllfvfsfpf qtyylldqwv fgtvmckvvs gfyyigfyss 121 mffitlmsvd rylavvhavy alkvrtirmg ttlclavwlt aimatipllv fyqvasedgv 181 lqcysfynqq tlkwkiftnf kmnilgllip ftifmfcyik ilhqlkrcqn hnktkairlv 241 livviasllf wvpfnvvlfl tslhsmhild gcsisqqlty athvteiisf thccvnpviy 301 afvgekfkkh lseifqkscs qifnylgrqm presceksss cqqhssrsss vdyil
36	AAI07160.1 人 CCR8 前体的示例性氨基酸序列	1 mdytldlsvt tvtdyyypdi fsspcdaeli qtngklllav fycllfvfsl lgnslvilvl 61 vvckklrsit dvyllnlals dllfvfsfpf qtyylldqwv fgtvmckvvs gfyyigfyss 121 mffitlmsvd rylavvhavy alkvrtirmg ttlclavwlt aimatipllv fyqvasedgv 181 lqcysfynqq tlkwkiftnf kmnilgllip ftifmfcyik ilhqlkrcqn hnktkairlv 241 livviasllf wvpfnvvlfl tslhsmhild gcsisqqlty athvteiisf thccvnpviy 301 afvgekfkkh lseifqkscs qifnylgrqm presceksss cqqhssrsss vdyil
37	NM_005508.4 编码人 CCR4 的示例性核酸序列	1 gctcacagga agccacgcac ccttgaaagg caccgggtcc ttcttagcat cgtgcttcct 61 gagcaagcct ggcattgcct cacagacctt cctcagagcc gctttcagaa aagcaagctg 121 cttctggttg ggcccagacc tgccttgagg agcctgtaga gttaaaaaat gaaccccacg 181 gatatagcag acaccaccct cgatgaaagc atatacagca attactatct gtatgaaagt 241 atccccaagc cttgcaccaa agaaggcatc aaggcatttg gggagctctt cctgccccca 301 ctgtattcct tggtttttgt atttggtctg cttggaaatt ctgtggtggt tctggtcctg 361 ttcaaataca agcggctcag gtccatgact gatgtgtacc tgctcaacct tgccatctcg 421 gatctgctct tcgtgttttc cctccctttt tggggctact atgcagcaga ccagtgggtt 481 tttgggctag gtctgtgcaa gatgatttcc tggatgtact tggtgggctt ttacagtggc 541 atattctttg tcatgctcat gagcattgat agatacctgg caattgtgca cgcggtgttt 601 tccttgaggg caaggacctt gacttatggg gtcatcacca gtttggctac atggtcagtg 661 gctgtgttcg cctcccttcc tggctttctg ttcagcactt gttatactga gcgcaaccat 721 acctactgca aaaccaagta ctctctcaac tccacgacgt ggaaggttct cagctccctg 781 gaaatcaaca ttctcggatt ggtgatcccc ttagggatca tgctgttttg ctactccatg 841 atcatcagga ccttgcagca ttgtaaaaat gagaagaaga acaaggcggt gaagatgatc 901 tttgccgtgg tggtcctctt ccttgggttc tggacacctt acaacatagt gctcttccta 961 gagaccctgg tggagctaga agtccttcag gactgcacct ttgaaagata cttggactat 1021 gccatccagg ccacagaaac tctggctttt gttcactgct gccttaatcc catcatctac 1081 ttttttctgg gggagaaatt tcgcaagtac atcctacagc tcttcaaaac ctgcaggggc 1141 ctttttgtgc tctgccaata ctgtgggctc ctccaaattt actctgctga cacccccagc 1201 tcatcttaca cgcagtccac catggatcat gatctccatg atgctctgta gaaaaatgaa 1261 atggtgaaat gcagagtcaa tgaactttcc acattcagag cttacttaaa attgtatttt 1321 agtaagagat tcctgagcca gtgtcaggag gaaggcttac acccacagtg gaaagacagc 1381 ttctcatcct gcaggcagct ttttctctcc cactagacaa gtccagcctg gcaagggttc 1441 acctgggctg aggcatcctt cctcacacca ggcttgcctg caggcatgag tcagtctgat 1501 gagaactctg agcagtgctt gaatgaagtt gtaggtaata ttgcaaggca aagactattc 1561 ccttctaacc tgaactgatg ggtttctcca gagggaattg cagagtactg gctgatggag 1621 taaatcgcta ccttttgctg tggcaaatgg gccctct
38	P51679.1 人 CCR4 前体的示例性氨基酸序列	1 mnptdiadtt ldesiysnyy lyesipkpct kegikafgel flpplyslvf vfgllgnsvv 61 vlvlfkykrl rsmtdvylln laisdllfvf slpfwgyyaa dqwvfglglc kmiswmylvg 121 fysgiffvml msidrylaiv havfslrart ltygvitsla twsvavfasl pgflfstcyt 181 ernhtycktk yslnsttwkv lssleinilg lviplgimlf cysmiirtlq hcknekknka 241 vkmifavvvl flgfwtpyni vlfletlvel evlqdctfer yldyaiqate tlafvhccln 301 piiyfflgek frkyilqlfk tcrglfvlcq ycgllqiysa dtpsssytqs tmdhdlhdal

[0465]

SEQ ID NO:	GenBank 登记号和描述	序列
39	NM_001206609.1 编码人CLA的示例性核酸序列	1 aatcatccga gaaccttgga gggtggacag tgccccttttacagatgaga aaactgaggc 61 ttgaagggga gaagcagctg cctctggcgg catggcttct ggctgcagga tgcccatgga 121 gttcgtggtg accctaggcc tgtgtctcgg cttcctttgc tgaacttgaa caggaagatg 181 gcagtggggg ccagtggtct agaaggagat aagatggctg gtgccatgcc tctgcaactc 241 ctcctgttgc tgatcctact gggccctggc aacagcttgc agctgtggga cacctgggca 301 gatgaagccg agaaagcctt gggtcccctg cttgcccggg accggagaca ggccaccgaa 361 tatgagtacc tagattatga tttcctgcca gaaacggagc ctccagaaat gctgaggaac 421 agcactgaca ccactcctct gactgggcct ggaacccctg agtctaccac tgtggagcct 481 gctgcaaggc gttctactgg cctggatgca ggaggggcag tcacagagct gaccacggag 541 ctggccaaca tggggaacct gtccacggat tcagcagcta tggagataca gaccactcaa 601 ccagcagcca cggaggcaca gaccactcaa ccagtgccca cggaggcaca gaccactcca 661 ctggcagcca cagaggcaca gacaactcga ctgacggcca cggaggcaca gaccactcca 721 ctggcagcca cagaggcaca gaccactcca ccagcagcca cggaagcaca gaccactcaa 781 cccacaggcc tggaggcaca gaccactgca ccagcagcca tggaggcaca gaccactgca 841 ccagcagcca tggaagcaca gaccactcca ccagcagcca tggaggcaca gaccactcaa 901 accacagcca tggaggcaca gaccactgca ccagaagcca cggaggcaca gaccactcaa 961 cccacagcca cggaggcaca gaccactcca ctggcagcca tggaggccct gtccacagaa 1021 cccagtgcca cagaggccct gtccatggaa cctactacca aaagaggtct gttcataccc 1081 ttttctgtgt cctctgttac tcacaagggc attcccatgg cagccagcaa tttgtccgtc 1141 aactacccag tgggggcccc agaccacatc tctgtgaagc agtgcctgct ggccatccta 1201 atcttggcgc tggtggccac tatcttcttc gtgtgcactg tggtgctggc ggtccgcctc 1261 tcccgcaagg gccacatgta ccccgtgcgt aattactccc ccaccgagat ggtctgcatc 1321 tcatccctgt tgcctgatgg gggtgagggg ccctctgcca cagccaatgg gggcctgtcc 1381 aaggccaaga gcccgggcct gacgccagag cccagggagg accgtgaggg ggatgacctc 1441 accctgcaca gcttcctccc ttagctcact ctgccatctg ttttggcaag accccacctc 1501 cacgggctct cctgggccac ccctgagtgc ccagacccca ttccacagct ctgggcttcc 1561 tcggagaccc ctggggatgg ggatcttcag ggaaggaact ctggccaccc aaacaggaca 1621 agagcagcct ggggccaagc agacgggcaa gtggagccac ctctttcctc cctccgcgga 1681 tgaagcccag ccacatttca gccgaggtcc aaggcaggag gccatttact tgagacagat 1741 tctctccttt ttcctgtccc ccatcttctc tgggtccctc taacatctcc catggctctc 1801 cccgcttctc ctggtcactg gagtctcctc cccatgtacc caaggaagat ggagctcccc 1861 catcccacac gcactgcact gccattgtct tttggttgcc atggtcacca aacaggaagt 1921 ggacattcta agggaggagt actgaagagt gacggacttc tgaggctgtt tcctgctgct 1981 cctctgactt ggggcagctt gggtcttctt gggcacctct ctgggaaaac ccagggtgag 2041 gttcagcctg tgagggctgg gatgggtttc gtgggcccaa gggcagacct ttctttggga 2101 ctgtgtggac caaggagctt ccatctagtg acaagtgacc cccagctatc gcctcttgcc 2161 ttcccctgtg gccactttcc agggtggact ctgtcttgtt cactgcagta tcccaactgc 2221 aggtccagtg caggcaataa atatgtgatg gacaaacgat agcggaatcc ttcaaggttt 2281 caaggctgtc tccttcaggc agccttcccg gaattctcca tccctcagtg caggatgggg

[0466]

SEQ ID NO:	GenBank 登记号和描述	序列
		2341 gctggtcctc agctgtctgc cctcagcccc tggcccccca ggaagcctct ttcatgggct 2401 gttaggttga cttcagtttt gcctcttgga caacaggggg tcttgtacat ccttgggtga 2461 ccaggaaaag ttcaggctat ggggggccaa agggagggct gccccttccc caccagtgac 2521 cactttattc cacttcctcc attacccagt tttggcccac agagtttggt ccccccaaa 2581 cctcggacca atatccctct aaacatcaat ctatcctcct gttaaagaaa aaaaaaaa
40	NM_003006.4 编码人CLA的示例性核酸序列	1 acacacagcc attgggggtt gctcggatcc gggactgccg caggggtgc cacagcagtg 61 cctggcagcg tgggctggga ccttgtcact aaagcagaga agccacttct tctgggccca 121 cgaggcagct gtcccatgct ctgctgagca cggtggtgcc atgcctctgc aactcctcct 181 gttgctgatc ctactgggcc ctggcaacag cttgcagctg tgggacacct gggcagatga 241 agccgagaaa gccttgggtc ccctgcttgc ccgggaccgg agacaggcca ccgaatatga 301 gtacctagat tatgatttcc tgccagaaac ggagcctcca gaaatgctga ggaacagcac 361 tgacaccact cctctgactg ggcctggaac ccctgagtct accactgtgg agcctgctgc 421 aaggcgttct actggcctgg atgcaggagg ggcagtcaca gagctgacca cggagctggc 481 caacatgggg aacctgtcca cggattcagc agctatggag atacagacca ctcaaccagc 541 agccacggag gcacagacca ctcaaccagt gcccacggag gcacagacca ctccactggc 601 agccacagag gcacagacaa ctcgactgac ggccacggag gcacagacca ctccactggc 661 agccacagag gcacagacca ctccaccagc agccacggaa gcacagacca ctcaacccac 721 aggcctggag gcacagacca ctgcaccagc agccatggag gcacagacca ctgcaccagc 781 agccatggaa gcacagacca ctccaccagc agccatggag gcacagacca ctcaaaccac 841 agccatggag gcacagacca ctgcaccaga agccacggag gcacagacca ctcaacccac 901 agccacggag gcacagacca ctccactggc agccatggag gccctgtcca cagaacccag 961 tgccacagag gccctgtcca tggaacctac taccaaaaga ggtctgttca tacccttttc 1021 tgtgtcctct gttactcaca agggcattcc catggcagcc agcaatttgt ccgtcaacta 1081 cccagtgggg gccccagacc acatctctgt gaagcagtgc ctgctggcca tcctaatctt 1141 ggcgctggtg gccactatct tcttcgtgtg cactgtggtg ctggcggtcc gcctctcccg 1201 caagggccac atgtaccccg tgcgtaatta ctcccccacc gagatggtct gcatctcatc 1261 cctgttgcct gatgggggtg aggggccctc tgccacagcc aatgggggcc tgtccaaggc 1321 caagagcccg ggcctgacgc cagagcccag ggaggaccgt gaggggatg acctcaccct 1381 gcacagcttc ctcccttagc tcactctgcc atctgttttg gcaagacccc acctccacgg 1441 gctctcctgg gccacccctg agtgcccaga ccccattcca cagctctggg cttcctcgga 1501 gaccctggg gatggggatc ttcagggaag gaactctggc cacccaaaca ggacaagagc 1561 agcctggggc caagcagacg ggcaagtgga gccacctctt tcctccctcc gcggatgaag 1621 cccagccaca tttcagccga ggtccaaggc aggaggccat ttacttgaga cagattctct 1681 ccttttcct gtcccccatc ttctctgggt ccctctaaca tctcccatgg ctctccccgc 1741 ttctcctggt cactggagtc tcctccccat gtacccaagg aagatggagc tcccccatcc 1801 cacacgcact gcactgccat tgtcttttgg ttgccatggt caccaaacag gaagtggaca

