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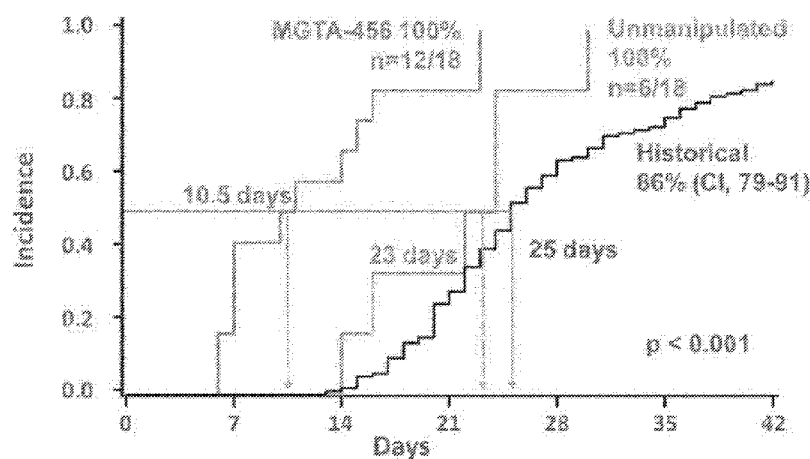
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(54) Title: COMPOSITIONS AND METHODS FOR THE EXPANSION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS

FIG. 1

Clinical evidence of AHR antagonist-mediated expansion of CB HSCs



(57) Abstract: Provided herein are compositions and methods useful for the expansion of hematopoietic stem and progenitor cells, such as those that have been genetically modified, for instance, to express a heterologous transgene. In accordance with the composition and methods described herein, hematopoietic stem and progenitor cells may be genetically modified, for example, to express transgenes encoding therapeutic proteins. Genetically modified hematopoietic stem and progenitor cells may be expanded, for instance, by treatment ex vivo with an aryl hydrocarbon receptor antagonist, and may be infused into a patient, such as a patient in need of hematopoietic stem cell transplant therapy. Thus, provided herein are methods for the treatment of various stem cell disorders, including hematopoietic diseases, metabolic disorders, cancers, and autoimmune diseases, among others.



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## COMPOSITIONS AND METHODS FOR THE EXPANSION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS

### Cross-Reference to Related Applications

This application claims priority to, and the benefit of U.S. Application Nos. 62/579,803, filed October 31, 2017, 62/596,676, filed December 8, 2017, 62/613,383, filed January 3, 2018, 62/625,917, filed February 2, 2018, 62/634,638, filed February 23, 2018, and 62/747,068, filed October 17, 2018, the entire contents of each of which are incorporated herein by reference.

### Field

The present disclosure relates to compositions and methods useful for expansion, for instance, by treatment ex vivo with an aryl hydrocarbon receptor antagonist, of hematopoietic stem and progenitor cells, such as those that have been genetically modified, for instance, to express transgenes encoding therapeutic proteins, as well as method of treating various related pathologies.

### Background

Despite advances in the medicinal arts, there remains a demand for treating pathologies of the hematopoietic system, such as diseases of a particular blood cell, metabolic disorders, cancers, and autoimmune conditions, among others. While hematopoietic stem cells have significant therapeutic potential, a limitation that has hindered their use in the clinic has been the difficulty associated with expanding populations of hematopoietic stem cells to achieve quantities sufficient for transplantation while preserving hematopoietic stem cell functional potential. There is currently a need for compositions and methods for effectuating the expansion of hematopoietic stem and progenitor cells.

### Summary

Provided herein are compositions and methods for expanding populations of hematopoietic stem or progenitor cells, such as hematopoietic stem or progenitor cells that are genetically modified, for instance, so as to disrupt a gene of interest or augment the expression of a gene of interest.

In a first aspect, provided herein is a method of producing an expanded population of genetically modified hematopoietic stem or progenitor cells ex vivo, the method including the steps of (a) disrupting an endogenous gene in a plurality of hematopoietic stem or progenitor cells, thereby producing a population of genetically modified hematopoietic stem or progenitor cells; and (b) contacting the population of genetically modified hematopoietic stem or progenitor cells with an expanding amount of an aryl hydrocarbon receptor antagonist (i.e., an amount of an aryl hydrocarbon receptor antagonist sufficient to increase the quantity of hematopoietic stem or progenitor cells in the population by, for example, 1.1-fold to about 1,000-fold, about 1.1-fold to about 5,000-fold, or more (e.g., about 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2-fold, 2.1-fold, 2.2-fold, 2.3-fold,

2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold, 3.5-fold, 3.6-fold, 3.7-fold, 3.8-fold, 3.9-fold, 4-fold, 4.1-fold, 4.2-fold, 4.3-fold, 4.4-fold, 4.5-fold, 4.6-fold, 4.7-fold, 4.8-fold, 4.9-fold, 5-fold, 5.1-fold, 5.2-fold, 5.3-fold, 5.4-fold, 5.5-fold, 5.6-fold, 5.7-fold, 5.8-fold, 5.9-fold, 6-fold, 6.1-fold, 6.2-fold, 6.3-fold, 6.4-fold, 6.5-fold, 6.6-fold, 6.7-fold, 6.8-fold, 6.9-fold, 7-fold, 7.1-fold, 7.2-fold, 7.3-fold, 7.4-fold, 7.5-fold, 7.6-fold, 7.7-fold, 7.8-fold, 7.9-fold, 8-fold, 8.1-fold, 8.2-fold, 8.3-fold, 8.4-fold, 8.5-fold, 8.6-fold, 8.7-fold, 8.8-fold, 8.9-fold, 9-fold, 9.1-fold, 9.2-fold, 9.3-fold, 9.4-fold, 9.5-fold, 9.6-fold, 9.7-fold, 9.8-fold, 9.9-fold, 10-fold, 50-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1,000-fold, or more), while maintaining hematopoietic stem cell functional potential).

In some embodiments, prior to (a), the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist.

In some embodiment, prior to (a), the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist during a period of time sufficient to induce cell cycling.

In some embodiment, prior to (a), the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist for at least about 1 day, preferably at least about 2 days, preferably at least about 3 days, preferably at least about 4 days, preferably at least about 5 days.

In another aspect, the provided herein is a method of expanding a population of genetically modified hematopoietic stem or progenitor cells ex vivo, wherein the cells have previously been genetically modified to disrupt an endogenous gene, the method including contacting a population of genetically modified hematopoietic stem or progenitor cells with an expanding amount of an aryl hydrocarbon receptor antagonist.

In yet another aspect, provided herein is a method of producing a population of genetically modified hematopoietic stem or progenitor cells, wherein the cells have previously been expanded ex vivo by contacting the population with an expanding amount of an aryl hydrocarbon receptor antagonist, the method including disrupting an endogenous gene in the expanded population of hematopoietic stem or progenitor cells.

In another aspect, provided herein is a method of producing an expanded population of genetically modified hematopoietic stem or progenitor cells ex vivo, the method including the steps of (a) introducing a polynucleotide into a plurality of hematopoietic stem or progenitor cells, thereby producing a population of genetically modified hematopoietic stem or progenitor cells that express the polynucleotide; and (b) contacting the population of genetically modified hematopoietic stem or progenitor cells with an expanding amount of an aryl hydrocarbon receptor antagonist (i.e., an amount of an aryl hydrocarbon receptor antagonist sufficient to increase the quantity of hematopoietic stem or progenitor cells in the population by, for example, 1.1-fold to about 1,000-fold, about 1.1-fold to about 5,000-fold, or more (e.g., about 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2-fold, 2.1-fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold, 3.5-fold, 3.6-fold, 3.7-fold, 3.8-fold, 3.9-fold, 4-fold, 4.1-fold, 4.2-fold, 4.3-fold, 4.4-fold, 4.5-fold, 4.6-fold, 4.7-fold, 4.8-fold, 4.9-fold, 5-fold, 5.1-fold, 5.2-fold, 5.3-fold, 5.4-fold, 5.5-fold, 5.6-fold, 5.7-fold, 5.8-



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In some embodiments, prior to (a), the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist.

In some embodiments, prior to (a), the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist during a period of time sufficient to induce cell cycling.

In some embodiments, prior to (a), the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist for at least about 1 day, preferably at least about 2 days, preferably at least about 3 days, preferably at least about 4 days, preferably at least about 5 days.

In another aspect, provided herein is a method of expanding a population of genetically modified hematopoietic stem or progenitor cells *ex vivo*, wherein the cells have previously been genetically modified by introducing a polynucleotide into the cells, the method including contacting a population of genetically modified hematopoietic stem or progenitor cells with an expanding amount of an aryl hydrocarbon receptor antagonist.

In yet another aspect, provided herein is a method of producing a population of genetically modified hematopoietic stem or progenitor cells, wherein the cells have previously been expanded *ex vivo* by contacting the population with an expanding amount of an aryl hydrocarbon receptor antagonist, the method including introducing a polynucleotide into the expanded population of hematopoietic stem or progenitor cells.

In some embodiments of any of the above aspects, the population of genetically modified hematopoietic stem or progenitor cells further includes hematopoietic stem or progenitor cells are not genetically modified.

The genetically modified hematopoietic stem or progenitor cells may expand at a rate that is proportional to the relative quantity of genetically modified hematopoietic stem or progenitor cells present in the population upon initial contact with the aryl hydrocarbon receptor antagonist. In some embodiments, the genetically modified hematopoietic stem or progenitor cells and the hematopoietic stem or progenitor cells that are not genetically modified may be expanded at a relative rate that is proportional to the ratio of genetically modified hematopoietic stem or progenitor cells to hematopoietic stem or progenitor cells that are not genetically modified present in the population upon initial contact with the aryl hydrocarbon receptor antagonist.

In some embodiments, the hematopoietic stem or progenitor cells that are not genetically modified do not out-compete the genetically modified hematopoietic stem or progenitor cells for expansion by the aryl hydrocarbon receptor antagonist.

In some embodiments, the genetically modified hematopoietic stem or progenitor cells expand more rapidly than the hematopoietic stem or progenitor cells that are not genetically modified.

In some embodiments of any of the above aspects, upon transplantation of the population of genetically modified hematopoietic stem or progenitor cells into a patient, the ratio of the genetically modified hematopoietic stem or progenitor cells, or progeny thereof, to the total quantity of hematopoietic stem cells in a sample (e.g., of bone marrow or peripheral blood) isolated from the patient is at least 75% (e.g., at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) of the ratio of genetically modified hematopoietic stem or progenitor cells to the total quantity of hematopoietic stem or progenitor cells present in the population at the time of administration of the cells to the patient.

In some embodiments, the population of hematopoietic stem or progenitor cells that have been genetically modified to disrupt an endogenous gene maintain disruption of the gene for at least 2 days (e.g., from about 2 days to about 30 days, such as from about 2 days to about 25 days, about 2 days to about 20 days, about 2 days to about 16 days, about 3 days to about 20 days, about 3 days to about 18 days, about 4 days to about 20 days, about 4 days to about 18 days, about 5 days to about 20 days, about 5 days to about 18 days, about 10 days to about 20 days, about 12 days to about 18 days, about 14 days to about 18 days, at least 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 14 days, 16 days, 18 days, 20 days, 25 days, or more) following the disrupting step and/or initial treatment with the aryl hydrocarbon receptor antagonist.

In some embodiments, the population of hematopoietic stem or progenitor cells that have been genetically modified to express a polynucleotide continue to exhibit expression of the polynucleotide for at least 2 days (e.g., from about 2 days to about 30 days, such as from about 2 days to about 25 days, about 2 days to about 20 days, about 2 days to about 16 days, about 3 days to about 20 days, about 3 days to about 18 days, about 4 days to about 20 days, about 4 days to about 18 days, about 5 days to about 20 days, about 5 days to about 18 days, about 10 days to about 20 days, about 12 days to about 18 days, about 14 days to about 18 days, at least 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 14 days, 16 days, 18 days, 20 days, 25 days, or more) following the introducing step and/or initial treatment with the aryl hydrocarbon receptor antagonist.

In some embodiments, the population of genetically modified hematopoietic stem or progenitor cells exhibits a higher engraftment potential relative to a population of hematopoietic stem or progenitor cells not treated with the aryl hydrocarbon receptor antagonist.

In some embodiments, step (a) includes contacting the hematopoietic stem or progenitor cells with the polynucleotide and a nuclease that catalyzes cleavage of an endogenous nucleic acid in the hematopoietic stem or progenitor cell.

In some embodiments, the nuclease is a CRISPR-associated protein, such as caspase 9. The nuclease may be, for example, a transcription activator-like effector nuclease, a meganuclease, or a zinc finger nuclease.

In some embodiments, step (a) includes contacting the hematopoietic stem or progenitor cells with a vector containing the polynucleotide to be expressed. The vector may be, e.g., a viral vector, such as adenovirus (Ad), retrovirus (e.g., retrovirus is a  $\gamma$ -retrovirus or a lentivirus), poxvirus, adeno-associated

virus, baculovirus, herpes simplex virus, or vaccinia virus. In some embodiments, the vector is a transposable element, such as piggybac transposon or sleeping beauty transposon.

In some embodiments, prior to expansion, the hematopoietic stem or progenitor cells are mobilized and isolated from a donor, such as a human. The mobilization may be conducted, e.g., by  
5 treating the donor with a mobilizing amount of a CXCR4 antagonist, such as plerixafor, and/or a CXCR2 agonist, such as Gro- $\beta$ , Gro- $\beta$  T, or a variant thereof. In some embodiments, the Gro- $\beta$ , Gro- $\beta$  T, or variant thereof has a purity that is at least 95% (e.g., from about 95% to about 99.99%, about 96%, to about 99.99%, about 97% to about 99.99%, about 98% to about 99.99%, about 99% to about 99.99%,  
10 about 95% to about 99.9%, about 97% to about 99.9%, about 99% to about 99.9%, such as 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, or more) relative to deamidated versions of these peptides.

In an additional aspect, provided herein is a method of treating a stem cell disorder in a patient (e.g., a human patient) by producing an expanded population of hematopoietic stem or progenitor cells in accordance with the method of any one of the above aspects or embodiments and infusing the resulting cells into the patient.

15 In another aspect, provided herein is a method of treating a stem cell disorder in a patient (e.g., a human patient) by infusing into the patient an expanded population of hematopoietic stem or progenitor cells produced according the method of any one of the above aspects or embodiments.

In yet another aspect, provided herein is a method of treating a stem cell disorder in a patient (e.g., a human patient) by contacting a population of hematopoietic stem or progenitor cells with an  
20 expanding amount of an aryl hydrocarbon receptor antagonist and infusing the resulting cells into the patient.

In another aspect, provided herein is a method of treating a stem cell disorder in a patient (e.g., a human patient) by infusing into the patient an expanded population of hematopoietic stem or progenitor cells produced by contacting a population of hematopoietic stem or progenitor cells with an expanding  
25 amount of an aryl hydrocarbon receptor antagonist.

In another aspect, provided herein is a method of treating a disorder in a patient (e.g., a human patient) in need thereof, comprising administering an expanded population of hematopoietic stem cells to the patient, wherein the expanded population of hematopoietic stem cells is prepared by contacting a first population of hematopoietic stem cells with an aryl hydrocarbon receptor antagonist for a time sufficient to  
30 produce the expanded population of hematopoietic stem cells.

In some embodiments, the stem cell disorder is a hemoglobinopathy disorder. The hemoglobinopathy disorder may be, for example, sickle cell anemia, thalassemia, Fanconi anemia, aplastic anemia, or Wiskott-Aldrich syndrome.

In some embodiments, the stem cell disorder is a myelodysplastic disorder. In some  
35 embodiments, the stem cell disorder is an immunodeficiency disorder, such as a congenital immunodeficiency or an acquired immunodeficiency, such as human immunodeficiency virus or acquired immune deficiency syndrome.

In some embodiments, the stem cell disorder is a metabolic disorder, such as glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurler syndrome or Hurler's Disease, sphingolipidoses, Mucopolipidosis II, or metachromatic leukodystrophy.

In some embodiments, the stem cell disorder is cancer, such as leukemia, lymphoma, multiple myeloma, or neuroblastoma. The cancer may be, for instance, a hematological cancer. In some embodiments, the cancer is myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, or non-Hodgkin's lymphoma.

In some embodiments, the stem cell disorder is adenosine deaminase deficiency and severe combined immunodeficiency, hyper immunoglobulin M syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, systemic sclerosis, systemic lupus erythematosus, multiple sclerosis, or juvenile rheumatoid arthritis.

In some embodiments, the stem cell disorder is an autoimmune disorder, such as multiple sclerosis, human systemic lupus, rheumatoid arthritis, inflammatory bowel disease, treating psoriasis, Type 1 diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, alopecia universalis, ankylosing spondylitis, antiphospholipid antibody syndrome, aplastic anemia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome, autoimmune oophoritis, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Chagas' disease, chronic fatigue immune dysfunction syndrome, chronic inflammatory demyelinating polyneuropathy, Crohn's disease, cicatricial pemphigoid, coeliac sprue-dermatitis herpetiformis, cold agglutinin disease, CREST syndrome, Degos disease, discoid lupus, dysautonomia, endometriosis, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Goodpasture's syndrome, Grave's disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, Hidradenitis suppurativa, idiopathic and/or acute thrombocytopenic purpura, idiopathic pulmonary fibrosis, IgA neuropathy, interstitial cystitis, juvenile arthritis, Kawasaki's disease, lichen planus, Lyme disease, Meniere disease, mixed connective tissue disease, myasthenia gravis, neuromyotonia, opsoclonus myoclonus syndrome, optic neuritis, Ord's thyroiditis, pemphigus vulgaris, pernicious anemia, polychondritis, polymyositis and dermatomyositis, primary biliary cirrhosis, polyarteritis nodosa, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, Raynaud phenomenon, Reiter's syndrome, rheumatic fever, sarcoidosis, scleroderma, Sjögren's syndrome, stiff person syndrome, Takayasu's arteritis, temporal arteritis, ulcerative colitis, uveitis, vasculitis, vitiligo, vulvodynia, and Wegener's granulomatosis.

In some embodiments, the stem cell disorder is a neurological disorder, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, Amyotrophic lateral sclerosis, Huntington's disease, mild cognitive impairment, amyloidosis, AIDS-related dementia, encephalitis, stroke, head trauma, epilepsy, mood disorders, or dementia.

In some embodiments, the hematopoietic stem cells are autologous with respect to the patient. For instance, autologous hematopoietic stem cells can be removed from a donor and the cells can subsequently be administered to (e.g., infused into) the patient so as to repopulate one or more cell types of the hematopoietic lineage.

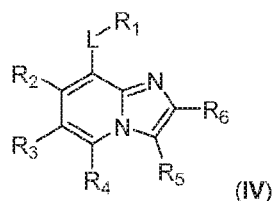
In some embodiments, the hematopoietic stem cells are allogeneic with respect to the patient. For instance, allogeneic hematopoietic stem cells can be removed from a donor, such as donor that is HLA-matched with respect to the patient, for instance, a closely related family member of the patient. In some embodiments, the allogeneic hematopoietic stem cells are HLA-mismatched with respect to the patient. Following withdrawal of the allogeneic hematopoietic stem cells from a donor, the cells can subsequently be administered to (e.g., infused into) the patient so as to repopulate one or more cell types of the hematopoietic lineage.

In some embodiments, the hematopoietic stem or progenitor cells, or progeny thereof, maintain hematopoietic stem cell functional potential after two or more days following infusion of the hematopoietic stem or progenitor cells into the patient. In some embodiments, the hematopoietic stem or progenitor cells, or progeny thereof, localize to hematopoietic tissue and/or reestablish hematopoiesis following infusion of the hematopoietic stem or progenitor cells into the patient. For instance, upon infusion into the patient, the hematopoietic stem or progenitor cells may give rise to recovery of a population of cells selected from the group consisting of megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeloblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T-lymphocytes, and B-lymphocytes.

In another aspect, provided herein is a method of producing microglia in the central nervous system of a human patient in need thereof, the method including administering an expanded population of hematopoietic stem cells to the patient, wherein the expanded population of hematopoietic stem cells is prepared by contacting a first population of hematopoietic stem cells with an aryl hydrocarbon receptor antagonist for a time sufficient to produce the expanded population of hematopoietic stem cells, and wherein administration of the expanded population of hematopoietic stem cells results in formation of microglia in the central nervous system of the patient.

In another aspect, provided herein is a kit containing a plurality of hematopoietic stem or progenitor cells and a package insert that instructs a user to perform the method of any of the above aspects or embodiments.

In some embodiments, the aryl hydrocarbon receptor antagonist is a compound represented by formula (IV)



wherein L is selected from the group consisting of  $-NR_{7a}(CR_{8a}R_{8b})_{n-}$ ,  $-O(CR_{8a}R_{8b})_{n-}$ ,  $-C(O)(CR_{8a}R_{8b})_{n-}$ ,  $-C(S)(CR_{8a}R_{8b})_{n-}$ ,  $-S(O)_{0-2}(CR_{8a}R_{8b})_{n-}$ ,  $-(CR_{8a}R_{8b})_{n-}$ ,  $-NR_{7a}C(O)(CR_{8a}R_{8b})_{n-}$ ,  $-NR_{7a}C(S)(CR_{8a}R_{8b})_{n-}$ ,  $-OC(O)(CR_{8a}R_{8b})_{n-}$ ,  $-OC(S)(CR_{8a}R_{8b})_{n-}$ ,  $-C(O)NR_{7a}(CR_{8a}R_{8b})_{n-}$ ,  $-C(S)NR_{7a}(CR_{8a}R_{8b})_{n-}$ ,  $-C(O)O(CR_{8a}R_{8b})_{n-}$ ,  $-C(S)O(CR_{8a}R_{8b})_{n-}$ ,  $-S(O)_2NR_{7a}(CR_{8a}R_{8b})_{n-}$ ,  $-NR_{7a}S(O)_2(CR_{8a}R_{8b})_{n-}$ ,  $-NR_{7a}C(O)NR_{7b}(CR_{8a}R_{8b})_{n-}$ , and  $-NR_{7a}C(O)O(CR_{8a}R_{8b})_{n-}$ , wherein  $R_{7a}$ ,  $R_{7b}$ ,  $R_{8a}$ ,

and  $R_{8b}$  are each independently selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl, and each  $n$  is independently an integer from 2 to 6;

$R_1$  is selected from the group consisting of  $-S(O)_2NR_{9a}R_{9b}$ ,  $-NR_{9a}C(O)R_{9b}$ ,  $-NR_{9a}C(S)R_{9b}$ ,  $-NR_{9a}C(O)NR_{9b}R_{9c}$ ,  $-C(O)R_{9a}$ ,  $-C(S)R_{9a}$ ,  $-S(O)_{0-2}R_{9a}$ ,  $-C(O)OR_{9a}$ ,  $-C(S)OR_{9a}$ ,  $-C(O)NR_{9a}R_{9b}$ ,  $-C(S)NR_{9a}R_{9b}$ ,  $-NR_{9a}S(O)_2R_{9b}$ ,  $-NR_{9a}C(O)OR_{9b}$ ,  $-OC(O)CR_{9a}R_{9b}R_{9c}$ ,  $-OC(S)CR_{9a}R_{9b}R_{9c}$ , optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl, wherein  $R_{9a}$ ,  $R_{9b}$ , and  $R_{9c}$  are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

$R_2$  is selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl;

$R_3$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

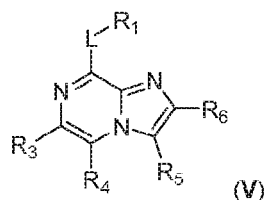
$R_4$  is selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl;

$R_5$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

$R_6$  is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

or a salt thereof.

In some embodiments, wherein the aryl hydrocarbon receptor antagonist is a compound represented by formula (V)



wherein  $L$  is selected from the group consisting of  $-NR_{7a}(CR_{8a}R_{8b})_n$ ,  $-O(CR_{8a}R_{8b})_n$ ,  $-C(O)(CR_{8a}R_{8b})_n$ ,  $-C(S)(CR_{8a}R_{8b})_n$ ,  $-S(O)_{0-2}(CR_{8a}R_{8b})_n$ ,  $-(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}C(O)(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}C(S)(CR_{8a}R_{8b})_n$ ,  $-OC(O)(CR_{8a}R_{8b})_n$ ,  $-OC(S)(CR_{8a}R_{8b})_n$ ,  $-C(O)NR_{7a}(CR_{8a}R_{8b})_n$ ,  $-C(S)NR_{7a}(CR_{8a}R_{8b})_n$ ,  $-C(O)O(CR_{8a}R_{8b})_n$ ,  $-C(S)O(CR_{8a}R_{8b})_n$ ,  $-S(O)_2NR_{7a}(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}S(O)_2(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}C(O)NR_{7b}(CR_{8a}R_{8b})_n$ , and  $-NR_{7a}C(O)O(CR_{8a}R_{8b})_n$ , wherein  $R_{7a}$ ,  $R_{7b}$ ,  $R_{8a}$ , and  $R_{8b}$  are each independently selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl, and each  $n$  is independently an integer from 2 to 6;

$R_1$  is selected from the group consisting of  $-S(O)_2NR_{9a}R_{9b}$ ,  $-NR_{9a}C(O)R_{9b}$ ,  $-NR_{9a}C(S)R_{9b}$ ,  $-NR_{9a}C(O)NR_{9b}R_{9c}$ ,  $-C(O)R_{9a}$ ,  $-C(S)R_{9a}$ ,  $-S(O)_{0-2}R_{9a}$ ,  $-C(O)OR_{9a}$ ,  $-C(S)OR_{9a}$ ,  $-C(O)NR_{9a}R_{9b}$ ,  $-C(S)NR_{9a}R_{9b}$ ,  $-NR_{9a}S(O)_2R_{9b}$ ,  $-NR_{9a}C(O)OR_{9b}$ ,  $-OC(O)CR_{9a}R_{9b}R_{9c}$ ,  $-OC(S)CR_{9a}R_{9b}R_{9c}$ , optionally substituted aryl,

optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl, wherein  $R_{9a}$ ,  $R_{9b}$ , and  $R_{9c}$  are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

$R_3$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

$R_4$  is selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl;

$R_5$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

$R_6$  is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; or a salt thereof.

In another aspect, the disclosure features a composition for uses in treating a disorder in a patient, said composition comprising hematopoietic stem or progenitor cells, or progeny thereof, prepared according to a method of any of the above aspects or embodiments.

In another aspect, the disclosure features use of a composition comprising hematopoietic stem or progenitor cells, or progeny thereof, prepared according to a method of any of the above aspects or embodiments in preparing a medicament for treating a disorder in a patient.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In the specification, the singular forms also include the plural unless the context clearly dictates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents and other references mentioned herein are incorporated by reference. The references cited herein are not admitted to be prior art to the claimed invention. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods and examples are illustrative only and are not intended to be limiting. In the case of conflict between the chemical structures and names of the compounds disclosed herein, the chemical structures will control.

Other features and advantages of the disclosure will be apparent from the following detailed description and claims.

#### Brief Description of the Figures

**FIG. 1** is a graph illustrating the clinical evidence of aryl hydrocarbon receptor (AHR) antagonist-mediated expansion of cord blood-derived hematopoietic stem cells. Particularly, the graph shows the

incidence of neutrophil recovery for patients transplanted with expanded hematopoietic stem cells (n=17) compared to that in the historical cohort (n=111). Neutrophil recovery was defined as the absolute neutrophil count greater than or equal to  $0.5 \times 10^9/L$  for three consecutive days.

**FIGS. 2A – 2D** are a series of graphs showing the expansion of CD34+ cells transduced with a lentiviral vector, as described in Example 2, below. Mobilized peripheral blood (mPB) CD34+ cells were thawed, transduced with a GFP-expressing lentivirus, and expanded for 7 days with an AHR antagonist. Cell numbers and percent GFP positive cells are shown from the culture after expansion. Transduction rates determined as %GFP positive cells. TD: transduced.

**FIGS. 3A – 3E** are a series of diagrams showing the expansion of lentivirally-transduced mobilized peripheral blood (mPB) CD34+ cells for transplantation into NSG mice, as described in Example 2, below. Mobilized peripheral blood (mPB) CD34+ cells were thawed, transduced, and expanded for 7 days with an AHR antagonist. **FIGS. 3A – 3C** show the absolute numbers of cells transplanted into each NSG mouse following mock transduction or GFP-lentiviral vector transduction as indicated. **FIGS. 3D and 3E** are flow cytometry plots for vehicle-cultured or expanded cells. Transduction rates (GFP+ cells) in the bulk, and CD34+ cell populations are shown.

**FIGS. 4A – 4C** are a series of graphs showing the engraftment of lentivirally-transduced, expanded mPB CD34+ cells, as described in Example 2, below. Mobilized peripheral blood (mPB) CD34+ cells expanded as shown in **FIGS. 3A – 3E** were transplanted into NSG mice and evaluated at 4 weeks post-transplant. **FIGS. 4A – 4C** show the engraftment and transduction rates in the peripheral blood of NSG mice transplanted with the cells at 4 weeks post-transplant as determined by flow cytometry, %Engraftment measured as %hCD45/%hCD45+%mCD45%. Editing rates determined as %B2M- cells. Each point represents one mouse. Bars at median values. Statistical significance was determined based on Student's t-test.

**FIGS. 5A – 5E** are a series of diagrams showing the expansion of edited mobilized peripheral blood (mPB) CD34+ cells for transplantation into NSG mice, as described in Example 2, below. Mobilized peripheral blood (mPB) CD34+ cells were thawed, edited, and expanded for 7 days with an AHR antagonist. **FIGS. 5A – 5C** show the absolute numbers of cells transplanted into each NSG mouse following culture and/or editing as indicated. **FIGS. 5D and 5E** are flow cytometry plots for vehicle-cultured or expanded cells. Editing rates in the bulk, CD34+ cells, and CD34+CD90+ cells are shown.

**FIGS. 6A – 6I** are a series of graphs showing the engraftment of gene-edited, expanded mPB CD34+ cells, as described in Example 2, below. Mobilized peripheral blood (mPB) CD34+ cells expanded as shown in **FIGS. 5A – 5E** were transplanted into NSG mice and evaluated at 16 weeks post-transplant. **FIGS. 6A – 6C** show the engraftment and editing rates in the peripheral blood of mice as determined by flow cytometry. **FIGS. 6D – 6F** show bone marrow engraftment and editing rates. **FIGS. 6G – 6I** show the frequency of CD34+ cells within the hCD45+ bone marrow (BM) cells and corresponding editing rates. %Engraftment measured as %hCD45/%hCD45+%mCD45. Editing rates determined as %B2M- cells. Each point represents one mouse. Bars at median values. Statistical significance was determined based on Student's t-test.



FIGS. 7A – 7N are a series of graphs showing the expansion and transplantation of edited BM CD34+ cells into NSG mice, as described in Example 2, below. BM-derived CD34+ cells were thawed, edited, and expanded for 7 days with an AHR antagonist. FIGS. 7A – 7C show the absolute numbers of cells transplanted into each NSG mouse following culture and/or editing as indicated. FIGS. 7D and 7E are flow cytometry plots for vehicle-cultured or expanded cells. Editing rates in the bulk, CD34+ cells, and CD34+CD90+ cells are shown. FIGS. 7F – 7H show the engraftment and editing rates in the peripheral blood of mice as determined by flow cytometry at 12 weeks post-transplant. FIGS. 7I – 7K show the engraftment and editing rates in the peripheral blood of mice as determined by flow cytometry at 16 weeks post-transplant. FIGS. 7L – 7N show the engraftment and editing rates in the bone marrow of mice as determined by flow cytometry at 16 weeks post-transplant. %Engraftment measured as %hCD45/%hCD45+%mCD45. Editing rates determined as %B2M- cells. Each point represents one mouse. Bars at median values. Statistical significance was determined based on Student's t-test.

FIG. 8 is a scheme showing the design of experiments aimed at investigating the ability of hematopoietic stem cells to migrate to central nervous system tissue and engraft as microglial cells in the brains of NSG mice, as described in Example 4, below.

FIGS. 9A and 9B are graphs showing the quantity of hCD45+ CD11b+ cells and Ku80+ Iba-1+ cells, respectively, in the brains of NSG mice, upon treatment of the mice with freshly isolated hematopoietic stem cells, vehicle, or MGTA-456, a hematopoietic stem cell composition obtained upon expansion of cord blood ex vivo using an aryl hydrocarbon receptor (AHR) antagonist. The graphs show the median values obtained upon observation of n=8 individual mice per group.

FIG. 10 is a graph showing the results of a second, independent experiment in which a second flow cytometry quantitation of microglial engraftment in NSG mice was conducted following transplantation of the mice with MGTA-456. Asterisk designates a p value of  $p < 0.05$  relative to freshly isolated hematopoietic stem cells. "###" notation designates a p value of  $p < 0.01$  relative to vehicle-expanded hematopoietic stem cells. Statistics were calculated using a one-tailed, two-sample equal variance Student's t-test.

FIG. 11 is a graph showing the proportion of Ku80+Iba1+ microglia in the brains of NSG mice transplanted with vehicle-expanded hematopoietic stem cells or MGTA-456. The frequency of Ku80+Iba1+ microglia in the brains of mice transplanted with vehicle-expanded or MGTA-456 were quantitated by IHC from selected sections. The majority of Ku80+Iba1+ microglia are non-perivascular. The bar graph shows the median values obtained upon observation of n=3 mice per group.

FIG. 12A is a graph showing the proportion of CD34+CD90+ mobilized peripheral blood cells in the G0 phase, G1 phase, or S-G2-M phase as a function of days in culture in the presence of cytokines and either vehicle (DMSO, dashed lines) or aryl hydrocarbon receptor antagonist (Compound 26, solid lines).

FIG. 12B is a graph showing the proportion of CD34+CD90+ cord blood cells in the G0 phase, G1 phase, or S-G2-M phase as a function of days in culture in the presence of cytokines and either vehicle (DMSO, dashed lines) or aryl hydrocarbon receptor antagonist (Compound 26, solid lines).

FIG. 13A and 13B are graphs showing rates of gene correction in cells from mobilized peripheral blood (FIG. 13A) and cord blood (FIG. 13B) when grown in culture for 1 day, 2 days, 3 days, or 4 days prior to electroporation in the presence of gene editing reagents and in the presence of vehicle (DMSO) or aryl hydrocarbon receptor antagonist (Compound 26).

FIG. 14A and 14B are graphs showing total number of corrected cells for mobilized peripheral blood (FIG. 14A) and umbilical cord blood (FIG. 14B) for various combinations of days of prestimulation (Pre-stim) and various combinations of days of post-electroporation (post EP) culture, wherein on the x-axis the first number in each pair of numbers refers to the number of days of prestimulation and the second number in each pair of numbers refers to the number of days of post-electroporation culture.

### Detailed Description

Provided herein are compositions and methods for the expansion of hematopoietic stem and progenitor cells, such as hematopoietic stem and progenitor cells that have been genetically modified, for instance, to disrupt an endogenous gene (e.g., a major histocompatibility complex gene) or to express a gene (e.g., a therapeutic transgene). It has presently been discovered that the use of an aryl hydrocarbon receptor antagonist to expand a population of hematopoietic stem or progenitor cells produces a population of cells that can be genetically modified while maintaining long-term engraftment potential and sustained expression of a transgene of interest.

Compositions and methods for expanding hematopoietic stem cells from various sources like bone marrow (BM) mobilized peripheral blood (mPB), or cord blood (CB) can have significant impact on patient outcomes by leading to faster engraftment, which allows for patients to leave the hospital sooner; allows for the expansion of usable CB inventory, which allows for more patients to receive a better matched graft, and improves outcomes in gene therapy by improving the capabilities of gene therapy by increasing the number of edited or transduced cells.

The sections that follow describe, in further detail, the compositions and methods that can be used to effectuate the expansion and genetic modification of hematopoietic stem and progenitor cells.

### Definitions

Listed below are definitions of various terms used in this application. These definitions apply to terms as they are used throughout this specification and claims, unless otherwise limited in specific instances, either individually or as part of a larger group.