[0467]

SEQ ID NO:	GenBank 登记号和描述	序列
		1861 ttctaaggga ggagtactga agagtgacgg acttctgagg ctgtttcctg ctgctcctct 1921 gacttggggc agcttgggtc ttcttgggca cctctctggg aaaacccagg gtgaggttca 1981 gcctgtgagg gctgggatgg gtttcgtggg cccaagggca gacctttctt tgggactgtg 2041 tggaccaagg agcttccatc tagtgacaag tgacccccag ctatcgcctc ttgccttccc 2101 ctgtggccac tttccagggt ggactctgtc ttgttcactg cagtatccca actgcaggtc 2161 cagtgcaggc aataaatatg tgatggacaa acgatagcgg aatccttcaa ggtttcaagg 2221 ctgtctcctt caggcagcct tcccggaatt ctccatccct cagtgcagga tgggggctgg 2281 tcctcagctg tctgccctca gcccctggcc ccccaggaag cctctttcat gggctgttag 2341 gttgacttca gttttgcctc ttggacaaca gggggtcttg tacatccttg ggtgaccagg 2401 aaaagttcag gctatggggg gccaaaggga gggctgcccc ttccccacca gtgaccactt 2461 tattccactt cctccattac ccagttttgg cccacagagt ttggtccccc ccaaacctcg 2521 gaccaatatc cctctaaaca tcaatctatc ctcctgttaa agaaaaaaaa aaa
41	NP_001193538.1 人CLA前体的示例性氨基酸序列	1 mavgasgleg dkmagamplq lllllillgp gnslqlwdtw adeaekalgp llardrrqat 61 eyeyldydfl peteppemlr nstdttpltg pgtpesttve paarrstgld aggavteltt 121 elanmgnlst dsaameiqtt qpaateaqtt qpvpteaqtt plaateaqtt rltateaqtt 181 plaateaqtt ppaateaqtt qptgleaqtt apaameaqtt apaameaqtt ppaameaqtt 241 qttameaqtt apeateaqtt qptateaqtt plaamealst epsatealsm epttkrglfi 301 pfsvssvthk gipmaasnls vnypvgapdh isvkqcllai lilalvatif fvctvvlavr 361 lsrkghmypv rnysptemvc issllpdgge gpsatanggl skakspgltp epredregdd 421 ltlhsflp
42	NP_002997.2 人CLA前体的示例性氨基酸序列	1 mplqllllli llgpgnslql wdtwadeaek algpllardr rqateyeyld ydflpetepp 61 emlrnstdtt pltgpgtpes ttvepaarrs tgldaggavt elttelanmg nlstdsaame 121 iqttqpaate aqttqpvpte aqttplaate aqttrltate aqttplaate aqttppaate 181 aqttqptgle aqttapaame aqttapaame aqttppaame aqttqttame aqttapeate 241 aqttqptate aqttplaame al 步骤 sate alsmepttkr glfipfsvss vthkgipmaa 301 snlsvnypvg apdhisvkqc llaililalv atiffvctvv lavrlsrkgh mypvrnyspt 361 emvcissllp dggegpsata ngglskaksp gltpepredr egddltlhsf lp

[0468] 5.3.6.用于产生CAR和/或归巢受体的多核苷酸

[0469] 本文描述了编码嵌合受体和归巢受体的多核苷酸序列(即核酸序列)。多核苷酸可包含于适于免疫细胞(例如NK细胞)转化的任何多核苷酸载体内。例如可使用含有编码第一和第二多肽(例如嵌合受体)的多核苷酸的合成载体、慢病毒或反转录病毒载体、自主复制质粒、病毒(例如反转录病毒、慢病毒、腺病毒或疱疹病毒)等转化NK细胞。适于NK细胞转化的慢病毒载体包括但不限于例如描述于美国专利号5,994,136、6,165,782、6,428,953、7,083,981和7,250,299中的慢病毒载体,所述专利的公开内容通过引用以其整体并入本文。适于NK细胞转化的HIV载体包括但不限于例如描述于美国专利号5,665,577的载体,其公开内容通过引用以其整体并入本文。

[0470] 可用于例如在NK细胞内产生本文所述多肽的核酸包括DNA、RNA或核酸类似物。核酸类似物可在碱基部分、糖部分或磷酸骨架上被修饰,并可包括脱氧尿苷取代脱氧胸苷、5-甲基-2'-脱氧胞苷或5-溴-2'-脱氧胞苷取代脱氧胞苷。糖部分的修饰可包括核糖2'羟基的修饰以形成2'-O-甲基或2'-O-烯丙基糖。可对脱氧核糖磷酸骨架进行修饰以产生吗啉代核酸(其中各碱基部分与6元吗啉代环连接)或肽核酸(其中脱氧磷酸骨架被假肽骨架置换,并保留4种碱基)。参见例如Summerton和Weller(1997)Antisense Nucleic Acid Drug

Dev.7:187-195;以及Hyrup等(1996)Bioorgan.Med.Chain.4:5-23。另外,脱氧磷酸骨架可被例如硫代磷酸酯或二硫代磷酸酯骨架、氨基磷酸酯或烷基磷酸三酯骨架置换。

[0471] 可将编码本文所述多肽的核酸作为载体(例如表达载体)的一部分导入宿主细胞。另外,本文所述多肽可通过用编码这类多肽的核酸转染宿主细胞来产生,且这类核酸可以是载体的一部分。在一个具体的实施方案中,载体是能够指导编码本文所述多肽的核酸表达的表达载体。表达载体的非限制性实例包括但不限于质粒和病毒载体,例如复制缺陷型反转录病毒、腺病毒、腺伴随病毒、新城疫病毒、痘病毒和杆状病毒。可采用标准分子生物学技术将编码本文所述多肽的核酸引入表达载体中。

[0472] 表达载体包含编码本文所述多肽的核酸,其呈适于在宿主细胞或非人对象中表达核酸的形式。在一个具体的实施方案中,表达载体包括根据待用于表达的宿主细胞选择的一个或多个调节序列,其与待表达的核酸有效连接。在表达载体内,"有效连接的"欲指目标核酸以允许核酸表达(例如在体外转录/翻译系统中或在当载体导入宿主细胞时在宿主细胞中)的方式与调节序列连接。调节序列包括启动子、增强子和其它表达控制元件(例如聚腺苷酸化信号)。调节序列包括在许多类型的宿主细胞中指导核酸组成型表达的调节序列、指导核酸只在某些宿主细胞中表达的调节序列(例如组织特异性调节序列)和在用特殊成分刺激时指导核酸表达的调节序列(例如诱导型调节序列)。本领域技术人员应认识到,表达载体的设计可取决于诸如例如待转化的宿主细胞的选择、所需蛋白质表达的水平等因素。

[0473] 可通过常规转化或转染技术将表达载体导入宿主细胞中。这类技术包括但不限于磷酸钙或氯化钙共沉淀、DEAE-葡聚糖介导的转染、脂转染和电穿孔。用于转化或转染宿主细胞的合适方法可见Sambrook等,1989,Molecular Cloning-A Laboratory Manual,2nd Edition,Cold Spring Harbor Press,New York和其它实验室手册。在某些实施方案中,宿主细胞用含有编码本文所述多肽的核酸的表达载体瞬时转染。在其它实施方案中,宿主细胞用含有编码本文所述多肽的核酸的表达载体稳定转染。

[0474] 含有任何多核苷酸的细胞可使用一个或多个选择标记选择。

[0475] 5.4.治疗血液病症或实体瘤的方法

[0476] 本文提供使用上文所述NK细胞或遗传修饰的NK细胞(例如包含CAR和/或归巢受体的NK细胞)治疗血液病症或实体瘤的方法。

[0477] 5.4.1.NK联合疗法

[0478] 一方面,本文提供治疗有需要的对象的血液病症或实体瘤的方法,所述方法包括:(a)给予所述对象分离的自然杀伤(NK)细胞群或其药物组合物,或分离的遗传修饰的NK细胞(例如包含CAR和/或归巢受体的NK细胞)群或其药物组合物;和(b)给予所述对象第二药剂或其药物组合物。第二药剂可以是可用于治疗血液病症或实体瘤的任何药学上可接受的药剂,包括但不限于抗体(例如单克隆抗体)、双特异性杀伤细胞衔接器(BiKE)、抗炎药、免疫调节剂(例如第5.2.7.1节所述免疫调节化合物)、细胞毒性剂、癌症疫苗、化疗剂、HDAC抑制剂或siRNA。

[0479] 5.4.1.1.NK与抗体的组合

[0480] 在某些实施方案中,第二药剂是抗体或其抗原结合片段。

[0481] 本文所用术语"抗体"和"免疫球蛋白"和"Ig"是技术术语,在本文可互换使用,是

指特异性结合抗原的抗原结合部位的分子。

[0482] 抗体可包括例如单克隆抗体、重组产生的抗体、单特异性抗体、多特异性抗体(包括双特异性抗体)、人抗体、人源化抗体例如复合人抗体或去免疫化抗体、鼠抗体(例如小鼠或大鼠抗体)、嵌合抗体、合成抗体和包含2条重链和2条轻链分子的四聚体抗体。在具体的实施方案中,抗体可包括但不限于抗体轻链单体、抗体重链单体、抗体轻链二聚体、抗体重链二聚体、抗体轻链-抗体重链对、胞内抗体(intrabody)、异型缀合抗体(heteroconjugate antibody)、单结构域抗体和单价抗体。在一个具体的实施方案中,抗体可包括抗原结合片段或表位结合片段,例如但不限于单链抗体或单链Fv(scFv)(例如包括单特异性、双特异性等)、骆驼化抗体、亲和体(affybodies)、Fab片段、F(ab')片段、$F(ab')_2$片段和二硫键连接Fv(sdFv)。在具体的实施方案中,所述抗体是指单克隆抗体。

[0483] 抗体可以是免疫球蛋白分子的任何类型(例如IgG、IgE、IgM、IgD、IgA或IgY)、任何类别(例如IgG_1、IgG_2、IgG_3、IgG_4、IgA_1或IgA_2)或任何亚类(例如IgG_{2a}或IgG_{2b})。在某些实施方案中,本文所述抗体是IgG抗体或类别(例如人IgG_1、IgG_2或IgG_4)或其亚类。在某些实施方案中,本文所述抗体是IgG_2抗体(例如人IgG_2)或其亚类(例如人IgG_{2a}或人IgG_{2b}或其混合物)。在某些实施方案中,本文所述抗体是IgG_1抗体(例如人IgG_1)或其亚类。在某些实施方案中,IgG_1所述抗体在恒定区包含一个或多个氨基酸取代和/或缺失。

[0484] 本文所用术语"单克隆抗体"是众所周知的技术术语,是指抗体获自一群均质或基本均质的抗体。术语"单克隆"不限于用于制备抗体的任何具体方法。一般而言,单克隆抗体群可通过细胞、细胞群或细胞系产生。在具体的实施方案中,本文所用的"单克隆抗体"是由单细胞或单一细胞系产生的抗体,其中抗体与表位免疫特异性结合,按例如通过ELISA或本领域已知的其它抗原结合或免疫结合测定法测定。在具体的实施方案中,单克隆抗体可以是嵌合抗体或人源化抗体。在某些实施方案中,单克隆抗体是单价抗体或多价(例如二价)抗体。