As used herein, the term "about" refers to a value that is within 10% above or below the value being described. For example, the term "about 5 nM" indicates a range of from 4.5 nM to 5.5 nM.

As used herein, the terms "conservative mutation," "conservative substitution," or "conservative amino acid substitution" refer to a substitution of one or more amino acids for one or more different amino acids that exhibit similar physicochemical properties, such as polarity, electrostatic charge, and steric volume. These properties are summarized for each of the twenty naturally-occurring amino acids in table 1 below.

Table 1. Representative physicochemical properties of naturally-occurring amino acids

Amino Acid	3 Letter Code	1 Letter Code	Side-chain Polarity	Electrostatic character at physiological pH (7.4)	Steric Volume <sup>†</sup>
Alanine	Ala	A	nonpolar	neutral	small
Arginine	Arg	R	polar	cationic	large
Asparagine	Asn	N	polar	neutral	intermediate
Aspartic acid	Asp	D	polar	anionic	intermediate
Cysteine	Cys	C	nonpolar	neutral	intermediate
Glutamic acid	Glu	E	polar	anionic	intermediate
Glutamine	Gln	Q	polar	neutral	intermediate
Glycine	Gly	G	nonpolar	neutral	small
Histidine	His	H	polar	Both neutral and cationic forms in equilibrium at pH 7.4	large
Isoleucine	Ile	I	nonpolar	neutral	large
Leucine	Leu	L	nonpolar	neutral	large
Lysine	Lys	K	polar	cationic	large
Methionine	Met	M	nonpolar	neutral	large
Phenylalanine	Phe	F	nonpolar	neutral	large
Proline	Pro	P	non-polar	neutral	intermediate
Serine	Ser	S	polar	neutral	small
Threonine	Thr	T	polar	neutral	intermediate
Tryptophan	Trp	W	nonpolar	neutral	bulky
Tyrosine	Tyr	Y	polar	neutral	large
Valine	Val	V	nonpolar	neutral	intermediate

<sup>†</sup>based on volume in Å<sup>3</sup>: 50-100 is small, 100-150 is intermediate, 150-200 is large, and >200 is bulky

5 From this table it is appreciated that the conservative amino acid families include, e.g., (i) G, A, V, L, I, P, and M; (ii) D and E; (iii) C, S and T; (iv) H, K and R; (v) N and Q; and (vi) F, Y and W. A conservative mutation or substitution is therefore one that substitutes one amino acid for a member of the same amino acid family (e.g., a substitution of Ser for Thr or Lys for Arg).

10 As used herein, "CRU (competitive repopulating unit)" refers to a unit of measure of long-term engrafting stem cells, which can be detected after in-vivo transplantation.

As used herein in the context of Gro- $\beta$ , Gro- $\beta$  T, or a variant thereof, the term "deamidated version" of one or more of these peptides refers to a form of the peptide in which the C-terminal asparagine residue that is located at position 69 in the amino acid sequence of Gro- $\beta$ , at position 65 in the amino acid sequence of Gro- $\beta$  T, and at equivalent positions in variant peptides, has been converted to an aspartic acid residue. Deamidated versions of Gro- $\beta$  and Gro- $\beta$  T (Gro- $\beta$  N69D and Gro- $\beta$  T N65D, respectively) are described in Table 2, herein.

As used herein, the term "disrupt" with respect to a gene refers to preventing the formation of a functional gene product. A gene product is functional only if it fulfills its normal (wild-type) functions. Disruption of the gene prevents expression of a functional factor encoded by the gene and comprises an insertion, deletion, or substitution of one or more bases in a sequence encoded by the gene and/or a promoter and/or an operator that is necessary for expression of the gene in the animal. The disrupted gene may be disrupted by, e.g., removal of at least a portion of the gene from a genome of the animal, alteration of the gene to prevent expression of a functional factor encoded by the gene, an interfering RNA, or expression of a dominant negative factor by an exogenous gene. Materials and methods of genetically modifying hematopoietic stem/progenitor cells are detailed in US 8,518,701; US 2010/0251395; and US 2012/0222143, the disclosures of each of which are incorporated herein by reference in their entirety (in case of conflict, the instant specification is controlling).

Various techniques known in the art can be used to inactivate genes to make knock-out animals and/or to introduce nucleic acid constructs into animals to produce founder animals and to make animal lines, in which the knockout or nucleic acid construct is integrated into the genome. Such techniques include, without limitation, pronuclear microinjection (U.S. Pat. No. 4,873,191), retrovirus mediated gene transfer into germ lines (Van der Puijten et al., Proc. Natl. Acad. Sci. USA, 82:6148-6152, 1985), gene targeting into embryonic stem cells (Thompson et al., Cell, 56:313-321, 1989), electroporation of embryos (Lo, Mol. Cell. Biol., 3:1803-1814, 1983), sperm-mediated gene transfer (Lavitrano et al., Proc. Natl. Acad. Sci. USA, 99:14230-14235, 2002; Lavitrano et al., Reprod. Fert. Develop., 18:19-23, 2006), and in vitro transformation of somatic cells, such as cumulus or mammary cells, or adult, fetal, or embryonic stem cells, followed by nuclear transplantation (Wilmut et al., Nature, 385:810-813, 1997; and Wakayama et al., Nature, 394:369-374, 1998). Pronuclear microinjection, sperm mediated gene transfer, and somatic cell nuclear transfer are particularly useful techniques. An animal that is genomically modified is an animal wherein all of its cells have the genetic modification, including its germ line cells. When methods are used that produce an animal that is mosaic in its genetic modification, the animals may be inbred and progeny that are genomically modified may be selected. Cloning, for instance, may be used to make a mosaic animal if its cells are modified at the blastocyst state, or genomic modification can take place when a single-cell is modified. Animals that are modified so they do not sexually mature can be homozygous or heterozygous for the modification, depending on the specific approach that is used. If a particular gene is inactivated by a knock out modification, homozygosity would normally be required. If a particular gene is inactivated by an RNA interference or dominant negative strategy, then heterozygosity is often adequate.

As used herein with respect to a hematopoietic stem cell, the term "progenitor" refers to a parent cell or an ancestor thereof that gave rise to the hematopoietic stem cell by way of cell division. For instance, a progenitor of a hematopoietic stem cell may be a parent cell that gave rise to the hematopoietic stem cell by mitotic reproduction, or an ancestor of the parent cell.

5 As used herein, the term "donor" refers to a subject, such as a mammalian subject (e.g., a human subject) from which one or more cells are isolated prior to administration of the cells, or progeny thereof, into a recipient. The one or more cells may be, for example, a population of hematopoietic stem or progenitor cells.

10 As used herein, the term "endogenous" describes a substance, such as a molecule, cell, tissue, or organ (e.g., a hematopoietic stem cell or a cell of hematopoietic lineage, such as a megakaryocyte, thrombocyte, platelet, erythrocyte, mast cell, myeloblast, basophil, neutrophil, eosinophil, microglial cell, granulocyte, monocyte, osteoclast, antigen-presenting cell, macrophage, dendritic cell, natural killer cell, T-lymphocyte, or B-lymphocyte) that is found naturally in a particular organism, such as a human patient.

15 As used herein, the term "engraftment potential" is used to refer to the ability of hematopoietic stem and progenitor cells to repopulate a tissue, whether such cells are naturally circulating or are provided by transplantation. The term encompasses all events surrounding or leading up to engraftment, such as tissue homing of cells and colonization of cells within the tissue of interest. The engraftment efficiency or rate of engraftment can be evaluated or quantified using any clinically acceptable parameter as known to those of skill in the art and can include, for example, assessment of competitive repopulating  
20 units (CRU); incorporation or expression of a marker in tissue(s) into which stem cells have homed, colonized, or become engrafted; or by evaluation of the progress of a subject through disease progression, survival of hematopoietic stem and progenitor cells, or survival of a recipient. Engraftment can also be determined by measuring white blood cell counts in peripheral blood during a post-transplant period. Engraftment can also be assessed by measuring recovery of marrow cells by donor cells in a  
25 bone marrow aspirate sample.

As used herein, the term "exogenous" describes a substance, such as a molecule, cell, tissue, or organ (e.g., a hematopoietic stem cell or a cell of hematopoietic lineage, such as a megakaryocyte, thrombocyte, platelet, erythrocyte, mast cell, myeloblast, basophil, neutrophil, eosinophil, microglial cell, granulocyte, monocyte, osteoclast, antigen-presenting cell, macrophage, dendritic cell, natural killer cell,  
30 T-lymphocyte, or B-lymphocyte) that is not found naturally in a particular organism, such as a human patient. Exogenous substances include those that are provided from an external source to an organism or to cultured matter extracted therefrom.

As used herein, the term "expanding amount" refers to a quantity or concentration of an agent, such as an aryl hydrocarbon receptor antagonist described herein, sufficient to induce the proliferation of  
35 a population of CD34+ cells (e.g., a CD34+ CD90+ cells), for example, by from about 1.1-fold to about 1,000-fold, about 1.1-fold to about 5,000-fold, or more (e.g., about 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2-fold, 2.1-fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold, 3.5-fold, 3.6-fold, 3.7-fold, 3.8-fold, 3.9-fold, 4-fold, 4.1-fold, 4.2-fold, 4.3-fold, 4.4-fold, 4.5-fold, 4.6-fold, 4.7-fold, 4.8-fold, 4.9-fold, 5-fold, 5.1-

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In one embodiment, the expanding amount, refers to a quantity or concentration of an agent, such as an aryl hydrocarbon receptor antagonist described herein, sufficient to induce the proliferation of a population of CD34<sup>+</sup> cells (e.g., a CD34<sup>+</sup> CD90<sup>+</sup> cells), for example, by from about 60-fold to about 900-fold, from about 80-fold to about 800-fold, from about 100-fold to about 700-fold, from about 150-fold to about 600-fold, from about 200-fold to about 500-fold, from about 250-fold to about 400-fold, from about 275-fold to about 350-fold, or about 325-fold.

As used herein, the term "hematopoietic progenitor cells" includes pluripotent cells capable of differentiating into several cell types of the hematopoietic system, including, without limitation, granulocytes, monocytes, erythrocytes, megakaryocytes, B-cells and T- cells, among others. Hematopoietic progenitor cells are committed to the hematopoietic cell lineage and generally do not self-renew. Hematopoietic progenitor cells can be identified, for example, by expression patterns of cell surface antigens, and include cells having the following immunophenotype: Lin<sup>-</sup> KLS<sup>+</sup> Fik2<sup>-</sup> CD34<sup>+</sup>. Hematopoietic progenitor cells include short-term hematopoietic stem cells, multi-potent progenitor cells, common myeloid progenitor cells, granulocyte-monocyte progenitor cells, and megakaryocyte-erythrocyte progenitor cells. The presence of hematopoietic progenitor cells can be determined functionally, for instance, by detecting colony-forming unit cells, e.g., in complete methylcellulose assays, or phenotypically through the detection of cell surface markers using flow cytometry and cell sorting assays described herein and known in the art.

As used herein, the term "hematopoietic stem cells" ("HSCs") refers to immature blood cells having the capacity to self-renew and to differentiate into mature blood cells containing diverse lineages including but not limited to granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells, microglia, osteoclasts, and lymphocytes (e.g., NK cells, B-cells and T-cells). Such cells may include CD34<sup>+</sup> cells. CD34<sup>+</sup> cells are immature cells that express the CD34 cell surface marker. In humans, CD34<sup>+</sup> cells are believed to include a subpopulation of cells with the stem cell properties defined above, whereas in mice, HSCs are CD34<sup>-</sup>. In addition, HSCs also refer to long term repopulating HSCs (LT-HSC) and short term repopulating HSCs (ST-HSC). LT-HSCs and ST-HSCs are differentiated, based on functional potential and on cell surface marker expression. For example, human HSCs are CD34<sup>+</sup>, CD38<sup>-</sup>, CD45RA<sup>-</sup>, CD90<sup>+</sup>, CD49F<sup>+</sup>, and lin<sup>-</sup> (negative for mature lineage markers including CD2, CD3, CD4, CD7, CD8, CD10, CD11B, CD19, CD20, CD56, CD235A). In mice, bone marrow LT-HSCs are CD34<sup>-</sup>, SCA-1<sup>+</sup>, C-kit<sup>+</sup>, CD135<sup>-</sup>, Slamf/CD150<sup>+</sup>, CD48<sup>-</sup>, and lin<sup>-</sup> (negative for mature lineage markers including Ter119, CD11b, Gr1, CD3, CD4, CD8, B220, IL7ra), whereas ST-HSCs are CD34<sup>+</sup>, SCA-1<sup>+</sup>, C-kit<sup>+</sup>, CD135<sup>-</sup>,

Slamf1/CD150+, and lin- (negative for mature lineage markers including Ter119, CD11b, Gr1, CD3, CD4, CD8, B220, IL7ra). In addition, ST-HSCs are less quiescent and more proliferative than LT-HSCs under homeostatic conditions. However, LT-HSC have greater self renewal potential (i.e., they survive throughout adulthood, and can be serially transplanted through successive recipients), whereas ST-HSCs have limited self renewal (i.e., they survive for only a limited period of time, and do not possess serial transplantation potential). Any of these HSCs can be used in the methods described herein. ST-HSCs are particularly useful because they are highly proliferative and thus, can more quickly give rise to differentiated progeny.

As used herein, the term "hematopoietic stem cell functional potential" refers to the functional properties of hematopoietic stem cells which include 1) multi-potency (which refers to the ability to differentiate into multiple different blood lineages including, but not limited to, granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells, microglia, osteoclasts, and lymphocytes (e.g., NK cells, B-cells and T-cells), 2) self-renewal (which refers to the ability of hematopoietic stem cells to give rise to daughter cells that have equivalent potential as the mother cell, and further that this ability can repeatedly occur throughout the lifetime of an individual without exhaustion), and 3) the ability of hematopoietic stem cells or progeny thereof to be reintroduced into a transplant recipient whereupon they home to the hematopoietic stem cell niche and re-establish productive and sustained hematopoiesis.

As used herein, the terms "Major histocompatibility complex antigens" ("MHC", also referred to as "human leukocyte antigens" ("HLA") in the context of humans) refer to proteins expressed on the cell surface that confer a unique antigenic identity to a cell. MHC/HLA antigens are target molecules that are recognized by T cells and NK cells as being derived from the same source of hematopoietic stem cells as the immune effector cells ("self") or as being derived from another source of hematopoietic reconstituting cells ("non-self"). Two main classes of HLA antigens are recognized: HLA class I and HLA class II. HLA class I antigens (A, B, and C in humans) render each cell recognizable as "self," whereas HLA class II antigens (DR, DP, and DQ in humans) are involved in reactions between lymphocytes and antigen presenting cells. Both have been implicated in the rejection of transplanted organs. An important aspect of the HLA gene system is its polymorphism. Each gene, MHC class I (A, B and C) and MHC class II (DP, DQ and DR) exists in different alleles. For example, two unrelated individuals may carry class I HLA-B, genes B5, and Bw41, respectively. Allelic gene products differ in one or more amino acids in the  $\alpha$  and/or  $\beta$  domain(s). Large panels of specific antibodies or nucleic acid reagents are used to type HLA haplotypes of individuals, using leukocytes that express class I and class II molecules. The genes commonly used for HLA typing are the six MHC Class I and Class II proteins, two alleles for each of HLA-A; HLA-B and HLA-DR. The HLA genes are clustered in a "super-locus" present on chromosome position 6p21, which encodes the six classical transplantation HLA genes and at least 132 protein coding genes that have important roles in the regulation of the immune system as well as some other fundamental molecular and cellular processes. The complete locus measures roughly 3.6 Mb, with at least 224 gene loci. One effect of this clustering is that "haplotypes", i.e. the set of alleles present on a single

chromosome, which is inherited from one parent, tend to be inherited as a group. The set of alleles inherited from each parent forms a haplotype, in which some alleles tend to be associated together. Identifying a patient's haplotypes can help predict the probability of finding matching donors and assist in developing a search strategy, because some alleles and haplotypes are more common than others and they are distributed at different frequencies in different racial and ethnic groups.

As used herein, the term "HLA-matched" refers to a donor-recipient pair in which none of the HLA antigens are mismatched between the donor and recipient, such as a donor providing a hematopoietic stem cell graft to a recipient in need of hematopoietic stem cell transplant therapy. HLA-matched (i.e., where all of the 6 alleles are matched) donor-recipient pairs have a decreased risk of graft rejection, as endogenous T cells and NK cells are less likely to recognize the incoming graft as foreign, and are thus less likely to mount an immune response against the transplant.

As used herein, the term "HLA-mismatched" refers to a donor-recipient pair in which at least one HLA antigen, in particular with respect to HLA-A, HLA-B, HLA-C, and HLA-DR, is mismatched between the donor and recipient, such as a donor providing a hematopoietic stem cell graft to a recipient in need of hematopoietic stem cell transplant therapy. In some embodiments, one haplotype is matched and the other is mismatched. HLA-mismatched donor-recipient pairs may have an increased risk of graft rejection relative to HLA-matched donor-recipient pairs, as endogenous T cells and NK cells are more likely to recognize the incoming graft as foreign in the case of an HLA-mismatched donor-recipient pair, and such T cells and NK cells are thus more likely to mount an immune response against the transplant.

As used herein, the term "aryl hydrocarbon receptor (AHR) modulator" refers to an agent that causes or facilitates a qualitative or quantitative change, alteration, or modification in one or more processes, mechanisms, effects, responses, functions, activities or pathways mediated by the AHR receptor. Such changes mediated by an AHR modulator, such as an inhibitor or a non-constitutive agonist of the AHR described herein, can refer to a decrease or an increase in the activity or function of the AHR, such as a decrease in, inhibition of, or diversion of, constitutive activity of the AHR.

An "AHR antagonist" refers to an AHR inhibitor that does not provoke a biological response itself upon specifically binding to the AHR polypeptide or polynucleotide encoding the AHR, but blocks or dampens agonist-mediated or ligand-mediated responses, i.e., an AHR antagonist can bind but does not activate the AHR polypeptide or polynucleotide encoding the AHR, and the binding disrupts the interaction, displaces an AHR agonist, and/or inhibits the function of an AHR agonist. Thus, as used herein, an AHR antagonist does not function as an inducer of AHR activity when bound to the AHR, i.e., they function as pure AHR inhibitors.

As used herein, patients that are "in need of" a hematopoietic stem cell transplant include patients that exhibit a defect or deficiency in one or more blood cell types, as well as patients having a stem cell disorder, autoimmune disease, cancer, or other pathology described herein. Hematopoietic stem cells generally exhibit 1) multi-potency, and can thus differentiate into multiple different blood lineages including, but not limited to, granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells,



microglia, osteoclasts, and lymphocytes (e.g., NK cells, B-cells and T-cells), 2) self-renewal, and can thus give rise to daughter cells that have equivalent potential as the mother cell, and 3) the ability to be reintroduced into a transplant recipient whereupon they home to the hematopoietic stem cell niche and re-establish productive and sustained hematopoiesis. Hematopoietic stem cells can thus be administered to a patient defective or deficient in one or more cell types of the hematopoietic lineage in order to re-constitute the defective or deficient population of cells in vivo. For example, the patient may be suffering from cancer, and the deficiency may be caused by administration of a chemotherapeutic agent or other medicament that depletes, either selectively or non-specifically, the cancerous cell population. Additionally or alternatively, the patient may be suffering from a hemoglobinopathy (e.g., a non-malignant hemoglobinopathy), such as sickle cell anemia, thalassemia, Fanconi anemia, aplastic anemia, and Wiskott-Aldrich syndrome. The subject may be one that is suffering from adenosine deaminase severe combined immunodeficiency (ADA SCID), HIV/AIDS, metachromatic leukodystrophy, Diamond-Blackfan anemia, and Schwachman-Diamond syndrome. The subject may have or be affected by an inherited blood disorder (e.g., sickle cell anemia) or an autoimmune disorder. Additionally or alternatively, the subject may have or be affected by a malignancy, such as neuroblastoma or a hematologic cancer. For instance, the subject may have a leukemia, lymphoma, or myeloma. In some embodiments, the subject has acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, or non-Hodgkin's lymphoma. In some embodiments, the subject has myelodysplastic syndrome. In some embodiments, the subject has an autoimmune disease, such as scleroderma, multiple sclerosis, ulcerative colitis, Crohn's disease, Type 1 diabetes, or another autoimmune pathology described herein. In some embodiments, the subject is in need of chimeric antigen receptor T-cell (CART) therapy. In some embodiments, the subject has or is otherwise affected by a metabolic storage disorder. The subject may suffer or otherwise be affected by a metabolic disorder selected from the group consisting of glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurler syndrome or Hurler's Disease, sphingolipidoses, Mucopolipidosis II, metachromatic leukodystrophy, or any other diseases or disorders which may benefit from the treatments and therapies disclosed herein and including, without limitation, severe combined immunodeficiency, Wiskott-Aldrich syndrome, hyper immunoglobulin M (IgM) syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, sickle cell disease, systemic sclerosis, systemic lupus erythematosus, multiple sclerosis, juvenile rheumatoid arthritis and those diseases, or disorders described in "Bone Marrow Transplantation for Non-Malignant Disease," ASH Education Book, 1:319-338 (2000), the disclosure of which is incorporated herein by reference in its entirety as it pertains to pathologies that may be treated by administration of hematopoietic stem cell transplant therapy. Additionally or alternatively, a patient "in need of" a hematopoietic stem cell transplant may one that is or is not suffering from one of the foregoing pathologies, but nonetheless exhibits a reduced level (e.g., as compared to that of an otherwise healthy subject) of one or more endogenous cell types within the hematopoietic lineage, such as megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeloblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages,

dendritic cells, natural killer cells, T-lymphocytes, and B-lymphocytes. One of skill in the art can readily determine whether one's level of one or more of the foregoing cell types, or other blood cell type, is reduced with respect to an otherwise healthy subject, for instance, by way of flow cytometry and fluorescence activated cell sorting (FACS) methods, among other procedures, known in the art.

5 As used herein, the terms "mobilize" and "mobilization" refer to processes by which a population of hematopoietic stem or progenitor cells is released from a stem cell niche, such as the bone marrow of a subject, into circulation in the peripheral blood. Mobilization of hematopoietic stem and progenitor cells can be monitored, for instance, by assessing the quantity or concentration of hematopoietic stem or progenitor cells in a peripheral blood sample isolated from a subject. For example, the peripheral blood  
10 sample may be withdrawn from the subject, and the quantity or concentration of hematopoietic stem or progenitor cells in the peripheral blood sample may subsequently be assessed, following the administration of a hematopoietic stem or progenitor cell mobilization regimen to the subject. The mobilization regimen may include, for instance, a CXCR4 antagonist, such as a CXCR4 antagonist described herein (e.g., plerixafor or a variant thereof), and a CXCR2 agonist, such as a CXCR2 agonist  
15 described herein (e.g., Gro- $\beta$  or a variant thereof, such as a truncation of Gro- $\beta$ , for instance, Gro- $\beta$  T). The quantity or concentration of hematopoietic stem or progenitor cells in the peripheral blood sample isolated from the subject following administration of the mobilization regimen may be compared to the quantity or concentration of hematopoietic stem or progenitor cells in a peripheral blood sample isolated from the subject prior to administration of the mobilization regimen. An observation that the quantity or  
20 concentration of hematopoietic stem or progenitor cells has increased in the peripheral blood of the subject following administration of the mobilization regimen is an indication that the subject is responding to the mobilization regimen, and that hematopoietic stem and progenitor cells have been released from one or more stem cell niches, such as the bone marrow, into peripheral blood circulation.

As used herein, the term "sample" refers to a specimen (e.g., blood, blood component (e.g.,  
25 serum or plasma), urine, saliva, amniotic fluid, cerebrospinal fluid, tissue (e.g., placental or dermal), pancreatic fluid, chorionic villus sample, and cells) taken from a subject.

As used herein, the phrase "stem cell disorder" broadly refers to any disease, disorder, or condition that may be treated or cured by engrafting or transplanting a population of hematopoietic stem or progenitor cells in a target tissue within a patient. For example, Type I diabetes has been shown to be  
30 cured by hematopoietic stem cell transplant, along with various other disorders. Diseases that can be treated by infusion of hematopoietic stem or progenitor cells into a patient include, sickle cell anemia, thalassemias, Fanconi anemia, aplastic anemia, Wiskott-Aldrich syndrome, ADA SCID, HIV/AIDS, metachromatic leukodystrophy, Diamond-Blackfan anemia, and Schwachman-Diamond syndrome. Additional diseases that may be treated by transplantation of hematopoietic stem and progenitor cells as  
35 described herein include blood disorders (e.g., sickle cell anemia) and autoimmune disorders, such as scleroderma, multiple sclerosis, ulcerative colitis, and Crohn's disease. Additional diseases that may be treated using hematopoietic stem and progenitor cell transplant therapy include cancer, such as a cancer described herein. Stem cell disorders include a malignancy, such as a neuroblastoma or a hematologic cancers, such as leukemia, lymphoma, and myeloma. For instance, the cancer may be acute myeloid

leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, or non-Hodgkin's lymphoma. Disorders that may be treated by transplanting a population of hematopoietic stem cells to a patient include neurological disorders, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, Amyotrophic lateral sclerosis, Huntington's disease, mild cognitive impairment, amyloidosis, AIDS-related dementia, encephalitis, stroke, head trauma, epilepsy, mood disorders, and dementia. As described herein, without being limited by mechanism, the ability of hematopoietic stem cell transplantation to treat such disorders may be due, in part, to the capacity of hematopoietic stem cells to migrate to the central nervous system and differentiate into microglial cells, thereby repopulating a hematopoietic cell line that may be damaged or deficient in patients having a neurological disorder. Additional diseases treatable using hematopoietic stem or progenitor cell transplant therapy include myelodysplastic syndrome. In some embodiments, the patient has or is otherwise affected by a metabolic storage disorder. For example, the patient may suffer or otherwise be affected by a metabolic disorder selected from the group consisting of glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurler syndrome or Hurler's Disease, sphingolipidoses, Mucopolipidosis II, metachromatic leukodystrophy, or any other diseases or disorders which may benefit from the treatments and therapies disclosed herein and including, without limitation, severe combined immunodeficiency, Wiscott-Aldrich syndrome, hyper immunoglobulin M (IgM) syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, sickle cell disease, systemic sclerosis, systemic lupus erythematosus, multiple sclerosis, juvenile rheumatoid arthritis and those diseases, or disorders described in "Bone Marrow Transplantation for Non-Malignant Disease," ASH Education Book, 1:319-338 (2000), the disclosure of which is incorporated herein by reference in its entirety as it pertains to pathologies that may be treated by administration of hematopoietic stem or progenitor cell transplant therapy.

As used herein, the terms "subject" and "patient" refer to an organism, such as a human, that receives treatment for a particular disease or condition as described herein. For instance, a patient, such as a human patient, that is in need of hematopoietic stem cell transplantation may receive treatment that includes a population of hematopoietic stem cells so as to treat a stem cell disorder, such as a cancer, autoimmune disease, or metabolic disorder described herein. A patient, such as a human patient suffering from a stem cell disorder, may, for instance, receive treatment in the form of a population of hematopoietic stem cells, such as a population of from about  $1 \times 10^6$  to about  $1 \times 10^9$  hematopoietic stem cells.

As used herein, the term "recipient" refers to a patient that receives a transplant, such as a transplant containing a population of hematopoietic stem cells. The transplanted cells administered to a recipient may be, e.g., autologous, syngeneic, or allogeneic cells.

As used herein, the term "transfection" refers to any of a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, such as electroporation, lipofection, calcium-phosphate precipitation, DEAE-dextran transfection and the like.

As used herein, the terms "treat", "treating" or "treatment" refer to a method of alleviating or abating a disease and/or its attendant symptoms. As used herein, the terms "preventing" or "prevent" describes reducing or eliminating the onset of the symptoms or complications of the disease, condition, or disorder. As used herein, the terms "disease(s)", "disorder(s)", and "condition(s)" are used interchangeably, unless the context clearly dictates otherwise.

"Treating" may refer to therapeutic treatment, in which the object is to prevent or slow down (lessen) an undesired physiological change or disorder or to promote a beneficial phenotype in the patient being treated. Beneficial or desired clinical results include, but are not limited to, promoting the engraftment of exogenous hematopoietic cells in a patient following hematopoietic stem or progenitor cell transplant therapy. Additional beneficial results include an increase in the cell count or relative concentration of hematopoietic stem cells in a patient in need of a hematopoietic stem or progenitor cell transplant following administration of an exogenous hematopoietic stem or progenitor cell graft to the patient. Beneficial results of therapy described herein may also include an increase in the cell count or relative concentration of one or more cells of hematopoietic lineage, such as a megakaryocyte, thrombocyte, platelet, erythrocyte, mast cell, myeloblast, basophil, neutrophil, eosinophil, microglial cell, granulocyte, monocyte, osteoclast, antigen-presenting cell, macrophage, dendritic cell, natural killer cell, T-lymphocyte, or B-lymphocyte, following and subsequent hematopoietic stem cell transplant therapy. Additional beneficial results may include the reduction in quantity of a disease-causing cell population, such as a population of cancer cells or autoimmune cells.

As used herein, the terms "variant" and "derivative" are used interchangeably and refer to naturally-occurring, synthetic, and semi-synthetic analogues of a compound, peptide, protein, or other substance described herein. A variant or derivative of a compound, peptide, protein, or other substance described herein may retain or improve upon the biological activity of the original material.

As used herein, the term "vector" includes a nucleic acid vector, such as a plasmid, a DNA vector, a plasmid, a RNA vector, virus, or other suitable replicon. Expression vectors described herein may contain a polynucleotide sequence as well as, for example, additional sequence elements used for the expression of proteins and/or the integration of these polynucleotide sequences into the genome of a mammalian cell. Certain vectors that can be used for the expression of peptides and proteins, such as those described herein, include plasmids that contain regulatory sequences, such as promoter and enhancer regions, which direct gene transcription. Other useful vectors for expression of peptides and proteins described herein contain polynucleotide sequences that enhance the rate of translation of these genes or improve the stability or nuclear export of the mRNA that results from gene transcription. These sequence elements may include, for example, 5' and 3' untranslated regions and a polyadenylation signal site in order to direct efficient transcription of the gene carried on the expression vector. The expression vectors described herein may also contain a polynucleotide encoding a marker for selection of cells that contain such a vector. Examples of a suitable marker include genes that encode resistance to antibiotics, such as ampicillin, chloramphenicol, kanamycin, and nourseothricin.

As used herein, the term "alkyl" refers to a straight- or branched-chain alkyl group having, for example, from 1 to 20 carbon atoms in the chain, or, in certain embodiments, from 1 to 6 carbon atoms in

the chain. Examples of alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, tert-pentyl, neopentyl, isopentyl, tert-pentyl, hexyl, isohexyl, and the like.

As used herein, the term "alkylene" refers to a straight- or branched-chain divalent alkyl group.  
5 The divalent positions may be on the same or different atoms within the alkyl chain. Examples of alkylene include methylene, ethylene, propylene, isopropylene, and the like.

As used herein, the term "heteroalkyl" refers to a straight or branched-chain alkyl group having, for example, from 1 to 20 carbon atoms in the chain, and further containing one or more heteroatoms (e.g., oxygen, nitrogen, or sulfur, among others) in the chain.

10 As used herein, the term "heteroalkylene" refers to a straight- or branched-chain divalent heteroalkyl group. The divalent positions may be on the same or different atoms within the heteroalkyl chain. The divalent positions may be one or more heteroatoms.

As used herein, the term "alkenyl" refers to a straight- or branched-chain alkenyl group having, for example, from 2 to 20 carbon atoms in the chain. It denotes a monovalent group derived from a  
15 hydrocarbon moiety containing, for example, from two to six carbon atoms having at least one carbon-carbon double bond. The double bond may or may not be the point of attachment to another group. Examples of alkenyl groups include, but are not limited to, vinyl, propenyl, isopropenyl, butenyl, tert-butulenyl, 1-methyl-2-buten-1-yl, hexenyl, and the like.

As used herein, the term "alkenylene" refers to a straight- or branched-chain divalent alkenyl  
20 group. The divalent positions may be on the same or different atoms within the alkenyl chain. Examples of alkenylene include ethenylene, propenylene, isopropenylene, butenylene, and the like.

As used herein, the term "heteroalkenyl" refers to a straight- or branched-chain alkenyl group having, for example, from 2 to 20 carbon atoms in the chain, and further containing one or more heteroatoms (e.g., oxygen, nitrogen, or sulfur, among others) in the chain.

25 As used herein, the term "heteroalkenylene" refers to a straight- or branched-chain divalent heteroalkenyl group. The divalent positions may be on the same or different atoms within the heteroalkenyl chain. The divalent positions may be one or more heteroatoms.

As used herein, the term "alkynyl" refers to a straight- or branched-chain alkynyl group having, for example, from 2 to 20 carbon atoms in the chain and at least one carbon-carbon triple bond. Examples  
30 of alkynyl groups include, but are not limited to, propargyl, butynyl, pentynyl, hexynyl, and the like.

As used herein, the term "alkynylene" refers to a straight- or branched-chain divalent alkynyl group. The divalent positions may be on the same or different atoms within the alkynyl chain.

As used herein, the term "heteroalkynyl" refers to a straight- or branched-chain alkynyl group having, for example, from 2 to 20 carbon atoms in the chain, and further containing one or more  
35 heteroatoms (e.g., oxygen, nitrogen, or sulfur, among others) in the chain.

As used herein, the term "heteroalkynylene" refers to a straight- or branched-chain divalent heteroalkynyl group. The divalent positions may be on the same or different atoms within the heteroalkynyl chain. The divalent positions may be one or more heteroatoms.

As used herein, the term "cycloalkyl" refers to a monocyclic, or fused, bridged, or spiro polycyclic ring structure that is saturated and has, for example, from 3 to 12 carbon ring atoms. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, bicyclo[3.1.0]hexane, and the like. Also contemplated is a monovalent group derived from a monocyclic or polycyclic carbocyclic ring compound having at least one carbon-carbon double bond by the removal of at least one or two hydrogen atoms. Examples of such groups include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl,

As used herein, the term "cycloalkylene" refers to a divalent cycloalkyl group. The divalent positions may be on the same or different atoms within the ring structure. Examples of cycloalkylene include cyclopropylene, cyclobutylene, cyclopentylene, cyclohexylene, and the like.

As used herein, the term "heterocycloalkyl" or "heterocyclyl" refers to a monocyclic, or fused, bridged, or spiro polycyclic ring structure that is saturated and has, for example, from 3 to 12 ring atoms per ring structure selected from carbon atoms and heteroatoms selected from, e.g., nitrogen, oxygen, and sulfur, among others. The ring structure may contain, for example, one or more oxo groups on carbon, nitrogen, or sulfur ring members. Exemplary heterocycloalkyl groups include, but are not limited to, [1,3] dioxolane, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperazinyl, piperidinyl, oxazolidinyl, isooxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, and tetrahydrofuryl.

As used herein, the term "heterocycloalkylene" refers to a divalent heterocycloalkyl group. The divalent positions may be on the same or different atoms within the ring structure.

As used herein, the term "aryl" refers to a monocyclic or polycyclic aromatic ring system containing, for example, from 6 to 19 carbon atoms. Aryl groups include, but are not limited to, phenyl, fluorenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl, and the like. The divalent positions may be one or more heteroatoms.

As used herein, the term "arylene" refers to a divalent aryl group. The divalent positions may be on the same or different atoms.