[0485] 在具体的实施方案中,抗体或其抗原结合片段与肿瘤相关抗原(TAA)特异性结合,这描述于第5.3.2节。在又一个具体的实施方案中,抗体或其抗原结合片段与CS-1结合。在一个更具体的实施方案中,抗体或其抗原结合片段是elotuzumab或其抗原结合片段。在又一个具体的实施方案中,抗体或其抗原结合片段与CD20结合。

[0486] 在具体的实施方案中,抗体或其抗原结合片与肿瘤微环境相关抗原(TMAA)段特异性结合,这描述于第5.3.2节。

[0487] 在具体的实施方案中,抗体或其抗原结合片段与免疫检查点蛋白特异性结合并拮抗其活性。在更具体的实施方案中,免疫检查点蛋白是CTLA-4、PD-1、PD-L1、PD-L2或LAG-3。在更具体的实施方案中,免疫检查点蛋白是BTLA、KIR、TIM-3、A2aR、B7-H3或B7-H4。在其它具体的实施方案中,抗体或其抗原结合片段与共刺激信号转导蛋白特异性结合,并拮抗其活性。在更具体的实施方案中,共刺激信号转导蛋白是ICOS、CD28、4-1BB、OX40、CD27或CD40。

[0488] 5.4.1.2.NK与双特异性杀伤细胞衔接器组合

[0489] 在某些实施方案中,第二药剂是双特异性杀伤细胞衔接器(BiKE)。

[0490] BiKE是含有2个单链可变片段(scFv)并特异性占用靶细胞(例如肿瘤细胞或感染细胞)和NK细胞两者以介导靶细胞杀伤的试剂。它们可用来使靶细胞(例如肿瘤细胞或感染

细胞)与NK细胞共定位,从而引发NK细胞介导的依赖抗体的细胞毒性(ADCC)。可通过本领域已知的任何方法,例如描述于Gleanson,M.K.等,Mol Cancer Ther,11:2674-2684(2012);Vallera,D.A.等,Cancer Biother Radiopharm,28:274-282(2013);Wiernik,A.等,Clin Cancer Res,19:3844-3855(2013);Reiners,K.S.等,Mol Ther,21:895-903(2013);Singer,H.等,J Immunother,33:599-608(2010);或Gleason,M.K.等,Blood,123:3016-3026(2014)的方法产生BiKE。BiKE的一个scFv与靶细胞(例如肿瘤细胞或感染细胞)表面上的抗原特异性结合,另一个scFv与NK细胞上的受体(例如Fc受体,例如CD16)特异性结合。

[0491] 在具体的实施方案中,BiKE包含与TAA特异性结合的第一scFv,这描述于第5.3.2节。在其它具体的实施方案中,BiKE包含与CD16特异性结合的第二scFv。

[0492] 5.4.1.3.其它抗癌药与NK组合

[0493] 可作为第二药剂给予的其它抗癌药是本领域众所周知的,包括抗炎药、免疫调节剂、细胞毒性剂、癌症疫苗、化疗剂、HDAC抑制剂和siRNA。除了采用本文所述方法产生的NK细胞和可选的灌注液、灌注液细胞、采用本文所述方法产生的NK细胞之外的自然杀伤细胞以外,还可给予患有癌症的个体(例如有肿瘤细胞的个体)的具体抗癌药包括但不限于:阿西维辛、阿柔比星、盐酸阿考达唑、阿克罗宁、阿多来新、阿霉素、adrucil、阿地白介素、六甲蜜胺、安波霉素、醋酸阿美蒽醌、安吖啶、阿那曲唑、安曲霉素、天冬酰胺酶(例如来自Erwinia chrysan、Erwinaze)、曲林菌素、阿瓦斯丁(贝伐单抗)、阿扎胞苷、阿扎替派、阿佐霉素、巴马司他、苯佐替派、比卡鲁胺、盐酸比生群、二甲磺酸双奈法德、比折来新、硫酸博莱霉素、布喹那钠、溴匹立明、白消安、放线菌素C、卡鲁睾酮、卡醋胺、卡贝替姆、卡铂、卡莫司汀、盐酸卡柔比星、卡折来新、西地芬戈、塞来考昔(COX-2抑制剂)、CC-122、CC-486(口服阿扎胞苷(azacididine))、Cerubidine、苯丁酸氮芥、西罗霉素、顺铂、克拉屈滨、甲磺酸克立那托、环磷酰胺、阿糖胞苷、达卡巴嗪、放线菌素D、盐酸柔红霉素、地西他滨、右奥马铂、地扎呱宁、甲磺酸地扎呱宁、地吖醌、多西他赛、多柔比星、盐酸多柔比星、屈洛昔芬、枸橼酸屈洛昔芬、丙酸屈他雄酮、达佐霉素、依达曲沙、盐酸依氟鸟氨酸(盐酸eflomithine)、依沙芦星、Elspar、恩洛铂、恩普氨酯、依匹哌啶、盐酸表柔比星、厄布洛唑、盐酸依索比星、雌莫司汀、雌莫司汀磷酸钠、依他硝唑、依托泊苷、磷酸依托泊苷、Etopophos、etoprine、盐酸法倔唑、法扎拉滨、芬维A胺、氟尿苷、磷酸氟达拉滨、氟尿嘧啶、氟西他滨、磷喹酮、福司曲星钠、吉西他滨、盐酸吉西他滨、羟基脲、Idamycin、盐酸伊达比星、异环磷酰胺、伊莫福新、异丙铂、伊立替康、盐酸伊立替康、醋酸兰瑞肽、来那度胺、来曲唑、醋酸亮丙瑞林、盐酸利阿唑、洛美曲索钠、洛莫司汀、盐酸洛索蒽醌、马索罗酚、美登素、盐酸氮芥、醋酸甲地孕酮、醋酸美仑孕酮、美法仑、美诺立尔、巯基嘌呤、甲氨蝶呤、甲氨蝶呤钠、氯苯氨啶、美妥替哌、米丁度胺、米托卡星、丝裂红素、米托洁林、米托马星、丝裂霉素、米托司培、米托坦、盐酸米托蒽醌、麦考酚酸、诺考达唑、诺拉霉素、奥马铂、奥昔舒仑、紫杉醇、培门冬酶、培利霉素、奈莫司汀、硫酸培洛霉素、培磷酰胺、哌泊溴烷、哌泊舒凡、盐酸吡罗蒽醌、普卡霉素、普洛美坦、泊马度胺、卟吩姆钠、紫菜霉素、泼尼莫司汀、盐酸丙卡巴肼、Proleukin、巯基嘌呤、嘌罗霉素、盐酸嘌罗霉素、吡唑呋林、Rheumatrex、利波腺苷、沙芬戈、盐酸沙芬戈、司莫司汀、辛曲秦、磷乙酰天冬氨酸钠、司帕霉素、盐酸锗螺胺、螺莫司汀、螺铂、链黑霉素、链佐星、磺氯苯脲、Tabloid、他利霉素、替可加兰钠、泰素帝、替加氟、盐酸替洛蒽醌、替莫泊芬、替尼泊苷、替罗昔隆、睾内酯、沙利度胺、thiamiprine、硫鸟嘌呤、塞替派、噻唑呋林、替拉扎明、拓扑杀、枸

橡酸托瑞米芬、醋酸曲托龙、Trexall、磷酸曲西立滨、三甲曲沙、葡糖醛酸三甲曲沙、曲普瑞林、盐酸妥布氯唑、乌拉莫司汀、乌瑞替派、伐普肽、维替泊芬、硫酸长春碱、硫酸长春新碱、长春地辛、硫酸长春地辛、硫酸长春匹定、硫酸长春甘酯、硫酸长春罗辛、酒石酸长春瑞滨、硫酸长春罗定、硫酸长春利定、伏氯唑、折尼铂、净司他丁和佐柔比星盐酸。

[0494] 其它抗癌药包括但不限于:20-epi-1,25-二羟基维生素D3;5-乙炔基尿嘧啶;阿比特龙;阿柔比星;酰夫文;adecypenol;阿多来新;阿地白介素;ALL-TK拮抗剂;六甲蜜胺;氨莫司汀;amidox;氨磷汀;氨基酮戊酸;氨柔比星;安吖啶;阿那格雷;阿那曲唑;穿心莲内酯;血管生成抑制剂;拮抗剂D;拮抗剂G;antarelix;抗背部化形态发生蛋白-1;前列腺癌抗雄激素药;抗雌激素药;抗瘤酮;反义寡核苷酸;阿非迪霉素甘氨酸盐;细胞凋亡基因调节剂;细胞凋亡调节剂;脱嘌呤核酸;ara-CDP-DL-PTBA;精氨酸脱氨酶;asulacrine;阿他美坦;阿莫司汀;axinastatin 1;axinastatin 2;axinastatin 3;阿扎司琼;阿扎毒素;重氮酪氨酸;巴卡丁III衍生物;balanol;巴马司他;BCR/ABL拮抗剂;benzochlorins;苯甲酰星形孢菌素;β内酰胺衍生物;β-alethine;亚阿克拉霉素B;桦木酸;bFGF抑制剂;比卡鲁胺;比生群;bisaziridinylspermine;双奈法德;bistratene A;比折来新;breflate;溴匹立明;布度钛;丁硫氨酸亚砜亚胺;卡泊三醇;抑激酶素C;camptosar(亦称Campto;伊立替康);喜树碱衍生物;卡培他滨;甲酰胺-氨基-三唑;羧基酰胺基三唑;CaRest M3;CARN 700;软骨衍生抑制剂;卡折来新;酪蛋白激酶抑制剂(ICOS);粟精胺;天蚕素B;西曲瑞克;chlorlns;氯喹喔啉磺酰胺(chloroquinoxaline sulfonamide);西卡前列素;顺式-吖啉;克拉屈滨;氯米芬类似物;克霉唑;collismycin A;collismycin B;康普瑞汀A4;康普瑞汀类似物;conagenin;crambescidin 816;克立那托;念珠藻环肽8;念珠藻环肽A衍生物;库拉素A;环戊蒽醌;cycloplatam;cypemycin;阿糖胞苷十八烷基磷酸钠(cytarabine ocfosfate);溶细胞因子;细胞生长抑素;达昔单抗;地西他滨;脱氢膜海鞘素B;地洛瑞林;地塞米松;右异环磷酰胺;右雷佐生;右维拉帕米;地吖醌;膜海鞘素B;didox;二乙基去甲精胺;二氢-5-氮杂胞苷;9-二氢泰素;dioxamycin;二苯基螺莫司汀;多西他赛;二十二醇;多拉司琼;去氧氟尿苷;多柔比星;屈洛昔芬;屈大麻酚;倍癌霉素SA;依布硒;依考莫司汀;依地福新;依决洛单抗;依氟鸟氨酸;榄香烯;乙嗪替氟;表柔比星;依立雄胺;雌莫司汀类似物;雌激素激动剂;雌激素拮抗剂;依他硝唑;依托泊苷磷酸;依西美坦;法倔唑;法扎拉滨;芬维A胺;非格司亭;非那雄胺;黄酮吡多;氟卓斯汀;fluasterone;氟达拉滨(例如Fludara);盐酸氟代柔红霉素(fluorodaunorunicin hydrochloride);福酚美克;福美坦;福司曲星;福莫司汀;钆替沙林;硝酸镓;加洛他滨;加尼瑞克;明胶酶抑制剂;吉西他滨;谷胱甘肽抑制剂;庚二醇二氨基磺酸酯;调蛋白;六亚甲基双乙酰胺;金丝桃素;伊班膦酸;伊达比星;艾多昔芬;伊决孟酮;伊莫福新;伊洛马司他;伊马替尼(例如GLEEVEC®);咪喹莫德;免疫刺激肽;胰岛素样生长因子-1受体抑制剂;干扰素激动剂;干扰素;白介素;碘苄胍;碘多柔比星(iododoxorubicin);4-依波米醇;伊罗普拉;伊索拉定;isobengazole;异高软海绵素B;伊他司琼;jasplakinolide;海蛞蝓提取物(kahalalide F);三醋酸层状素N;兰瑞肽;leinamycin;来格司亭;硫酸香菇多糖;leptolstatin;来曲唑;白血病抑制因子;白细胞α干扰素;亮丙瑞林+雌激素+黄体酮;亮丙瑞林;左旋咪唑;利阿唑;线性聚胺类似物;亲脂性二糖肽;亲脂性铂化合物;lissoclinamide7;洛铂;蚯蚓磷脂;洛美曲索;氯尼达明;洛索蒽醌;洛索立宾;勒托替康;替沙林镥;lysofylline;裂解肽;美坦新;制甘糖酶素A;马立马司他;

马索罗酚;乳腺丝抑蛋白;溶基质蛋白抑制剂;基质基质蛋白酶抑制剂;美诺立尔;merbarone;美替瑞林;甲硫氨酸酶;甲氧氯普胺;MIF抑制剂;米非司酮;米替福新;米立司亭;米托胍腙;二溴卫矛醇;丝裂霉素类似物;米托萘胺;分裂毒素成纤维细胞生长因子-肥皂草蛋白;米托蒽醌;莫法罗汀;莫拉司亭;抗EGFR抗体(例如Erbitux(西妥昔单抗));抗CD19抗体;抗CD20抗体(例如利妥昔单抗);抗二唾液酸神经节苷脂(GD2)抗体(例如单克隆抗体3F8或ch14>18);抗ErbB2抗体(例如赫赛汀);人绒促性素;单磷酰脂质A+分枝杆菌细胞壁sk;莫哌达醇;氮芥抗癌药;mycaperoxide B;分枝杆菌细胞壁提取物;myriaporone;N-乙酰地那林;N-取代的苯甲酰胺;那法瑞林;nagrestip;纳洛酮+喷他佐辛;napavin;naphterpin;那托司亭;奈达铂;奈莫柔比星;奈立膦酸;尼鲁米特;nisamycin;一氧化氮调节剂;硝基氧抗氧化剂;nitrullyn;奥利美生(GENASENSE®);O^6-苄基鸟嘌呤;奥曲肽;okicenone;寡核苷酸;奥那司酮;昂丹司琼;昂丹司琼;oracin;口服细胞因子诱导剂;奥马铂;奥沙特隆;奥沙利铂(例如Floxatin);oxaunomycin;紫杉醇;紫杉醇类似物;紫杉醇衍生物;palauamine;棕榈酰利索新(palmitoylrhizoxin);帕米膦酸;人参三醇;帕诺米芬;副球菌素;帕折普汀;培门冬酶;培得星;戊聚硫钠;喷司他丁;pentrozole;全氟溴烷;培磷酰胺;紫苏子醇;phenazinomycin;苯乙酸盐;磷酸酶抑制剂;溶血性链球菌制剂;盐酸毛果芸香碱;吡柔比星;吡曲克辛;placetin A;placetin B;纤溶酶原激活物抑制剂;铂络合物;铂化合物;铂三胺络合物;卟吩姆钠;紫菜霉素;泼尼松;丙基双吖啶酮;前列腺素J2;蛋白酶体抑制剂;基于蛋白A的免疫调节剂;蛋白激酶C抑制剂;微藻蛋白激酶C抑制剂;蛋白质酪氨酸磷酸酶抑制剂;嘌呤核苷磷酸化酶抑制剂;红紫素;吡唑啉吖啶;吡啶氧基化血红蛋白聚氧乙烯缀合物(pyridoxylated hemoglobin polyoxyethylene conjugate);raf拮抗剂;雷替曲塞;雷莫司琼;ras法呢基蛋白质转移酶抑制剂;ras抑制剂;ras-GAP抑制剂;脱甲基化瑞替普汀;依替膦酸铼Re 186;利索新;核酶;RII视黄酰胺;罗希吐碱;罗莫肽;罗喹美克;rubiginone B1;ruboxyl;沙芬戈;saintopin;SarCNU;sarcophytol A;沙格司亭;Sdi 1模拟物;司莫司汀;老化衍生抑制剂1;有义寡核苷酸;信号转导抑制剂;西佐喃;索布佐生;硼卡钠;苯乙酸钠;solverol;生长调节素结合蛋白;索纳明;膦门冬酸;穗霉素D;螺莫司汀;splenopentin;spongistatin 1;司夸胺;stipiamide;基质溶素抑制剂;sulfinosine;超活性血管活性肠肽拮抗剂;suradista;苏拉明;苦马豆素;他莫司汀;他莫昔芬甲碘化物;牛磺莫司汀;他扎罗汀;替可加兰钠;替加氟;tellurapyrylium;端粒酶抑制剂;替莫泊芬;替尼泊苷;tetrachlorodecaoxide;tetrazomine;thaliblastine;噻可拉林;血小板生成素;血小板生成素模拟物;胸腺法新;胸腺生成素受体激动剂;胸腺曲南;甲状腺刺激激素;乙基锡初红紫素(tin ethyl etiopurpurin);替拉扎明;二氯二茂钛;topsentin;托瑞米芬;翻译抑制剂;维A酸;三乙酰尿苷;曲西立滨;三甲曲沙;曲普瑞林;托烷司琼;妥罗雄脲;酪氨酸激酶抑制剂;酪氨酸磷酸化抑制剂;UBC抑制剂;乌苯美司;泌尿生殖窦衍生生长抑制性因子;尿激酶受体拮抗剂;伐普肽;variolin B;Vectibix(帕尼单抗)维拉雷琐;veramine;verdins;维替泊芬;长春瑞滨;vinxaltine;αVβ3人源化抗单抗;伏氯唑;Welcovorin(亚叶酸);Xeloda(卡培他滨);扎诺特隆;折尼铂;亚苄维和净司他丁斯酯。

[0495] 在一个具体的实施方案中,作为第二药剂给予的抗癌药是沙利度胺、来那度胺、泊马度胺、CC-122、阿扎胞苷、地西他滨或CC-486(口服阿扎胞苷)。在一个更具体的实施方案中,作为第二药剂给予的抗癌药是来那度胺或泊马度胺。在一个具体的实施方案中,作为第

二药剂给予的抗癌药是免疫调节化合物(例如第5.2.7.1节所述免疫调节化合物)。在一个具体的实施方案中,作为第二药剂给予的抗癌药是罗米地新。

[0496] 5.4.2.使用遗传修饰的NK细胞的治疗

[0497] 另一方面,本文提供治疗有需要的对象的血液病症或实体瘤的方法,所述方法包括给予所述对象分离的NK细胞群或其药物组合物,其中NK细胞是遗传修饰的(例如包含嵌合抗原受体(CAR)和/或归巢受体,其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和可选的共刺激结构域)。

[0498] 遗传修饰的NK细胞(例如包含CAR和/或归巢受体的NK细胞)描述于第5.3节。

[0499] 5.4.3.血液病症和实体瘤

[0500] 在具体的实施方案中,血液病症是血液过度增殖病症。在具体的实施方案中,血液病症是血液癌症,例如白血病或淋巴瘤。在更具体的实施方案中,血液癌症是急性白血病,例如急性T细胞白血病、急性髓细胞白血病(AML)、急性前髓细胞白血病、急性原始粒细胞白血病、急性成巨核细胞性白血病、前体B急性成淋巴细胞性白血、前体T急性成淋巴细胞性白血、伯基特白血病(伯基特淋巴瘤)或急性双表型白血病;慢性白血病,例如慢性髓系淋巴瘤、慢性髓细胞白血病(CML)、慢性单核细胞白血病、慢性淋巴细胞白血病(CLL)/小淋巴细胞淋巴瘤或B细胞幼淋巴细胞白血病;毛细胞淋巴瘤;T细胞幼淋巴细胞白血病;或淋巴瘤,例如组织细胞淋巴瘤、淋巴浆细胞淋巴瘤(例如瓦尔登斯特伦巨球蛋白血症)、脾边缘区淋巴瘤、浆细胞瘤变(例如浆细胞骨髓瘤、浆细胞瘤、单克隆免疫球蛋白沉积病或重链病)、结外边缘区B细胞淋巴瘤(MALT淋巴瘤)、节边缘区B细胞淋巴瘤(NMZL)、滤泡性淋巴瘤、套细胞淋巴瘤、弥散性大B细胞淋巴瘤、纵隔(胸腺)大B细胞淋巴瘤、血管内大B细胞淋巴瘤、原发性渗出性淋巴瘤、T细胞大颗粒淋巴细胞白血病、侵袭性NK细胞白血病、成人T细胞白血病/淋巴瘤、鼻型结外NK/T细胞淋巴瘤、肠病型T细胞淋巴瘤、肝脾T细胞淋巴瘤、母细胞性NK细胞淋巴瘤、蕈样肉芽肿病(塞扎里综合征)、原发性皮肤CD30-阳性T细胞淋巴细胞增生性病症(例如原发性皮肤间变性大细胞淋巴瘤或淋巴瘤样丘疹病)、血管免疫母细胞性T细胞淋巴瘤、未分类外周T细胞淋巴瘤、间变性大细胞淋巴瘤、霍奇金淋巴瘤或结节性淋巴细胞为主型霍奇金淋巴瘤。在另一个具体的实施方案中,血液癌症是急性髓细胞白血病(AML)。在另一个具体的实施方案中,血液癌症是慢性淋巴细胞白血病(CLL)。在另一个具体的实施方案中,血液癌症是多发性骨髓瘤或骨髓增生异常综合征。

[0501] 实体瘤可以是但不限于例如癌,例如腺癌、肾上腺皮质癌、结肠腺癌、结肠直肠腺癌、结肠直肠癌、导管细胞癌、肺癌、甲状腺癌、鼻咽癌、黑素瘤(例如恶性黑素瘤)、非黑素瘤皮肤癌或未分类癌;带状瘤;促结缔组织增生性小圆细胞肿瘤;内分泌肿瘤;尤因肉瘤;生殖细胞肿瘤(例如睾丸癌、卵巢癌、绒毛膜癌、内胚层窦瘤、生殖细胞瘤等);肝胚细胞瘤;肝细胞癌;成神经细胞瘤;非横纹肌肉瘤软组织肉瘤;骨肉瘤;成视网膜细胞瘤;横纹肌肉瘤或维尔姆斯瘤。在另一个实施方案中,实体瘤为胰腺癌或乳腺癌。在其它实施方案中,实体瘤是听神经瘤;星形细胞瘤(例如I级纤维性星形细胞瘤、II级低级星形细胞瘤;III级多形性成胶质细胞瘤;或IV级多形性成胶质细胞瘤);脊索瘤;颅咽管瘤;神经胶质瘤(例如脑干神经胶质瘤;室管膜瘤;混合性神经胶质瘤;视神经神经胶质瘤;或亚室管膜瘤);成胶质细胞瘤;成神经管细胞瘤;脑膜瘤;转移性脑肿瘤;少突神经胶质瘤;成松果体细胞瘤;垂体肿瘤;原发性外胚层肿瘤或神经鞘瘤。在另一个实施方案中,实体瘤是前列腺癌。

[0502] 在某些实施方案中，患有血液癌症或实体瘤的个体，例如患有自然杀伤细胞缺乏的个体，是在所述给予前曾接受骨髓移植的个体。在某些实施方案中，骨髓移植是在所述血液癌症或所述实体瘤的治疗中。在某些其它实施方案中，骨髓移植是在所述血液癌症或所述实体瘤以外的病况的治疗中。在某些实施方案中，除所述骨髓移植以外，个体还接受免疫抑制剂。在某些实施方案中，接受骨髓移植的个体在所述给予时显示移植物抗宿主病(GVHD)的一个或多个症状。在某些其它实施方案中，在表现出移植物抗宿主病(GVHD)的症状之前给予接受骨髓移植的个体所述细胞。

[0503] 在某些具体实施方案中，患有血液癌症或实体瘤的个体在所述给予之前曾接受至少一剂TNFα抑制剂，例如ETANERCEPT®(Enbrel)。在具体的实施方案中，所述个体在所述血液癌症或所述实体瘤诊断的1、2、3、4、5、6、7、8、9、10、11或12个月内接受所述剂量的TNFα抑制剂。在一个具体的实施方案中，已接受某一剂量的TNFα抑制剂的个体表现出急性髓系淋巴瘤白血病。在一个更具体的实施方案中，已接受某一剂量的TNFα抑制剂并表现出急性髓系淋巴瘤白血病的个体进一步表现出血细胞5号染色体长臂缺失。在另一个实施方案中，患有血液癌症或实体瘤(例如血液癌症)的个体显示费城染色体。