As used herein, the term "heteroaryl" refers to a monocyclic heteroaromatic, or a bicyclic or a tricyclic fused-ring heteroaromatic group. In certain embodiments, the heteroaryl group contains five to ten ring atoms of which one ring atom is selected from S, O, and N; zero, one, or two ring atoms are additional heteroatoms independently selected from S, O, and N; and the remaining ring atoms are carbon. Heteroaryl groups include, but are not limited to, pyridyl, pyrrolyl, furyl, thienyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,3,4-triazinyl, 1,2,3-triazinyl, benzofuryl, [2,3-dihydro]benzofuryl, isobenzofuryl, benzothienyl, benzotriazolyl, isobenzothienyl, indolyl, isoindolyl, 3H-indolyl, benzimidazolyl, imidazo[1,2-a]pyridyl, benzothiazolyl, benzoxazolyl, quinoliziny, quinazolinyl, pthalazinyl, quinoxaliny, cinnolinyl, naphthyridinyl, pyrido[3,4-b]pyridyl, pyrido[3,2-b]pyridyl, pyrido[4,3-b]pyridyl, quinolyl, isoquinolyl, tetrazolyl, 5,6,7,8-tetrahydroquinolyl, 5,6,7,8-tetrahydroisoquinolyl, purinyl, pteridinyl, carbazolyl, xanthenyl, benzoquinolyl, and the like.

As used herein, the term "heteroarylene" refers to a divalent heteroaryl group. The divalent positions may be on the same or different atoms. The divalent positions may be one or more heteroatoms.

Unless otherwise constrained by the definition of the individual substituent, the foregoing  
5 chemical moieties, such as "alkyl", "alkylene", "heteroalkyl", "heteroalkylene", "alkenyl", "alkenylene",  
"heteroalkenyl", "heteroalkenylene", "alkynyl", "alkynylene", "heteroalkynyl", "heteroalkynylene",  
"cycloalkyl", "cycloalkylene", "heterocycloalkyl", "heterocycloalkylene", "aryl", "arylene", "heteroaryl", and  
"heteroarylene" groups can optionally be substituted. As used herein, the term "optionally substituted"  
10 refers to a compound or moiety containing one or more (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more)  
substituents, as permitted by the valence of the compound or moiety or a site thereof, such as a  
substituent selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, alkyl  
aryl, alkyl heteroaryl, alkyl cycloalkyl, alkyl heterocycloalkyl, amino, ammonium, acyl, acyloxy, acylamino,  
aminocarbonyl, alkoxycarbonyl, ureido, carbamate, aryl, heteroaryl, sulfinyl, sulfonyl, alkoxy, sulfanyl,  
15 halogen, carboxy, trihalomethyl, cyano, hydroxy, mercapto, nitro, and the like. The substitution may  
include situations in which neighboring substituents have undergone ring closure, such as ring closure of  
vicinal functional substituents, to form, for instance, lactams, lactones, cyclic anhydrides, acetals,  
hemiacetals, thioacetals, amins, and hemiaminals, formed by ring closure, for example, to furnish a  
protecting group.

As used herein, the term "optionally substituted" refers to a chemical moiety that may have one or  
20 more chemical substituents, as valency permits, such as C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-10  
cycloalkyl, C3-10 heterocycloalkyl, aryl, alkylaryl, heteroaryl, alkylheteroaryl, amino, ammonium, acyl,  
acyloxy, acylamino, aminocarbonyl, alkoxycarbonyl, ureido, carbamate, sulfinyl, sulfonyl, alkoxy, sulfanyl,  
halogen, carboxy, trihalomethyl, cyano, hydroxy, mercapto, nitro, and the like. An optionally substituted  
chemical moiety may contain, e.g., neighboring substituents that have undergone ring closure, such as  
25 ring closure of vicinal functional substituents, thus forming, e.g., lactams, lactones, cyclic anhydrides,  
acetals, thioacetals, or amins formed by ring closure, for instance, in order to generate protecting group.

In accordance with the application, any of the aryls, substituted aryls, heteroaryls and substituted  
heteroaryls described herein, can be any aromatic group.

The terms "hal," "halo," and "halogen," as used herein, refer to an atom selected from fluorine,  
30 chlorine, bromine and iodine.

As described herein, compounds of the application and moieties present in the compounds may  
optionally be substituted with one or more substituents, such as are illustrated generally above, or as  
exemplified by particular classes, subclasses, and species of the application. It will be appreciated that  
the phrase "optionally substituted" is used interchangeably with the phrase "substituted or unsubstituted."  
35 In general, the term "substituted", whether preceded by the term "optionally" or not, refers to the  
replacement of hydrogen radicals in a given structure with the radical of a specified substituent. Unless  
otherwise indicated, an optionally substituted group may have a substituent at each substitutable position  
of the group, and when more than one position in any given structure may be substituted with more than  
one substituent selected from a specified group, the substituent may be either the same or different at

every position. The terms "optionally substituted", "optionally substituted alkyl", "optionally substituted alkenyl", "optionally substituted alkynyl", "optionally substituted cycloalkyl", "optionally substituted cycloalkenyl", "optionally substituted aryl", "optionally substituted heteroaryl", "optionally substituted aralkyl", "optionally substituted heteroaralkyl", "optionally substituted heterocycloalkyl", and any other optionally substituted group as used herein, refer to groups that are substituted or unsubstituted by independent replacement of one, two, or three or more of the hydrogen atoms thereon with substituents including, but not limited to:

-F, -Cl, -Br, -I, -OH, protected hydroxy, -NO<sub>2</sub>, -CN, -NH<sub>2</sub>, protected amino, -NH-C<sub>1</sub>-C<sub>12</sub>-alkyl, -NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NH-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl,  
 10 -NH-aryl, -NH-heteroaryl, -NH-heterocycloalkyl, -dialkylamino, -diarylmino, -diheteroarylmino, -O-C<sub>1</sub>-C<sub>12</sub>-alkyl, -O-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -O-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -O-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -O-aryl, -O-heteroaryl, -O-heterocycloalkyl, -C(O)-C<sub>1</sub>-C<sub>12</sub>-alkyl, -C(O)-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -C(O)-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -C(O)-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -C(O)-aryl, -C(O)-heteroaryl, -C(O)-heterocycloalkyl, -CONH<sub>2</sub>, -CONH-C<sub>1</sub>-C<sub>12</sub>-alkyl, -CONH-C<sub>2</sub>-C<sub>12</sub>-alkenyl,  
 15 -CONH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -CONH-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -CONH-aryl, -CONH-heteroaryl, -CONH-heterocycloalkyl, -OCO<sub>2</sub>-C<sub>1</sub>-C<sub>12</sub>-alkyl, -OCO<sub>2</sub>-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -OCO<sub>2</sub>-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -OCO<sub>2</sub>-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -OCO<sub>2</sub>-aryl, -OCO<sub>2</sub>-heteroaryl, -OCO<sub>2</sub>-heterocycloalkyl, -OCONH<sub>2</sub>, -OCONH-C<sub>1</sub>-C<sub>12</sub>-alkyl, -OCONH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -OCONH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -OCONH-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -OCONH-aryl, -OCONH-heteroaryl, -OCONH-heterocycloalkyl,  
 20 -NHC(O)-C<sub>1</sub>-C<sub>12</sub>-alkyl, -NHC(O)-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHC(O)-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHC(O)-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -NHC(O)-aryl, -NHC(O)-heteroaryl, -NHC(O)-heterocycloalkyl, -NHCO<sub>2</sub>-C<sub>1</sub>-C<sub>12</sub>-alkyl, -NHCO<sub>2</sub>-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHCO<sub>2</sub>-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHCO<sub>2</sub>-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -NHCO<sub>2</sub>-aryl, -NHCO<sub>2</sub>-heteroaryl, -NHCO<sub>2</sub>-heterocycloalkyl, NHC(O)NH<sub>2</sub>, -NHC(O)NH-C<sub>1</sub>-C<sub>12</sub>-alkyl, -NHC(O)NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl,  
 25 -NHC(O)NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHC(O)NH-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -NHC(O)NH-aryl, -NHC(O)NH-heteroaryl, NHC(O)NH-heterocycloalkyl, -NHC(S)NH<sub>2</sub>, -NHC(S)NH-C<sub>1</sub>-C<sub>12</sub>-alkyl, -NHC(S)NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHC(S)NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHC(S)NH-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -NHC(S)NH-aryl, -NHC(S)NH-heteroaryl, -NHC(S)NH-heterocycloalkyl, -NHC(NH)NH<sub>2</sub>,  
 30 -NHC(NH)NH-C<sub>1</sub>-C<sub>12</sub>-alkyl, -NHC(NH)NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHC(NH)NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHC(NH)NH-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -NHC(NH)NH-aryl, -NHC(NH)NH-heteroaryl, -NHC(NH)NH-heterocycloalkyl, -NHC(NH)-C<sub>1</sub>-C<sub>12</sub>-alkyl, -NHC(NH)-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHC(NH)-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHC(NH)-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -NHC(NH)-aryl, -NHC(NH)-heteroaryl, -NHC(NH)-heterocycloalkyl, -C(NH)NH-C<sub>1</sub>-C<sub>12</sub>-alkyl,  
 35 -C(NH)NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -C(NH)NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, C(NH)NH-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -C(NH)NH-aryl, -C(NH)NH-heteroaryl, -C(NH)NH-heterocycloalkyl, -S(O)-C<sub>1</sub>-C<sub>12</sub>-alkyl, -S(O)-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -S(O)-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -S(O)-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -S(O)-aryl, -S(O)-heteroaryl, -S(O)-heterocycloalkyl -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NH-C<sub>1</sub>-C<sub>12</sub>-alkyl, -SO<sub>2</sub>NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -SO<sub>2</sub>NH-C<sub>2</sub>-C<sub>12</sub>-alkenyl,



-SO<sub>2</sub>NH-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -SO<sub>2</sub>NH-aryl, -SO<sub>2</sub>NH-heteroaryl, -SO<sub>2</sub>NH-heterocycloalkyl,  
 -NHSO<sub>2</sub>-C<sub>1</sub>-C<sub>12</sub>-alkyl, -NHSO<sub>2</sub>-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -NHSO<sub>2</sub>-C<sub>2</sub>-C<sub>12</sub>-alkenyl,  
 -NHSO<sub>2</sub>-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -NHSO<sub>2</sub>-aryl, -NHSO<sub>2</sub>-heteroaryl, -NHSO<sub>2</sub>-heterocycloalkyl,  
 -CH<sub>2</sub>NH<sub>2</sub>, -CH<sub>2</sub>SO<sub>2</sub>CH<sub>3</sub>, -aryl, -arylalkyl, -heteroaryl, -heteroarylalkyl, -heterocycloalkyl,  
 5 -C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, polyalkoxyalkyl, polyalkoxy, -methoxymethoxy, -methoxyethoxy, -SH,  
 -S-C<sub>1</sub>-C<sub>12</sub>-alkyl, -S-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -S-C<sub>2</sub>-C<sub>12</sub>-alkenyl, -S-C<sub>3</sub>-C<sub>12</sub>-cycloalkyl, -S-aryl,  
 -S-heteroaryl, -S-heterocycloalkyl, or methylthiomethyl.

Where the number of any given substituent is not specified, there may be one or more substituents present. For example, "halo-substituted C1-4 alkyl" may include one or more of the same or  
 10 different halogens.

When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both *E* and *Z* geometric isomers. Likewise, all tautomeric forms of carbonyl-containing compounds are also intended to be included.

15 It is to be understood that the compounds provided herein may contain chiral centers. Such chiral centers may be of either the (*R*) or (*S*) configuration, or may be a mixture thereof. Thus, the compounds provided herein may be enantiomerically pure, or may be stereoisomeric or diastereomeric mixtures. As such, one of skill in the art will recognize that administration of a compound in its (*R*) form is equivalent, for compounds that undergo epimerization *in vivo*, to administration of the compound in its (*S*) form.

20 Compounds described herein include, but are not limited to, those set forth above, as well as any of their isomers, such as diastereomers and enantiomers, as well as salts, esters, amides, thioesters, solvates, and polymorphs thereof, as well as racemic mixtures and pure isomers of the compounds set forth above.

## 25 Stem Cells

In some embodiments, the stem cells of which the population is modified (e.g., expanded) with the compositions and methods described are capable of being expanded upon contacting the aryl hydrocarbon receptor antagonist. In some embodiments, the stem cells are genetically modified stem cells. In some embodiments, the stem cells are not genetically modified stem cells.

30 In some embodiments, the stem cells are embryonic stem cells or adult stem cells. In some embodiments, the stem cells are totipotent stem cells, pluripotent stem cells, multipotent stem cells, oligopotent stem cells, or unipotent stem cells. In some embodiments, the stem cells are tissue-specific stem cells.

In some embodiments, the stem cells are hematopoietic stem cells, intestinal stem cells,  
 35 osteoblastic stem cells, mesenchymal stem cells (i.e., lung mesenchymal stem cells, bone marrow-derived mesenchymal stromal cells, or bone marrow stromal cells), neural stem cells (i.e., neuronal dopaminergic stem cells or motor-neuronal stem cells), epithelial stem cells (i.e., lung epithelial stem cells, breast epithelial stem cells, vascular epithelial stem cells, or intestinal epithelial stem cells), cardiac myocyte progenitor stem cells, skin stem cells (i.e., epidermal stem cells or follicular stem cells (hair

follicle stem cells)), skeletal muscle stem cells, adipose stem cells, liver stem cells, induced pluripotent stem cells, umbilical cord stem cells, amniotic fluid stem cells, limbal stem cells, dental pulp stem cells, placental stem cells, myoblasts, endothelial progenitor cells, exfoliated teeth derived stem cells, or hair follicle stem cells.

5 In some embodiments, the stem cells are hematopoietic stem cells.

In some embodiments, the stem cells are primary stem cells. For example, the stem cells are obtained from bone marrow, adipose tissue, or blood. In some embodiments, the the stem cells are cultured stem cells.

10 In some embodiments, the stem cells are CD34+ cells. In some embodiments, the stem cells are CD90+ cells. In some embodiments, the stem cells are CD45RA- cells. In some embodiments, the stem cells are CD34+CD90+ cells. In some embodiments, the stem cells are CD34+CD45RA- cells. In some embodiments, the stem cells are CD90+CD45RA- cells. In some embodiments, the stem cells are CD34+CD90+CD45RA- cells.

15 In some embodiments, the hematopoietic stem cells are extracted from the bone marrow, mobilized into the peripheral blood and then collected by apheresis, or isolated from umbilical cord blood units.

20 In some embodiments, the hematopoietic stem cells are CD34+ hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD90+ hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD45RA- hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD34+CD90+ hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD34+CD45RA- hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD90+CD45RA- hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD34+CD90+CD45RA- hematopoietic stem cells.

## 25 **Methods of Genetic Modification of Hematopoietic Stem and Progenitor Cells**

The compositions and methods described herein provide strategies for disrupting a gene of interest and for promoting the expression of target genes in populations of hematopoietic stem and progenitor cells, as well as for expanding these cells. For instance, a population of hematopoietic stem cells may be expanded according to the methods described herein and may be genetically modified, e.g.,

30 so as to exhibit an altered gene expression pattern. Alternatively, a population of cells may be enriched with hematopoietic stem cells, or a population of hematopoietic stem cells may be maintained in a multi-potent state, and the cells may further be modified using established genome editing techniques known in the art. For instance, one may use a genome editing procedure to promote the expression of an exogenous gene or inhibit the expression of an endogenous gene within a hematopoietic stem cell.

35 Populations of hematopoietic stem cells may be expanded, enriched, or maintained in a multi-potent state according to the methods described herein and subsequently genetically modified so as to express a desired target gene, or populations of these cells may be genetically modified first and then expanded, enriched, or maintained in a multi-potent state.

In some embodiments, the populations (e.g., plurality) of hematopoietic stem cells are expanded, enriched, or maintained in a multi-potent state according to the methods described herein by being contacted with an aryl hydrocarbon receptor antagonist as described herein and subsequently genetically modified so as to express a desired target gene and substantially maintain the engraftable properties of the hematopoietic stem cells cells. In some embodiments, the populations (e.g., plurality) of hematopoietic stem cells are expanded, enriched, or maintained in a multi-potent state according to the methods described herein by being contacted with an aryl hydrocarbon receptor antagonist as described herein and subjected to conditions during a period of time sufficient to induce cell cycling, and subsequently genetically modified so as to express a desired target gene and substantially maintain the engraftable properties of the hematopoietic stem cells cells. In one non-limiting embodiment, the conditions sufficient to induce cell cycling may comprise contacting the hematopoietic stem cells with one or more cytokines in amounts sufficient to induce cell cycling. Non-limiting examples of cytokines include SCF, IL6, TPO, FLT3L, and combinations thereof. Other agents or methods may also be used to induce cell cycling.

In some embodiments, the period of time sufficient to induce cell cycling may be at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, or at least about 5 days. In some embodiments, the period of time sufficient to induce cell cycling is about 1 to about 5 days, about 1 to about 4 days, about 2 to about 4 days, about 1 to about 3 days, or about 2 to about 3 days. In some embodiments, the period of time sufficient to induce cell cycling may vary depending on the lineage of the cells.

In some embodiments, contacting the hematopoietic stem cells with an aryl hydrocarbon receptor antagonist does not affect cell cycling. Advantageously, actively cycling cells may be more easily genetically modified so as to express a desired target gene than a non-cycling cell. Additionally, in some embodiments, contacting the hematopoietic stem cells with an aryl hydrocarbon receptor antagonist does not prevent stem cells from entering the cell cycle, and allows the stem cells to remain as stem cells (e.g., including dividing so as to multiply in number without substantially differentiating), delaying differentiation and prolonging engraftment potential relative to cells (e.g., hematopoietic stem cells) not contacted with an aryl hydrocarbon receptor antagonist.

In some embodiments, the populations (e.g., plurality) of hematopoietic stem cells are expanded, enriched, or maintained in a multi-potent state according to the methods described herein by being contacted with an aryl hydrocarbon receptor antagonist as described herein during at least a period of time sufficient to induce cell cycling and subsequently genetically modified so as to express a desired target gene resulting in improved genetic modification relative to a comparable method wherein the populations (e.g., plurality) of hematopoietic stem cells are not contacted with an aryl hydrocarbon receptor antagonist as described herein during a period of time sufficient to induce cell cycling prior to being subsequently genetically modified.

In some embodiments, the populations of hematopoietic stem cells are expanded, enriched, or maintained in a multi-potent state according to the methods described herein by being contacted with an aryl hydrocarbon receptor antagonist as described herein during a period of time sufficient to induce cell

cycling and subsequently genetically modified so as to express a desired target gene resulting in improved engraftment potential relative to a comparable method wherein the the populations of hematopoietic stem cells are not contacted with an aryl hydrocarbon receptor antagonist as described herein during a period of time sufficient to induce cell cycling prior to being subsequently genetically modified.

In some embodiments, hematopoietic stem cells are expanded, enriched, or maintained in a multi-potent state according to the methods described herein by being contacted with an aryl hydrocarbon receptor antagonist as described herein during a period of time sufficient to induce cell cycling in substantially all of the hematopoietic stem cells.

In some embodiments, the populations (e.g., plurality) of hematopoietic stem cells are expanded subsequently to being genetically modified. For example, the hematopoietic stem cells may be expanded in the presence of an aryl hydrocarbon receptor antagonist subsequently to being genetically modified. Expansion of the genetically modified hematopoietic stem cells may be performed, for example, to increase the number of engraftable genetically modified cells in a hematopoietic stem cell graft.

A wide array of methods has been established for the incorporation of target genes into the genome of a cell (e.g., a mammalian cell, such as a murine or human cell) so as to facilitate the expression of such genes.

#### *Polynucleotides encoding target genes*

One example of a platform that can be used to facilitate the expression of a target gene in a hematopoietic stem cell is by the integration of the polynucleotide encoding a target gene into the nuclear genome of the cell. A variety of techniques have been developed for the introduction of exogenous genes into a eukaryotic genome. One such technique involves the insertion of a target gene into a vector, such as a viral vector. Vectors for use with the compositions and methods described herein can be introduced into a cell by a variety of methods, including transformation, transfection, direct uptake, projectile bombardment, and by encapsulation of the vector in a liposome. Examples of suitable methods of transfecting or transforming cells include calcium phosphate precipitation, electroporation, microinjection, infection, lipofection and direct uptake. Such methods are described in more detail, for example, in Green, et al., *Molecular Cloning: A Laboratory Manual*, Fourth Edition, Cold Spring Harbor University Press, New York (2014); and Ausubel, et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (2015), the disclosures of each of which are incorporated herein by reference.

Exogenous genes can also be introduced into a mammalian cell through the use of a vector containing the gene of interest to cell membrane phospholipids. For example, vectors can be targeted to the phospholipids on the extracellular surface of the cell membrane by linking the vector molecule to a VSV-G protein, a viral protein with affinity for all cell membrane phospholipids. Viral vectors containing the VSV-G protein are described in further detail, e.g., in US 5,512,421; and in US 5,670,354, the disclosures of each of which are incorporated by reference herein.

Recognition and binding of the polynucleotide encoding a target gene by mammalian RNA polymerase is an important molecular event for gene expression to occur. As such, one may include

sequence elements within the polynucleotide that exhibit a high affinity for transcription factors that recruit RNA polymerase and promote the assembly of the transcription complex at the transcription initiation site. Such sequence elements include, e.g., a mammalian promoter, the sequence of which can be recognized and bound by specific transcription initiation factors and ultimately RNA polymerase. Alternatively, promoters derived from viral genomes can be used for the stable expression of target genes in mammalian cells. Examples of functional viral promoters that can be used to promote mammalian expression of these enzymes include adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, cytomegalovirus promoter, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein barr virus (EBV) promoter, Rous sarcoma virus (RSV) promoter, and the cytomegalovirus (CMV) promoter. Additional viral promoters include the SV40 late promoter from simian virus 40, the Baculovirus polyhedron enhancer/promoter element, Herpes Simplex Virus thymidine kinase (HSV tk) promoter, and the 35S promoter from Cauliflower Mosaic Virus. Suitable phage promoters for use with the compositions and methods described herein include, but are not limited to, the E. coli T7 and T3 phage promoters, the S. typhimurium phage SP6 promoter, B. subtilis SP01 phage and B. subtilis phage phi 29 promoters, and N4 phage and K11 phage promoters as described in US 5,547,892, the disclosure of which is incorporated herein by reference.

Upon incorporation of a polynucleotide encoding a target gene has been incorporated into the genome of a cell (e.g., the nuclear genome of a hematopoietic stem cell), the transcription of this polynucleotide can be induced by methods known in the art. For example expression can be induced by exposing the mammalian cell to an external chemical reagent, such as an agent that modulates the binding of a transcription factor and/or RNA polymerase to the mammalian promoter and thus regulate gene expression. The chemical reagent can serve to facilitate the binding of RNA polymerase and/or transcription factors to the mammalian promoter, e.g., by removing a repressor protein that has bound the promoter. Alternatively, the chemical reagent can serve to enhance the affinity of the mammalian promoter for RNA polymerase and/or transcription factors such that the rate of transcription of the gene located downstream of the promoter is increased in the presence of the chemical reagent. Examples of chemical reagents that potentiate polynucleotide transcription by the above mechanisms include tetracycline and doxycycline. These reagents are commercially available (Life Technologies, Carlsbad, CA) and can be administered to a mammalian cell in order to promote gene expression according to established protocols.

Other DNA sequence elements that may be included in polynucleotides for use with the compositions and methods described herein include enhancer sequences. Enhancers represent another class of regulatory elements that induce a conformational change in the polynucleotide comprising the gene of interest such that the DNA adopts a three-dimensional orientation that is favorable for binding of transcription factors and RNA polymerase at the transcription initiation site. Thus, polynucleotides for use with the compositions and methods described herein include those that encode a target gene and additionally include a mammalian enhancer sequence. Many enhancer sequences are now known from mammalian genes, and examples include enhancers from the genes that encode mammalian globin, elastase, albumin,  $\alpha$ -fetoprotein, and insulin. Enhancers for use with the compositions and methods

described herein also include those that are derived from the genetic material of a virus capable of infecting a eukaryotic cell. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. Additional enhancer sequences that induce activation of eukaryotic gene transcription are disclosed in Yaniv et al. Nature 297:17 (1982), the disclosure of which is incorporated herein by reference. An enhancer may be spliced into a vector containing a polynucleotide encoding a target gene, for example, at a position 5' or 3' to this gene. In a preferred orientation, the enhancer is positioned at the 5' side of the promoter, which in turn is located 5' relative to the polynucleotide encoding the target gene.

In addition to promoting high rates of transcription and translation, stable expression of an exogenous gene in a hematopoietic stem cell can be achieved by integration of the polynucleotide comprising the gene into the nuclear DNA of the cell. A variety of vectors for the delivery and integration of polynucleotides encoding exogenous proteins into the nuclear DNA of a mammalian cell have been developed. Examples of expression vectors are disclosed in, e.g., WO94/11026, the disclosure of which is incorporated herein by reference. Expression vectors for use with the compositions and methods described herein contain a polynucleotide sequence that encodes a target gene, as well as, e.g., additional sequence elements used for the expression of these enzymes and/or the integration of these polynucleotide sequences into the genome of a mammalian cell. Certain vectors that can be used for the expression of target genes include plasmids that contain regulatory sequences, such as promoter and enhancer regions, which direct gene transcription. Other useful vectors for expression of target genes contain polynucleotide sequences that enhance the rate of translation of these genes or improve the stability or nuclear export of the mRNA that results from gene transcription. These sequence elements often encode features within RNA transcripts that enhance the nuclear export, cytosolic half-life, and ribosomal affinity of these molecules, e.g., 5' and 3' untranslated regions, an internal ribosomal entry site (IRES), and polyadenylation signal site in order to direct efficient transcription of the gene carried on the expression vector. Exemplary expression vectors may also contain a polynucleotide encoding a marker for selection of cells that contain such a vector. Non-limiting examples of a suitable marker include genes that encode resistance to antibiotics, such as ampicillin, chloramphenicol, kanamycin, or nourseothricin.

### *Vectors for the expression of target genes*

Viral genomes provide a rich source of vectors that can be used for the efficient delivery of exogenous genes into a mammalian cell. Viral genomes are particularly useful vectors for gene delivery because the polynucleotides contained within such genomes are typically incorporated into the nuclear genome of a mammalian cell by generalized or specialized transduction. These processes occur as part of the natural viral replication cycle, and often do not require added proteins or reagents in order to induce gene integration. Examples of viral vectors include a retrovirus, adenovirus (e.g., Ad5, Ad26, Ad34, Ad35, and Ad48), parvovirus (e.g., adeno-associated viruses), coronavirus, negative strand RNA viruses such as orthomyxovirus (e.g., influenza virus), rhabdovirus (e.g., rabies and vesicular stomatitis virus), paramyxovirus (e.g. measles and Sendai), positive strand RNA viruses, such as picornavirus and

alphavirus, and double stranded DNA viruses including herpes virus (e.g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (e.g., vaccinia, modified vaccinia Ankara (MVA), fowlpox and canarypox). Other viruses include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Examples of retroviruses include: avian leukosis-sarcoma, mammalian C-type, B-type viruses, D-type viruses, HTLV-BLV group, lentivirus, spumavirus (Coffin, J. M., *Retroviridae: The viruses and their replication*, In *Fundamental Virology*, Third Edition, B. N. Fields, et al., Eds., Lippincott-Raven Publishers, Philadelphia, 1996, the disclosure of which is incorporated herein by reference). Other examples of viral vectors include murine leukemia viruses, murine sarcoma viruses, mouse mammary tumor virus, bovine leukemia virus, feline leukemia virus, feline sarcoma virus, avian leukemia virus, human T-cell leukemia virus, baboon endogenous virus, Gibbon ape leukemia virus, Mason Pfizer monkey virus, simian immunodeficiency virus, simian sarcoma virus, Rous sarcoma virus and lentiviruses. Other examples of vectors are described in, e.g., US 5,801,030, the disclosure of which is incorporated herein by reference.

#### 15 *Additional transfection methods*

Other techniques that can be used to introduce a polynucleotide, such as DNA or RNA (e.g., mRNA, tRNA, siRNA, miRNA, shRNA, chemically modified RNA) into a mammalian cell are well known in the art. For instance, electroporation can be used to permeabilize mammalian cells by the application of an electrostatic potential. Mammalian cells, such as hematopoietic stem cells, subjected to an external electric field in this manner are subsequently predisposed to the uptake of exogenous nucleic acids. Electroporation of mammalian cells is described in detail, e.g., in Chu et al. *Nucleic Acids Research* 15:1311 (1987), the disclosure of which is incorporated herein by reference. A similar technique, Nucleofection™, utilizes an applied electric field in order to stimulate the uptake of exogenous polynucleotides into the nucleus of a eukaryotic cell. Nucleofection™ and protocols useful for performing this technique are described in detail, e.g., in Distler et al. *Experimental Dermatology* 14:315 (2005), as well as in US 2010/0317114, the disclosures of each of which are incorporated herein by reference.

Additional techniques useful for the transfection of hematopoietic stem cells include the squeeze-poration methodology. This technique induces the rapid mechanical deformation of cells in order to stimulate the uptake of exogenous DNA through membranous pores that form in response to the applied stress. This technology is advantageous in that a vector is not required for delivery of nucleic acids into a cell, such as a hematopoietic stem cell. Squeeze-poration is described in detail, e.g., in Sharei et al. *Journal of Visualized Experiments* 81:e50980 (2013), the disclosure of which is incorporated herein by reference.

Lipofection represents another technique useful for transfection of hematopoietic stem cells. This method involves the loading of nucleic acids into a liposome, which often presents cationic functional groups, such as quaternary or protonated amines, towards the liposome exterior. This promotes electrostatic interactions between the liposome and a cell due to the anionic nature of the cell membrane, which ultimately leads to uptake of the exogenous nucleic acids, e.g., by direct fusion of the liposome with the cell membrane or by endocytosis of the complex. Lipofection is described in detail, e.g., in US

7,442,386, the disclosure of which is incorporated herein by reference. Similar techniques that exploit ionic interactions with the cell membrane to provoke the uptake of foreign nucleic acids include contacting a cell with a cationic polymer-nucleic acid complex. Cationic molecules that associate with polynucleotides so as to impart a positive charge favorable for interaction with the cell membrane include activated dendrimers (described, e.g., in Dennig, *Topics in Current Chemistry* 228:227 (2003), the disclosure of which is incorporated herein by reference) and diethylaminoethyl (DEAE)-dextran, the use of which as a transfection agent is described in detail, e.g., in Gulick et al. *Current Protocols in Molecular Biology* 40:1:9.2:9.2.1 (1997), the disclosure of which is incorporated herein by reference. Magnetic beads are another tool that can be used to transfect hematopoietic stem cells in a mild and efficient manner, as this methodology utilizes an applied magnetic field in order to direct the uptake of nucleic acids. This technology is described in detail, e.g., in US 2010/0227406, the disclosure of which is incorporated herein by reference.

Another useful tool for inducing the uptake of exogenous nucleic acids by hematopoietic stem cells is laserfection, a technique that involves exposing a cell to electromagnetic radiation of a particular wavelength in order to gently permeabilize the cells and allow polynucleotides to penetrate the cell membrane. This technique is described in detail, e.g., in Rhodes et al. *Methods in Cell Biology* 82:309 (2007), the disclosure of which is incorporated herein by reference.

Microvesicles represent another potential vehicle that can be used to modify the genome of a hematopoietic stem cell according to the methods described herein. For instance, microvesicles that have been induced by the co-overexpression of the glycoprotein VSV-G with, e.g., a genome-modifying protein, such as a nuclease, can be used to efficiently deliver proteins into a cell that subsequently catalyze the site-specific cleavage of an endogenous polynucleotide sequence so as to prepare the genome of the cell for the covalent incorporation of a polynucleotide of interest, such as a gene or regulatory sequence. The use of such vesicles, also referred to as Gesicles, for the genetic modification of eukaryotic cells is described in detail, e.g., in Quinn et al. *Genetic Modification of Target Cells by Direct Delivery of Active Protein* [abstract]. In: *Methylation changes in early embryonic genes in cancer* [abstract], in: *Proceedings of the 18th Annual Meeting of the American Society of Gene and Cell Therapy*; 2015 May 13, Abstract No. 122.

### **Modulation of Gene Expression using Gene Editing Techniques**

In addition to viral vectors, a variety of additional tools have been developed that can be used for the incorporation of exogenous genes into hematopoietic stem cells. One such method that can be used for incorporating polynucleotides encoding target genes into hematopoietic stem cells involves the use of transposons. Transposons are polynucleotides that encode transposase enzymes and contain a polynucleotide sequence or gene of interest flanked by 5' and 3' excision sites. Once a transposon has been delivered into a cell, expression of the transposase gene commences and results in active enzymes that cleave the gene of interest from the transposon. This activity is mediated by the site-specific recognition of transposon excision sites by the transposase. In certain cases, these excision sites may be terminal repeats or inverted terminal repeats. Once excised from the transposon, the gene of interest can



be integrated into the genome of a mammalian cell by transposase-catalyzed cleavage of similar excision sites that exist within the nuclear genome of the cell. This allows the gene of interest to be inserted into the cleaved nuclear DNA at the complementary excision sites, and subsequent covalent ligation of the phosphodiester bonds that join the gene of interest to the DNA of the mammalian cell genome completes the incorporation process. In certain cases, the transposon may be a retrotransposon, such that the gene encoding the target gene is first transcribed to an RNA product and then reverse-transcribed to DNA before incorporation in the mammalian cell genome. Transposon systems include the piggybac transposon (described in detail in, e.g., WO 2010/085699) and the sleeping beauty transposon (described in detail in, e.g., US2005/0112764), the disclosures of each of which are incorporated herein by reference.

Another useful tool for the disruption and integration of target genes into the genome of a hematopoietic stem cell is the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system, a system that originally evolved as an adaptive defense mechanism in bacteria and archaea against viral infection. The CRISPR/Cas system includes palindromic repeat sequences within plasmid DNA and an associated Cas9 nuclease. This ensemble of DNA and protein directs site specific DNA cleavage of a target sequence by first incorporating foreign DNA into CRISPR loci. Polynucleotides containing these foreign sequences and the repeat-spacer elements of the CRISPR locus are in turn transcribed in a host cell to create a guide RNA, which can subsequently anneal to a target sequence and localize the Cas9 nuclease to this site. In this manner, highly site-specific cas9-mediated DNA cleavage can be engendered in a foreign polynucleotide because the interaction that brings cas9 within close proximity of the target DNA molecule is governed by RNA:DNA hybridization. As a result, one can theoretically design a CRISPR/Cas system to cleave any target DNA molecule of interest. This technique has been exploited in order to edit eukaryotic genomes (Hwang et al. Nature Biotechnology 31:227 (2013), the disclosure of which is incorporated herein by reference) and can be used as an efficient means of site-specifically editing hematopoietic stem cell genomes in order to cleave DNA prior to the incorporation of a gene encoding a target gene. The use of CRISPR/Cas to modulate gene expression has been described in, e.g., US 8,697,359, the disclosure of which is incorporated herein by reference.

The CRISPR/Cas system can be used to create one or more double stranded breaks in a target DNA sequence, which can then be repaired by either the homologous recombination (HR) or non-homologous end joining (NHEJ) DNA repair pathways. The Cas9 enzyme, together with a guide RNA specific to the target DNA (gRNA), can be supplied to a cell to induce one or more double strand break. The Cas9 enzyme can be supplied as a protein, as a ribonucleoprotein complexed with the guide RNA, or as an RNA or DNA encoding the Cas9 protein that is then used by the cell to synthesize the Cas9 protein. The gRNA may comprise both tracrRNA and crRNA sequences in a chimeric RNA. Alternatively, or in addition, the gRNA may comprise a scaffold region that binds to the Cas9 protein, and a complementary base pairing region, also sometimes called a spacer, that targets the gRNA Cas9 protein complex to a particular DNA sequence. In some cases, the complementary base pairing region can be about 20 nucleotides in length, and is complementary to target DNA sequence immediately adjacent to a protospacer adjacent motif (e.g., a PAM motif). In some cases, the PAM comprises a sequence of NGG,

NGA or NAG. The complementary base pairing region of the gRNA hybridizes to the target DNA sequence, and guides the gRNA Cas9 protein complex to the target sequence where the Cas9 endonuclease domains then cut within the target sequence, generating a double strand break that may be 3-4 nucleotides upstream of the PAM. Thus, by altering the complementary base pairing region, almost any DNA sequence can be targeted for the generation of a double stranded break. Methods for selecting an appropriate complementary base pairing region will be known to those skilled in the art. For example, gRNAs can be selected to minimize the number of off-target binding sites of the gRNA in the target DNA sequence. In some cases, modified Cas9 genome editing systems may be used to, for example, increase DNA targeting specificity. An example of a modified Cas9 genome editing system comprises split Cas9 systems such as the Dimeric Cas9-FokI genome editing system.

The double strand break or breaks generated by CRISPR/Cas9 genome editing system may be repaired by the non homologous end joining pathway (NHEJ), which ligates the ends of the double strand break together. NHEJ may result in deletions in the DNA around or near the site of the double strand break. Alternatively, the double strand break generated by CRISPR/Cas9 genome editing system may be repaired through a homology directed repair, also called homologous recombination (HR) repair pathway. In the HR pathway, the double strand break is repaired by exchanging sequences between two similar or identical DNA molecules. The HR repair pathway can therefore be used to introduce exogenous DNA sequences into the genome. In using the HR pathway for genome editing, a DNA template is supplied to the cell along with the Cas9 and gRNA. In some cases, the template may contain exogenous sequences to be introduced into the genome via genome editing flanked by homology arms that comprise DNA sequences on either side of the site of the Cas9 induced double strand break. These homology arms may be, for example, between about 50 or 1000 nucleotides, or in other cases up to several kilobases in length or longer. The template may be a linear DNA, or a circular DNA such as a plasmid, or may be supplied using a viral vector or other means of delivery. The template DNA may comprise double stranded or single stranded DNA. All manner of delivering the template DNA, the gRNA and the Cas9 protein to the cell to achieve the desired genome editing are envisaged as being within the scope of the invention.

The CRISPR/Cas9 and HR based genome editing systems of the disclosure provide not only methods of introducing exogenous DNA sequences into a genome or DNA sequence of interest, but also a platform for correcting mutations in genes. An altered or corrected version of a mutated sequence, for example a sequence changing one or more point mutations back to the wild type consensus sequence, inserting a deleted sequence, or deleting an inserted sequence, could be supplied to the cell as a template sequence, and that template sequence used by the cell to fix a CRISPR/Cas9 induced double strand break via the HR pathway. For example, in a patient with one or more disease causing mutations, hematopoietic stem and/or progenitor cells such as the hematopoietic stem and/or progenitor cells of the patient, can be removed from the body. The mutation can then corrected by CRISPR/Cas9 and HR mediated genome editing in the genome of one or more of these hematopoietic stem and/or progenitor cells, the corrected hematopoietic stem and/or progenitor cell(s) expanded with the methods of the disclosure, and then the edited cell population infused back into the patient, thereby supplying a source of

the wild type version of the gene and curing the patient of the disease caused by the mutation or mutations in that gene. Mutations that can cause genetic diseases include not only point mutations, but also insertions, deletions and inversions. These mutations can be in protein coding sequence and affect the amino acid sequence of the protein, or they may be in non-coding sequences such as untranslated regions, promoters, cis regulatory elements required for gene expression, sequences required for splicing, or sequences required for DNA structure. All mutations are potentially editable by CRISPR/Cas9 mediated genome editing methods of the disclosure. In some cases, the patient may be conditioned to eliminate or reduce the native hematopoietic stem and/or progenitor cells that carry the mutant version of the gene, thus enriching for the exogenously supplied genome edited hematopoietic stem and/or progenitor cells. Both autologous and allogeneic genome edited hematopoietic stem and/or progenitor cells can be used to treat a genetic disease of a patient of the disclosure.

In addition to the CRISPR/Cas9 system, alternative methods for disruption of a target DNA by site-specifically cleaving genomic DNA prior to the incorporation of a gene of interest in a hematopoietic stem and/or progenitor cell include the use of zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). Unlike the CRISPR/Cas system, these enzymes do not contain a guiding polynucleotide to localize to a specific target sequence. Target specificity is instead controlled by DNA binding domains within these enzymes. The use of ZFNs and TALENs in genome editing applications is described, e.g., in Umov et al. *Nature Reviews Genetics* 11:636 (2010); and in Joung et al. *Nature Reviews Molecular Cell Biology* 14:49 (2013), the disclosure of both of which are incorporated herein by reference. As with the CRISPR/Cas9 genome editing systems, double strand breaks introduced by TALENs or ZFNs can also be repaired via the HR pathway, and this pathway can be used to introduce exogenous DNA sequences or repair mutations in the DNA.

Additional genome editing techniques that can be used to disrupt or incorporate polynucleotides encoding target genes into the genome of a hematopoietic stem cell include the use of ARCUS<sup>TM</sup> meganucleases that can be rationally designed so as to site-specifically cleave genomic DNA. The use of these enzymes for the incorporation of genes encoding target genes into the genome of a mammalian cell is advantageous in view of the defined structure-activity relationships that have been established for such enzymes. Single chain meganucleases can be modified at certain amino acid positions in order to create nucleases that selectively cleave DNA at desired locations, enabling the site-specific incorporation of a target gene into the nuclear DNA of a hematopoietic stem cell. These single-chain nucleases have been described extensively in, e.g., US 8,021,867 and US 8,445,251, the disclosures of each of which are incorporated herein by reference.

#### Methods for Expanding Hematopoietic Stem Cells

In another aspect, the disclosure features a method of producing an expanded population of hematopoietic stem cells ex vivo, the method including contacting a population of hematopoietic stem cells with the compound of any one of the above aspects or embodiments in an amount sufficient to produce an expanded population of hematopoietic stem cells.

In another aspect, the disclosure features a method of enriching a population of cells with hematopoietic stem cells ex vivo, the method including contacting a population of hematopoietic stem cells with the compound of any one of the above aspects or embodiments in an amount sufficient to produce a population of cells enriched with hematopoietic stem cells.

5 In another aspect, the disclosure features a method of maintaining the hematopoietic stem cell functional potential of a population of hematopoietic stem cells ex vivo for two or more days, the method including contacting a first population of hematopoietic stem cells with the compound of any one of the above aspects or embodiments, wherein the first population of hematopoietic stem cells exhibits a hematopoietic stem cell functional potential after two or more days that is greater than that of a control  
10 population of hematopoietic stem cells cultured under the same conditions and for the same time as the first population of hematopoietic stem cells but not contacted with the compound.

In one embodiment, said method for expanding hematopoietic stem cells, comprises (a) providing a starting cell population comprising hematopoietic stem cells and (b) culturing said starting cell population ex vivo in the presence of an AHR antagonist agent compound of any one of the above  
15 aspects or embodiments.

The starting cell population comprising hematopoietic stem cells will be selected by the person skilled in the art depending on the envisaged use. Various sources of cells comprising hematopoietic stem cells have been described in the art, including bone marrow, peripheral blood, neonatal umbilical cord blood, placenta or other sources such as liver, particularly fetal liver.

20 The cell population may first be subjected to enrichment or purification steps, including negative and/or positive selection of cells based on specific cellular markers in order to provide the starting cell population. Methods for isolating said starting cell population based on specific cellular markers may use fluorescent activated cell sorting (FACS) technology also called flow cytometry or solid or insoluble substrate to which is bound antibodies or ligands that interact with specific cell surface markers. For  
25 example, cells may be contacted with a solid substrate (e.g., column of beads, flasks, magnetic particles) containing the antibodies and any unbound cells are removed. When a solid substrate comprising magnetic or paramagnetic beads is used, cells bound to the beads can be readily isolated by a magnetic separator.

In one embodiment, said starting cell population is enriched in a desirable cell marker phenotype  
30 (e.g., CD34+, CD133+, CD90+) or based on efflux of dyes such as rhodamine, Hoechst or aldehyde dehydrogenase activity. In one specific embodiment, said starting cell population is enriched in CD34+ cells. Methods for enriching blood cell population in CD34+ cells include kits commercialized by Miltenyi Biotec (CD34+ direct isolation kit, Miltenyi Biotec, Bergisch, Gladbach, Germany) or by Baxter (Isolex 3000).

35 In some embodiments, the hematopoietic stem cells are CD34+ hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD90+ hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD45RA- hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD34+CD90+ hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD34+CD45RA- hematopoietic stem cells. In some

embodiments, the hematopoietic stem cells are CD90+CD45RA- hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD34+CD90+CD45RA- hematopoietic stem cells.

In some embodiments, the hematopoietic stem cells are mammalian cells, such as human cells. In some embodiments, the human cells are CD34+ cells, such as CD34+ cells are CD34+, CD34+CD38-,  
 5 CD34+CD38-CD90+, CD34+CD38-CD90+CD45RA-, CD34+CD38-CD90+CD45RA-CD49F+, or CD34+CD90+CD45RA- cells.

In some embodiments, the hematopoietic stem cells are obtained from human cord blood, mobilized human peripheral blood, or human bone marrow. The hematopoietic stem cells may, for example, be freshly isolated from the human or may have been previously cryopreserved.

10 The amount of cord blood from a single birth is often inadequate to treat an adult or an older child. One advantage of the expansion methods using the compounds of the invention, or an agent capable of down-regulating the activity and/or expression of aryl hydrocarbon receptor and/or a downstream effector of aryl hydrocarbon receptor pathway, is that it enables the production of a sufficient amount of hematopoietic stem cells from only one cord blood unit.

15 Accordingly, in one embodiment, the starting cell population is derived from neonatal umbilical cord blood cells which have been enriched in CD34+ cells. In one related embodiment, said starting cell population is derived from one or two umbilical cord blood units.

In another embodiment, the starting cell population is derived from human mobilized peripheral blood cells which have been enriched in CD34+ cells. In one related embodiment, said starting cell  
 20 population is derived from human mobilized peripheral blood cells isolated from only one patient.

Said starting cell population enriched in CD34+ cells may preferably contain at least about 50% CD34+ cells, in some embodiments, more than about 90% CD34+ cells, and may comprise between  $10^5$  and  $10^9$  nucleated cells.

The starting cell population may be used directly for expansion or frozen and stored for use at a  
 25 later date.

Conditions for culturing the starting cell population for hematopoietic stem cell expansion will vary depending, inter alia, on the starting cell population, the desired final number of cells, and desired final proportion of HSCs.

In one embodiment, the culturing conditions comprises the use of other cytokines and growth  
 30 factors, generally known in the art for hematopoietic stem cell expansion. Such cytokines and growth factors include without limitation IL-1, IL-3, IL-6, IL-11, G-CSF, GM-CSF, SCF, Flt3-L, thrombopoietin (TPO), erythropoietin, and analogs thereof. As used herein, "analogs" include any structural variants of the cytokines and growth factors having the biological activity as the naturally occurring forms, including without limitation, variants with enhanced or decreased biological activity when compared to the naturally  
 35 occurring forms or cytokine receptor agonists such as an agonist antibody against the TPO receptor (for example, VB22B sc(Fv)2 as detailed in patent publication WO 2007/145227, and the like). Cytokine and growth factor combinations are chosen to expand HSC and progenitor cells while limiting the production of terminally differentiated cells. In one specific embodiment, one or more cytokines and growth factors are selected from the group consisting of SCF, Flt3-L and TPO. In one specific embodiment, at least TPO

is used in a serum-free medium under suitable conditions for HSC expansion. In one related embodiment, a mixture of IL6, SCF, Flt3-L and TPO is used in the method for expanding HSCs in combination with the compound of the present disclosure.

The expansion of HSC may be carried out in a basal medium, which may be supplemented with mixtures of cytokines and growth factors. A basal medium typically comprises amino acids, carbon sources, vitamins, serum proteins (e.g. albumin), inorganic salts, divalent cations, buffers and any other element suitable for use in expansion of HSC. Examples of such basal medium appropriate for a method of expanding HSC include, without limitation, StemSpan® SFEM—Serum-Free Expansion Medium (StemCell Technologies, Vancouver, Canada), StemSpan® H3000—Defined Medium (StemCell Technologies, Vancouver, Canada), CellGro® SCGM (CellGenix, Freiburg Germany), StemPro®-34 SFM (Invitrogen).

In one embodiment, the compound of the present disclosure is administered during the expansion method of said starting cell population under a concentration appropriate for HSC expansion. In one specific embodiment, said compound or AHR modulating agent is administered at a concentration comprised between 1 pM and 100 µM, for example between 10 pM and 10 µM, or between 100 pM and 1 µM.

In one embodiment where starting cell population essentially consists of CD34+ enriched cells from one or two cord blood units, the cells are grown under conditions for HSC expansion from about 3 days to about 90 days, for example between 7 and 28 days and/or until the indicated fold expansion and the characteristic cell populations are obtained. In one specific embodiment, the cells are grown under conditions for HSC expansion not more than 21 days, 14 days or 7 days.

In one embodiment, the starting cell population is cultured during a time sufficient to reach an absolute number of CD34+ cells of at least  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$  or  $10^9$  cells. In another embodiment, said starting cell population is cultured during a time sufficient for a 10 to 50000 fold expansion of CD34+ cells, for example between 100 and 10000 fold expansion, for examples between 50 and 1000 fold expansion.

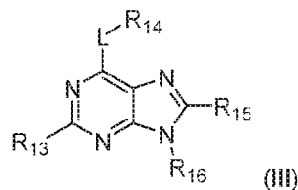
The cell population obtained after the expansion method may be used without further purification or may be subject to further purification or selection steps.

The cell population may then be washed to remove the compound of the present disclosure and/or any other components of the cell culture and resuspended in an appropriate cell suspension medium for short term use or in a long-term storage medium, for example a medium suitable for cryopreservation.

#### **Aryl Hydrocarbon Receptor Antagonists**

Prior to infusion into a patient, hematopoietic and progenitor cells may be expanded ex vivo, for example, by contacting the cells with an aryl hydrocarbon receptor antagonist. Aryl hydrocarbon receptor antagonists useful in conjunction with the compositions and methods described herein include those described in US Patent No. 9,580,426, the disclosure of which is incorporated herein by reference in its entirety.

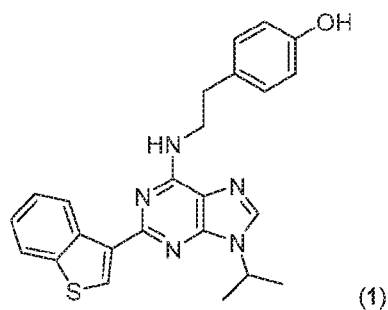
In some embodiments, aryl hydrocarbon receptor antagonists include those represented by formula (III)



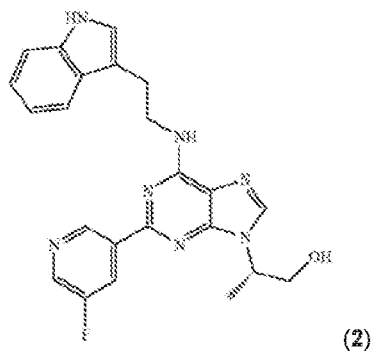
in which:

- 5           L is selected from  $\text{---NR}_{17a}(\text{CH}_2)_{2-3}$ ,  $\text{---NR}_{17a}(\text{CH}_2)_2\text{NR}_{17b}\text{---}$ ,  $\text{---NR}_{17a}(\text{CH}_2)_2\text{S---}$ ,  $\text{---NR}_{17a}\text{CH}_2\text{CH}(\text{OH})\text{---}$  and  $\text{---NR}_{17a}\text{CH}(\text{CH}_3)\text{CH}_2\text{---}$ ; wherein  $\text{R}_{17a}$  and  $\text{R}_{17b}$  are independently selected from hydrogen and  $\text{C}_{1-4}$  alkyl;
- $\text{R}_{13}$  is selected from thiophenyl, 1H-benzoimidazolyl, isoquinolinyl, 1H-imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, pyrazinyl, pyridazinyl, and thiazolyl; In some embodiments,
- 10          wherein the thiophenyl, 1H-benzoimidazolyl, isoquinolinyl, 1H-imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, pyrazinyl, pyridazinyl, or thiazolyl of  $\text{R}_{13}$  can be optionally substituted by 1 to 3 radicals independently selected from cyano, hydroxy,  $\text{C}_{1-4}$  alkyl,  $\text{C}_{1-4}$  alkoxy, halo, halo-substituted- $\text{C}_{1-4}$  alkyl, halo-substituted- $\text{C}_{1-4}$  alkoxy, amino,  $\text{---C(O)R}_{20a}$ ,  $\text{---S(O)}_{0-2}\text{R}_{20a}$ ,  $\text{---C(O)OR}_{20a}$  and  $\text{---C(O)NR}_{20a}\text{R}_{20b}$ ; wherein  $\text{R}_{20a}$  and  $\text{R}_{20b}$  are independently selected from hydrogen and  $\text{C}_{1-4}$  alkyl;
- 15           $\text{R}_{14}$  is selected from  $\text{---S(O)}_2\text{NR}_{18a}\text{R}_{18b}$ ,  $\text{---NR}_{18a}\text{C(O)R}_{18b}\text{---}$ ,  $\text{---NR}_{18a}\text{C(O)NR}_{18b}\text{R}_{18c}$ , phenyl, 1H-pyrrolopyridin-3-yl, 1H-pyrrolopyridin-5-yl, 1H-indolyl thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl and 1H-indazolyl; wherein  $\text{R}_{18a}$ ,  $\text{R}_{18b}$  and  $\text{R}_{18c}$  are independently selected from hydrogen and  $\text{C}_{1-4}$  alkyl; and the phenyl, 1H-pyrrolopyridin-3-yl, 1H-pyrrolo[2,3-b]pyridin-5-yl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl,
- 20          1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl or 1H-indazolyl of  $\text{R}_{14}$  is optionally substituted with 1 to 3 radicals independently selected from hydroxy, halo, methyl, methoxy, amino,  $\text{---O}(\text{CH}_2)_2\text{NR}_{19a}\text{R}_{19b}$ ,  $\text{---S(O)}_2\text{NR}_{19a}\text{R}_{19b}$ ,  $\text{---OS(O)}_2\text{NR}_{19a}\text{R}_{19b}$  and  $\text{---NR}_{19a}\text{S(O)}_2\text{R}_{19b}$ ; wherein  $\text{R}_{19a}$  and  $\text{R}_{19b}$  are independently selected from hydrogen and  $\text{C}_{1-4}$  alkyl;
- $\text{R}_{15}$  is selected from hydrogen,  $\text{C}_{1-4}$  alkyl and biphenyl; and
- 25           $\text{R}_{16}$  is selected from  $\text{C}_{1-10}$  alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, and benzyl, (4-pentylphenyl)(phenyl)methyl and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl wherein said alkyl, cyclopropyl, cyclohexyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-3-yl, oxetan-2-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-
- 30          pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl can be optionally substituted with 1 to 3 radicals independently selected from hydroxy,  $\text{C}_{1-4}$  alkyl and halo-substituted- $\text{C}_{1-4}$  alkyl; or a salt thereof.

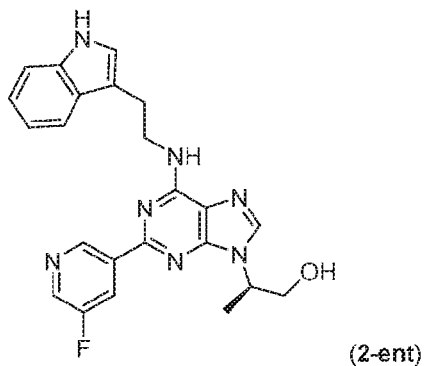
In some embodiments, aryl hydrocarbon receptor antagonists useful in conjunction with the compositions and methods described herein include SR-1, represented by formula (1), below.



In some embodiments, aryl hydrocarbon receptor antagonists useful in conjunction with the compositions and methods described herein Compound 2, represented by formula (2), below.

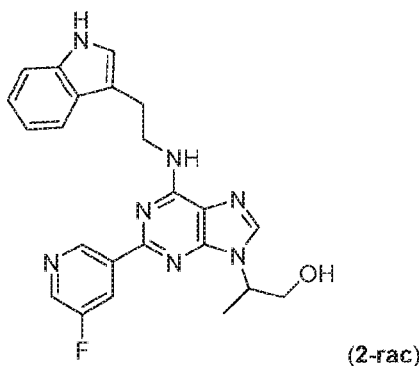


5 In some embodiments, aryl hydrocarbon receptor antagonists useful in conjunction with the compositions and methods described herein include Compound 2-ent, represented by formula (2-ent), below.

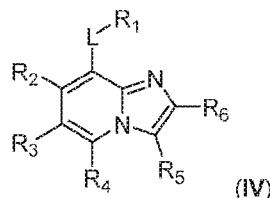


10 In some embodiments, aryl hydrocarbon receptor antagonists useful in conjunction with the compositions and methods described herein include Compound 2-rac, represented by formula (2-rac), below.





In some embodiments, aryl hydrocarbon receptor antagonists include those represented by formula (IV)



- 5 wherein L is a linker selected from the group consisting of  $-NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-O(CR_{8a}R_{8b})_n-$ ,  $-C(O)(CR_{8a}R_{8b})_n-$ ,  $-C(S)(CR_{8a}R_{8b})_n-$ ,  $-S(O)_{0-2}(CR_{8a}R_{8b})_n-$ ,  $-(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}C(O)(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}C(S)(CR_{8a}R_{8b})_n-$ ,  $-OC(O)(CR_{8a}R_{8b})_n-$ ,  $-OC(S)(CR_{8a}R_{8b})_n-$ ,  $-C(O)NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-C(S)NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-C(O)O(CR_{8a}R_{8b})_n-$ ,  $-C(S)O(CR_{8a}R_{8b})_n-$ ,  $-S(O)_2NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}S(O)_2(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}C(O)NR_{7b}(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}(CR_{8a}R_{8b})_nNR_{7a}-$ ,  $-NR_{7a}(CR_{8a}R_{8b})_nO-$ ,  $-NR_{7a}(CR_{8a}R_{8b})_nS-$ ,  $-O(CR_{8a}R_{8b})_nNR_{7a}-$ ,  $-O(CR_{8a}R_{8b})_nO-$ ,  $-O(CR_{8a}R_{8b})_nS-$ ,  $-S(CR_{8a}R_{8b})_nNR_{7a}-$ ,  $-S(CR_{8a}R_{8b})_nO-$ ,  $-S(CR_{8a}R_{8b})_nS-$ , and  $-NR_{7a}C(O)O(CR_{8a}R_{8b})_n-$ , wherein  $R_{7a}$ ,  $R_{7b}$ ,  $R_{8a}$ , and  $R_{8b}$  are each independently selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl, and each n is independently an integer from 2 to 6;

- $R_1$  is selected from the group consisting of  $-S(O)_2NR_{9a}R_{9b}$ ,  $-NR_{9a}C(O)R_{9b}$ ,  $-NR_{9a}C(S)R_{9b}$ ,  $-NR_{9a}C(O)NR_{9b}R_{9c}$ ,  $-C(O)R_{9a}$ ,  $-C(S)R_{9a}$ ,  $-S(O)_{0-2}R_{9a}$ ,  $-C(O)OR_{9a}$ ,  $-C(S)OR_{9a}$ ,  $-C(O)NR_{9a}R_{9b}$ ,  $-C(S)NR_{9a}R_{9b}$ ,  $-NR_{9a}S(O)_2R_{9b}$ ,  $-NR_{9a}C(O)OR_{9b}$ ,  $-OC(O)CR_{9a}R_{9b}R_{9c}$ ,  $-OC(S)CR_{9a}R_{9b}R_{9c}$ , optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl, wherein  $R_{9a}$ ,  $R_{9b}$ , and  $R_{9c}$  are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

$R_2$  is selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl;

$R_3$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

- 25  $R_4$  is selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl;

$R_5$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

$R_6$  is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

or a salt thereof.

As used herein to describe linkers (represented by "L" in formulas (IV), (V), and the like), the notation "- (Linker) -" (wherein "linker" is represented using chemical symbols such as  $NR_{7a}(CR_{8a}R_{8b})_n$ ,  $O(CR_{8a}R_{8b})_n$ ,  $C(O)(CR_{8a}R_{8b})_n$ ,  $C(S)(CR_{8a}R_{8b})_n$ ,  $S(O)_{0-2}(CR_{8a}R_{8b})_n$ ,  $(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}C(O)(CR_{8a}R_{8b})_n$ ,  $NR_{7a}C(S)(CR_{8a}R_{8b})_n$ ,  $OC(O)(CR_{8a}R_{8b})_n$ ,  $OC(S)(CR_{8a}R_{8b})_n$ ,  $C(O)NR_{7a}(CR_{8a}R_{8b})_n$ ,  $C(S)NR_{7a}(CR_{8a}R_{8b})_n$ ,  $C(O)O(CR_{8a}R_{8b})_n$ ,  $C(S)O(CR_{8a}R_{8b})_n$ ,  $S(O)_2NR_{7a}(CR_{8a}R_{8b})_n$ ,  $NR_{7a}S(O)_2(CR_{8a}R_{8b})_n$ , and  $NR_{7a}C(O)NR_{7b}(CR_{8a}R_{8b})_n$ ) designates that the left hyphen represents a covalent bond to the indicated position on the imidazopyridine or imidazopyrazine ring system, while the right hyphen represents a covalent bond to  $R_1$ .

In some embodiments,  $R_1$  is selected from the group consisting of  $-S(O)_2NR_{9a}R_{9b}$ ,  $-NR_{9a}C(O)R_{9b}$ ,  $-NR_{9a}C(S)R_{9b}$ ,  $-NR_{9a}C(O)NR_{9b}R_{9c}$ ,  $-C(O)R_{9a}$ ,  $-C(S)R_{9a}$ ,  $-S(O)_{0-2}R_{9a}$ ,  $-C(O)OR_{9a}$ ,  $-C(S)OR_{9a}$ ,  $-C(O)NR_{9a}R_{9b}$ ,  $-C(S)NR_{9a}R_{9b}$ ,  $-NR_{9a}S(O)_2R_{9b}$ ,  $-NR_{9a}C(O)OR_{9b}$ ,  $-OC(O)CR_{9a}R_{9b}R_{9c}$ ,  $-OC(S)CR_{9a}R_{9b}R_{9c}$ , phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl, or 1H-indazolyl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino,  $-O(CH_2)_2NR_{10a}R_{10b}$ ,  $-S(O)_2NR_{10a}R_{10b}$ ,  $-OS(O)_2NR_{10a}R_{10b}$ , and  $-NR_{10a}S(O)_2R_{10b}$ , wherein  $R_{10a}$  and  $R_{10b}$  are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl.

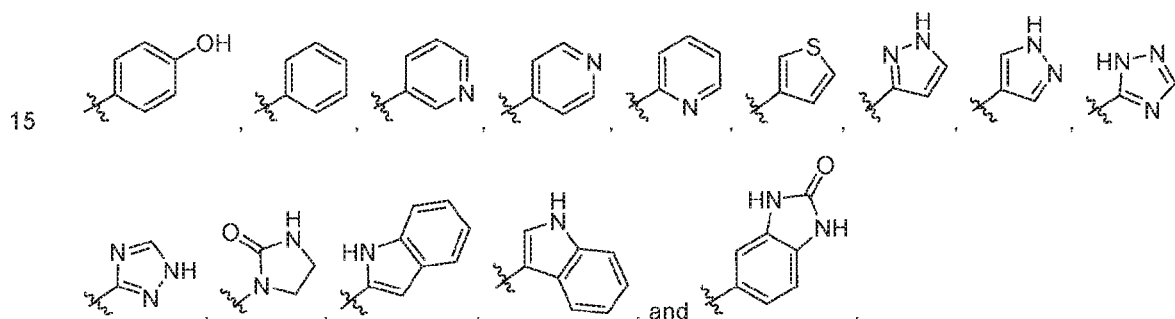
In some embodiments,  $R_1$  is selected from the group consisting of  $-S(O)_2NR_{9a}R_{9b}$ ,  $-NR_{9a}C(O)R_{9b}$ ,  $-NR_{9a}C(S)R_{9b}$ ,  $-NR_{9a}C(O)NR_{9b}R_{9c}$ ,  $-C(O)R_{9a}$ ,  $-C(S)R_{9a}$ ,  $-S(O)_{0-2}R_{9a}$ ,  $-C(O)OR_{9a}$ ,  $-C(S)OR_{9a}$ ,  $-C(O)NR_{9a}R_{9b}$ ,  $-C(S)NR_{9a}R_{9b}$ ,  $-NR_{9a}S(O)_2R_{9b}$ ,  $-NR_{9a}C(O)OR_{9b}$ ,  $-OC(O)CR_{9a}R_{9b}R_{9c}$ , and  $-OC(S)CR_{9a}R_{9b}R_{9c}$ .

In some embodiments,  $R_1$  is selected from the group consisting of phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl, or 1H-indazolyl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino,  $-O(CH_2)_2NR_{10a}R_{10b}$ ,  $-S(O)_2NR_{10a}R_{10b}$ ,  $-OS(O)_2NR_{10a}R_{10b}$ , and  $-NR_{10a}S(O)_2R_{10b}$ .

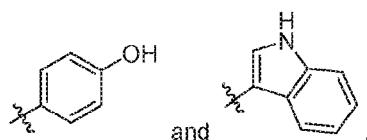
In some embodiments,  $R_1$  is selected from the group consisting of phenyl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, and 2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl, wherein the phenyl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, or 2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino,  $-\text{O}(\text{CH}_2)_2\text{NR}_{10a}\text{R}_{10b}$ ,  $-\text{S}(\text{O})_2\text{NR}_{10a}\text{R}_{10b}$ ,  $-\text{OS}(\text{O})_2\text{NR}_{10a}\text{R}_{10b}$ , and  $-\text{NR}_{10a}\text{S}(\text{O})_2\text{R}_{10b}$ .

In some embodiments,  $R_1$  is selected from the group consisting of phenyl, phenol-4-yl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, and 2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl.

In some embodiments,  $R_1$  is selected from the group consisting of:



In some embodiments,  $R_1$  is selected from the group consisting of:



In some embodiments,  $R_1$  is selected from the group consisting of phenol-4-yl and 1H-indol-3-yl.

In some embodiments,  $L$  is selected from the group consisting of  $-\text{NR}_{7a}(\text{CR}_{8a}\text{R}_{8b})_{n-}$  and  $-\text{O}(\text{CR}_{8a}\text{R}_{8b})_{n-}$ .

In some embodiments,  $L$  is selected from the group consisting of  $-\text{NH}(\text{CH}_2)_2-$  and  $-\text{O}(\text{CH}_2)_2-$ .

In some embodiments,  $R_2$  is hydrogen.

In some embodiments,  $R_3$  is selected from the group consisting of optionally substituted aryl and optionally substituted heteroaryl.

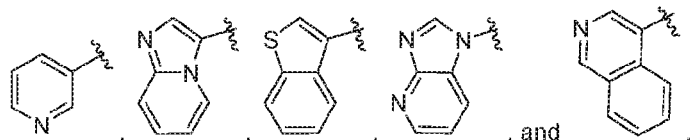
In some embodiments,  $R_3$  is selected from the group consisting of phenyl, thiophenyl, furanyl, 1H-benzimidazolyl, quinolinyl, isoquinolinyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, and thiazolyl, wherein the phenyl, thiophenyl, furanyl, 1H-benzimidazolyl, quinolinyl, isoquinolinyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-

imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, or thiazolyl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, and wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl.

In some embodiments, R<sub>3</sub> is selected from the group consisting of thiophen-2-yl, thiophen-3-yl, furan-3-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, imidazo[1,2-a]pyridin-3-yl, benzo[b]thiophen-3-yl, pyrimidin-5-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-imidazol-1-yl, pyrazin-2-yl, pyridazin-4-yl, 1H-pyrrol-2-yl and thiazol-5-yl, wherein the thiophen-2-yl, thiophen-3-yl, furan-3-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, benzo[b]thiophen-3-yl, pyrimidin-5-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-imidazol-1-yl, pyrazin-2-yl, pyridazin-4-yl, 1H-pyrrol-2-yl, or thiazol-5-yl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>.

In some embodiments, R<sub>3</sub> is selected from the group consisting of thiophen-3-yl, benzo[b]thiophen-3-yl, pyridin-3-yl, pyrimidin-5-yl, 1H-imidazol-1-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, and imidazo[1,2-a]pyridin-3-yl, wherein the thiophen-3-yl, benzo[b]thiophen-3-yl, pyridin-3-yl, pyrimidin-5-yl, 1H-imidazol-1-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, or imidazo[1,2-a]pyridin-3-yl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>.

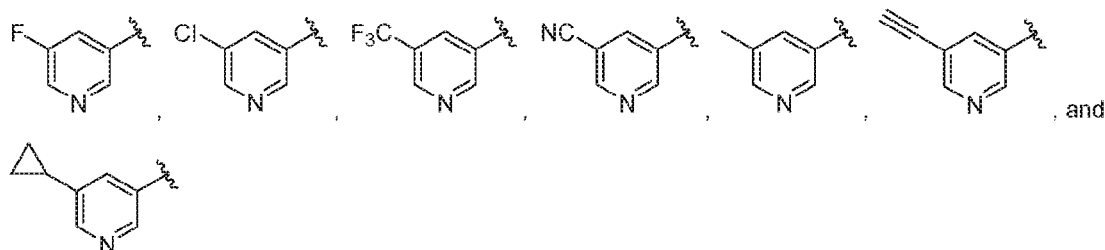
In some embodiments, R<sub>3</sub> is selected from the group consisting of optionally substituted:



In some embodiments, R<sub>3</sub> is pyridin-3-yl, wherein the pyridin-3-yl is optionally substituted at C5, for example, with a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>.

In some embodiments, the pyridin-3-yl is substituted at C5 with a substituent selected from the group consisting of ethoxycarbonyl, methoxy, cyano, methyl, methylsulfonyl, fluoro, chloro, trifluoromethyl, ethynyl, and cyclopropyl.

In some embodiments, R<sub>3</sub> is selected from the group consisting of:



In some embodiments,  $R_3$  is imidazo[1,2-a]pyridin-3-yl, wherein the imidazo[1,2-a]pyridin-3-yl is optionally substituted, for example, with a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O) $R_{11a}$ , -S(O) $_{0-2}R_{11a}$ , -C(O)OR $_{11a}$ , and -C(O)NR $_{11a}R_{11b}$ .

In some embodiments,  $R_3$  is benzo[b]thiophen-3-yl, wherein the benzo[b]thiophen-3-yl is optionally substituted, for example, with a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O) $R_{11a}$ , -S(O) $_{0-2}R_{11a}$ , -C(O)OR $_{11a}$ , and -C(O)NR $_{11a}R_{11b}$ .

In some embodiments,  $R_3$  is 1H-imidazo[4,5-b]pyridin-1-yl, wherein the 1H-imidazo[4,5-b]pyridin-1-yl is optionally substituted, for example, with a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O) $R_{11a}$ , -S(O) $_{0-2}R_{11a}$ , -C(O)OR $_{11a}$ , and -C(O)NR $_{11a}R_{11b}$ .

In some embodiments,  $R_3$  is isoquinolin-4-yl, wherein the isoquinolin-4-yl is optionally substituted, for example, with a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O) $R_{11a}$ , -S(O) $_{0-2}R_{11a}$ , -C(O)OR $_{11a}$ , and -C(O)NR $_{11a}R_{11b}$ .

In some embodiments,  $R_4$  is hydrogen.

In some embodiments,  $R_5$  is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl.

In some embodiments,  $R_5$  is selected from the group consisting of isopropyl, methyl, ethyl, prop-1-en-2-yl, isobutyl, cyclohexyl, sec-butyl, (S)-sec-butyl, (R)-sec-butyl, 1-hydroxypropan-2-yl, (S)-1-hydroxypropan-2-yl, (R)-1-hydroxypropan-2-yl, and nonan-2-yl.

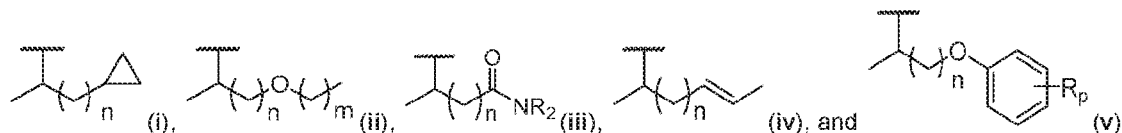
In some embodiments,  $R_5$  is (S)-1-hydroxypropan-2-yl.