[0504] 在某些其它实施方案中，所述个体中的血液癌症或实体瘤对一种或多种抗癌药不应。在一个具体的实施方案中，血液癌症或实体瘤对GLEEVEC®(甲磺酸伊马替尼)不应。

[0505] 在某些实施方案中，所述个体中的血液癌症或实体瘤对至少一种抗癌药有反应；在该实施方案中，加入本文所述的胎盘灌注液、分离的胎盘灌注液细胞、分离的自然杀伤细胞(例如胎盘自然杀伤细胞、例如胎盘衍生中间体自然杀伤细胞)、分离的混合自然杀伤细胞或激活的NK或TSPNK细胞和/或其组合和可选的免疫调节化合物(例如第5.2.7.1节所述免疫调节化合物)作为辅助治疗或与所述抗癌药的联合疗法。在某些其它实施方案中，患有血液癌症或实体瘤的个体在所述给予之前已用至少一种抗癌药治疗，并且复发。在某些实施方案中，待治疗的个体患有难治性癌症。在一个实施方案中，用本文所述细胞癌症治疗方法防止(例如阻止或延迟)癌症复发。在一个实施方案中，本文所述癌症治疗方法导致癌症缓解11个月以上、2、3、4、5、6、7、8、9、10、11或121个月以上、1年以上、2年以上、3年以上或4年以上。

[0506] 在某些实施方案中，NK细胞从肿瘤病变中分离，例如是肿瘤浸润性淋巴细胞；预期这类NK细胞对肿瘤相关抗原(TAA)或肿瘤微环境相关抗原(TMAA)有特异性。

[0507] 在一个实施方案中，本文提供治疗患有多发性骨髓瘤的个体的方法，所述方法包括给予个体(1)来那度胺或泊马度胺和(2)CAR NK细胞，其中所述CAR NK细胞在治疗所述个体的多发性骨髓瘤时是有效的。在一个具体的实施方案中，所述CAR NK细胞是脐带血NK细胞，或自脐带血造血细胞(例如造血干细胞)产生的NK细胞。在另一个实施方案中，所述CAR NK细胞通过本文所述用于产生NK细胞的二阶段或三阶段方法产生。在另一个实施方案中，所述来那度胺或泊马度胺和CAR NK细胞彼此分别给予。在治疗患有多发性骨髓瘤的个体的方法的某些具体实施方案中，所述CAR NK细胞包含CAR胞外域，所述胞外域是CS-1结合域。在具体的实施方案中，CS-1结合域包含结合CS-1的抗体的scFv或抗原结合片段。在某些具体实施方案中，CS-1结合域包含elotuzumab的单链形式和/或elotuzumab的抗原结合片段。

[0508] 在一个实施方案中，本文提供治疗患有多发性骨髓瘤的个体的方法，所述方法包

括给予个体(1)来那度胺或泊马度胺;(2)elotuzumab;和(3)CAR NK细胞,其中所述CAR NK细胞在治疗所述个体的多发性骨髓瘤时是有效的。在一个具体的实施方案中,所述CAR NK细胞是脐带血NK细胞,或自脐带血造血细胞(例如造血干细胞)产生的NK细胞。在另一个实施方案中,所述CAR NK细胞通过本文所述用于产生NK细胞的二阶段或三阶段方法产生。在另一个实施方案中,所述来那度胺或泊马度胺、elotuzumab和/或CAR NK细胞彼此分别给予。在治疗患有多发性骨髓瘤的个体的方法的某些具体实施方案中,所述CAR NK细胞包含CAR胞外域,所述胞外域是CS-1结合域。在具体的实施方案中,CS-1结合域包含结合CS-1的抗体的scFv或抗原结合片段。

[0509] 在另一个实施方案中,本文提供治疗患有血液癌症(例如伯基特淋巴瘤)的个体的方法,所述方法包括给予个体(1)罗米地新和(2)CAR NK细胞,其中所述CAR NK细胞对治疗所述个体的血液癌症(例如伯基特淋巴瘤)是有效的。在治疗患有血液癌症(例如伯基特淋巴瘤)的个体的方法的某些具体实施方案中,所述CAR NK细胞包含CAR胞外域,所述胞外域是CD20结合域。在具体的实施方案中,CD20结合域包含结合CD20的抗体的scFv或抗原结合片段。

[0510] 5.5.治疗感染性疾病的方法

[0511] 本文提供使用上文所述NK细胞或遗传修饰的NK细胞(例如包含CAR和/或归巢受体的NK细胞)治疗感染性疾病的方法。

[0512] 5.5.1.使用NK联合疗法治疗感染性疾病

[0513] 另一方面,本文提供治疗有需要的对象的感染性疾病的方法,所述方法包括:(a)给予所述对象分离的自然杀伤(NK)细胞群或其药物组合物,或分离的遗传修饰的NK细胞(例如包含CAR和/或归巢受体的NK细胞)群或其药物组合物;和(b)给予所述对象第二药剂或其药物组合物。第二药剂可以是可用于治疗感染性疾病的任何药学上可接受的药剂,包括但不限于抗体(例如单克隆抗体)、双特异性杀伤细胞衔接器(BiKE)或抗病毒剂。

[0514] 5.5.1.1.与免疫检查点蛋白结合的抗体

[0515] 在某些实施方案中,第二药剂是抗体或其抗原结合片段(有关抗体的描述参见第5.4.1.1节)。在具体的实施方案中,如第5.4.1.1节所述,抗体与免疫检查点蛋白、免疫检查点蛋白或共刺激信号转导蛋白特异性结合并拮抗其活性。

[0516] 5.5.1.2.双特异性杀伤细胞衔接器

[0517] 在某些实施方案中,如第5.4.1.2节所述,第二药剂是BiKE。

[0518] 5.5.1.3.抗病毒剂

[0519] 在某些实施方案中,第二药剂是抗病毒剂,其包括但不限于:咪喹莫德、普达非洛、鬼臼树脂、干扰素α(IFNα)、reticolos、壬苯醇醚-9、阿昔洛韦、泛昔洛韦、伐昔洛韦、更昔洛韦、西多福韦、金刚烷胺、金刚乙胺、利巴韦林、扎那米韦和奥塞米韦;蛋白酶抑制剂例如茚地那韦、奈非那韦、利托那韦或沙奎那韦;核苷反转录酶抑制剂例如去羟肌苷、拉米夫定、司他夫定、扎西他滨或齐多夫定;或非核苷反转录酶抑制剂例如奈韦拉平或依法韦仑。

[0520] 5.5.2.使用遗传修饰的NK细胞治疗感染性疾病

[0521] 另一方面,本文提供治疗有需要的对象的感染性疾病的方法,所述方法包括给予所述对象分离的NK细胞群或其药物组合物,其中NK细胞是遗传修饰的(例如包含嵌合抗原受体(CAR)和/或归巢受体,其中所述CAR包含胞外域、跨膜结构域、胞内刺激结构域和可选

的共刺激结构域)。

[0522] 遗传修饰的NK细胞(例如包含CAR和/或归巢受体的NK细胞)描述于第5.3节。

[0523] 5.5.3.感染性疾病

[0524] 在某些实施方案中,感染性疾病是由病毒、细菌、真菌或寄生虫引起的感染。在具体的实施方案中,感染性疾病是病毒感染。

[0525] 在具体的实施方案中,病毒感染是由腺病毒科(Adenoviridae)、微小RNA病毒科(Picornaviridae)、疱疹病毒科(Herpesviridae)、嗜肝DNA病毒科(Hepadnaviridae)、黄病毒科(Flaviviridae)、反转录病毒科(Retroviridae)、正黏病毒科(Orthomyxoviridae)、副粘病毒科(Paramyxoviridae)、乳头状瘤病毒科(Papilommaviridae)、弹状病毒科(Rhabdoviridae)或披膜病毒科(Togaviridae)的病毒引起的感染。在更具体的实施方案中,所述病毒是人类免疫缺陷病毒(HIV)、柯萨奇病毒、甲型肝炎病毒(HAV)、脊髓灰质炎病毒、EB病毒(EBV)、单纯疱疹1型(HSV1)、单纯疱疹2型(HSV2)、人巨细胞病毒(CMV)、人疱疹病毒8型(HHV8)、带状疱疹病毒(水痘带状病毒(VZV)或带状疱疹病毒)、乙型肝炎病毒(HBV)、丙型肝炎病毒(HCV)、丁型肝炎病毒(HDV)、戊型肝炎病毒(HEV)、流感病毒(例如甲型流感病毒、乙型流感病毒、丙型流感病毒或托高土病毒)、麻疹病毒、腮腺炎病毒、副流感病毒、乳头状瘤病毒、狂犬病病毒或风疹病毒。

[0526] 在其它更具体的实施方案中,所述病毒是腺病毒A种,12、18或31血清型;腺病毒B种,3、7、11、14、16、34、35或50血清型;腺病毒C种,1、2、5或6血清型;D种,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或51血清型;E种,4血清型;或F种,血清型40或41。

[0527] 在某些其它更具体的实施方案中,病毒是阿波衣病毒(Apoi virus,APOIV)、Aroa病毒(AROAV)、巴格扎病毒(bagaza virus,BAGV)、斑齐病毒(Banzi virus,BANV)、博博衣病毒(Bouboui virus,BOUV)、卡西帕科利病毒(Cacipacore virus,CPCV)、凯里岛病毒(Carey Island virus,CIV)、牛骨山脊病毒(Cowbone Ridge virus,CRV)、登革热病毒(DENV)、埃杰山病毒(Edge Hill virus,EHV)、加德格兹谷病毒(Gadgets Gully virus,GGYV)、伊列乌斯病毒(Ilheus virus,ILHV)、以色列-土耳其脑脊膜脑脊髓炎病毒(ITV)、日本脑炎病毒(JEV)、朱格拉病毒(Jugra virus,JUGV)、朱格拉病毒(Jutiapa virus,JUTV)、凯丹姆病毒(kadam virus,KADV)、凯多各病毒(Kedougou virus,KEDV)、科科贝拉病毒(Kokobera virus,KOKV)、科坦戈病毒(Koutango virus,KOUV)、卡萨努森林病病毒(KFDV)、兰加特病毒(Langat virus,LGTV)、米班病毒(Meaban virus,MEAV)、摩多克病毒(Modoc virus,MODV)、蒙大拿鼠耳蝙蝠白质脑炎病毒(MMLV)、澳洲墨莱溪谷脑炎病毒(MVEV)、恩塔亚病毒(Ntaya virus,NTAV)、鄂木斯克出血热病毒(OHFV)、波瓦桑病毒(Powassan virus,POWV)、里奥布拉伏病毒(Rio Bravo virus,RBV)、罗亚尔农场病毒(Royal Farm virus,RFV)、萨博亚病毒(Saboya virus,SABV)、圣路易斯脑炎病毒(SLEV)、萨尔别霍病毒(Sal Vieja virus,SVV)、圣帕利塔病毒(San Perlita virus,SPV)、索马里滋里夫病毒(Saumarez Reef virus,SREV)、塞皮克病毒(Sepik virus,SEPV)、坦布苏病毒(Tembusu virus,TMUV)、蜱传脑炎病毒(TBEV)、秋列尼病毒(Tyuleniy virus,TYUV)、乌干达S病毒(UGSV)、乌苏土病毒(Usutu virus,USUV)、韦塞尔斯布朗病毒(Wesselsbron virus,WESSV)、西尼罗河病毒(WNV)、雅温德病毒(Yaounde virus,YAOV)、黄热病毒(YFV)、横须贺病毒(Yokose virus,YOKV)或寨卡

病毒(Zika virus,ZIKV)。

[0528] 在其它实施方案中,将NK细胞给予患有病毒感染的对象作为包括一种或多种其它抗病毒剂的抗病毒治疗方案的一部分。可以给予患有病毒感染的个体的具体抗病毒剂包括但不限于:咪喹莫德、普达非洛、鬼臼树脂、干扰素α(IFNα)、reticolos、壬苯醇醚-9、阿昔洛韦、泛昔洛韦、伐昔洛韦、更昔洛韦、西多福韦、金刚烷胺、金刚乙胺、利巴韦林、扎那米韦和奥塞米韦;蛋白酶抑制剂例如茚地那韦、奈非那韦、利托那韦、或沙奎那韦;核苷反转录酶抑制剂例如去羟肌苷、拉米夫定、司他夫定、扎西他滨、或齐多夫定和非核苷反转录酶抑制剂例如奈韦拉平,或依法韦仑。