In some embodiments,  $R_5$  is (R)-1-hydroxypropan-2-yl

In some embodiments,  $R_5$  is (S)-sec-butyl.

In some embodiments,  $R_5$  is (R)-sec-butyl.

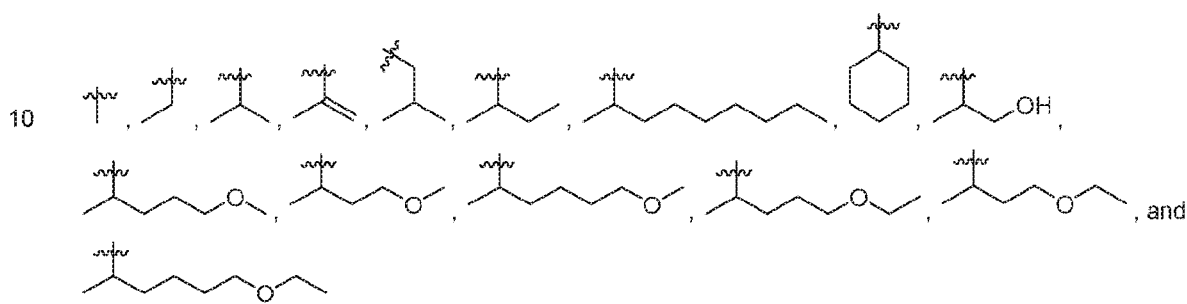
In some embodiments,  $R_5$  is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein  $n$  is an integer from 1 to 6,  $m$  is an integer from 0 to 6,  $p$  is an integer from 0 to 5, and

- 5 each  $R$  is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino,  $-C(O)R_{12a}$ ,  $-S(O)_{0-2}R_{12a}$ ,  $-C(O)OR_{12a}$ , and  $-C(O)NR_{12a}R_{12b}$ , and wherein  $R_{12a}$  and  $R_{12b}$  are each independently selected from the group consisting of hydrogen and C1-4 alkyl.

In some embodiments,  $R_5$  is selected from the group consisting of:



In some embodiments,  $R_5$  is (ii).

- 15 In some embodiments,  $R_5$  is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl.

- 20 In some embodiments,  $R_5$  is (S)-4-methoxybutan-2-yl.

In some embodiments,  $R_5$  is (R)-4-methoxybutan-2-yl.

In some embodiments,  $R_5$  is (S)-5-methoxypentan-2-yl.

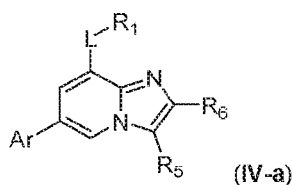
In some embodiments,  $R_5$  is (R)-5-methoxypentan-2-yl.

In some embodiments,  $R_5$  is (S)-4-ethoxybutan-2-yl.

- 25 In some embodiments,  $R_5$  is (R)-4-ethoxybutan-2-yl.

In some embodiments,  $R_5$  is hydrogen.

In some embodiments, the disclosure features a compound represented by formula (IV-a)



wherein L is a linker selected from the group consisting of -NR<sub>7a</sub>(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -O(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -C(O)(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -C(S)(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -S(O)<sub>0-2</sub>(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -NR<sub>7a</sub>C(O)(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -NR<sub>7a</sub>C(S)(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -OC(O)(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -OC(S)(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -C(O)NR<sub>7a</sub>(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -C(S)NR<sub>7a</sub>(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -C(O)O(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -C(S)O(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -S(O)<sub>2</sub>NR<sub>7a</sub>(CR<sub>8a</sub>R<sub>8b</sub>)<sub>n</sub>-, -

5  $\text{NR}_{7a}\text{S}(\text{O})_2(\text{CR}_{8a}\text{R}_{8b})_{n-}$ ,  $-\text{NR}_{7a}\text{C}(\text{O})\text{NR}_{7b}(\text{CR}_{8a}\text{R}_{8b})_{n-}$ , and  $-\text{NR}_{7a}\text{C}(\text{O})\text{O}(\text{CR}_{8a}\text{R}_{8b})_{n-}$ , wherein  $\text{R}_{7a}$ ,  $\text{R}_{7b}$ ,  $\text{R}_{8a}$ , and  $\text{R}_{8b}$  are each independently selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl, and each  $n$  is independently an integer from 2 to 6;

R<sub>1</sub> is selected from the group consisting of -S(O)<sub>2</sub>NR<sub>9a</sub>R<sub>9b</sub>, -NR<sub>9a</sub>C(O)R<sub>9b</sub>, -NR<sub>9a</sub>C(S)R<sub>9b</sub>, -NR<sub>9a</sub>C(O)NR<sub>9b</sub>R<sub>9c</sub>, -C(O)R<sub>9a</sub>, -C(S)R<sub>9a</sub>, -S(O)<sub>0-2</sub>R<sub>9a</sub>, -C(O)OR<sub>9a</sub>, -C(S)OR<sub>9a</sub>, -C(O)NR<sub>9a</sub>R<sub>9b</sub>, -C(S)NR<sub>9a</sub>R<sub>9b</sub>, -NR<sub>9a</sub>S(O)<sub>2</sub>R<sub>9b</sub>, -NR<sub>9a</sub>C(O)OR<sub>9b</sub>, -OC(O)CR<sub>9a</sub>R<sub>9b</sub>R<sub>9c</sub>, -OC(S)CR<sub>9a</sub>R<sub>9b</sub>R<sub>9c</sub>, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl, wherein R<sub>9a</sub>, R<sub>9b</sub>, and R<sub>9c</sub> are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl (for example, R<sub>1</sub> may be selected from the group consisting of phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzoimidazolyl, or 1H-indazolyl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, halo, halo-substituted-C<sub>1-4</sub> alkyl, halo-substituted-C<sub>1-4</sub> alkoxy, amino, -O(CH<sub>2</sub>)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -S(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -OS(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, and -NR<sub>10a</sub>S(O)<sub>2</sub>R<sub>10b</sub>, wherein R<sub>10a</sub> and R<sub>10b</sub> are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl);

Ar is selected from the group consisting of optionally substituted monocyclic aryl and heteroaryl, such as optionally substituted thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, and thiazolyl;

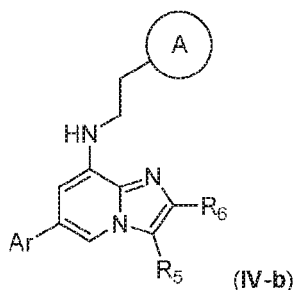
30  $R_3$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

$R_a$  is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

35 or a salt thereof.

In some embodiments, Ar is pyridin-3-yl, wherein the pyridin-3-yl is optionally substituted at C5, for example, with a substituent selected from the group consisting of ethoxycarbonyl, methoxy, cyano, methyl, methylsulfonyl, fluoro, chloro, trifluoromethyl, ethynyl, and cyclopropyl.

In some embodiments, the disclosure features a compound represented by formula (IV-b)



wherein A is an optionally substituted ring system selected from the group consisting of phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, or 1H-indazolyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, halo, halo-substituted-C<sub>1-4</sub> alkyl, halo-substituted-C<sub>1-4</sub> alkoxy, amino, -O(CH<sub>2</sub>)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -S(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -OS(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, and -NR<sub>10a</sub>S(O)<sub>2</sub>R<sub>10b</sub>, wherein R<sub>10a</sub> and R<sub>10b</sub> are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

Ar is selected from the group consisting of optionally substituted monocyclic aryl and heteroaryl, such as optionally substituted thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, and thiazolyl;

R<sub>5</sub> is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

R<sub>6</sub> is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

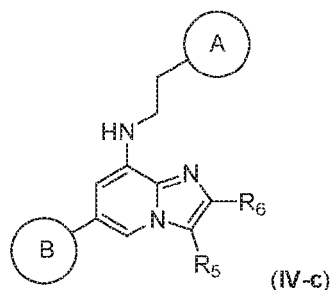
or a salt thereof.

In some embodiments, A is selected from the group consisting of phenyl, phenol-4-yl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, and 2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl.

In some embodiments, A is selected from the group consisting of phenol-4-yl and 1H-indol-3-yl.

In some embodiments, the disclosure features a compound represented by formula (IV-c)





wherein A is an optionally substituted ring system selected from the group consisting of phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, or 1H-indazolyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, halo, halo-substituted-C<sub>1-4</sub> alkyl, halo-substituted-C<sub>1-4</sub> alkoxy, amino, -O(CH<sub>2</sub>)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -S(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -OS(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, and -NR<sub>10a</sub>S(O)<sub>2</sub>R<sub>10b</sub>, wherein R<sub>10a</sub> and R<sub>10b</sub> are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

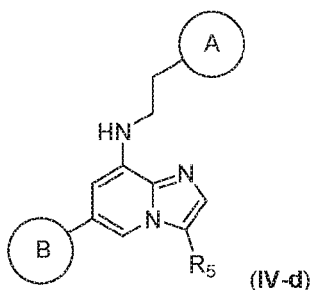
B is an optionally substituted ring system selected from the group consisting of thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, and thiazolyl, wherein the thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, 1H-imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, or thiazolyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C<sub>1-4</sub> alkyl, C<sub>2-4</sub> alkenyl, C<sub>2-4</sub> alkynyl, C<sub>3-6</sub> cycloalkyl, C<sub>1-4</sub> alkoxy, halo, halo-substituted-C<sub>1-4</sub> alkyl, halo-substituted-C<sub>1-4</sub> alkoxy, amino, -C(O)R<sub>11a</sub>, -S(O)<sub>2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

R<sub>5</sub> is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

R<sub>6</sub> is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; or a salt thereof.

In some embodiments, B is pyridin-3-yl, wherein the pyridin-3-yl is optionally substituted at C5, for example, with a substituent selected from the group consisting of ethoxycarbonyl, methoxy, cyano, methyl, methylsulfonyl, fluoro, chloro, trifluoromethyl, ethynyl, and cyclopropyl.

In some embodiments, the disclosure features a compound represented by formula (IV-d)



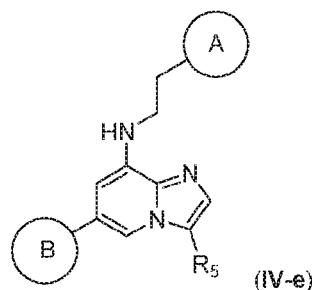
wherein A is an optionally substituted ring system selected from the group consisting of phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, or 1H-indazolyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, halo, halo-substituted-C<sub>1-4</sub> alkyl, halo-substituted-C<sub>1-4</sub> alkoxy, amino, -O(CH<sub>2</sub>)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -S(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -OS(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, and -NR<sub>10a</sub>S(O)<sub>2</sub>R<sub>10b</sub>, wherein R<sub>10a</sub> and R<sub>10b</sub> are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

B is an optionally substituted ring system selected from the group consisting of thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, and thiazolyl, wherein the thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, 1H-imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, or thiazolyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C<sub>1-4</sub> alkyl, C<sub>2-4</sub> alkenyl, C<sub>2-4</sub> alkynyl, C<sub>3-6</sub> cycloalkyl, C<sub>1-4</sub> alkoxy, halo, halo-substituted-C<sub>1-4</sub> alkyl, halo-substituted-C<sub>1-4</sub> alkoxy, amino, -C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

R<sub>5</sub> is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

or a salt thereof.

In some embodiments, the disclosure features a compound represented by formula (IV-e)

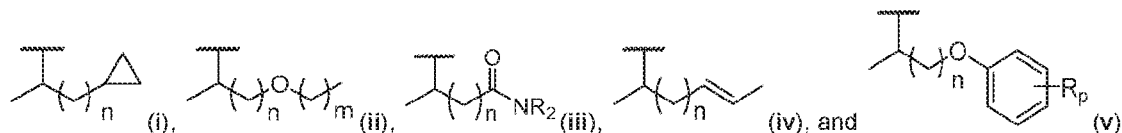


wherein A is an optionally substituted ring system selected from the group consisting of phenyl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, and 2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl, wherein the phenyl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, or 2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -O(CH<sub>2</sub>)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -S(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -OS(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, and -NR<sub>10a</sub>S(O)<sub>2</sub>R<sub>10b</sub>, wherein R<sub>10a</sub> and R<sub>10b</sub> are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

B is an optionally substituted ring system selected from the group consisting of thiophen-2-yl, thiophen-3-yl, furan-3-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, imidazo[1,2-a]pyridin-3-yl, benzo[b]thiophen-3-yl, pyrimidin-5-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-imidazol-1-yl, pyrazin-2-yl, pyridazin-4-yl, 1H-pyrrol-2-yl and thiazol-5-yl, wherein the thiophen-2-yl, thiophen-3-yl, furan-3-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, benzo[b]thiophen-3-yl, pyrimidin-5-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-imidazol-1-yl, pyrazin-2-yl, pyridazin-4-yl, 1H-pyrrol-2-yl, or thiazol-5-yl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>11a</sub>, -S(O)<sub>2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

R<sub>5</sub> is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted with

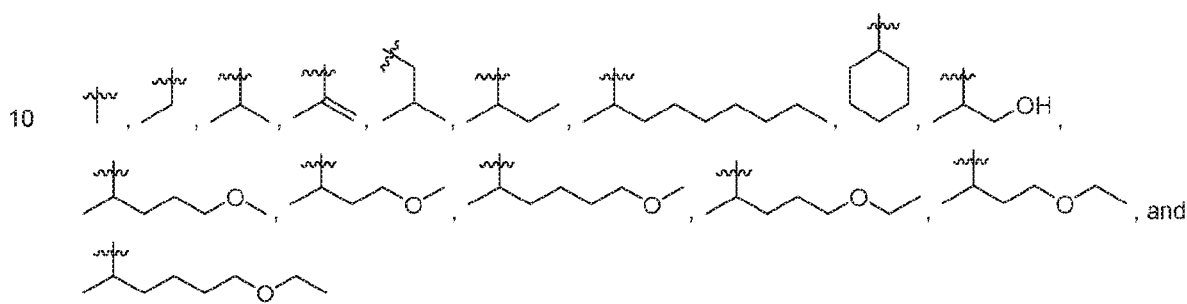
from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl, or  $R_5$  is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein  $n$  is an integer from 1 to 6,  $m$  is an integer from 0 to 6,  $p$  is an integer from 0 to 5, and

- 5 each  $R$  is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino,  $-C(O)R_{12a}$ ,  $-S(O)_{0-2}R_{12a}$ ,  $-C(O)OR_{12a}$ , and  $-C(O)NR_{12a}R_{12b}$ , and wherein  $R_{12a}$  and  $R_{12b}$  are each independently selected from the group consisting of hydrogen and C1-4 alkyl;

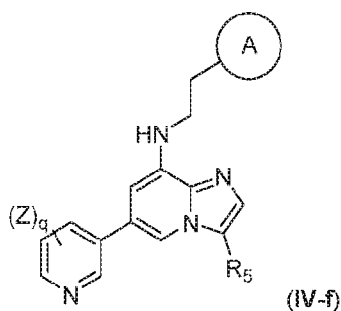
In some embodiments,  $R_5$  is selected from the group consisting of:



In some embodiments,  $R_5$  is (ii);

- 15 In some embodiments,  $R_5$  is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl; or a salt thereof.

- 20 In some embodiments, the disclosure features a compound represented by formula (IV-f)

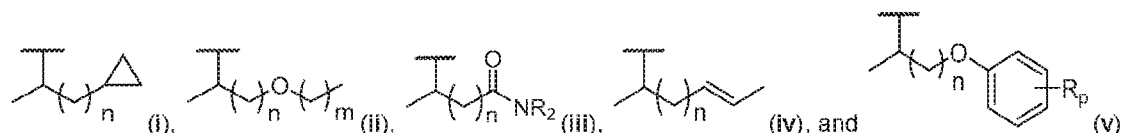


wherein  $A$  is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;

$q$  is an integer from 0 to 4;

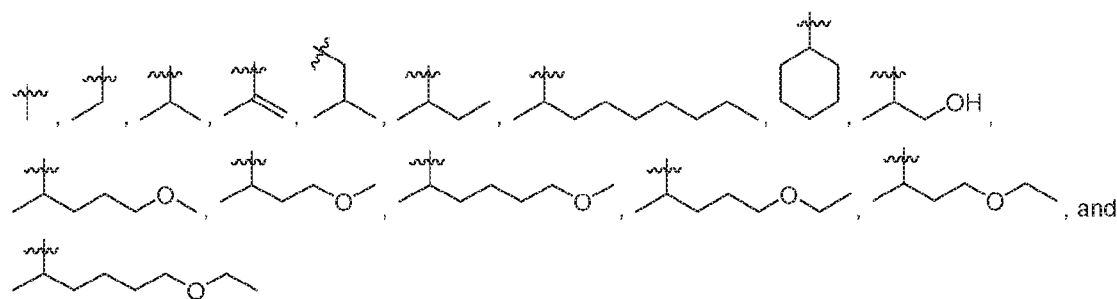
each Z is independently a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

- 5 R<sub>5</sub> is selected from the group consisting of isopropyl, methyl, ethyl, prop-1-en-2-yl, isobutyl, cyclohexyl, sec-butyl, (S)-sec-butyl, (R)-sec-butyl, 1-hydroxypropan-2-yl, (S)-1-hydroxypropan-2-yl, (R)-1-hydroxypropan-2-yl, and nonan-2-yl, or R<sub>5</sub> is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



- 10 wherein n is an integer from 1 to 6, m is an integer from 0 to 6, p is an integer from 0 to 5, and each R is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>12a</sub>, -S(O)<sub>0-2</sub>R<sub>12a</sub>, -C(O)OR<sub>12a</sub>, and -C(O)NR<sub>12a</sub>R<sub>12b</sub>, and wherein R<sub>12a</sub> and R<sub>12b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

- 15 In some embodiments, R<sub>5</sub> is selected from the group consisting of:



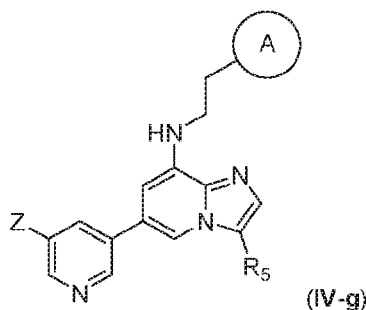
in some embodiments, R<sub>5</sub> is (ii);

- 20 in some embodiments, R<sub>5</sub> is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;

or a salt thereof.

In some embodiments, each Z is independently a substituent selected from the group consisting of ethoxycarbonyl, methoxy, cyano, methyl, methylsulfonyl, fluoro, chloro, trifluoromethyl, ethynyl, and cyclopropyl.

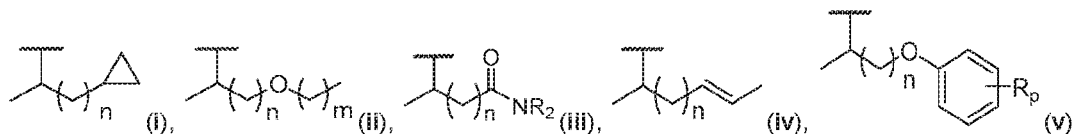
- 30 In some embodiments, the disclosure features a compound represented by formula (IV-g)



wherein A is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;

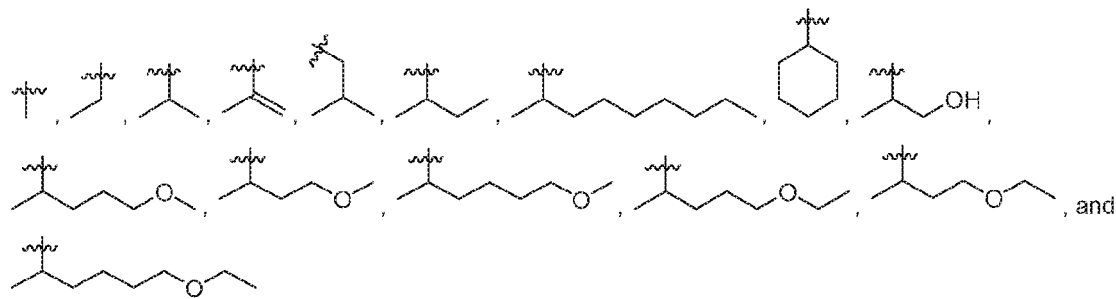
Z is a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

R<sub>5</sub> is selected from the group consisting of isopropyl, methyl, ethyl, prop-1-en-2-yl, isobutyl, cyclohexyl, sec-butyl, (S)-sec-butyl, (R)-sec-butyl, 1-hydroxypropan-2-yl, (S)-1-hydroxypropan-2-yl, (R)-1-hydroxypropan-2-yl, and nonan-2-yl, or R<sub>5</sub> is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein n is an integer from 1 to 6, m is an integer from 0 to 6, p is an integer from 0 to 5, and each R is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>12a</sub>, -S(O)<sub>0-2</sub>R<sub>12a</sub>, -C(O)OR<sub>12a</sub>, and -C(O)NR<sub>12a</sub>R<sub>12b</sub>, and wherein R<sub>12a</sub> and R<sub>12b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

In some embodiments, R<sub>5</sub> is selected from the group consisting of:



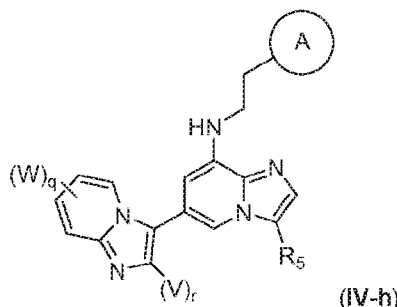
in some embodiments, R<sub>5</sub> is (ii);

in some embodiments, R<sub>5</sub> is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-

ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;

or a salt thereof.

5 In some embodiments, the disclosure features a compound represented by formula (IV-h)



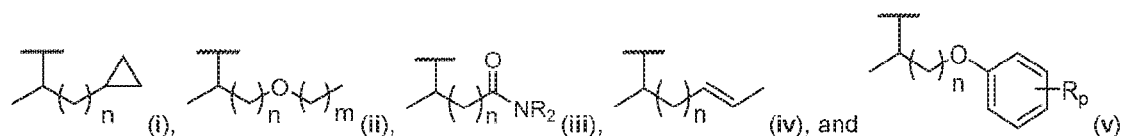
wherein A is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;

q is an integer from 0 to 4;

10 r is 0 or 1;

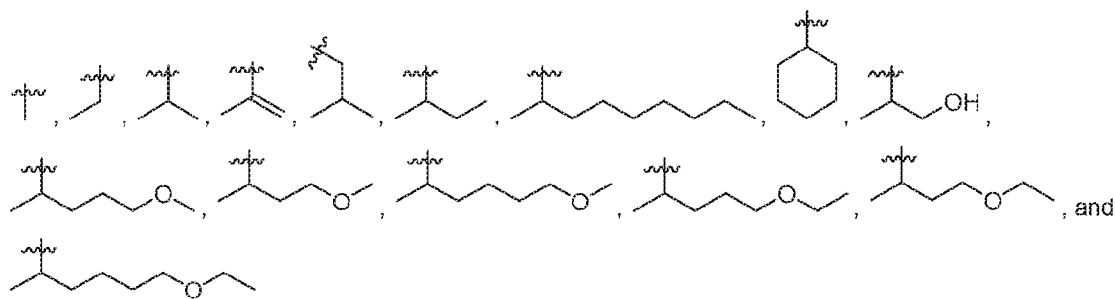
W and V are each independently a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

15 R<sub>5</sub> is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl, or R<sub>5</sub> is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



25 wherein n is an integer from 1 to 6, m is an integer from 0 to 6, p is an integer from 0 to 5, and each R is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>12a</sub>, -S(O)<sub>0-2</sub>R<sub>12a</sub>, -C(O)OR<sub>12a</sub>, and -C(O)NR<sub>12a</sub>R<sub>12b</sub>, and wherein R<sub>12a</sub> and R<sub>12b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

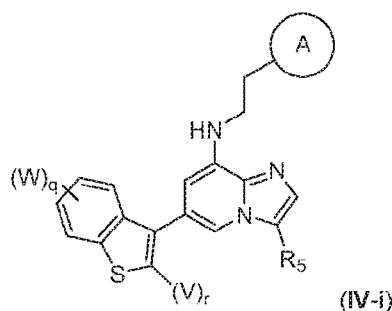
30 In some embodiments, R<sub>5</sub> is selected from the group consisting of:



in some embodiments,  $R_5$  is (ii);

- 5 in some embodiments,  $R_5$  is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;
- 10 or a salt thereof.

In some embodiments, the disclosure features a compound represented by formula (IV-i)



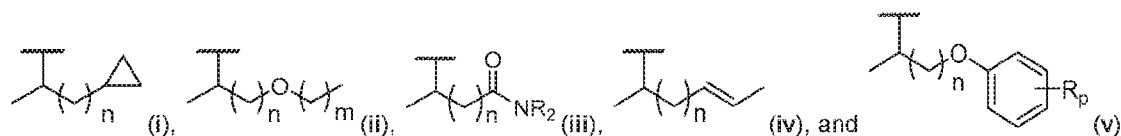
- wherein A is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;
- 15 q is an integer from 0 to 4;
- r is 0 or 1;

- W and V are each independently a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O) $R_{11a}$ , -S(O) $_{0-2}R_{11a}$ , -C(O)OR $_{11a}$ , and -C(O)NR $_{11a}R_{11b}$ , wherein  $R_{11a}$  and  $R_{11b}$  are each independently
- 20 selected from the group consisting of hydrogen and C $_{1-4}$  alkyl; and

- $R_5$  is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl,
- 25

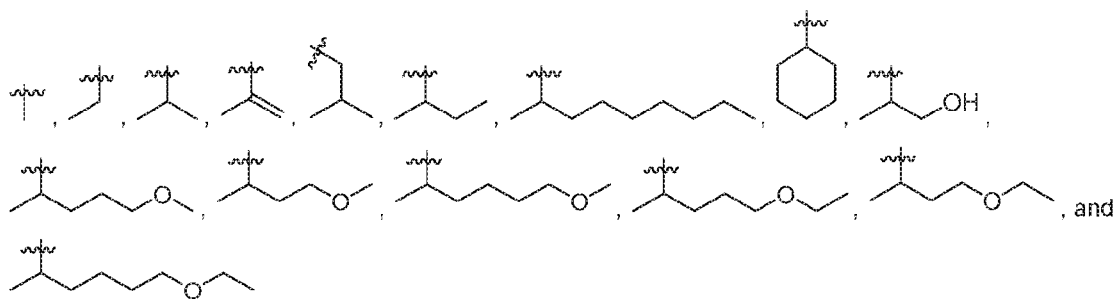


or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl, or  $R_5$  is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein  $n$  is an integer from 1 to 6,  $m$  is an integer from 0 to 6,  $p$  is an integer from 0 to 5, and each  $R$  is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino,  $-C(O)R_{12a}$ ,  $-S(O)_{0-2}R_{12a}$ ,  $-C(O)OR_{12a}$ , and  $-C(O)NR_{12a}R_{12b}$ , and wherein  $R_{12a}$  and  $R_{12b}$  are each independently selected from the group consisting of hydrogen and C1-4 alkyl;

In some embodiments,  $R_5$  is selected from the group consisting of:

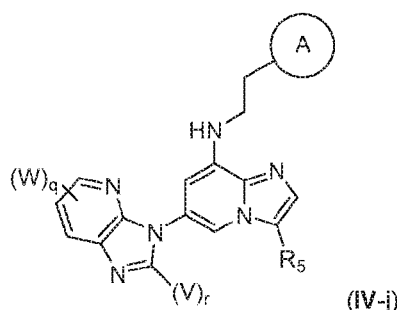


in some embodiments,  $R_5$  is (ii);

in some embodiments,  $R_5$  is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;

or a salt thereof.

In some embodiments, the disclosure features a compound represented by formula (IV-j)



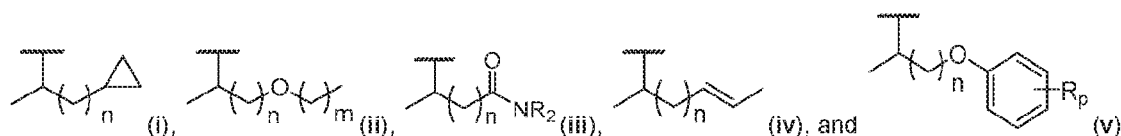
wherein  $A$  is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;

q is an integer from 0 to 4;

r is 0 or 1;

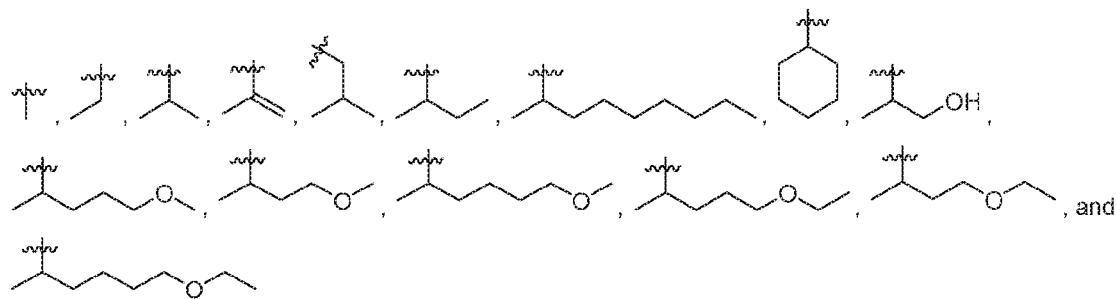
W and V are each independently a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

R<sub>5</sub> is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl, or R<sub>5</sub> is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein n is an integer from 1 to 6, m is an integer from 0 to 6, p is an integer from 0 to 5, and each R is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>12a</sub>, -S(O)<sub>0-2</sub>R<sub>12a</sub>, -C(O)OR<sub>12a</sub>, and -C(O)NR<sub>12a</sub>R<sub>12b</sub>, and wherein R<sub>12a</sub> and R<sub>12b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

In some embodiments, R<sub>5</sub> is selected from the group consisting of:

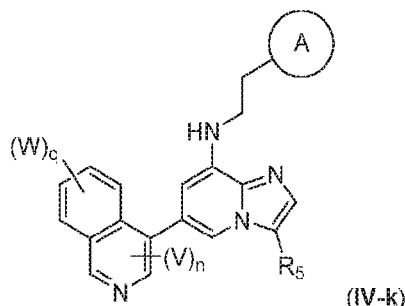


in some embodiments, R<sub>5</sub> is (ii);

in some embodiments, R<sub>5</sub> is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;

or a salt thereof.

In some embodiments, the disclosure features a compound represented by formula (IV-k)



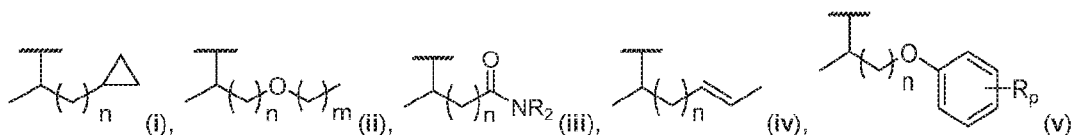
wherein A is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;

q is an integer from 0 to 4;

r is 0 or 1;

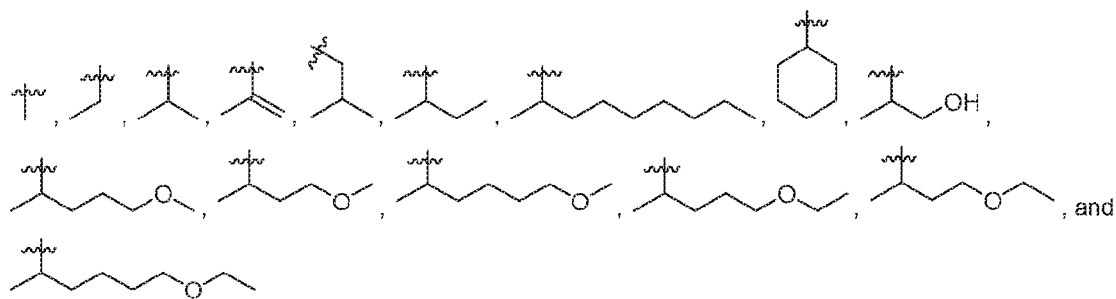
W and V are each independently a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

R<sub>5</sub> is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl, or R<sub>5</sub> is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein n is an integer from 1 to 6, m is an integer from 0 to 6, p is an integer from 0 to 5, and each R is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>12a</sub>, -S(O)<sub>0-2</sub>R<sub>12a</sub>, -C(O)OR<sub>12a</sub>, and -C(O)NR<sub>12a</sub>R<sub>12b</sub>, and wherein R<sub>12a</sub> and R<sub>12b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

In some embodiments, R<sub>5</sub> is selected from the group consisting of:

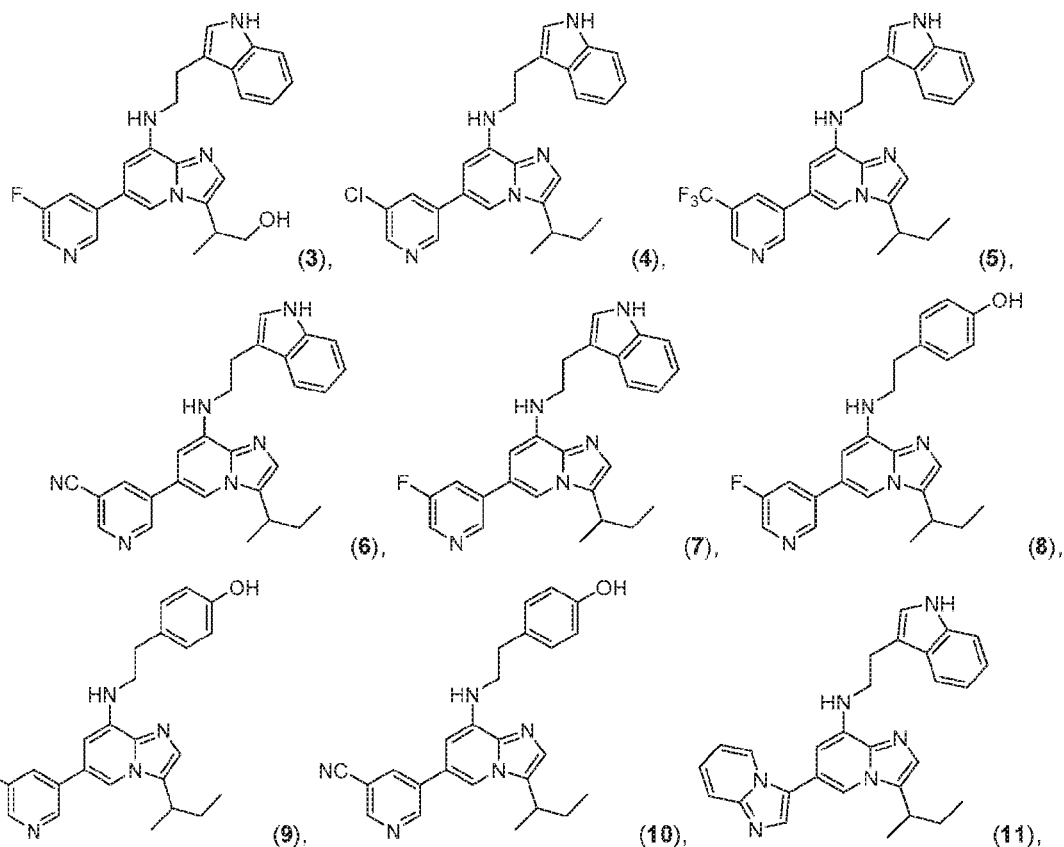


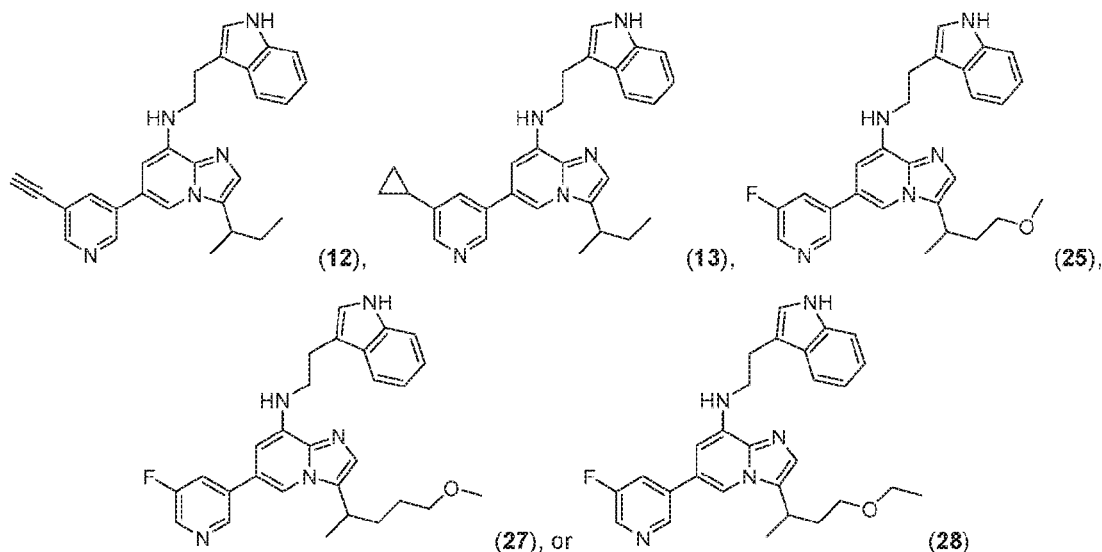
in some embodiments,  $R_5$  is (ii):

5 in some embodiments,  $R_5$  is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;

or a salt thereof.

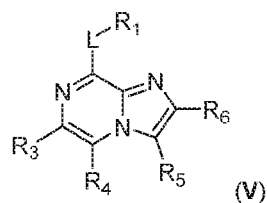
In some embodiments, the aryl hydrocarbon receptor antagonist is compound (3), compound (4), compound (5), compound (6), compound (7), compound (8), compound (9), compound (10), compound (11), compound (12), compound (13), compound (25), compound (27), or compound (28)





or salts thereof.

In some embodiments, aryl hydrocarbon receptor antagonists include those represented by  
5 formula (V)



wherein L is a linker selected from the group consisting of  $-NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-O(CR_{8a}R_{8b})_n-$ ,  $-C(O)(CR_{8a}R_{8b})_n-$ ,  $-C(S)(CR_{8a}R_{8b})_n-$ ,  $-S(O)_{0-2}(CR_{8a}R_{8b})_n-$ ,  $-(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}C(O)(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}C(S)(CR_{8a}R_{8b})_n-$ ,  $-OC(O)(CR_{8a}R_{8b})_n-$ ,  $-OC(S)(CR_{8a}R_{8b})_n-$ ,  $-C(O)NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-C(S)NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-C(O)O(CR_{8a}R_{8b})_n-$ ,  $-C(S)O(CR_{8a}R_{8b})_n-$ ,  $-S(O)_2NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}S(O)_2(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}C(O)NR_{7b}(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}(CR_{8a}R_{8b})_nNR_{7a}-$ ,  $-NR_{7a}(CR_{8a}R_{8b})_nO-$ ,  $-NR_{7a}(CR_{8a}R_{8b})_nS-$ ,  $-O(CR_{8a}R_{8b})_nNR_{7a}-$ ,  $-O(CR_{8a}R_{8b})_nO-$ ,  $-O(CR_{8a}R_{8b})_nS-$ ,  $-S(CR_{8a}R_{8b})_nNR_{7a}-$ ,  $-S(CR_{8a}R_{8b})_nO-$ ,  $-S(CR_{8a}R_{8b})_nS-$ , and  $-NR_{7a}C(O)O(CR_{8a}R_{8b})_n-$ , wherein  $R_{7a}$ ,  $R_{7b}$ ,  $R_{8a}$ , and  $R_{8b}$  are each independently selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl, and  
15 each n is independently an integer from 2 to 6;

$R_i$  is selected from the group consisting of  $-S(O)_2NR_{9a}R_{9b}$ ,  $-NR_{9a}C(O)R_{9b}$ ,  $-NR_{9a}C(S)R_{9b}$ ,  $-NR_{9a}C(O)NR_{9b}R_{9c}$ ,  $-C(O)R_{9a}$ ,  $-C(S)R_{9a}$ ,  $-S(O)_{0-2}R_{9a}$ ,  $-C(O)OR_{9a}$ ,  $-C(S)OR_{9a}$ ,  $-C(O)NR_{9a}R_{9b}$ ,  $-C(S)NR_{9a}R_{9b}$ ,  $-NR_{9a}S(O)_2R_{9b}$ ,  $-NR_{9a}C(O)OR_{9b}$ ,  $-OC(O)CR_{9a}R_{9b}R_{9c}$ ,  $-OC(S)CR_{9a}R_{9b}R_{9c}$ , optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted  
20 heterocycloalkyl, wherein  $R_{9a}$ ,  $R_{9b}$ , and  $R_{9c}$  are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

R<sub>3</sub> is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

R<sub>4</sub> is selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl;

R<sub>5</sub> is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

R<sub>6</sub> is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

or a salt thereof.

In some embodiments, R<sub>1</sub> is selected from the group consisting of -S(O)<sub>2</sub>NR<sub>9a</sub>R<sub>9b</sub>, -NR<sub>9a</sub>C(O)R<sub>9b</sub>, -NR<sub>9a</sub>C(S)R<sub>9b</sub>, -NR<sub>9a</sub>C(O)NR<sub>9b</sub>R<sub>9c</sub>, -C(O)R<sub>9a</sub>, -C(S)R<sub>9a</sub>, -S(O)<sub>0-2</sub>R<sub>9a</sub>, -C(O)OR<sub>9a</sub>, -C(S)OR<sub>9a</sub>, -C(O)NR<sub>9a</sub>R<sub>9b</sub>, -C(S)NR<sub>9a</sub>R<sub>9b</sub>, -NR<sub>9a</sub>S(O)<sub>2</sub>R<sub>9b</sub>, -NR<sub>9a</sub>C(O)OR<sub>9b</sub>, -OC(O)CR<sub>9a</sub>R<sub>9b</sub>R<sub>9c</sub>, -OC(S)CR<sub>9a</sub>R<sub>9b</sub>R<sub>9c</sub>, phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, or 1H-indazolyl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -O(CH<sub>2</sub>)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -S(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -OS(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, and -NR<sub>10a</sub>S(O)<sub>2</sub>R<sub>10b</sub>; wherein R<sub>10a</sub> and R<sub>10b</sub> are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl.

In some embodiments, R<sub>1</sub> is selected from the group consisting of -S(O)<sub>2</sub>NR<sub>9a</sub>R<sub>9b</sub>, -NR<sub>9a</sub>C(O)R<sub>9b</sub>, -NR<sub>9a</sub>C(S)R<sub>9b</sub>, -NR<sub>9a</sub>C(O)NR<sub>9b</sub>R<sub>9c</sub>, -C(O)R<sub>9a</sub>, -C(S)R<sub>9a</sub>, -S(O)<sub>0-2</sub>R<sub>9a</sub>, -C(O)OR<sub>9a</sub>, -C(S)OR<sub>9a</sub>, -C(O)NR<sub>9a</sub>R<sub>9b</sub>, -C(S)NR<sub>9a</sub>R<sub>9b</sub>, -NR<sub>9a</sub>S(O)<sub>2</sub>R<sub>9b</sub>, -NR<sub>9a</sub>C(O)OR<sub>9b</sub>, -OC(O)CR<sub>9a</sub>R<sub>9b</sub>R<sub>9c</sub>, and -OC(S)CR<sub>9a</sub>R<sub>9b</sub>R<sub>9c</sub>.

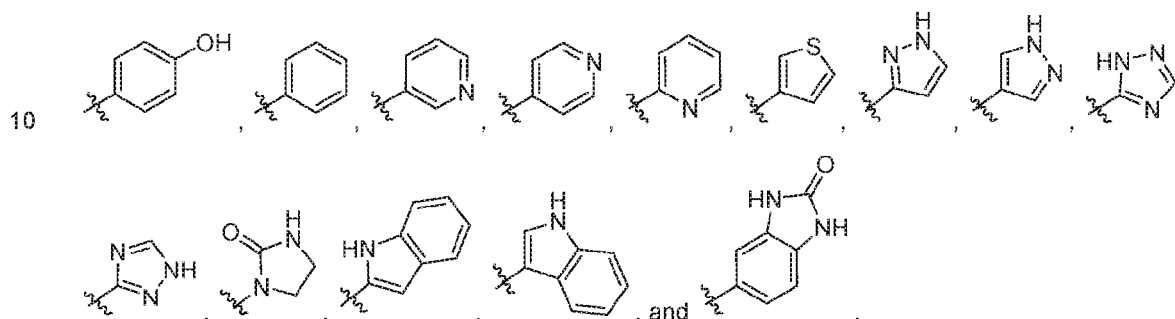
In some embodiments, R<sub>1</sub> is selected from the group consisting of phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, or 1H-indazolyl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -O(CH<sub>2</sub>)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -S(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -OS(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, and -NR<sub>10a</sub>S(O)<sub>2</sub>R<sub>10b</sub>.

In some embodiments, R<sub>1</sub> is selected from the group consisting of phenyl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, and 2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl, wherein the phenyl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, or 2-oxo-

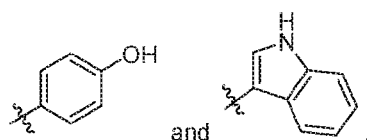
2,3-dihydro-1H-benzo[d]imidazol-5-yl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino,  $-\text{O}(\text{CH}_2)_2\text{NR}_{10a}\text{R}_{10b}$ ,  $-\text{S}(\text{O})_2\text{NR}_{10a}\text{R}_{10b}$ ,  $-\text{OS}(\text{O})_2\text{NR}_{10a}\text{R}_{10b}$ , and  $-\text{NR}_{10a}\text{S}(\text{O})_2\text{R}_{10b}$ .

5 In some embodiments,  $\text{R}_1$  is selected from the group consisting of phenyl, phenol-4-yl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, and 2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl.

In some embodiments,  $\text{R}_1$  is selected from the group consisting of:



In some embodiments,  $\text{R}_1$  is selected from the group consisting of:



In some embodiments,  $\text{R}_1$  is selected from the group consisting of phenol-4-yl and 1H-indol-3-yl.

15 In some embodiments,  $\text{L}$  is selected from the group consisting of  $-\text{NR}_{7a}(\text{CR}_{8a}\text{R}_{8b})_{n-}$  and  $-\text{O}(\text{CR}_{8a}\text{R}_{8b})_{n-}$ .

In some embodiments,  $\text{L}$  is selected from the group consisting of  $-\text{NH}(\text{CH}_2)_2-$  and  $-\text{O}(\text{CH}_2)_2-$ .

In some embodiments,  $\text{R}_3$  is selected from the group consisting of optionally substituted aryl and optionally substituted heteroaryl.

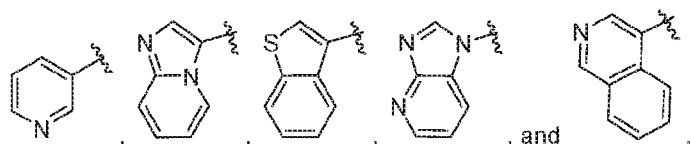
20 In some embodiments,  $\text{R}_3$  is selected from the group consisting of phenyl, thiophenyl, furanyl, 1H-benzimidazolyl, quinolyl, isoquinolyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, and thiazolyl, wherein the phenyl, thiophenyl, furanyl, 1H-benzimidazolyl, quinolyl, isoquinolyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, or thiazolyl is optionally substituted, for example, with from 1  
25 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino,  $-\text{C}(\text{O})\text{R}_{11a}$ ,  $-\text{S}(\text{O})_2\text{R}_{11a}$ ,  $-\text{C}(\text{O})\text{OR}_{11a}$ , and  $-\text{C}(\text{O})\text{NR}_{11a}\text{R}_{11b}$ , and wherein  $\text{R}_{11a}$  and  $\text{R}_{11b}$  are each independently selected from the group consisting of hydrogen and C1-4 alkyl.

30 In some embodiments,  $\text{R}_3$  is selected from the group consisting of thiophen-2-yl, thiophen-3-yl, furan-3-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, imidazo[1,2-a]pyridin-

3-yl, benzo[b]thiophen-3-yl, pyrimidin-5-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-imidazol-1-yl, pyrazin-2-yl, pyridazin-4-yl, 1H-pyrrol-2-yl and thiazol-5-yl, wherein the thiophen-2-yl, thiophen-3-yl, furan-3-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, benzo[b]thiophen-3-yl, pyrimidin-5-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-imidazol-1-yl, pyrazin-2-yl, pyridazin-4-yl, 1H-pyrrol-2-yl, or thiazol-5-yl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>.

In some embodiments, R<sub>3</sub> is selected from the group consisting of thiophen-3-yl, benzo[b]thiophen-3-yl, pyridin-3-yl, pyrimidin-5-yl, 1H-imidazol-1-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, and imidazo[1,2-a]pyridin-3-yl, wherein the thiophen-3-yl, benzo[b]thiophen-3-yl, pyridin-3-yl, pyrimidin-5-yl, 1H-imidazol-1-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, or imidazo[1,2-a]pyridin-3-yl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>.

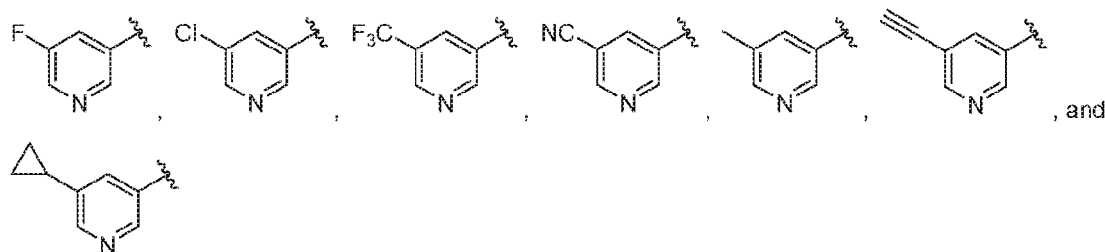
In some embodiments, R<sub>3</sub> is selected from the group consisting of optionally substituted:



In some embodiments, R<sub>3</sub> is pyridin-3-yl, wherein the pyridin-3-yl is optionally substituted at C5, for example, with a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>.

In some embodiments, the pyridin-3-yl is substituted at C5 with a substituent selected from the group consisting of ethoxycarbonyl, methoxy, cyano, methyl, methylsulfonyl, fluoro, chloro, trifluoromethyl, ethynyl, and cyclopropyl.

In some embodiments, R<sub>3</sub> is selected from the group consisting of:



In some embodiments, R<sub>3</sub> is imidazo[1,2-a]pyridin-3-yl, wherein the imidazo[1,2-a]pyridin-3-yl is optionally substituted, for example, with a substituent selected from the group consisting of C1-4 alkyl,



halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>.

In some embodiments, R<sub>3</sub> is benzo[b]thiophen-3-yl, wherein the benzo[b]thiophen-3-yl is optionally substituted, for example, with a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>.

In some embodiments, R<sub>3</sub> is 1H-imidazo[4,5-b]pyridin-1-yl, wherein the 1H-imidazo[4,5-b]pyridin-1-yl is optionally substituted, for example, with a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>.

In some embodiments, R<sub>3</sub> is isoquinolin-4-yl, wherein the isoquinolin-4-yl is optionally substituted, for example, with a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>.

In some embodiments, R<sub>4</sub> is hydrogen.

In some embodiments, R<sub>5</sub> is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl.

In some embodiments, R<sub>5</sub> is selected from the group consisting of isopropyl, methyl, ethyl, prop-1-en-2-yl, isobutyl, cyclohexyl, sec-butyl, (S)-sec-butyl, (R)-sec-butyl, 1-hydroxypropan-2-yl, (S)-1-hydroxypropan-2-yl, (R)-1-hydroxypropan-2-yl, and nonan-2-yl.

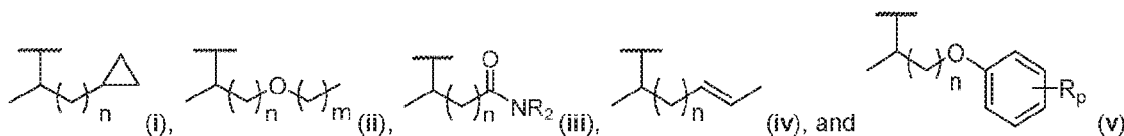
In some embodiments, R<sub>5</sub> is (S)-1-hydroxypropan-2-yl.

In some embodiments, R<sub>5</sub> is (R)-1-hydroxypropan-2-yl.

In some embodiments, R<sub>5</sub> is (S)-sec-butyl.

In some embodiments, R<sub>5</sub> is (R)-sec-butyl.

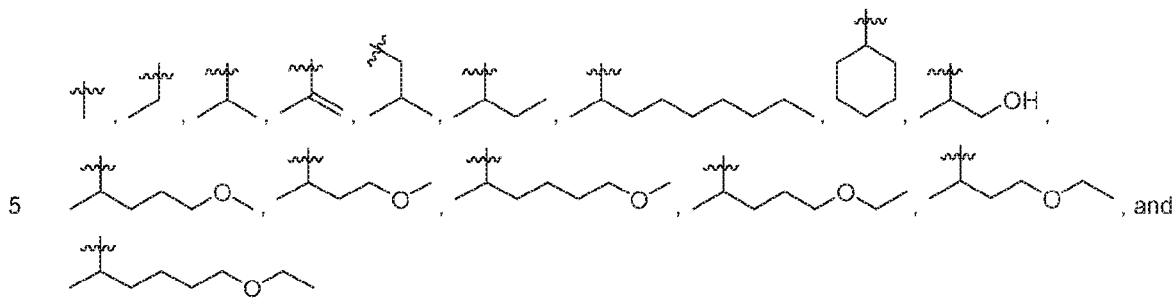
In some embodiments, R<sub>5</sub> is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein n is an integer from 1 to 6, m is an integer from 0 to 6, p is an integer from 0 to 5, and each R is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4

alkoxy, amino,  $-C(O)R_{12a}$ ,  $-S(O)_{0-2}R_{12a}$ ,  $-C(O)OR_{12a}$ , and  $-C(O)NR_{12a}R_{12b}$ , and wherein  $R_{12a}$  and  $R_{12b}$  are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl.

In some embodiments,  $R_5$  is selected from the group consisting of:



In some embodiments,  $R_5$  is (ii).

10 In some embodiments,  $R_5$  is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl.

15 In some embodiments,  $R_5$  is (S)-4-methoxybutan-2-yl.

In some embodiments,  $R_5$  is (R)-4-methoxybutan-2-yl.

In some embodiments,  $R_5$  is (S)-5-methoxypentan-2-yl.

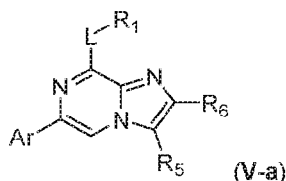
In some embodiments,  $R_5$  is (R)-5-methoxypentan-2-yl.

In some embodiments,  $R_5$  is (S)-4-ethoxybutan-2-yl.

In some embodiments,  $R_5$  is (R)-4-ethoxybutan-2-yl.

20 In some embodiments,  $R_5$  is hydrogen.

In some embodiments, the disclosure features a compound represented by formula (V-a)



25 wherein L is a linker selected from the group consisting of  $-NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-O(CR_{8a}R_{8b})_n-$ ,  $-C(O)(CR_{8a}R_{8b})_n-$ ,  $-C(S)(CR_{8a}R_{8b})_n-$ ,  $-S(O)_{0-2}(CR_{8a}R_{8b})_n-$ ,  $-(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}C(O)(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}C(S)(CR_{8a}R_{8b})_n-$ ,  $-OC(O)(CR_{8a}R_{8b})_n-$ ,  $-OC(S)(CR_{8a}R_{8b})_n-$ ,  $-C(O)NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-C(S)NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-C(O)O(CR_{8a}R_{8b})_n-$ ,  $-C(S)O(CR_{8a}R_{8b})_n-$ ,  $-S(O)_2NR_{7a}(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}S(O)_2(CR_{8a}R_{8b})_n-$ ,  $-NR_{7a}C(O)NR_{7b}(CR_{8a}R_{8b})_n-$ , and  $-NR_{7a}C(O)O(CR_{8a}R_{8b})_n-$ , wherein  $R_{7a}$ ,  $R_{7b}$ ,  $R_{8a}$ , and  $R_{8b}$  are each independently selected from the group consisting of hydrogen and optionally substituted C<sub>1-4</sub> alkyl, and each n is independently an integer from 2 to 6;

$R_1$  is selected from the group consisting of  $-S(O)_2NR_{9a}R_{9b}$ ,  $-NR_{9a}C(O)R_{9b}$ ,  $-NR_{9a}C(S)R_{9b}$ ,  $-NR_{9a}C(O)NR_{9b}R_{9c}$ ,  $-C(O)R_{9a}$ ,  $-C(S)R_{9a}$ ,  $-S(O)_{0-2}R_{9a}$ ,  $-C(O)OR_{9a}$ ,  $-C(S)OR_{9a}$ ,  $-C(O)NR_{9a}R_{9b}$ ,  $-C(S)NR_{9a}R_{9b}$ ,  $-NR_{9a}S(O)_2R_{9b}$ ,  $-NR_{9a}C(O)OR_{9b}$ ,  $-OC(O)CR_{9a}R_{9b}R_{9c}$ ,  $-OC(S)CR_{9a}R_{9b}R_{9c}$ , optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl, wherein  $R_{9a}$ ,  $R_{9b}$ , and  $R_{9c}$  are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl (for example,  $R_1$  may be selected from the group consisting of phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, or 1H-indazolyl is optionally substituted, for example, with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino,  $-O(CH_2)_2NR_{10a}R_{10b}$ ,  $-S(O)_2NR_{10a}R_{10b}$ ,  $-OS(O)_2NR_{10a}R_{10b}$ , and  $-NR_{10a}S(O)_2R_{10b}$ , wherein  $R_{10a}$  and  $R_{10b}$  are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl);

$Ar$  is selected from the group consisting of optionally substituted monocyclic aryl and heteroaryl, such as optionally substituted thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, and thiazolyl;

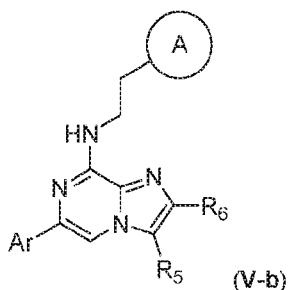
$R_5$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

$R_6$  is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

or a salt thereof.

In some embodiments,  $Ar$  is pyridin-3-yl, wherein the pyridin-3-yl is optionally substituted at C5, for example, with a substituent selected from the group consisting of ethoxycarbonyl, methoxy, cyano, methyl, methylsulfonyl, fluoro, chloro, trifluoromethyl, ethynyl, and cyclopropyl.

In some embodiments, the disclosure features a compound represented by formula (V-b)



wherein A is an optionally substituted ring system selected from the group consisting of phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, or 1H-indazolyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, halo, halo-substituted-C<sub>1-4</sub> alkyl, halo-substituted-C<sub>1-4</sub> alkoxy, amino, -O(CH<sub>2</sub>)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -S(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -OS(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, and -NR<sub>10a</sub>S(O)<sub>2</sub>R<sub>10b</sub>, wherein R<sub>10a</sub> and R<sub>10b</sub> are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

Ar is selected from the group consisting of optionally substituted monocyclic aryl and heteroaryl, such as optionally substituted thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, and thiazolyl;

R<sub>5</sub> is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

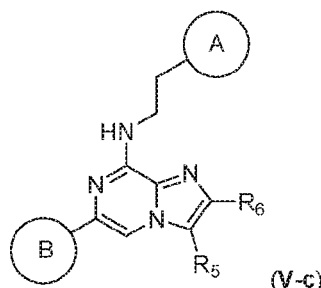
R<sub>6</sub> is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

or a salt thereof.

In some embodiments, A is selected from the group consisting of phenyl, phenol-4-yl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, and 2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl.

In some embodiments, A is selected from the group consisting of phenol-4-yl and 1H-indol-3-yl.

In some embodiments, the disclosure features a compound represented by formula (V-c)



wherein A is an optionally substituted ring system selected from the group consisting of phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, or 1H-indazolyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, halo, halo-substituted-C<sub>1-4</sub> alkyl, halo-substituted-C<sub>1-4</sub> alkoxy, amino, -O(CH<sub>2</sub>)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -S(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -OS(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, and -NR<sub>10a</sub>S(O)<sub>2</sub>R<sub>10b</sub>, wherein R<sub>10a</sub> and R<sub>10b</sub> are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

B is an optionally substituted ring system selected from the group consisting of thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, and thiazolyl, wherein the thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, 1H-imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, or thiazolyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C<sub>1-4</sub> alkyl, C<sub>2-4</sub> alkenyl, C<sub>2-4</sub> alkynyl, C<sub>3-6</sub> cycloalkyl, C<sub>1-4</sub> alkoxy, halo, halo-substituted-C<sub>1-4</sub> alkyl, halo-substituted-C<sub>1-4</sub> alkoxy, amino, -C(O)R<sub>11a</sub>, -S(O)<sub>2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

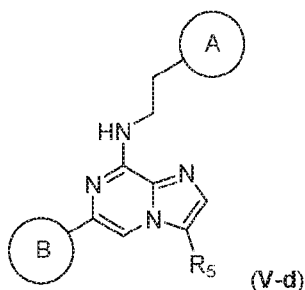
R<sub>5</sub> is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

R<sub>6</sub> is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

or a salt thereof.

In some embodiments, B is pyridin-3-yl, wherein the pyridin-3-yl is optionally substituted at C5, for example, with a substituent selected from the group consisting of ethoxycarbonyl, methoxy, cyano, methyl, methylsulfonyl, fluoro, chloro, trifluoromethyl, ethynyl, and cyclopropyl.

In some embodiments, the disclosure features a compound represented by formula (V-d)



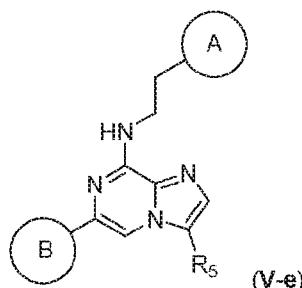
wherein A is an optionally substituted ring system selected from the group consisting of phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, and 1H-indazolyl, wherein the phenyl, 1H-pyrrolopyridinyl, 1H-indolyl, thiophenyl, pyridinyl, 1H-1,2,4-triazolyl, 2-oxoimidazolidinyl, 1H-pyrazolyl, 2-oxo-2,3-dihydro-1H-benzimidazolyl, or 1H-indazolyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, halo, halo-substituted-C<sub>1-4</sub> alkyl, halo-substituted-C<sub>1-4</sub> alkoxy, amino, -O(CH<sub>2</sub>)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -S(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -OS(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, and -NR<sub>10a</sub>S(O)<sub>2</sub>R<sub>10b</sub>, wherein R<sub>10a</sub> and R<sub>10b</sub> are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

B is an optionally substituted ring system selected from the group consisting of thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, and thiazolyl, wherein the thiophenyl, furanyl, 1H-benzimidazolyl, isoquinolinyl, 1H-imidazopyridinyl, benzothiophenyl, pyrimidinyl, pyridinyl, 1H-imidazolyl, pyrazinyl, pyridazinyl, 1H-pyrrolyl, or thiazolyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C<sub>1-4</sub> alkyl, C<sub>2-4</sub> alkenyl, C<sub>2-4</sub> alkynyl, C<sub>3-6</sub> cycloalkyl, C<sub>1-4</sub> alkoxy, halo, halo-substituted-C<sub>1-4</sub> alkyl, halo-substituted-C<sub>1-4</sub> alkoxy, amino, -C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

R<sub>5</sub> is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

or a salt thereof.

In some embodiments, the disclosure features a compound represented by formula (V-e)

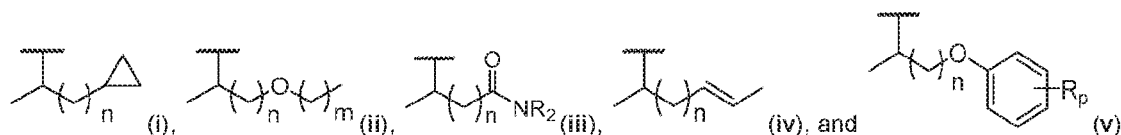


wherein A is an optionally substituted ring system selected from the group consisting of phenyl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, and 2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl, wherein the phenyl, 1H-indol-2-yl, 1H-indol-3-yl, thiophen-3-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-1,2,4-triazol-3-yl, 1H-1,2,4-triazol-5-yl, 2-oxoimidazolidin-1-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, or 2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -O(CH<sub>2</sub>)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -S(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, -OS(O)<sub>2</sub>NR<sub>10a</sub>R<sub>10b</sub>, and -NR<sub>10a</sub>S(O)<sub>2</sub>R<sub>10b</sub>, wherein R<sub>10a</sub> and R<sub>10b</sub> are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

B is an optionally substituted ring system selected from the group consisting of thiophen-2-yl, thiophen-3-yl, furan-3-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, imidazo[1,2-a]pyridin-3-yl, benzo[b]thiophen-3-yl, pyrimidin-5-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-imidazol-1-yl, pyrazin-2-yl, pyridazin-4-yl, 1H-pyrrol-2-yl and thiazol-5-yl, wherein the thiophen-2-yl, thiophen-3-yl, furan-3-yl, 1H-benzo[d]imidazol-1-yl, isoquinolin-4-yl, 1H-imidazo[4,5-b]pyridin-1-yl, benzo[b]thiophen-3-yl, pyrimidin-5-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 1H-imidazol-1-yl, pyrazin-2-yl, pyridazin-4-yl, 1H-pyrrol-2-yl, or thiazol-5-yl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>11a</sub>, -S(O)<sub>2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

R<sub>5</sub> is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted

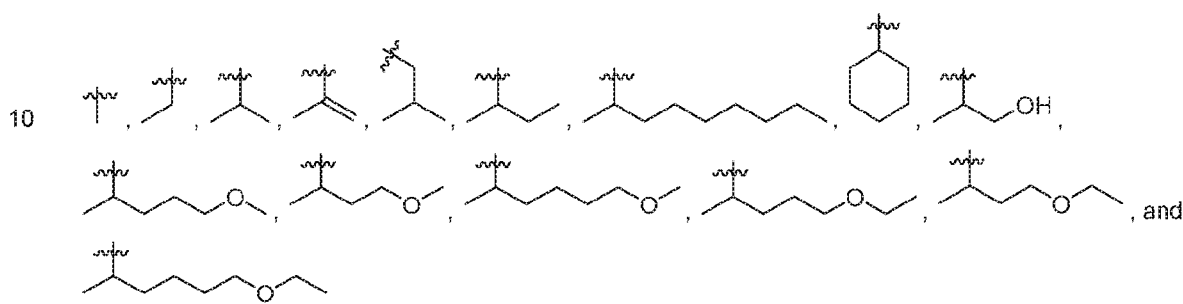
with from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl, or  $R_5$  is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein  $n$  is an integer from 1 to 6,  $m$  is an integer from 0 to 6,  $p$  is an integer from 0 to 5, and

- 5 each  $R$  is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino,  $-C(O)R_{12a}$ ,  $-S(O)_{0-2}R_{12a}$ ,  $-C(O)OR_{12a}$ , and  $-C(O)NR_{12a}R_{12b}$ , and wherein  $R_{12a}$  and  $R_{12b}$  are each independently selected from the group consisting of hydrogen and C1-4 alkyl;

In some embodiments,  $R_5$  is selected from the group consisting of:

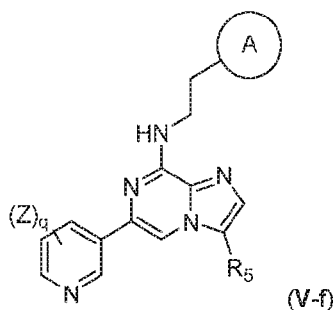


In some embodiments,  $R_5$  is (ii);

- 15 in some embodiments,  $R_5$  is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;

- 20 or a salt thereof.

In some embodiments, the disclosure features a compound represented by formula (V-f)



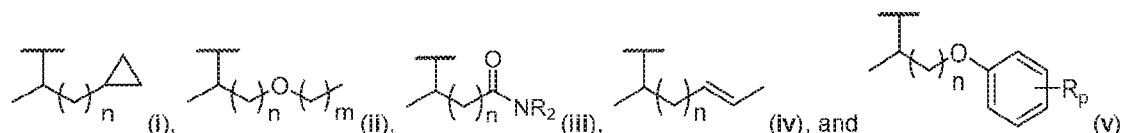
wherein  $A$  is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;

- 25  $q$  is an integer from 0 to 4;



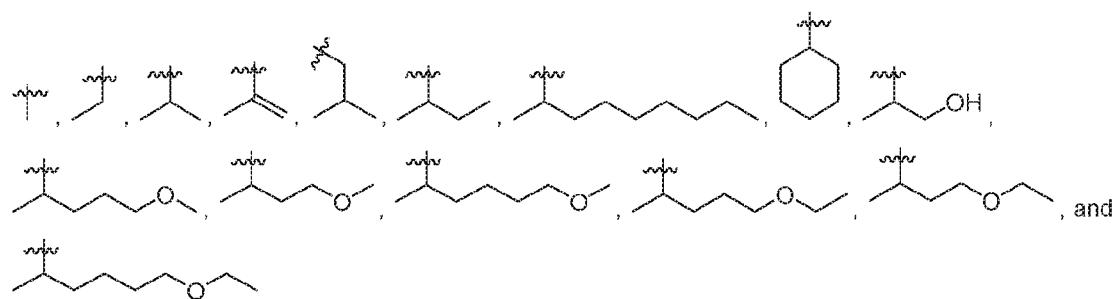
each Z is independently a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

- 5 R<sub>5</sub> is selected from the group consisting of isopropyl, methyl, ethyl, prop-1-en-2-yl, isobutyl, cyclohexyl, sec-butyl, (S)-sec-butyl, (R)-sec-butyl, 1-hydroxypropan-2-yl, (S)-1-hydroxypropan-2-yl, (R)-1-hydroxypropan-2-yl, and nonan-2-yl, or R<sub>5</sub> is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



- 10 wherein n is an integer from 1 to 6, m is an integer from 0 to 6, p is an integer from 0 to 5, and each R is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>12a</sub>, -S(O)<sub>0-2</sub>R<sub>12a</sub>, -C(O)OR<sub>12a</sub>, and -C(O)NR<sub>12a</sub>R<sub>12b</sub>, and wherein R<sub>12a</sub> and R<sub>12b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

- 15 In some embodiments, R<sub>5</sub> is selected from the group consisting of:



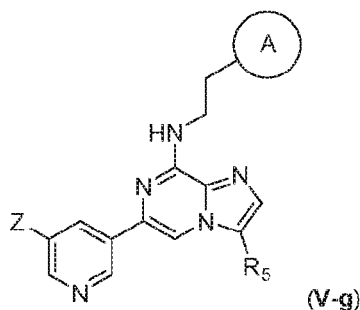
in some embodiments, R<sub>5</sub> is (ii);

- 20 in some embodiments, R<sub>5</sub> is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;

or a salt thereof.

In some embodiments, each Z is independently a substituent selected from the group consisting of ethoxycarbonyl, methoxy, cyano, methyl, methylsulfonyl, fluoro, chloro, trifluoromethyl, ethynyl, and cyclopropyl.