[0529] 5.6.给药

[0530] 可通过本领域已知的适于活细胞或第二药剂给药的任何医学上可接受的途径,将本文所述NK细胞、遗传修饰的NK细胞或第二药剂给予个体,例如带有肿瘤细胞或感染细胞的个体。在不同的实施方案中,细胞可例如借助导管或注射器经外科植入、注射、输注或以别的方式直接或间接给予有需要的部位。在不同的实施方案中,第二药剂可例如借助导管或注射器注射、输注或以别的方式直接或间接给予有需要的部位。在一个实施方案中,细胞或第二药剂静脉内给予个体。在另一个实施方案中,将细胞或第二药剂给予个体的肿瘤(例如实体瘤)或感染的部位。在其中个体在一个以上部位患有肿瘤或感染的一个具体的实施方案中,将细胞或第二药剂给予至少2个或全部肿瘤/感染部位。在某些其它实施方案中,细胞或第二药剂或其组合物经口服、经鼻、动脉内、胃肠外、经眼、肌内、皮下、腹膜内、大脑内、心室内、脑室内、鞘内、脑池内、脊柱内和/或脊柱周给予。在具体的实施方案中,细胞或第二药剂或其组合物通过注射、输注、静脉内(IV)给药、股骨内给药或肿瘤内给药给予。在某些具体实施方案中,细胞或第二药剂通过颅内或椎管内针和/或导管(有或无泵装置)递送。

[0531] 在具体的实施方案中,给予所述对象分离的NK细胞群或其药物组合物的步骤是通过注射。在具体的实施方案中,NK细胞的注射是局部注射。在更具体的实施方案中,局部注射直接进入实体瘤(例如肉瘤)中。在具体的实施方案中,NK细胞的给药经注射器注射。在具体的实施方案中,NK细胞经注射给予借助于腹腔镜检查、内窥镜检查、超声、计算机体层摄影术、磁共振或放射检查。

[0532] 可将NK细胞、遗传修饰的NK细胞或第二药剂在组合物(例如基质、水凝胶、支架等)中给予个体。

[0533] 在一个实施方案中,将该细胞接种在天然基质,例如胎盘生物材料例如羊膜材料中。这类羊膜材料可以是例如直接从哺乳动物胎盘切下的羊膜;固定或热处理的羊膜、基本干的(即<20%H_2O)羊膜、绒毛膜、基本干的绒毛膜、基本干的羊膜和绒毛膜等。胎盘干细胞可接种于其上的优选的胎盘生物材料描述于Hariri,美国申请公布号2004/0048796,其公开内容通过引用以其整体并入本文。

[0534] 在另一个实施方案中,将细胞悬浮于适于例如注射的水凝胶溶液中。用于这类组合物的合适水凝胶包括自组装肽,例如RAD16。在一个实施方案中,可允许包含细胞的水凝胶溶液例如在模子中硬化,以形成细胞分散在其中供植入用的基质。还可培养这类基质中的细胞使得细胞在植入前经有丝分裂扩增。水凝胶可以是例如有机聚合物(天然或合成),其通过共价健、离子键或氢键交联产生三维开放晶格(open-lattice)结构,该结构捕获水分子以形成凝胶。水凝胶形成材料包括多糖例如海藻酸及其盐、肽、聚膦腈和聚丙烯酸脂

(其是离子交联的)或嵌段共聚物例如聚环氧乙烷-聚丙二醇嵌共聚物(其分别是通过温度或pH交联)。在一些实施方案中,水凝胶或基质是生物可降解的。

[0535] 在一些实施方案中,用于本发明的制剂包含原位可聚合凝胶(参见例如美国专利申请公布号2002/0022676;Anseth等,J.Control Release,78(1-3):199-209(2002);Wang等,Biomaterials,24(22):3969-80(2003)。

[0536] 在一些实施方案中,聚合物至少部分溶于水溶液,例如水、缓冲盐溶液或具有带电荷的侧基的水醇溶液或其单价离子盐。具有可与阳离子反应的酸性侧基的聚合物的实例是聚(磷腈)、聚(丙烯酸)、聚(甲基丙烯酸)、丙烯酸和甲基丙烯酸的共聚物、聚(乙酸乙烯酯)和磺化聚合物,例如磺化聚苯乙烯。也可以使用由丙烯酸或甲基丙烯酸和乙烯基醚单体或聚合物反应形成的具有酸性侧基的共聚物。酸性基团的实例是羧酸基团、磺酸基团、卤化(优选氟化)醇基团、酚OH7基团和酸性OH基团。

[0537] 可将细胞接种于三维构架或支架中和植入体内。这类构架可与刺激组织形成或另增强或改进本文所述方法实践的任一种或多种生长因子、细胞、药物或其它组分结合植入。

[0538] 可用于本发明的支架的实例包括非织毡、多孔泡沫或自组装肽。非织毡使用由乙醇酸和乳酸的合成可吸收共聚物(例如PGA/PLA)构成的纤维形成(VICRYL,Ethicon,Inc.,Somerville,N.J.)。还可使用通过例如冷冻干燥或冻干等方法形成的由例如聚(ε-己内酯)/聚(乙醇酸)(PCL/PGA)共聚物组成的泡沫(参见例如美国专利号6,355,699)作为支架。

[0539] 细胞还可接种在包括但不限于磷酸一、二、三、α-三、β-三和四-钙、羟基磷灰石、氟磷灰石、硫酸钙、氟化钙、氧化钙、碳酸钙、磷酸镁钙、生物活性玻璃(例如BIOGLASS®)等生理上可接受的陶瓷材料及其混合物或与之接触。目前市购可获得的多孔生物相容性陶瓷材料包括SURGIBONE®(CanMedica Corp.,Canada)、ENDOBON®(Merck Biomaterial France,France)、CEROS®(Mathys,AG,Bettlach,Switzerland)和矿化胶原骨移植产品例如HEALOS™(DePuy,Inc.,Raynham,MA)和VITOSS®、RHAKOSS™和CORTOSS®(Orthovita,Malvern,Pa.)。构架可以是天然和/或合成材料的混合物、共混物或复合材料。

[0540] 在另一个实施方案中,可将细胞接种在由复丝(可由例如PGA、PLA、PCL共聚物或共混物等生物可吸收材料制造)组成的毛毡或透明质酸上或与之接触。

[0541] 在另一个实施方案中,可将细胞接种在可为复合结构的泡沫支架上。可将这类泡沫支架模制成有用形状,例如待修复、置换或强化的身体内特定结构的一部分的形状。在一些实施方案中,在本文所述细胞孵育之前,将构架用例如0.1M乙酸处理,接着在聚赖氨酸、PBS和/或胶原中孵育以增强细胞附着。基质的外表面可经修饰以改进细胞的附着或生长和组织的分化,例如通过血浆包被的基质,或添加一种或多种蛋白质(例如胶原、弹性纤维、网状纤维)、糖蛋白、糖胺聚糖(例如硫酸肝素、4-硫酸软骨素、6-硫酸软骨素、硫酸皮肤素、硫酸角质素等)、细胞基质和/或其它材料,例如但不限于明胶、海藻酸、琼脂糖、琼脂糖和植物胶等。

[0542] 在一些实施方案中,支架包含赋予其非血栓形成的材料或用所述材料处理。这些处理和材料还可促进和维持内皮生长、迁移和胞外基质沉淀。这些材料和处理的实例包括但不限于天然材料(例如基底膜蛋白例如层粘连蛋白和IV型胶原)、合成材料(例如EPTFE)

和链段聚氨酯脲硅酮，例如PURSPAN™(The Polymer Technology Group,Inc.,Berkeley,Calif.)。支架可包含抗血栓剂例如肝素；支架还可经处理以在接种胎盘干细胞之前改变表面电荷(例如用血浆包被)。

[0543] 在具体的实施方案中，将NK细胞、遗传修饰的NK细胞或第二药剂与药用载体一起给予。药用载体可以是本领域已知的任一种。在具体的实施方案中，NK细胞或遗传修饰的NK细胞在细胞表面上被岩藻糖基化。

[0544] NK细胞或遗传修饰的NK细胞(例如包含CAR和/或归巢受体的NK细胞)的数目或第二药剂的量的测定可独立进行。这类测定可取决于对象的病况，并可由医生确定。

[0545] 在某些实施方案中，可使用NK细胞、遗传修饰的NK细胞或第二药剂，例如以导致对个体有可检测益处的任何量或数目(例如有效量)给予个体，其中个体患有病毒感染、癌症或肿瘤细胞，例如患有肿瘤细胞、实体瘤或血液癌症的个体，例如癌症患者。可以细胞绝对数将细胞给予这类个体，例如，可以约、至少约或至多约$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$、$1x10^9$、$5x10^9$、$1x10^{10}$、$5x10^{10}$或$1x10^{11}$个细胞给予所述个体。在其它实施方案中，可以细胞相对数将细胞给予这类个体，例如，可以约、至少约或至多约$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$、$1x10^9$、$5x10^9$、$1x10^{10}$、$5x10^{10}$或$1x10^{11}$个细胞给予所述个体。在其它实施方案中，可以细胞相对数将细胞给予这类个体，例如，可以约、至少约或至多约$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$或$5x10^8$个细胞给予所述个体。可按NK细胞或遗传修饰的NK细胞和可选的胎盘灌注液细胞的数目与所述个体中肿瘤/感染细胞的数目(例如估计数)之间适当的比率将细胞给予这类个体。例如可按NK细胞或遗传修饰的NK与个体中肿瘤/感染细胞数目约、至少约或至多约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或100:1的比率将细胞给予所述个体。可通过例如清点来自个体组织样品(例如血样、活检样品等)中肿瘤/感染细胞的数目，来估计这类个体中肿瘤/感染细胞的数目。在具体的实施方案中，例如，对于实体瘤，所述高清点数目结合肿瘤或肿瘤成像进行以获取大致的肿瘤体积。

[0546] 在一个具体的实施方案中，NK细胞(或遗传修饰的NK细胞)补充了胎盘灌注液细胞或胎盘灌注液。在一个具体的实施方案中，约$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$或更多个NK细胞(或遗传修饰的NK细胞)/毫升，或$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$、$1x10^9$、$5x10^9$、$1x10^{10}$、$5x10^{10}$、$1x10^{11}$或更多个NK细胞(或遗传修饰的NK细胞)/毫升，补充了约或至少约$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$或更多个分离的胎盘灌注液细胞/毫升，或$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$、$1x10^9$、$5x10^9$、$1x10^{10}$、$5x10^{10}$、$1x10^{11}$或更多个分离的胎盘灌注液细胞/毫升。在其它更具体的实施方案中，约$1x10^4$，$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$或更多个NK细胞(或遗传修饰的NK细胞)/毫升，或$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$、$1x10^9$、$5x10^9$、$1x10^{10}$、$5x10^{10}$、$1x10^{11}$或更多个NK细胞(或遗传修饰的NK细胞)/毫升补充了约或至少约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或1000mL灌注液，或约1单位灌注液。

[0547] 在另一个具体的实施方案中，NK细胞(或遗传修饰的NK细胞)补充了贴壁胎盘细胞，例如贴壁胎盘干细胞或多能细胞，例如$CD34^-$、$CD10^+$、$CD105^+$、$CD200^+$组织培养塑料贴壁胎盘细胞。在具体的实施方案中，NK细胞补充了约$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$更多个贴壁胎盘干细胞/毫升，或$1x10^4$、$5x10^4$、$1x10^5$、$5x10^5$、$1x10^6$、$5x10^6$、$1x10^7$、$5x10^7$、$1x10^8$、$5x10^8$、$1x10^9$、$5x10^9$、$1x10^{10}$、$5x10^{10}$、$1x10^{11}$更多个贴壁胎盘细胞，例如，贴壁胎盘干细胞或多能细胞。