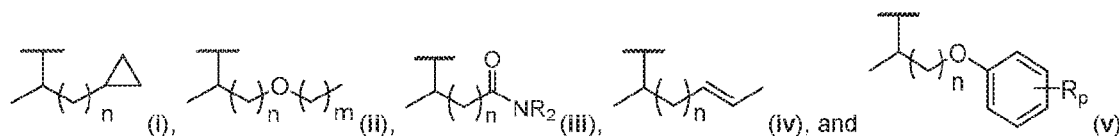
- 30 In some embodiments, the disclosure features a compound represented by formula (V-g)



wherein A is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;

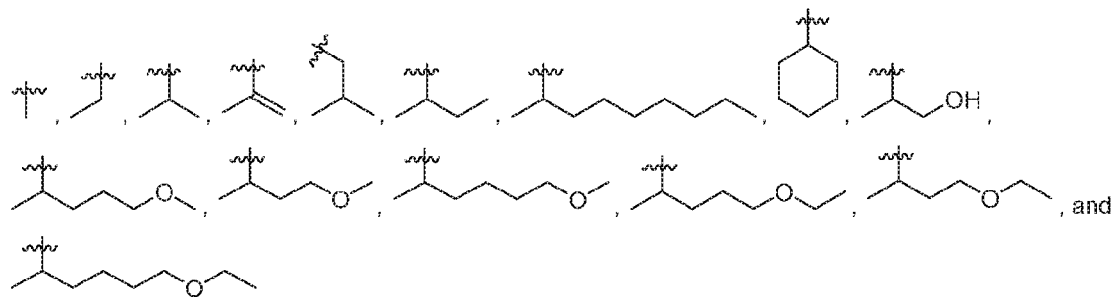
Z is a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

R<sub>5</sub> is selected from the group consisting of isopropyl, methyl, ethyl, prop-1-en-2-yl, isobutyl, cyclohexyl, sec-butyl, (S)-sec-butyl, (R)-sec-butyl, 1-hydroxypropan-2-yl, (S)-1-hydroxypropan-2-yl, (R)-1-hydroxypropan-2-yl, and nonan-2-yl, or R<sub>5</sub> is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein n is an integer from 1 to 6, m is an integer from 0 to 6, p is an integer from 0 to 5, and each R is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>12a</sub>, -S(O)<sub>0-2</sub>R<sub>12a</sub>, -C(O)OR<sub>12a</sub>, and -C(O)NR<sub>12a</sub>R<sub>12b</sub>, and wherein R<sub>12a</sub> and R<sub>12b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

In some embodiments, R<sub>5</sub> is selected from the group consisting of:



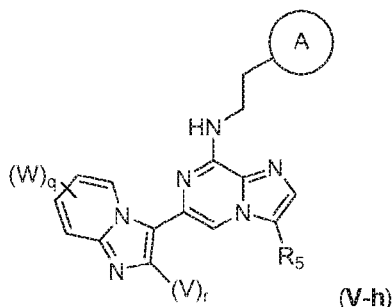
in some embodiments, R<sub>5</sub> is (ii);

in some embodiments, R<sub>5</sub> is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-

ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;

or a salt thereof.

5 In some embodiments, the disclosure features a compound represented by formula (V-h)



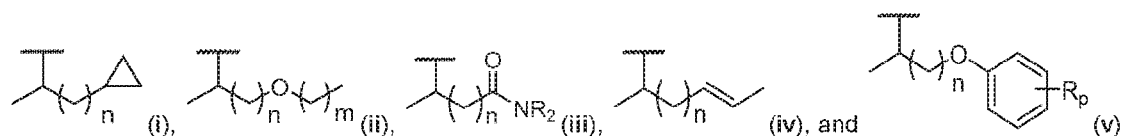
wherein A is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;

q is an integer from 0 to 4;

10 r is 0 or 1;

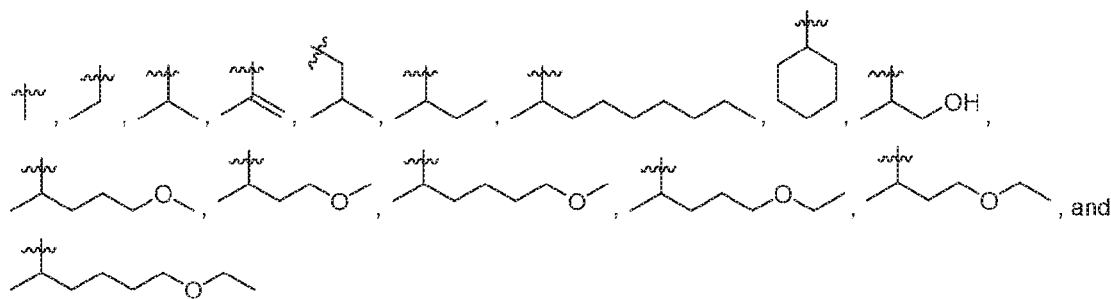
W and V are each independently a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

15 R<sub>5</sub> is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl, or R<sub>5</sub> is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



25 wherein n is an integer from 1 to 6, m is an integer from 0 to 6, p is an integer from 0 to 5, and each R is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>12a</sub>, -S(O)<sub>0-2</sub>R<sub>12a</sub>, -C(O)OR<sub>12a</sub>, and -C(O)NR<sub>12a</sub>R<sub>12b</sub>, and wherein R<sub>12a</sub> and R<sub>12b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

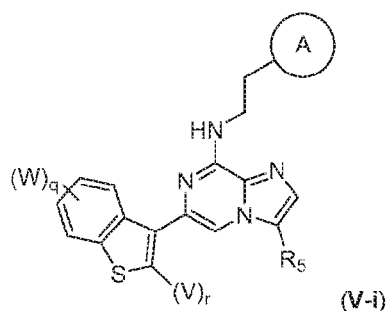
30 In some embodiments, R<sub>5</sub> is selected from the group consisting of:



in some embodiments,  $R_5$  is (ii);

- 5 in some embodiments,  $R_5$  is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;
- 10 or a salt thereof.

In some embodiments, the disclosure features a compound represented by formula (V-i)

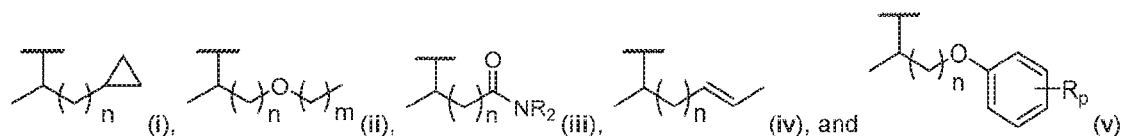


- wherein A is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;
- 15 q is an integer from 0 to 4;
- r is 0 or 1;

- W and V are each independently a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O) $R_{11a}$ , -S(O) $_{0-2}R_{11a}$ , -C(O)OR $_{11a}$ , and -C(O)NR $_{11a}R_{11b}$ , wherein  $R_{11a}$  and  $R_{11b}$  are each independently
- 20 selected from the group consisting of hydrogen and C $_{1-4}$  alkyl; and

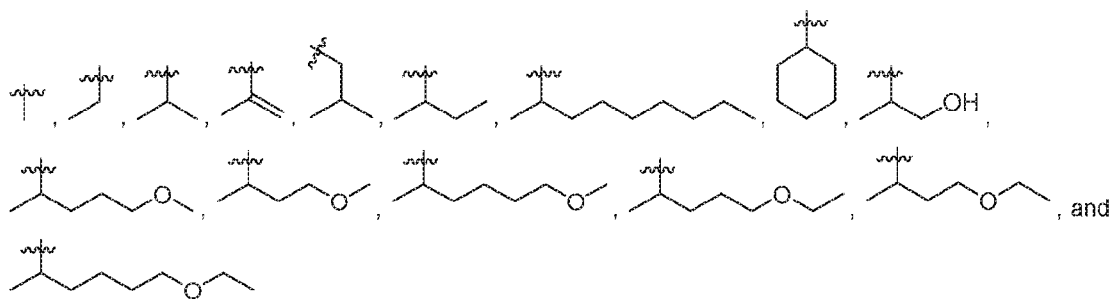
- $R_5$  is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl,
- 25

or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl, or  $R_5$  is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein  $n$  is an integer from 1 to 6,  $m$  is an integer from 0 to 6,  $p$  is an integer from 0 to 5, and each  $R$  is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino,  $-C(O)R_{12a}$ ,  $-S(O)_{0-2}R_{12a}$ ,  $-C(O)OR_{12a}$ , and  $-C(O)NR_{12a}R_{12b}$ , and wherein  $R_{12a}$  and  $R_{12b}$  are each independently selected from the group consisting of hydrogen and C1-4 alkyl;

In some embodiments,  $R_5$  is selected from the group consisting of:

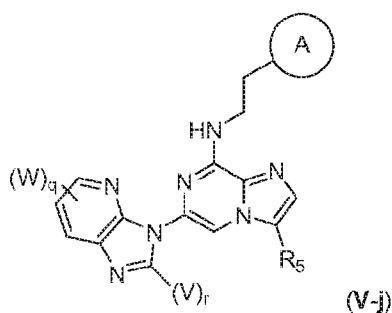


in some embodiments,  $R_5$  is (ii);

in some embodiments,  $R_5$  is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;

or a salt thereof.

In some embodiments, the disclosure features a compound represented by formula (V-j)



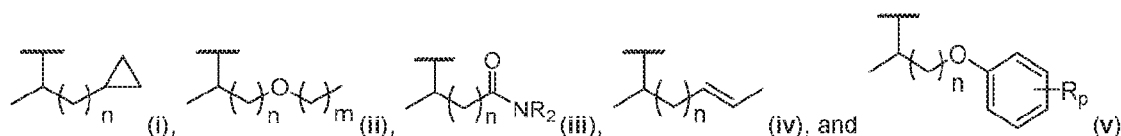
wherein  $A$  is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;

q is an integer from 0 to 4;

r is 0 or 1;

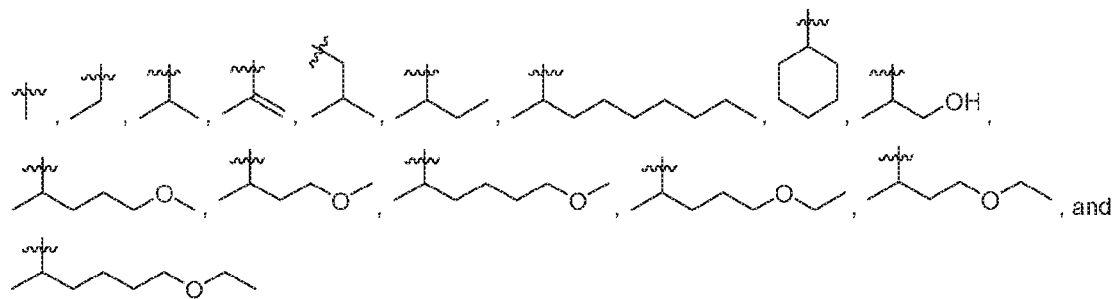
W and V are each independently a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

R<sub>5</sub> is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl, or R<sub>5</sub> is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein n is an integer from 1 to 6, m is an integer from 0 to 6, p is an integer from 0 to 5, and each R is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>12a</sub>, -S(O)<sub>0-2</sub>R<sub>12a</sub>, -C(O)OR<sub>12a</sub>, and -C(O)NR<sub>12a</sub>R<sub>12b</sub>, and wherein R<sub>12a</sub> and R<sub>12b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

In some embodiments, R<sub>5</sub> is selected from the group consisting of:

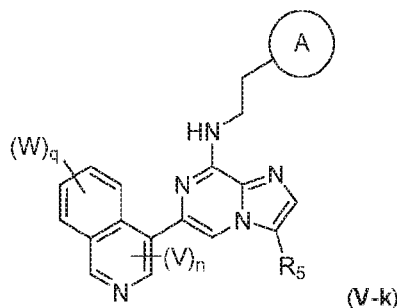


in some embodiments, R<sub>5</sub> is (ii);

in some embodiments, R<sub>5</sub> is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;

or a salt thereof.

In some embodiments, the disclosure features a compound represented by formula (V-k)



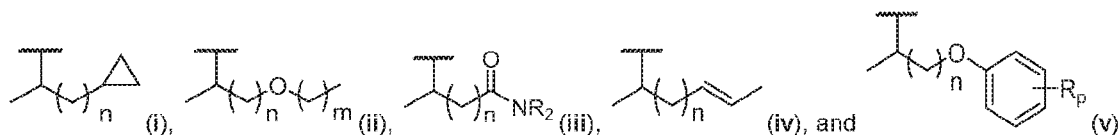
wherein A is an optionally substituted ring system selected from the group consisting of phenol-4-yl and 1H-indol-3-yl;

q is an integer from 0 to 4;

r is 0 or 1;

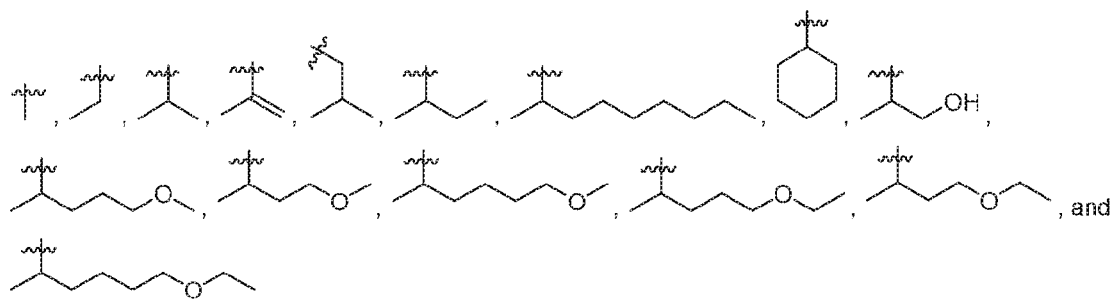
W and V are each independently a substituent selected from the group consisting of C1-4 alkyl, halo, halo-substituted-C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, cyano, amino, C(O)R<sub>11a</sub>, -S(O)<sub>0-2</sub>R<sub>11a</sub>, -C(O)OR<sub>11a</sub>, and -C(O)NR<sub>11a</sub>R<sub>11b</sub>, wherein R<sub>11a</sub> and R<sub>11b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl; and

R<sub>5</sub> is selected from the group consisting of C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, and 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl, wherein the C1-10 alkyl, prop-1-en-2-yl, cyclohexyl, cyclopropyl, 2-(2-oxopyrrolidin-1-yl)ethyl, oxetan-2-yl, oxetan-3-yl, benzhydryl, tetrahydro-2H-pyran-2-yl, tetrahydro-2H-pyran-3-yl, phenyl, tetrahydrofuran-3-yl, benzyl, (4-pentylphenyl)(phenyl)methyl, or 1-(1-(2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1H-1,2,3-triazol-4-yl)ethyl is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of hydroxy, C1-4 alkyl, and halo-substituted-C1-4alkyl, or R<sub>5</sub> is selected from the group consisting of (i), (ii), (iii), (iv), and (v)



wherein n is an integer from 1 to 6, m is an integer from 0 to 6, p is an integer from 0 to 5, and each R is independently selected from the group consisting of cyano, hydroxy, C1-4 alkyl, C2-4 alkenyl, C2-4 alkynyl, C3-6 cycloalkyl, C1-4 alkoxy, halo, halo-substituted-C1-4 alkyl, halo-substituted-C1-4 alkoxy, amino, -C(O)R<sub>12a</sub>, -S(O)<sub>0-2</sub>R<sub>12a</sub>, -C(O)OR<sub>12a</sub>, and -C(O)NR<sub>12a</sub>R<sub>12b</sub>, and wherein R<sub>12a</sub> and R<sub>12b</sub> are each independently selected from the group consisting of hydrogen and C<sub>1-4</sub> alkyl;

In some embodiments, R<sub>5</sub> is selected from the group consisting of:

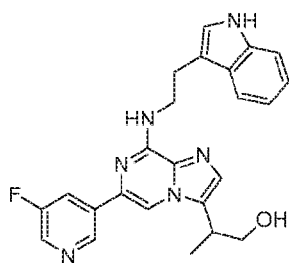


in some embodiments,  $R_5$  is (ii);

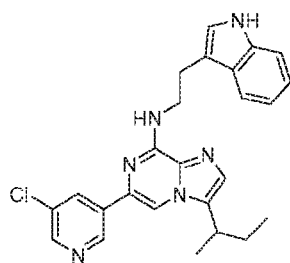
5 in some embodiments,  $R_5$  is selected from the group consisting of 4-methoxybutan-2-yl, (S)-4-methoxybutan-2-yl, (R)-4-methoxybutan-2-yl, 4-ethoxybutan-2-yl, (S)-4-ethoxybutan-2-yl, (R)-4-ethoxybutan-2-yl, 5-methoxypentan-2-yl, (S)-5-methoxypentan-2-yl, (R)-5-methoxypentan-2-yl, 5-ethoxypentan-2-yl, (S)-5-ethoxypentan-2-yl, (R)-5-ethoxypentan-2-yl, 6-methoxyhexan-2-yl, (S)-6-methoxyhexan-2-yl, (R)-6-methoxyhexan-2-yl, 6-ethoxyhexan-2-yl, (S)-6-ethoxyhexan-2-yl, and (R)-6-ethoxyhexan-2-yl;

or a salt thereof.

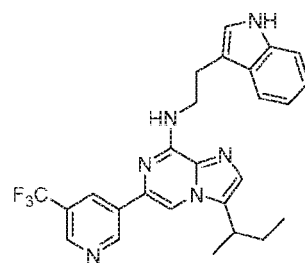
In some embodiments, the aryl hydrocarbon receptor antagonist is compound (14), compound (15), compound (16), compound (17), compound (18), compound (19), compound (20), compound (21), compound (22), compound (23), compound (24), compound (26), compound (29), or compound (30).



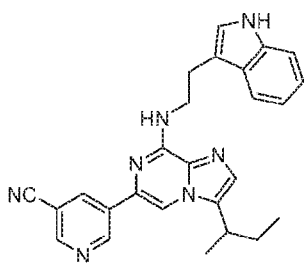
(14).



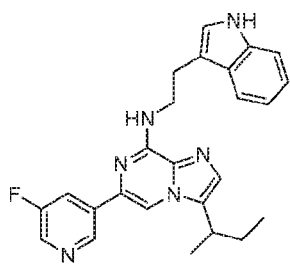
(15).



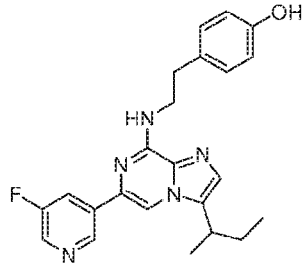
(16).



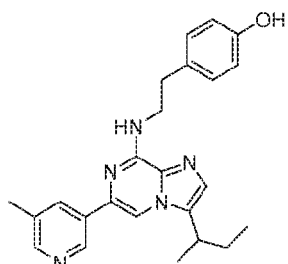
(17).



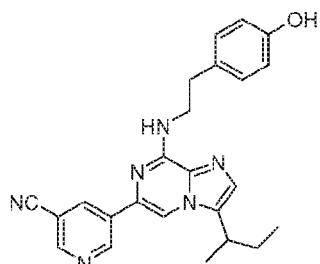
(18).



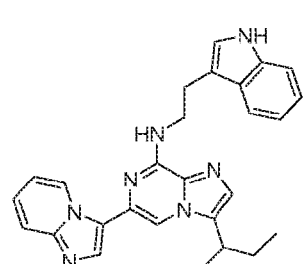
(19).



(20).

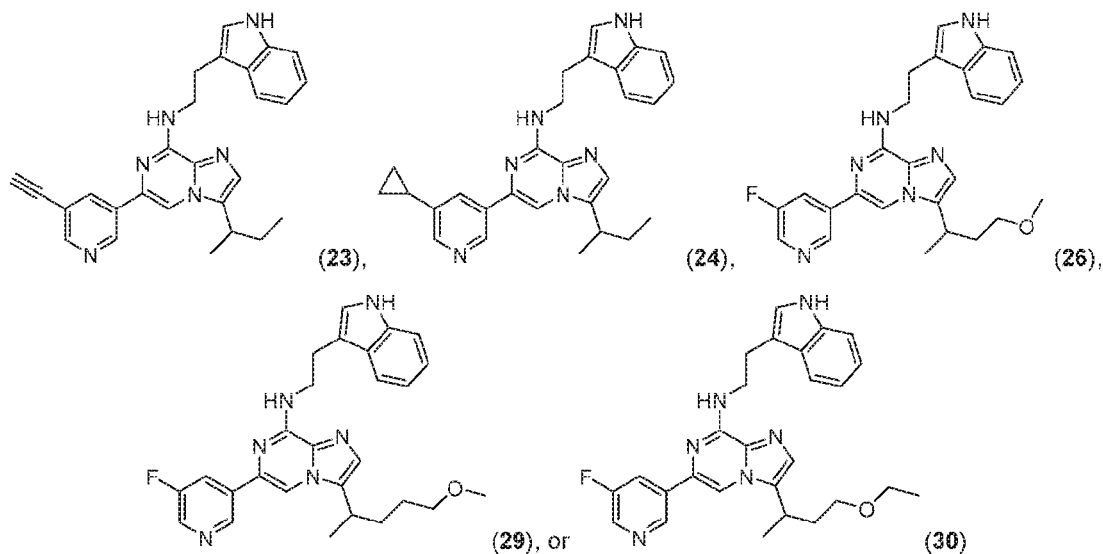


(21).



(22),





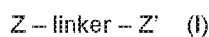
or salts thereof.

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### CXCR4 Antagonists

Exemplary CXCR4 antagonists for use in conjunction with the compositions and methods described herein are compounds represented by formula (I)

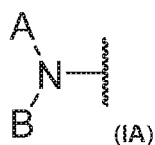
10



or a pharmaceutically acceptable salt thereof, wherein Z is:

- (i) a cyclic polyamine containing from 9 to 32 ring members, wherein from 2 to 8 of the ring members are nitrogen atoms separated from one another by 2 or more carbon atoms; or
- (ii) an amine represented by formula (IA)

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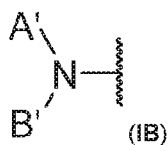


wherein A includes a monocyclic or bicyclic fused ring system including at least one nitrogen atom and B is H or a substituent of from 1 to 20 atoms;

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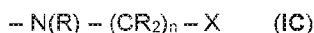
and wherein Z' is:

- (i) a cyclic polyamine containing from 9 to 32 ring members, wherein from 2 to 8 of the ring members are nitrogen atoms separated from one another by 2 or more carbon atoms;
- (ii) an amine represented by formula (IB)



wherein A' includes a monocyclic or bicyclic fused ring system including at least one nitrogen atom and B' is H or a substituent of from 1 to 20 atoms; or

(iii) a substituent represented by formula (IC)

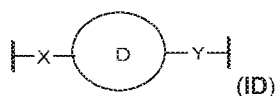


wherein each R is independently H or C<sub>1</sub>-C<sub>6</sub> alkyl, n is 1 or 2, and X is an aryl or heteroaryl group or a mercaptan;

wherein the linker is a bond, optionally substituted alkylene (e.g., optionally substituted C<sub>1</sub>-C<sub>6</sub> alkylene), optionally substituted heteroalkylene (e.g., optionally substituted C<sub>1</sub>-C<sub>6</sub> heteroalkylene), optionally substituted alkenylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenylene), optionally substituted heteroalkenylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkenylene), optionally substituted alkynylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> alkynylene), optionally substituted heteroalkynylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkynylene), optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene.

In some embodiments, Z and Z' may each independently a cyclic polyamine containing from 9 to 32 ring members, of which from 2 to 8 are nitrogen atoms separated from one another by 2 or more carbon atoms. In some embodiments, Z and Z' are identical substituents. As an example, Z may be a cyclic polyamine including from 10 to 24 ring members. In some embodiments, Z may be a cyclic polyamine that contains 14 ring members. In some embodiments, Z includes 4 nitrogen atoms. In some embodiments, Z is 1,4,8,11-tetraazocyclotetradecane.

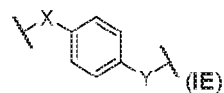
In some embodiments, the linker is represented by formula (ID)



wherein ring D is an optionally substituted aryl group, an optionally substituted heteroaryl group, an optionally substituted cycloalkyl group, or an optionally substituted heterocycloalkyl group; and

X and Y are each independently optionally substituted alkylene (e.g., optionally substituted C<sub>1</sub>-C<sub>6</sub> alkylene), optionally substituted heteroalkylene (e.g., optionally substituted C<sub>1</sub>-C<sub>6</sub> heteroalkylene), optionally substituted alkenylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenylene), optionally substituted heteroalkenylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkenylene), optionally substituted alkynylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> alkynylene), or optionally substituted heteroalkynylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkynylene).

As an example, the linker may be represented by formula (IE)

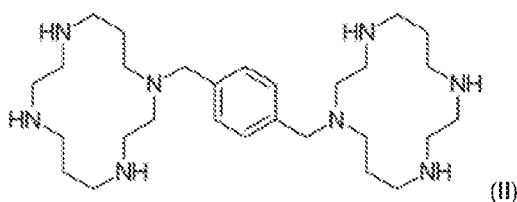


wherein ring D is an optionally substituted aryl group, an optionally substituted heteroaryl group, an optionally substituted cycloalkyl group, or an optionally substituted heterocycloalkyl group; and

X and Y are each independently optionally substituted alkylene (e.g., optionally substituted C<sub>1</sub>-C<sub>6</sub> alkylene), optionally substituted heteroalkylene (e.g., optionally substituted C<sub>1</sub>-C<sub>6</sub> heteroalkylene), optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> alkenylene), optionally substituted heteroalkenylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkenylene), optionally substituted alkynylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> alkynylene), or optionally substituted heteroalkynylene (e.g., optionally substituted C<sub>2</sub>-C<sub>6</sub> heteroalkynylene). In some embodiments, X and Y are each independently optionally substituted C<sub>1</sub>-C<sub>6</sub> alkylene. In some embodiments, X and Y are identical substituents. In some embodiments, X and Y may be each be methylene, ethylene, n-propylene, n-butylene, n-pentylene, or n-hexylene groups. In some embodiments, X and Y are each methylene groups.

The linker may be, for example, 1,3-phenylene, 2,6-pyridine, 3,5-pyridine, 2,5-thiophene, 4,4'-(2,2'-bipyrimidine), 2,9-(1,10-phenanthroline), or the like. In some embodiments, the linker is 1,4-phenylene-bis-(methylene).

CXCR4 antagonists useful in conjunction with the compositions and methods described herein include plerixafor (also referred to herein as "AMD3100" and "Mozobil"), or a pharmaceutically acceptable salt thereof, represented by formula (II), 1,1'-[1,4-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane.



Additional CXCR4 antagonists that may be used in conjunction with the compositions and methods described herein include variants of plerixafor, such as a compound described in US Patent No. 5,583,131, the disclosure of which is incorporated herein by reference as it pertains to CXCR4 antagonists. In some embodiments, the CXCR4 antagonist may be a compound selected from the group consisting of: 1,1'-[1,3-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[1,4-phenylene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; bis-zinc or bis-copper complex of 1,1'-[1,4-phenylene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[3,3'-biphenylene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[1,4-phenylene-bis-(methylene)]-bis-1,4,7,11-

tetraazacyclotetradecane; 1,11'-[1,4-phenylene-bis-(methylene)]-1,4,8,11-tetraazacyclotetradecane-1,4,7,11-tetraazacyclotetradecane; 1,1'-[2,6-pyridine-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[3,5-pyridine-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[2,5-thiophene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[4,4'-(2,2'-bipyridine)-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[2,9-(1,10-phenanthroline)-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[1,3-phenylene-bis-(methylene)]-bis-1,4,7,10-tetraazacyclotetradecane; 1,1'-[1,4-phenylene-bis-(methylene)]-bis-1,4,7,10-tetraazacyclotetradecane; 1'-[5-nitro-1,3-phenylenebis(methylene)]bis-1,4,8,11-tetraazacyclotetradecane; 1',1'-[2,4,5,6-tetrachloro-1,3-phenyleneis(methylene)]bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[2,3,5,6-tetra-fluoro-1,4-phenylenebis(methylene)]bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[1,4-naphthylene-bis-(methylene)]bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[1,3-phenylenebis-(methylene)]bis-1,5,9-triazacyclododecane; 1,1'-[1,4-phenylene-bis-(methylene)]-1,5,9-triazacyclododecane; 1,1'-[2,5-dimethyl-1,4-phenylenebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[2,5-dichloro-1,4-phenylenebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; 1,1'-[2-bromo-1,4-phenylenebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane; and 1,1'-[6-phenyl-2,4-pyridinebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane.

In some embodiments, the CXCR4 antagonist is a compound described in US 2006/0035829, the disclosure of which is incorporated herein by reference as it pertains to CXCR4 antagonists. In some embodiments, the CXCR4 antagonist may be a compound selected from the group consisting of: 3,7,11,17-tetraazabicyclo(13.3.1)heptadeca-1(17),13,15-triene; 4,7,10,17-tetraazabicyclo(13.3.1)heptadeca-1(17),13,15-triene; 1,4,7,10-tetraazacyclotetradecane; 1,4,7-triazacyclotetradecane; and 4,7,10-triazabicyclo(13.3.1)heptadeca-1(17),13,15-triene.

The CXCR4 antagonist may be a compound described in WO 2001/044229, the disclosure of which is incorporated herein by reference as it pertains to CXCR4 antagonists. In some embodiments, the CXCR4 antagonist may be a compound selected from the group consisting of: N-[4-(11-fluoro-1,4,7-triazacyclotetradecanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine; N-[4-(11,11-difluoro-1,4,7-triazacyclotetradecanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine; N-[4-(1,4,7-triazacyclotetradecan-2-onyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine; N-[12-(5-oxa-1,9-diazacyclotetradecanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine; N-[4-(11-oxa-1,4,7-triazacyclotetradecanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine; N-[4-(11-thia-1,4,7-triazacyclotetradecanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine; N-[4-(11-sulfoxo-1,4,7-triazacyclotetradecanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine; N-[4-(11-sulfonyl-1,4,7-triazacyclotetradecanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine; and N-[4-(3-carboxy-1,4,7-triazacyclotetradecanyl)-1,4-phenylenebis(methylene)]-2-(aminomethyl)pyridine.

Additional CXCR4 antagonists useful in conjunction with the compositions and methods described herein include compounds described in WO 2000/002870, the disclosure of which is incorporated herein by reference as it pertains to CXCR4 antagonists. In some embodiments, the CXCR4 antagonist may be a compound selected from the group consisting of: N-[1,4,8,11-

tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-2-(aminomethyl)pyridine; N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-N-methyl-2-(aminomethyl)pyridine; N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-4-(aminomethyl)pyridine; N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-3-(aminomethyl)pyridine; N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-(2-aminomethyl-5-methyl)pyrazine; N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-2-(aminoethyl)pyridine; N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-2-(aminomethyl)thiophene; N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-2-(aminomethyl)mercaptan; N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-2-amino benzylamine; N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-4-amino benzylamine; N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-4-(aminoethyl)imidazole; N-[1,4,8,11-tetraazacyclotetra-decanyl-1,4-phenylenebis(methylene))-benzylamine; N-[4-(1,4,7-triazacyclotetra-decanyl)-1,4-phenylenebis(methylene))-2-(aminomethyl)pyridine; N-[7-(4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene))-2-(aminomethyl)pyridine; N-[7-(4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene))-2-(aminomethyl)pyridine; N-[1-(1,4,7-triazacyclotetra-decanyl)-1,4-phenylenebis(methylene))-2-(aminomethyl)pyridine; N-[4-[4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene))-2-(aminomethyl)pyridine; N-[4-[4,7,10-triazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl]-1,4-phenylenebis(methylene))-2-(aminomethyl)pyridine; N-[1,4,8,11-tetraazacyclotetradecanyl-1,4-phenylenebis(methylene))-purine; 1-[1,4,8,11-tetraazacyclotetradecanyl-1,4-phenylenebis(methylene))-4-phenylpiperazine; N-[4-(1,7-diazacyclotetradecanyl)-1,4-phenylenebis(methylene))-2-(aminomethyl)pyridine; and N-[7-(4,10-diazabicyclo[13.3.1]heptadeca-1(17),13,15-trienyl)-1,4-phenylenebis(methylene))-2-(aminomethyl)pyridine.

In some embodiments, the CXCR4 antagonist is a compound selected from the group consisting of: 1-[2,6-dimethoxypyrid-4-yl(methylene))-1,4,8,11-tetraazacyclotetradecane; 1-[2-chloropyrid-4-yl(methylene))-1,4,8,11-tetraazacyclotetradecane; 1-[2,6-dimethylpyrid-4-yl(methylene))-1,4,8,11-tetraazacyclotetradecane; 1-[2-methylpyrid-4-yl(methylene))-1,4,8,11-tetraazacyclotetradecane; 1-[2,6-dichloropyrid-4-yl(methylene))-1,4,8,11-tetraazacyclotetradecane; 1-[2-chloropyrid-5-yl(methylene))-1,4,8,11-tetraazacyclotetradecane; and 7-[4-methylphenyl(methylene))-4,7,10,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene.

In some embodiments, the CXCR4 antagonist is a compound described in US Patent No. 5,698,546, the disclosure of which is incorporated herein by reference as it pertains to CXCR4 antagonists. In some embodiments, the CXCR4 antagonist may be a compound selected from the group consisting of: 7,7'-[1,4-phenylene-bis(methylene)]bis-3,7,11,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene; 7,7'-[1,4-phenylene-bis(methylene)]bis[15-chloro-3,7,11,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene]; 7,7'-[1,4-phenylene-bis(methylene)]bis[15-methoxy-3,7,11,17-tetraazabicyclo[13.3.1]heptadeca-1(17),13,15-triene]; 7,7'-[1,4-phenylene-bis(methylene)]bis-3,7,11,17-tetraazabicyclo[13.3.1]-heptadeca-

13,16-triene-15-one; 7,7'-[1,4-phenylene-bis(methylene)]bis-4,7,10,17-tetraazabicyclo[13.3.1]-heptadeca-1(17),13,15-triene;

8,8'-[1,4-phenylene-bis(methylene)]bis-4,8,12,19-tetraazabicyclo[15.3.1]nonadeca-1(19),15,17-triene;

6,6'-[1,4-phenylene-bis(methylene)]bis-3,6,9,15-tetraazabicyclo[11.3.1]pentadeca-1(15),11,13-triene;

- 5 6,6'-[1,3-phenylene-bis(methylene)]bis-3,6,9,15-tetraazabicyclo[11.3.1]pentadeca-1(15),11,13-triene; and  
17,17'-[1,4-phenylene-bis(methylene)]bis-3,6,14,17,23,24-hexaazatricyclo[17.3.1.1<sup>8,12</sup>]tetracosa-  
1(23),8,10,12(24),19,21-hexaene.

In some embodiments, the CXCR4 antagonist is a compound described in US Patent No.

5,021,409, the disclosure of which is incorporated herein by reference as it pertains to CXCR4

- 10 antagonists. In some embodiments, the CXCR4 antagonist may be a compound selected from the group  
consisting of: 2,2'-bicyclam, 6,6'-bicyclam; 3,3'-(bis-1,5,9,13-tetraaza cyclohexadecane); 3,3'-(bis-  
1,5,8,11,14-pentaazacyclohexadecane); methylene (or polymethylene) di-1-N-1,4,8,11-tetraaza  
cyclotetradecane; 3,3'-bis-1,5,9,13-tetraazacyclohexadecane; 3,3'-bis-1,5,8,11,14-  
pentaazacyclohexadecane; 5,5'-bis-1,4,8,11-tetraazacyclotetradecane; 2,5'-bis-1,4,8,11-  
15 tetraazacyclotetradecane; 2,6'-bis-1,4,8,11-tetraazacyclotetradecane; 11,11'-(1,2-ethanediyl)bis-1,4,8,11-  
tetraazacyclotetradecane; 11,11'-(1,2-propanediyl)bis-1,4,8,11-tetraazacyclotetradecane; 11,11'-(1,2-  
butanediyl)bis-1,4,8,11-tetraazacyclotetradecane; 11,11'-(1,2-pentanediyl)bis-1,4,8,11-  
tetraazacyclotetradecane; and 11,11'-(1,2-hexanediyl)bis-1,4,8,11-tetraazacyclotetradecane.