[0548] 在另一个具体的实施方案中，NK细胞(或遗传修饰的NK细胞)补充了条件培养基，例如被$CD34^-$、$CD10^+$、$CD105^+$、$CD200^+$组织培养塑料贴壁胎盘细胞调节的培养基，例如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、10mL干细胞条件培养基/单位灌注液，或每10^4、10^5、10^6、10^7、10^8、10^9、10^{10}或10^{11}个NK细胞(或遗传修饰的NK细胞)。在某些实施方案中，组织培养塑料贴壁胎盘细胞是描述于美国专利号7,468,276和美国专利申请公布号2007/0275362(其公开内容通过引用以其整体并入本文)的多能贴壁胎盘细胞。在另一个具体的实施方案中，该方法另包括使免疫调节化合物(例如第5.2.7.1节所述免疫调节化合物)或沙利度胺与肿瘤细胞接近或给予个体。

[0549] 在另一个具体的实施方案中，NK细胞(或遗传修饰的NK细胞)补充了胎盘灌注液细胞，在所述使之接触之前，使灌注液细胞与白介素-2(IL-2)接近一段时间。在某些实施方案中，所述一段时间为在所述使之接近前大约、至少或至多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或48小时。

[0550] 可在治疗进程期间将NK细胞、遗传修饰的NK细胞或第二药剂一次性给予(即呈单剂量)患有病毒感染、血液病症或实体瘤的个体；或可多次给予(即呈多剂量)，例如，在治疗进程中每1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22或23小时一次，或1、2、3、4、5、6或7天一次，或每1、2、3、4、5、6、7、8、9、10、24、36或更多周一次。其中在使用NK细胞(或遗传修饰的NK细胞)和第二药剂两者的实施方案中，可一起例如在同一制剂中；分别，例如在不同制剂中，在大致同一时间将第二药剂和NK细胞(或遗传修饰的NK细胞)给予个体；或可分别例如按不同的给药方案或在一天的不同时间给予。第二药剂可在NK细胞(或遗传修饰的NK细胞)之前、之后或与之同一时间给予。不论过去曾将NK细胞(或遗传修饰的NK细胞)或第二药剂给予个体与否，均可给予NK细胞(或遗传修饰的NK细胞)或第二药剂。

[0551] 5.7.患者

[0552] 本公开内容中提及的患者可以是但不限于人或非人脊椎动物，例如野生动物、驯养动物或农场动物。在某些实施方案中，患者是哺乳动物，例如人、牛、狗、猫、山羊、马、绵羊、猪、大鼠或小鼠。在一个实施方案中，患者是人患者。

[0553] 5.8.药盒

[0554] 本文提供包含装满包含本文所述NK细胞或遗传修饰的NK细胞(例如包含CAR和/或归巢受体的NK细胞)的组合物的一个或多个容器和装满包含本文所述第二药剂的组合物的一个或多个容器的药包或药盒。本文还提供包含含有包含CAR和/或归巢受体的本文所述NK细胞的组合物的一个或多个容器的药包或药盒。任选与容器相关的可以是监管生产、使用或销售药品或生物制品的政府机构规定形式的说明书，该说明书反映了获得生产、使用或销售机构许可用于人类给药。

[0555] 本文包括的药盒可按照本文提供的治疗方法，例如治疗血液癌症、实体瘤或病毒

感染的方法使用。

6.实施例

[0556] 6.1.实施例1:使用利妥昔单抗的依赖抗体的细胞毒性(ADCC)

[0557] 本文提供的实施例表明共同给予NK细胞(此为PiNK细胞)和对细胞表面抗原(在此情况下为CD20)有特异性的抗体,例如肿瘤相关抗原增加NK细胞的NK抗体依赖性细胞介导的细胞毒性(ADCC)。

[0558] 本文提供的实验利用抗CD20抗体、利妥昔单抗和Daudi细胞(目录号:CCL-213,ATCC),其是CD20的高表达细胞。收获Daudi细胞,并用PKH26(目录号:PKH26GL-1KT,Sigma-Aldrich)标记(Ferlazzo,G.等,J Immunol,172:1455-1462(2004);Lehmann,D.等,Stem Cells Dev,21:2926-2938(2012)),其亲脂性脂族残基插入细胞质膜中。细胞经洗涤,与以图1所示不同浓度的利妥昔单抗(和人IgG作为同种型对照)一起在室温下孵育1小时。在洗涤3次后,将10^4个靶细胞置于96孔U型底组织培养板中,与以不同的效应子-靶标(E:T)比率(50:1、20:1、10:1和2.5:1)在200μl补充10%FBS的RPMI 1640中与培养的NK细胞一起孵育。将培养物在5%CO_2中于37℃孵育4小时。在孵育后,收获细胞和TO-PRO-3(目录号T3605,Invitrogen),将不可透过膜的DNA染剂加入培养物中至0.25μM终浓度,接着使用BD FACSCanto II进行FACS分析。细胞毒性(图1中的"%细胞毒性")表示为总$PKH26^+$靶标肿瘤细胞内死细胞($PKH26^+$TO-PRO-3^+)减去自然细胞死亡的的百分比。

[0559] 与人IgG对照相比,使Daudi细胞与利妥昔单抗一起孵育增加(PiNK)细胞的细胞毒性,因此表明当伴以共同给予抗CD20抗体时,提高PiNK细胞的细胞溶解活性(图1)。

[0560] 6.2.实施例2:三阶段NK细胞针对多发性骨髓瘤的细胞毒性

[0561] MM细胞系和原代MM样品的表型表征。将原发性多发性骨髓瘤(MM)细胞(组织溶液,供体ID:MM285、MM293)或MM肿瘤细胞系:RPMI8226(ATCC,目录号CCL-155)和OPM2(DSMZ,目录号ACC-50)细胞(各1x10^6)用于该测定法。按照生产商的方案,将细胞用抗PD-L1APC(Biolegend,目录号329708)、抗CS1PE-Cy7(Biolegend,目录号331816)和7-AAD(BD Bioscience,目录号559925)染色。数据在BD LSRFortessa(BD Biosciences)上获取,并应用FLOWJO®软件(Tree Star)分析。数据表示为根据7-AAD-单细胞选通的%阳性细胞。%阳性门控的设置使用未染色样品作为对照进行。

[0562] 结果。PD-L1和CS-1在MM细胞系上的表达见图2。图2中最左边的峰表示对照,最右边的峰表示样品。对PD-L1是阳性的细胞的百分比如下:71.6%MM285、70.7%MM293、66.2%OPM-2和94.4%RPMI8226。对CS-1是阳性的细胞的百分比如下:31.8%MM285、58.8%MM293、93.4%OPM-2和29.5%RPMI8226。

[0563] 三阶段NK细胞针对MM细胞系和原代MM样品的24小时细胞毒性测定法。在以3:1(分别为3x10^5和1x10^5个三阶段NK和OPM2细胞)的效应子-靶标(E:T)比率在1mL补充10%FBS和抗生素的RPMI1640(基础培养基)中,或实验条件:IL-15(5ng/mL)(Invitrogen,目录号PHC9153);IL-2(200IU/mL)(Invitrogen,目录号PHC0023);抗PD-L1(10ng/mL)(Affymetrix,目录号16-5983-82);抗IgG(10ng/mL)(Affymetrix,目录号16-4714-82);REVLIMID®(来那度胺;1uM)或DMSO(0.1%)在48孔板中与三阶段NK细胞一起共培养之前,将OPM2细胞用10μM PKH26荧光染料(Sigma-Aldrich,目录号PKH26-GL)标记。只接种靶

细胞作为对照。在37℃和5％CO_2下孵育24小时后，收获细胞，接着用1μM TO-PRO-3染色以识别死细胞。按照生产商提供的方案，使用计数珠粒，通过流式细胞术，定量测定各样品中有活力的靶细胞(PKH26$^+$TO-PRO-3$^-$)的数目(Invitrogen，目录号C36950)。将计数珠粒引入该测定法中以计算在持续24小时培养期间肿瘤细胞的任何潜在增殖。

[0564] 简单地说，如下计算各样品中有活力的靶细胞的数目：(％PKH26$^+$TO-PRO-3$^-$活的靶标)/(％计数珠粒)x(计数珠粒批次的指定珠粒计数)。如下计算样品(靶细胞与三阶段NK细胞共培养物)中的百分比存活率(％存活率)：将24小时后具有三阶段NK细胞的共培养物中余留的活的PKH26$^+$靶细胞的绝对数除以仅靶细胞的培养物中余留的活的PKH26$^+$靶细胞的绝对数。24小时报告的百分比细胞毒性计算为：100-％存活率。结果表示为均值±均值的标准差。

[0565] 结果。三阶段NK细胞显示针对不同MM细胞系的细胞毒活性。三阶段NK细胞以3:1的E:T比率针对4种原代MM样品发挥20-60％特异性裂溶解(图3)。观察到来自不同供体的MM靶标对NK杀伤的不同敏感性。另外，三阶段NK细胞针对OPM2的细胞毒性的最初评价表明通过加入细胞因子、免疫调节化合物和用于这些实验的单克隆抗体，细胞溶解活性得到提高(图4)。

[0566] 等同物

[0567] 本发明的范围不受限于本文所述的具体实施方案。实际上，从前面的描述和附图来看，除所描述的以外本发明的各种改动对于本领域技术人员而言应是显而易见的。这类改动欲落入随附权利要求书的范围内。

[0568] 本文引用的所有参考文献均通过引用以其整体并入本文并用于所有目的，程度就像各单独的出版物、专利或专利申请明确而具体地表明通过引用以其整体并入用于所有目的一样。任何出版物的引用是针对其在申请日之前的公开内容，不应解释为承认由于在先发明，本发明无权先于这类在先出版物。

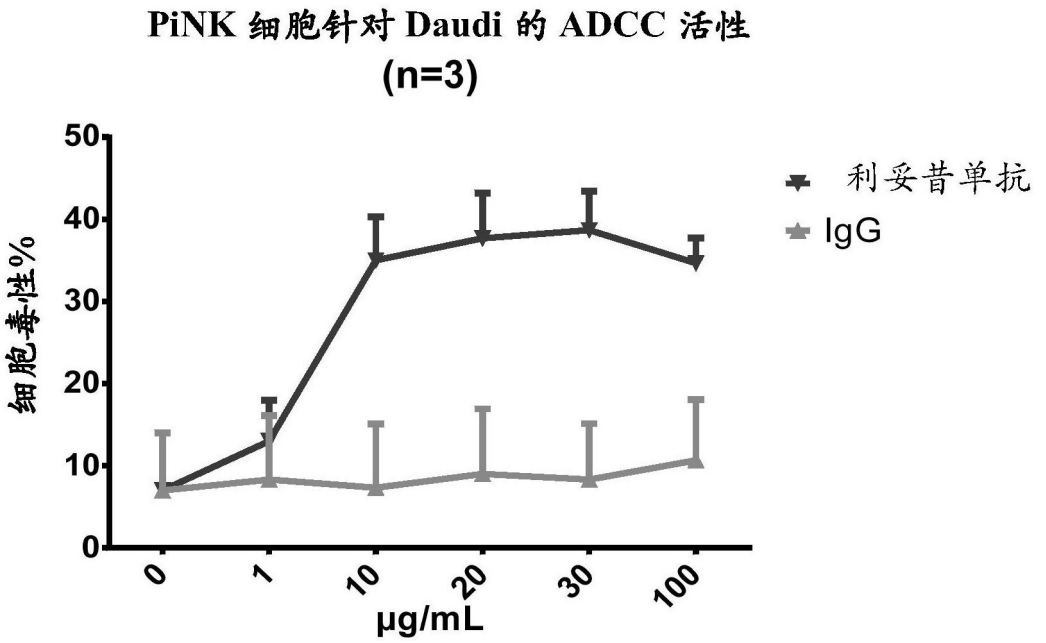
PiNK 细胞针对 Daudi 的 ADCC 活性
(n=3)
细胞毒性%
50
40
30
20
10
0
0
1
10
20
30
100
μg/mL
利妥昔单抗
IgG

图1

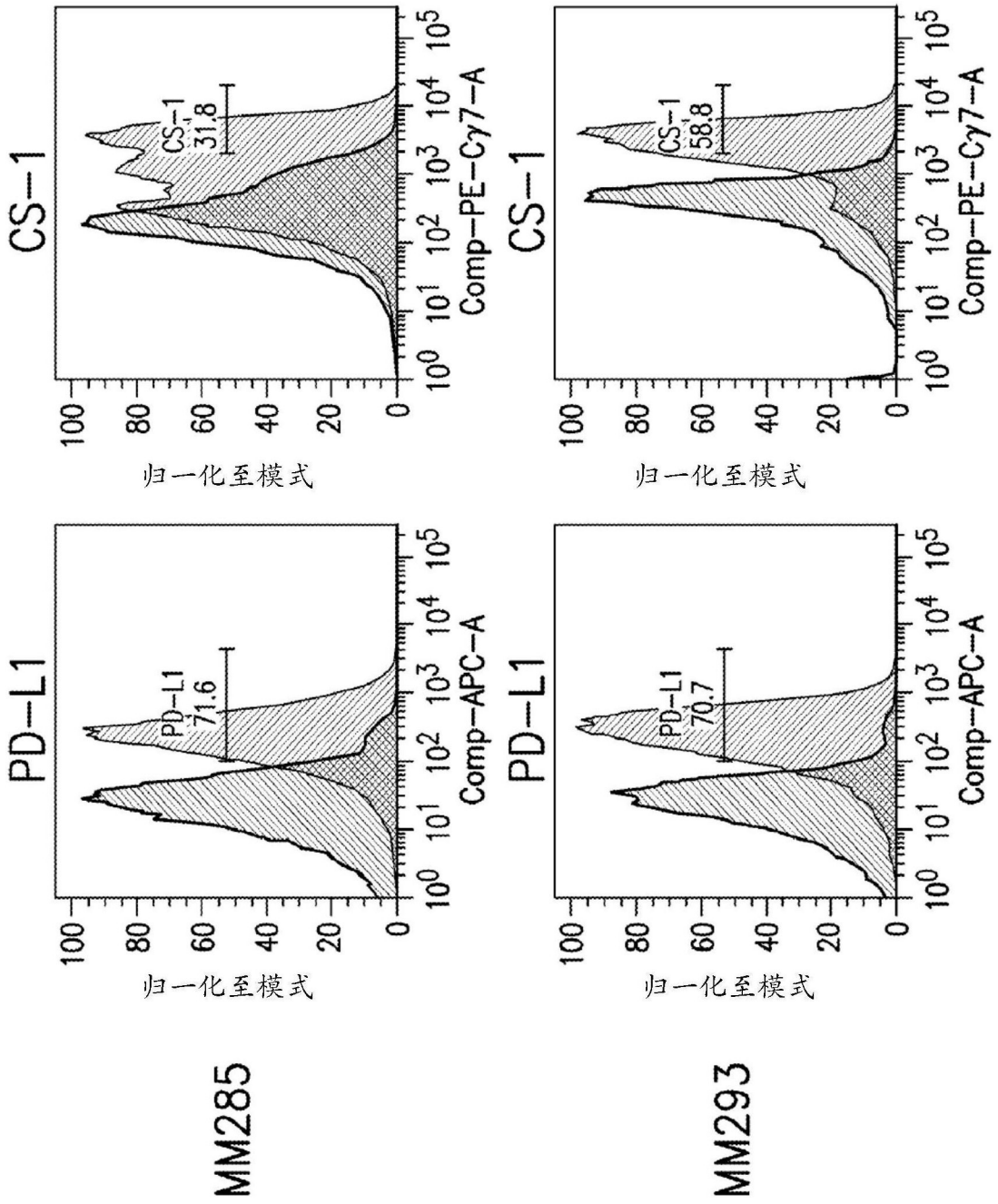
CS-1
CS-1
31.8
归一化至模式
Comp-PE-Cy7-A
CS-1
CS-1
58.8
归一化至模式
Comp-PE-Cy7-A
PD-L1
PD-L1
71.6
归一化至模式
Comp-APC-A
PD-L1
PD-L1
70.7
归一化至模式
Comp-APC-A
MM285
MM293

图2

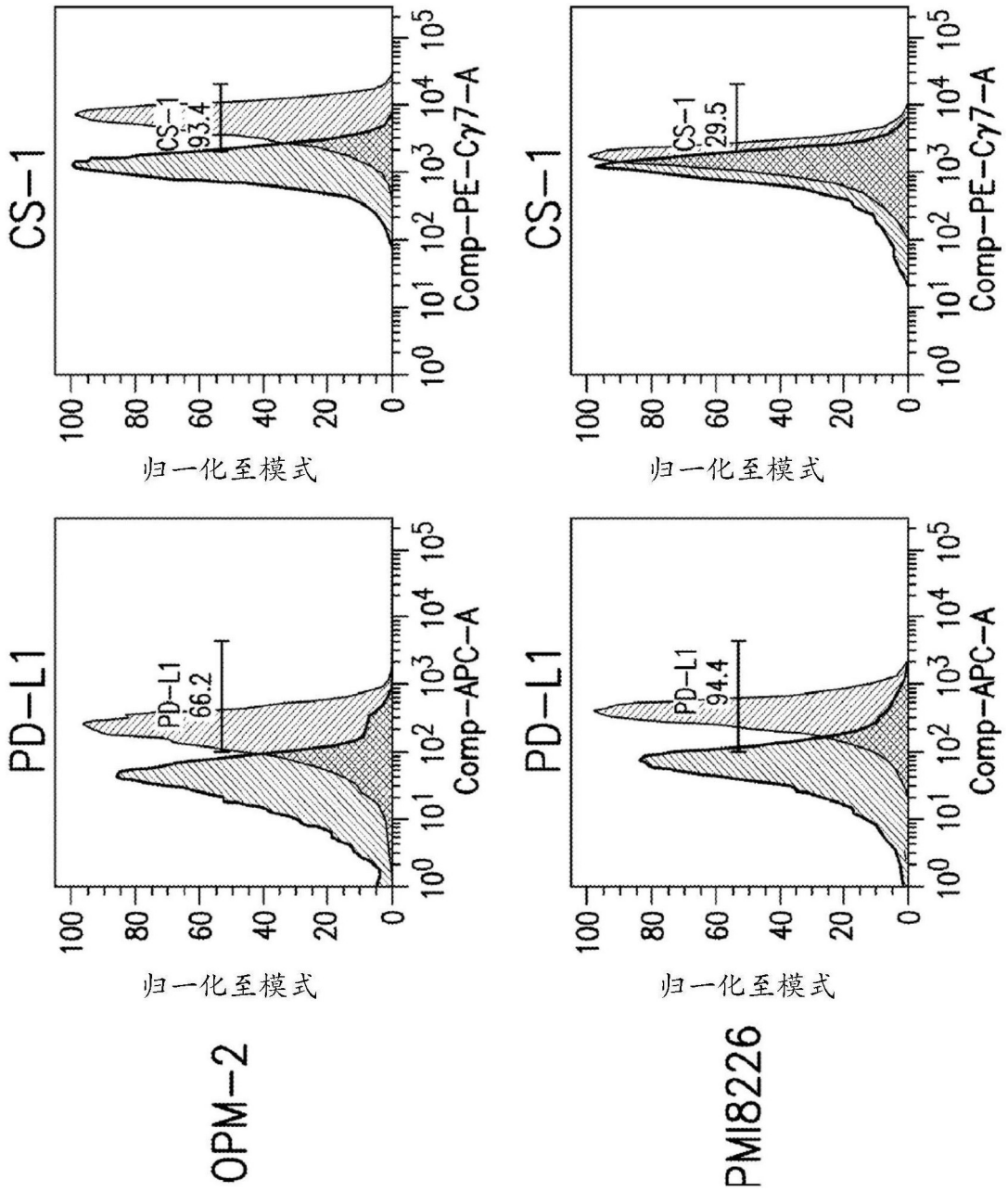
CS-1
CS-1
93.4
Comp-PE-Cy7-A
归一化至模式
CS-1
CS-1
29.5
Comp-PE-Cy7-A
归一化至模式
PD-L1
PD-L1
66.2
Comp-APC-A
归一化至模式
PD-L1
PD-L1
94.4
Comp-APC-A
归一化至模式
OPM-2
PMI8226

图2(续)

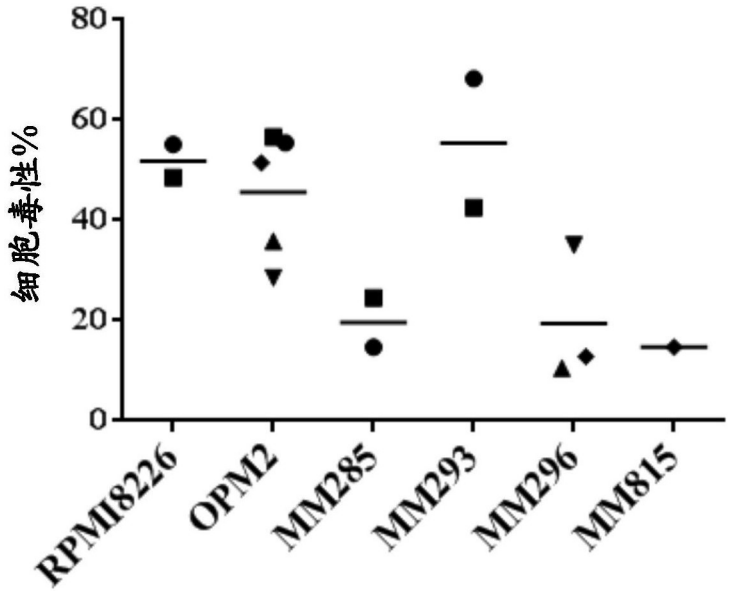
细胞毒性%
80
60
40
20
0
RPMI8226
OPM2
MM285
MM293
MM296
MM815

图3

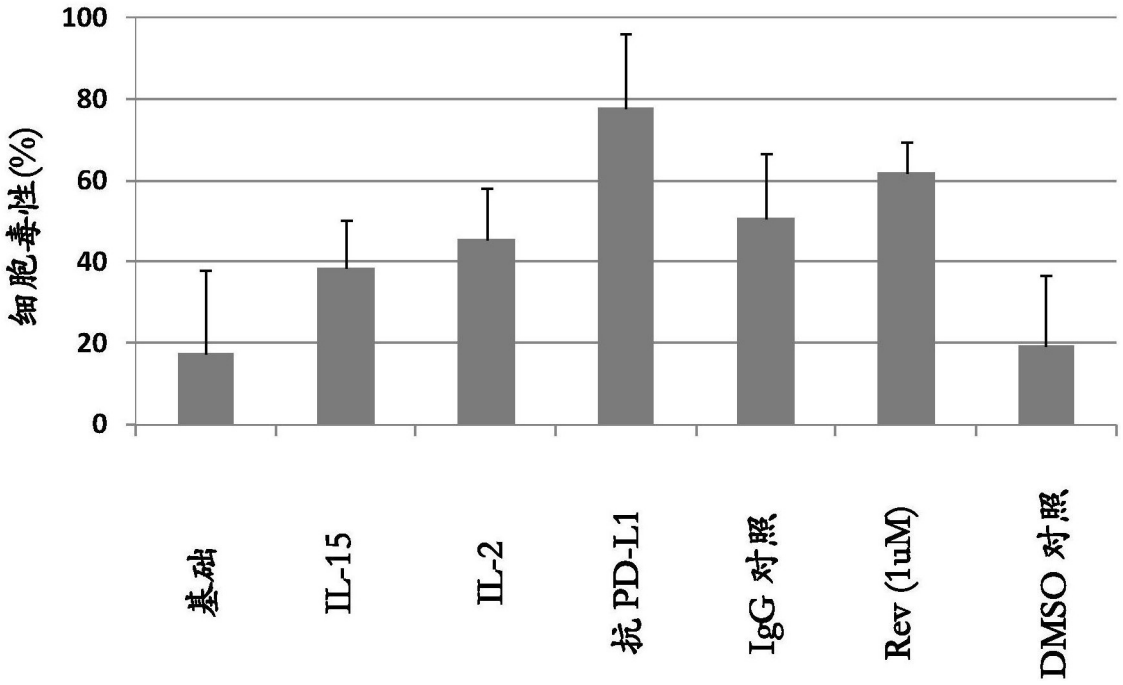
细胞毒性(%)
100
80
60
40
20
0
基础
IL-15
IL-2
抗 PD-L1
IgG 对照
Rev (1uM)
DMSO 对照

图4