In some embodiments, the CXCR4 antagonist is a compound described in WO 2000/056729, the

- 20 disclosure of which is incorporated herein by reference as it pertains to CXCR4 antagonists. In some  
embodiments, the CXCR4 antagonist may be a compound selected from the group consisting of: N-(2-  
pyridinylmethyl)-N'-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-(2-  
pyridinylmethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-  
(6,7-dihydro-5H-cyclopenta[b]pyridin-7-yl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(1,2,3,4-  
25 tetrahydro-1-naphthalenyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(1-naphthalenyl)-1,4-  
benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(8-quinoliny)-1,4-benzenedimethanamine; N-(2-  
pyridinylmethyl)-N'-[2-[(2-pyridinylmethyl)amino]ethyl]-N'-(1-methyl-1,2,3,4-tetrahydro-8-quinoliny)-1,4-  
benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-[(1H-imidazol-2-ylmethyl)amino]ethyl]-N'-(1-methyl-  
1,2,3,4-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(1,2,3,4-tetrahydro-  
30 8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-[(1H-imidazol-2-  
ylmethyl)amino]ethyl]-N'-(1,2,3,4-tetrahydro-1-naphthalenyl)-1,4-benzenedimethanamine; N-(2-  
pyridinylmethyl)-N'-(2-phenyl-5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N,N'-bis(2-  
pyridinylmethyl)-N'-(2-phenyl-5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine;  
N-(2-pyridinylmethyl)-N'-(5,6,7,8-tetrahydro-5-quinoliny)-1,4-benzenedimethanamine; N-(2-  
35 pyridinylmethyl)-N'-(1H-imidazol-2-ylmethyl)-N'-(5,6,7,8-tetrahydro-5-quinoliny)-1,4-  
benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(1H-imidazol-2-ylmethyl)-N'-(5,6,7,8-tetrahydro-8-  
quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[(2-amino-3-phenyl)propyl]-N'-(5,6,7,8-  
tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(1H-imidazol-4-ylmethyl)-N'-  
(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(2-

- quinolinylmethyl)-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(2-(2-naphthoyl)aminoethyl)-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[(S)-(2-acetylamino-3-phenyl)propyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[(S)-(2-acetylamino-3-phenyl)propyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine;
- 5 N-(2-pyridinylmethyl)-N'-[3-((2-naphthalenylmethyl)amino)propyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-(S)-pyrrolidinylmethyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-(R)-pyrrolidinylmethyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine;
- 10 N-(2-pyridinylmethyl)-N'-[3-pyrazolylmethyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-pyrrolylmethyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-thiophenylmethyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-thiazolylmethyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-furanylmethyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-[(phenylmethyl)amino]ethyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(2-aminoethyl)-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-3-pyrrolidinyl-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-4-piperidinyl-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-[(phenyl)amino]ethyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(7-methoxy-1,2,3,4-tetrahydro-2-naphthalenyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(6-methoxy-1,2,3,4-tetrahydro-2-naphthalenyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(1-methyl-1,2,3,4-tetrahydro-2-naphthalenyl)-1,4-benzenedimethanamine;
- 25 N-(2-pyridinylmethyl)-N'-(7-methoxy-3,4-dihydronaphthalenyl)-1-(aminomethyl)-4-benzamide; N-(2-pyridinylmethyl)-N'-(6-methoxy-3,4-dihydronaphthalenyl)-1-(aminomethyl)-4-benzamide; N-(2-pyridinylmethyl)-N'-(1H-imidazol-2-ylmethyl)-N'-(7-methoxy-1,2,3,4-tetrahydro-2-naphthalenyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(8-hydroxy-1,2,3,4-tetrahydro-2-naphthalenyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(1H-imidazol-2-ylmethyl)-N'-(8-hydroxy-1,2,3,4-tetrahydro-2-naphthalenyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(8-Fluoro-1,2,3,4-tetrahydro-2-naphthalenyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(1H-imidazol-2-ylmethyl)-N'-(8-Fluoro-1,2,3,4-tetrahydro-2-naphthalenyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(5,6,7,8-tetrahydro-7-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(1H-imidazol-2-ylmethyl)-N'-(5,6,7,8-tetrahydro-7-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-[(2-naphthalenylmethyl)amino]ethyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-(isobutylamino)ethyl]-N'-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine;
- 35

N-(2-pyridinylmethyl)-N'-[2-[(2-pyridinylmethyl)amino]ethyl]-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-[(2-furanylmethyl)amino]ethyl]-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine;

N-(2-pyridinylmethyl)-N'-(2-guanidinoethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-

5 benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-[bis-[(2-methoxy)phenylmethyl]amino]ethyl]-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-[(1H-imidazol-4-ylmethyl)amino]ethyl]-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-[(1H-imidazol-2-ylmethyl)amino]ethyl]-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-(phenylureido)ethyl]-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[[N'-(n-butyl)carboxamido]methyl]-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine;

N-(2-pyridinylmethyl)-N'-(carboxamidomethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-

15 benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[(N'-phenyl)carboxamidomethyl]-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(carboxymethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine;

N-(2-pyridinylmethyl)-N'-(phenylmethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine;

N-(2-pyridinylmethyl)-N'-(1H-benzimidazol-2-ylmethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-

20 benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(5,6-dimethyl-1H-benzimidazol-2-ylmethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine (hydrobromide salt); N-(2-pyridinylmethyl)-N'-(5-nitro-1H-benzimidazol-2-ylmethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[(1H)-5-azabenzimidazol-2-ylmethyl]-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine;

N-(2-pyridinylmethyl)-N-(4-phenyl-1H-imidazol-2-ylmethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-

25 benzenedimethanamine; N-(2-pyridinylmethyl)-N'-[2-(2-pyridinyl)ethyl]-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(2-benzoxazolyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine;

N-(2-pyridinylmethyl)-N'-(trans-2-aminocyclohexyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-

30 benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(2-phenylethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(3-phenylpropyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N'-(trans-2-aminocyclopentyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine;

N-[[4-[[[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-N-(5,6,7,8-tetrahydro-8-quinoliny)-glycinamide; N-

[[4-[[[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-N-(5,6,7,8-tetrahydro-8-quinoliny)-(L)-alaninamide;

N-[[4-[[[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-N-(5,6,7,8-tetrahydro-8-quinoliny)-(L)-

35 aspartamide; N-[[4-[[[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-N-(5,6,7,8-tetrahydro-8-quinoliny)-pyrazinamide; N-[[4-[[[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-N-(5,6,7,8-tetrahydro-8-quinoliny)-(L)-prolinamide; N-[[4-[[[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-N-(5,6,7,8-tetrahydro-8-quinoliny)-(L)-lysινamide; N-[[4-[[[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-N-(5,6,7,8-tetrahydro-8-quinoliny)-benzamide; N-[[4-[[[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-N-(5,6,7,8-tetrahydro-8-



- quinoliny]-picolinamide; N'-Benzyl-N-[[4-[[[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-N-(5,6,7,8-tetrahydro-8-quinoliny)]-urea;
- N'-phenyl-N-[[4-[[[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-N-(5,6,7,8-tetrahydro-8-quinoliny)]-urea;
- N-(6,7,8,9-tetrahydro-5H-cyclohepta[bacteriapyridin-9-yl]-4-[[[(2-pyridinylmethyl)amino]methyl]benzamide;
- 5 N-(5,6,7,8-tetrahydro-8-quinoliny)-4-[[[(2-pyridinylmethyl)amino]methyl]benzamide; N,N'-bis(2-pyridinylmethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N,N'-bis(2-pyridinylmethyl)-N'-(6,7,8,9-tetrahydro-5H-cyclohepta[bacteriapyridin-9-yl]-1,4-benzenedimethanamine; N,N'-bis(2-pyridinylmethyl)-N'-(6,7-dihydro-5H-cyclopenta[bacteriapyridin-7-yl]-1,4-benzenedimethanamine; N,N'-bis(2-pyridinylmethyl)-N'-(1,2,3,4-tetrahydro-1-naphthalenyl)-1,4-benzenedimethanamine;
- 10 N,N'-bis(2-pyridinylmethyl)-N'-[(5,6,7,8-tetrahydro-8-quinoliny)methyl]-1,4-benzenedimethanamine; N,N'-bis(2-pyridinylmethyl)-N'[(6,7-dihydro-5H-cyclopenta[bacteriapyridin-7-yl)methyl]-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N-(2-methoxyethyl)-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(2-pyridinylmethyl)-N-[2-(4-methoxyphenyl)ethyl]-N'-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N,N'-bis(2-pyridinylmethyl)-1,4-(5,6,7,8-tetrahydro-8-quinoliny)benzenedimethanamine; N-[(2,3-dimethoxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N,N'-bis(2-pyridinylmethyl)-N-[1-(N'-phenyl-N'-methylureido)-4-piperidiny]-1,3-benzenedimethanamine; N,N'-bis(2-pyridinylmethyl)-N-[N'-p-toluenesulfonylphenylalanyl]-4-piperidiny]-1,3-benzenedimethanamine; N,N'-bis(2-pyridinylmethyl)-N-[1-[3-(2-chlorophenyl)-5-methyl-isoxazol-4-oyl]-4-piperidiny]-1,3-benzenedimethanamine; N-[(2-hydroxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[bacteriapyridin-9-yl]-1,4-benzenedimethanamine; N-[(4-cyanophenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[bacteriapyridin-9-yl]-1,4-benzenedimethanamine; N-[(4-cyanophenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[(4-acetamidophenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[(4-phenoxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[bacteriapyridin-9-yl]-1,4-benzenedimethanamine; N-[(1-methyl-2-carboxamido)ethyl]-N,N'-bis(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[(4-benzoyloxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[bacteriapyridin-9-yl]-1,4-benzenedimethanamine; N-[(thiophene-2-yl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[bacteriapyridin-9-yl]-1,4-benzenedimethanamine; N-[1-(benzyl)-3-pyrrolidiny]-N,N'-bis(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[[1-methyl-3-(pyrazol-3-yl)]propyl]-N,N'-bis(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[1-(phenyl)ethyl]-N,N'-bis(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[(3,4-methylenedioxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[1-benzyl-3-carboxymethyl-4-piperidiny]-N,N'-bis(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[(3,4-methylenedioxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(3-pyridinylmethyl)-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[[1-methyl-2-(2-tolyl)carboxamido]ethyl]-N,N'-bis(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[(1,5-dimethyl-2-phenyl-3-pyrazolinone-4-yl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-

- benzenedimethanamine; N-[(4-propoxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-(1-phenyl-3,5-dimethylpyrazolin-4-ylmethyl)-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[H-imidazol-4-ylmethyl]-N,N'-bis(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[(3-methoxy-4,5-methylenedioxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[(3-cyanophenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[(3-cyanophenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(5-ethylthiophene-2-ylmethyl)-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-(5-ethylthiophene-2-ylmethyl)-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[(2,6-difluorophenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[(2,6-difluorophenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[(2-difluoromethoxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-(2-difluoromethoxyphenylmethyl)-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(1,4-benzodioxan-6-ylmethyl)-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N,N'-bis(2-pyridinylmethyl)-N-[1-(N"-phenyl-N"-methylureido)-4-piperidiny]-1,4-benzenedimethanamine; N,N'-bis(2-pyridinylmethyl)-N-[N"-p-toluenesulfonylphenylalanyl]-4-piperidiny]-1,4-benzenedimethanamine; N-[1-(3-pyridinecarboxamido)-4-piperidiny]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[1-(cyclopropylcarboxamido)-4-piperidiny]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[1-(1-phenylcyclopropylcarboxamido)-4-piperidiny]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-(1,4-benzodioxan-6-ylmethyl)-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[1-[3-(2-chlorophenyl)-5-methyl-isoxazol-4-carboxamido]-4-piperidiny]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[1-(2-thiomethylpyridine-3-carboxamido)-4-piperidiny]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[(2,4-difluorophenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(1-methylpyrrol-2-ylmethyl)-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[(2-hydroxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[(3-methoxy-4,5-methylenedioxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(3-pyridinylmethyl)-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[2-(N"-morpholinomethyl)-1-cyclopentyl]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[(1-methyl-3-piperidiny)propyl]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-(1-methylbenzimidazol-2-ylmethyl)-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[1-(benzyl)-3-pyrrol idiny]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[[1-(1-phenyl-3-(N"-morpholino)]propyl]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[1-(iso-propyl)-4-piperidiny]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[1-(ethoxycarbonyl)-4-piperidiny]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-

- 8-quinoliny)-1,4-benzenedimethanamine; N-[(1-methyl-3-pyrazolyl)propyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[1-methyl-2-(N'',N''-diethylcarboxamido)ethyl]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[(1-methyl-2-phenylsulfonyl)ethyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[(2-chloro-4,5-methylenedioxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[1-methyl-2-[N''-(4-chlorophenyl)carboxamido]ethyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(1-acetoxyindol-3-ylmethyl)-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[(3-benzyloxy-4-methoxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-(3-quinolylmethyl)-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-[(8-hydroxy)-2-quinolylmethyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-(2-quinolylmethyl)-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[(4-acetamidophenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[1H-imidazol-2-ylmethyl]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-(3-quinolylmethyl)-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-(2-thiazolylmethyl)-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-(4-pyridinylmethyl)-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[(5-benzyloxy)benzo[b]pyrrol-3-ylmethyl]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-(1-methylpyrazol-2-ylmethyl)-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[(4-methyl)-1H-imidazol-5-ylmethyl]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[(4-dimethylamino)-1-naphthalenyl]methyl]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[1,5-dimethyl-2-phenyl-3-pyrazolinone-4-ylmethyl]-N,N'-bis(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[1-[(1-acetyl-2-(R)-prolinyl)-4-piperidinyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[1-[2-acetamidobenzoyl-4-piperidinyl]-4-piperidinyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[(2-cyano-2-phenyl)ethyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[(N''-acetyltryptophanyl)-4-piperidinyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[(N''-benzoylvalinyl)-4-piperidinyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[(4-dimethylaminophenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-(4-pyridinylmethyl)-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinoliny)-1,4-benzenedimethanamine; N-(1-methylbenzimidazol-2-ylmethyl)-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,4-benzenedimethanamine; N-[1-butyl-4-piperidinyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[1-benzoyl-4-piperidinyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[1-(benzyl)-3-pyrrolidinyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,3-benzenedimethanamine;

N-[(1-methyl)benzo[b]pyrrol-3-ylmethyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[1H-imidazol-4-ylmethyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,3-benzenedimethanamine; N-[1-(benzyl)-4-piperidinyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[1-methylbenzimidazol-2-ylmethyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[(2-phenyl)benzo[b]pyrrol-3-ylmethyl]-N-[2-(2-pyridinyl)ethyl]-N'-(2-pyridinylmethyl)-1,4-benzenedimethanamine; N-[(6-methylpyridin-2-yl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinolinyl)-1,4-benzenedimethanamine; N-(3-methyl-1H-pyrazol-5-ylmethyl)-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinolinyl)-1,3-benzenedimethanamine; N-[(2-methoxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinolinyl)-1,3-benzenedimethanamine; N-[(2-ethoxyphenyl)methyl]-N'-(2-pyridinylmethyl)-N-(6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl)-1,3-benzenedimethanamine; N-(benzyloxyethyl)-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinolinyl)-1,3-benzenedimethanamine; N-[(2-ethoxy-1-naphthalenyl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinolinyl)-1,3-benzenedimethanamine; N-[(6-methylpyridin-2-yl)methyl]-N'-(2-pyridinylmethyl)-N-(5,6,7,8-tetrahydro-8-quinolinyl)-1,3-benzenedimethanamine; 1-[[4-[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]guanidine; N-(2-pyridinylmethyl)-N-(8-methyl-8-azabicyclo[3.2.1]octan-3-yl)-1,4-benzenedimethanamine; 1-[[4-[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]homopiperazine; 1-[[3-[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]homopiperazine; trans and cis-1-[[4-[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-3,5-piperidinediamine; N,N'-[1,4-Phenylenebis(methylene)]bis-4-(2-pyrimidyl)piperazine; 1-[[4-[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-1-(2-pyridinyl)methylamine; 2-(2-pyridinyl)-5-[[[(2-pyridinylmethyl)amino]methyl]-1,2,3,4-tetrahydroisoquinoline]; 1-[[4-[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-3,4-diaminopyrrolidine; 1-[[4-[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-3,4-diacetylaminopyrrolidine; 8-[[4-[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-2,5,8-triaza-3-oxabicyclo[4.3.0]nonane; and 8-[[4-[(2-pyridinylmethyl)amino]methyl]phenyl]methyl]-2,5,8-triazabicyclo[4.3.0]nonane.

Additional CXCR4 antagonists that may be used in conjunction with the compositions and methods described herein include those described in WO 2001/085196, WO 1999/050461, WO 2001/094420, and WO 2003/090512, the disclosures of each of which are incorporated herein by reference as they pertain to compounds that inhibit CXCR4 activity or expression.

### CXCR2 Agonists

#### *Gro-β, Gro-β T, and variants thereof*

Exemplary CXCR2 agonists that may be used in conjunction with the compositions and methods described herein are Gro-β and variants thereof. Gro-β (also referred to as growth-regulated protein β, chemokine (C-X-C motif) ligand 2 (CXCL2), and macrophage inflammatory protein 2-α (MIP2-α)) is a cytokine capable of mobilizing hematopoietic stem and progenitor cells, for example, by stimulating the release of proteases, and particularly MMP9, from peripheral neutrophils. Without being limited by mechanism, MMP9 may induce mobilization of hematopoietic stem and progenitor cells from stem cell

niches, such as the bone marrow, to circulating peripheral blood by stimulating the degradation of proteins such as stem cell factor, its corresponding receptor, CD117, and CXCL12, all of which generally maintain hematopoietic stem and progenitor cells immobilized in bone marrow.

In addition to Gro- $\beta$ , exemplary CXCR2 agonists that may be used in conjunction with the compositions and methods described herein are truncated forms of Gro- $\beta$ , such as those that feature a deletion at the N-terminus of Gro- $\beta$  of from 1 to 8 amino acids (e.g., peptides that feature an N-terminal deletion of 1 amino acids, 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, or 8 amino acids). In some embodiments, CXCR2 agonists that may be used in conjunction with the compositions and methods described herein include Gro- $\beta$  T, which is characterized by a deletion of the first four amino acids from the N-terminus of Gro- $\beta$ . Gro- $\beta$  and Gro- $\beta$  T are described, for example, in US Patent No. 6,080,398, the disclosure of which is incorporated herein by reference in its entirety.

In addition, exemplary CXCR2 agonists that may be used in conjunction with the compositions and methods described herein are variants of Gro- $\beta$  containing an aspartic acid residue in place of the asparagine residue at position 69 of SEQ ID NO: 1. This peptide is referred to herein as Gro- $\beta$  N69D. Similarly, CXCR2 agonists that may be used with the compositions and methods described herein include variants of Gro- $\beta$  T containing an aspartic acid residue in place of the asparagine residue at position 65 of SEQ ID NO: 2. This peptide is referred to herein as Gro- $\beta$  T N65D T. Gro- $\beta$  N69D and Gro- $\beta$  T N65D are described, for example, in US Patent No. 6,447,766.

The amino acid sequences of Gro- $\beta$ , Gro- $\beta$  T, Gro- $\beta$  N69D, and Gro- $\beta$  T N65D are set forth in Table 2, below.

Table 2. Amino acid sequences of Gro- $\beta$  and select variants thereof

SEQ ID NO.	Description	Amino Acid Sequence
1	Gro- $\beta$	APLATELRCQCLQTLQGIHLKNIQSVK VKSPGPHCAQTEVIATLKNQKACLN PASPMVKKIIEKMLKNGKSN
2	Gro- $\beta$ -T	TELRQCLQTLQGIHLKNIQSVKVK PGPHCAQTEVIATLKNQKACLNPAS PMVKKIIEKMLKNGKSN
3	Gro- $\beta$ N69D	APLATELRCQCLQTLQGIHLKNIQSVK VKSPGPHCAQTEVIATLKNQKACLN PASPMVKKIIEKMLKDQKSN
4	Gro- $\beta$ -T N65D	TELRQCLQTLQGIHLKNIQSVKVK PGPHCAQTEVIATLKNQKACLNPAS PMVKKIIEKMLKDQKSN

Additional CXCR2 agonists that may be used in conjunction with the compositions and methods described herein include other variants of Gro- $\beta$ , such as peptides that have one or more amino acid substitutions, insertions, and/or deletions relative to Gro- $\beta$ . In some embodiments, CXCR2 agonists that may be used in conjunction with the compositions and methods described herein include peptides having at least 85% sequence identity to the amino acid sequence of SEQ ID NO: 1 (e.g., a peptide having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 1). In some embodiments, the amino acid sequence of the CXCR2 agonist differs from that of SEQ ID NO: 1 only by way of one or more conservative amino acid substitutions. In some embodiments, in some embodiments, the amino acid sequence of the CXCR2 agonist differs from that of SEQ ID NO: 1 by no more than 20, no more than 15, no more than 10, no more than 5, or no more than 1 nonconservative amino acid substitutions.

Additional examples of CXCR2 agonists useful in conjunction with the compositions and methods described herein are variants of Gro- $\beta$  T, such as peptides that have one or more amino acid substitutions, insertions, and/or deletions relative to Gro- $\beta$  T. In some embodiments, the CXCR2 agonist may be a peptide having at least 85% sequence identity to the amino acid sequence of SEQ ID NO: 2 (e.g., a peptide having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 2). In some embodiments, the amino acid sequence of the CXCR2 agonist differs from that of SEQ ID NO: 2 only by way of one or more conservative amino acid substitutions. In some embodiments, in some embodiments, the amino acid sequence of the CXCR2 agonist differs from that of SEQ ID NO: 2 by no more than 20, no more than 15, no more than 10, no more than 5, or no more than 1 nonconservative amino acid substitutions.

Additional examples of CXCR2 agonists useful in conjunction with the compositions and methods described herein are variants of Gro- $\beta$  N69D, such as peptides that have one or more amino acid substitutions, insertions, and/or deletions relative to Gro- $\beta$  N69D. In some embodiments, the CXCR2 agonist may be a peptide having at least 85% sequence identity to the amino acid sequence of SEQ ID NO: 3 (e.g., a peptide having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 3). In some embodiments, the amino acid sequence of the CXCR2 agonist differs from that of SEQ ID NO: 3 only by way of one or more conservative amino acid substitutions. In some embodiments, in some embodiments, the amino acid sequence of the CXCR2 agonist differs from that of SEQ ID NO: 3 by no more than 20, no more than 15, no more than 10, no more than 5, or no more than 1 nonconservative amino acid substitutions.

Additional examples of CXCR2 agonists useful in conjunction with the compositions and methods described herein are variants of Gro- $\beta$  T N65D, such as peptides that have one or more amino acid substitutions, insertions, and/or deletions relative to Gro- $\beta$  T N65D. In some embodiments, the CXCR2 agonist may be a peptide having at least 85% sequence identity to the amino acid sequence of SEQ ID NO: 4 (e.g., a peptide having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 4). In some embodiments, the amino acid sequence of the CXCR2 agonist differs from that of SEQ ID NO: 4 only by way of one or more conservative amino acid substitutions. In some embodiments, in some embodiments, the amino acid sequence of the

CXCR2 agonist differs from that of SEQ ID NO: 4 by no more than 20, no more than 15, no more than 10, no more than 5, or no more than 1 nonconservative amino acid substitutions.

*Agonistic anti-CXCR2 antibodies and antigen-binding fragments thereof*

5 In some embodiments, the CXCR2 agonist is an antibody or antigen-binding fragment thereof that binds CXCR2 and activates CXCR2 signal transduction. In some embodiments, the CXCR2 agonist may be an antibody or antigen-binding fragment thereof that binds the same epitope on CXCR2 as Gro- $\beta$  or a variant or truncation thereof, such as Gro- $\beta$  T, as assessed, for example, by way of a competitive CXCR2 binding assay. In some embodiments, the CXCR2 agonist is an antibody or an antigen-binding  
10 fragment thereof that competes with Gro- $\beta$  or a variant or truncation thereof, such as Gro- $\beta$  T, for binding to CXCR2.

In some embodiments of any of the above aspects, the antibody or antigen-binding fragment thereof is selected from the group consisting of a monoclonal antibody or antigen-binding fragment thereof, a polyclonal antibody or antigen-binding fragment thereof, a humanized antibody or antigen-  
15 binding fragment thereof, a bispecific antibody or antigen-binding fragment thereof, a dual-variable immunoglobulin domain, a single-chain Fv molecule (scFv), a diabody, a triabody, a nanobody, an antibody-like protein scaffold, a Fv fragment, a Fab fragment, a F(ab')<sub>2</sub> molecule, and a tandem di-scFv. In some embodiments, the antibody has an isotype selected from the group consisting of IgG, IgA, IgM, IgD, and IgE.

20 **Synthetic CXCR2 Agonists**

The peptidic CXCR2 agonists described herein, such as Gro- $\beta$ , Gro- $\beta$  T, and variants thereof, may be prepared synthetically, for instance, using solid phase peptide synthesis techniques. Systems and processes for performing solid phase peptide synthesis include those that are known in the art and have been described, for instance, in US Patent Nos. 9,169,287; 9,388,212; 9,206,222; 6,028,172; and  
25 5,233,044, among others, the disclosures of each of which are incorporated herein by reference as they pertain to protocols and techniques for the synthesis of peptides on solid support. Solid phase peptide synthesis is a process in which amino acid residues are added to peptides that have been immobilized on a solid support, such as a polymeric resin (e.g., a hydrophilic resin, such as a polyethylene-glycol-containing resin, or hydrophobic resin, such as a polystyrene-based resin).

30 Peptides, such as those containing protecting groups at amino, hydroxy, thiol, and carboxy substituents, among others, may be bound to a solid support such that the peptide is effectively immobilized on the solid support. For example, the peptides may be bound to the solid support via their C termini, thereby immobilizing the peptides for subsequent reaction in at a resin-liquid interface.

The process of adding amino acid residues to immobilized peptides can include exposing a  
35 deprotection reagent to the immobilized peptides to remove at least a portion of the protection groups from at least a portion of the immobilized peptides. The deprotection reagent exposure step can be configured, for instance, such that side-chain protection groups are preserved, while N-terminal protection groups are removed. For instance, an exemplary amino protecting contains a fluorenylmethyloxycarbonyl (Fmoc) substituent. A deprotection reagent containing a strongly basic substance, such as piperidine

(e.g., a piperidine solution in an appropriate organic solvent, such as dimethyl formamide (DMF)) may be exposed to the immobilized peptides such that the Fmoc protecting groups are removed from at least a portion of the immobilized peptides. Other protecting groups suitable for the protection of amino substituents include, for instance, the tert-butyloxycarbonyl (Boc) moiety. A deprotection reagent comprising a strong acid, such as trifluoroacetic acid (TFA) may be exposed to immobilized peptides containing a Boc-protected amino substituent so as to remove the Boc protecting group by an ionization process. In this way, peptides can be protected and deprotected at specific sites, such as at one or more side-chains or at the N- or C-terminus of an immobilized peptide so as to append chemical functionality regioselectively at one or more of these positions. This can be used, for instance, to derivatize a side-chain of an immobilized peptide, or to synthesize a peptide, e.g., from the C-terminus to the N-terminus.

The process of adding amino acid residues to immobilized peptides can include, for instance, exposing protected, activated amino acids to the immobilized peptides such that at least a portion of the activated amino acids are bonded to the immobilized peptides to form newly-bonded amino acid residues. For example, the peptides may be exposed to activated amino acids that react with the deprotected N-termini of the peptides so as to elongate the peptide chain by one amino acid. Amino acids can be activated for reaction with the deprotected peptides by reaction of the amino acid with an agent that enhances the electrophilicity of the backbone carbonyl carbon of the amino acid. For example, phosphonium and uronium salts can, in the presence of a tertiary base (e.g., diisopropylethylamine (DIPEA) and triethylamine (TEA), among others), convert protected amino acids into activated species (for example, BOP, PyBOP, HBTU, and TBTU all generate HOBT esters). Other reagents can be used to help prevent racemization that may be induced in the presence of a base. These reagents include carbodiimides (for example, DCC or WSCDI) with an added auxiliary nucleophile (for example, 1-hydroxy-benzotriazole (HOBt), 1-hydroxy-azabenzotriazole (HOAt), or HOSu) or derivatives thereof. Another reagent that can be utilized to prevent racemization is TBTU. The mixed anhydride method, using isobutyl chloroformate, with or without an added auxiliary nucleophile, can also be used, as well as the azide method, due to the low racemization associated with this reagent. These types of compounds can also increase the rate of carbodiimide-mediated couplings, as well as prevent dehydration of Asn and Gln residues. Typical additional reagents include also bases such as N,N-diisopropylethylamine (DIPEA), triethylamine (TEA) or N-methylmorpholine (NMM). These reagents are described in detail, for instance, in US Patent No. 8,546,350, the disclosure of which is incorporated herein in its entirety.

During the recombinant expression and folding of Gro- $\beta$  and Gro- $\beta$  T in aqueous solution, a particular C-terminal asparagine residue (Asn69 within Gro- $\beta$  and Asn65 within Gro- $\beta$  T) is prone to deamidation. This process effectuates the conversion of the asparagine residue to aspartic acid. Without wishing to be bound by any theory, the chemical synthesis of Gro- $\beta$  and Gro- $\beta$  T may overcome this problem, for instance, by providing conditions that reduce the exposure of this asparagine residue to nucleophilic solvent. When prepared synthetically (i.e., chemically synthesized), for instance, using, e.g., the solid phase peptide synthesis techniques described above, synthetic Gro- $\beta$ , Gro- $\beta$  T, and variants thereof that may be used in conjunction with the compositions and methods described herein may have a purity of, e.g., at least about 95% relative to the deamidated versions of these peptides (i.e., contain less



than 5% of the corresponding deamidated peptide). For instance, synthetic Gro- $\beta$ , Gro- $\beta$  T, and variants thereof that may be used in conjunction with the compositions and methods described herein may have a purity of about 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, 99.99%, or more, relative to the deamidated versions of these peptides (e.g., the Asn69 deamidated version of SEQ ID NO: 1 or the Asn65 deamidated version of SEQ ID NO: 2). For instance, s\Synthetic Gro- $\beta$ , Gro- $\beta$  T, and variants thereof may have, for instance, a purity of from about 95% to about 99.99%, such as a purity of from about 95% to about 99.99%, about 96% to about 99.99%, about 97% to about 99.99%, about 98% to about 99.99%, about 99% to about 99.99%, about 99.9% to about 99.99%, about 95% to about 99.5%, about 96% to about 99.5%, about 95% to about 99%, or about 97% to about 99% relative to the deamidated versions of these peptides (e.g., the Asn69 deamidated version of SEQ ID NO: 1 or the Asn65 deamidated version of SEQ ID NO: 2).

#### **Cell Population with Expanded Hematopoietic Stem Cells as Obtained by the Expansion Method and Therapeutic Compositions**

In another aspect, the disclosure features a composition comprising a population of hematopoietic stem cells, wherein the hematopoietic stem cells or progenitors thereof have been contacted with the compound of any one of the above aspects or embodiments, thereby expanding the hematopoietic stem cells or progenitors thereof.

The invention further provides a cell population with expanded hemapoetic stem cells obtainable or obtained by the expansion method described above. In one embodiment, such cell population is resuspended in a pharmaceutically acceptable medium suitable for administration to a mammalian host, thereby providing a therapeutic composition.

The compound as defined in the present disclosure enables the expansion of HSCs, for example from only one or two cord blood units, to provide a cell population quantitatively and qualitatively appropriate for efficient short and long term engraftment in a human patient in need thereof. In one embodiment, the present disclosure relates to a therapeutic composition comprising a cell population with expanded HSCs derived from not more than one or two cord blood units. In one embodiment, the present disclosure relates to a therapeutic composition containing a total amount of cells of at least about  $10^5$ , at least about  $10^6$ , at least about  $10^7$ , at least about  $10^8$  or at least about  $10^9$  cells with about 20% to about 100%, for example between about 43% to about 80%, of total cells being CD34+ cells. In certain embodiments, said composition contains between 20-100%, for example between 43-80%, of total cells being CD34+CD90+CD45RA-.

In some embodiments, the hematopoietic stem cells are CD34+ hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD90+ hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD45RA- hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD34+CD90+ hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD34+CD45RA- hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD90+CD45RA- hematopoietic stem cells. In some embodiments, the hematopoietic stem cells are CD34+CD90+CD45RA- hematopoietic stem cells.

In some embodiments, the hematopoietic stem cells of the therapeutic composition are mammalian cells, such as human cells. In some embodiments, the human cells are CD34+ cells, such as CD34+ cells are CD34+, CD34+CD38-, CD34+CD38-CD90+, CD34+CD38-CD90+CD45RA-, CD34+CD38-CD90+CD45RA-CD49F+, or CD34+CD90+CD45RA- cells.

In some embodiments, the hematopoietic stem cells of the therapeutic composition are obtained from human cord blood, mobilized human peripheral blood, or human bone marrow. The hematopoietic stem cells may, for example, be freshly isolated from the human or may have been previously cryopreserved.

## Methods of Treatment

As described herein, hematopoietic stem cell transplant therapy can be administered to a subject in need of treatment so as to populate or repopulate one or more blood cell types, such as a blood cell lineage that is deficient or defective in a patient suffering from a stem cell disorder. Hematopoietic stem and progenitor cells exhibit multi-potency, and can thus differentiate into multiple different blood lineages including, but not limited to, granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), dendritic cells, microglia, osteoclasts, and lymphocytes (e.g., NK cells, B-cells and T-cells). Hematopoietic stem cells are additionally capable of self-renewal, and can thus give rise to daughter cells that have equivalent potential as the mother cell, and also feature the capacity to be reintroduced into a transplant recipient whereupon they home to the hematopoietic stem cell niche and re-establish productive and sustained hematopoiesis. Thus, hematopoietic stem and progenitor cells represent a useful therapeutic modality for the treatment of a wide array of disorders in which a patient has a deficiency or defect in a cell type of the hematopoietic lineage. The deficiency or defect may be caused, for example, by depletion of a population of endogenous cells of the hematopoietic system due to administration of a chemotherapeutic agent (e.g., in the case of a patient suffering from a cancer, such as a hematologic cancer described herein). The deficiency or defect may be caused, for example, by depletion of a population of endogenous hematopoietic cells due to the activity of self-reactive immune cells, such as T lymphocytes or B lymphocytes that cross-react with self antigens (e.g., in the case of a patient suffering from an autoimmune disorder, such as an autoimmune disorder described herein). Additionally or alternatively, the deficiency or defect in cellular activity may be caused by aberrant expression of an enzyme (e.g., in the case of a patient suffering from various metabolic disorders, such as a metabolic disorder described herein).

Thus, hematopoietic stem cells can be administered to a patient defective or deficient in one or more cell types of the hematopoietic lineage in order to re-constitute the defective or deficient population of cells in vivo, thereby treating the pathology associated with the defect or depletion in the endogenous blood cell population. Hematopoietic stem and progenitor cells can be used to treat, e.g., a non-malignant hemoglobinopathy (e.g., a hemoglobinopathy selected from the group consisting of sickle cell anemia, thalassemia, Fanconi anemia, aplastic anemia, and Wiskott-Aldrich syndrome). In these cases,

for example, a CXCR4 antagonist and/or a CXCR2 agonist may be administered to a donor, such as a donor identified as likely to exhibit release of a population of hematopoietic stem and progenitor cells from a stem cell niche, such as the bone marrow, into circulating peripheral blood in response to such treatment. The hematopoietic stem and progenitor cells thus mobilized may then be withdrawn from the donor and administered to a patient, where the cells may home to a hematopoietic stem cell niche and re-constitute a population of cells that are damaged or deficient in the patient.

Hematopoietic stem or progenitor cells mobilized to the peripheral blood of a subject may be withdrawn (e.g., harvested or collected) from the subject by any suitable technique. For example, the hematopoietic stem or progenitor cells may be withdrawn by a blood draw. In some embodiments, hematopoietic stem or progenitor cells mobilized to a subject's peripheral blood as contemplated herein may be harvested (i.e., collected) using apheresis. In some embodiments, apheresis may be used to enrich a donor's blood with mobilized hematopoietic stem or progenitor cells.

A dose of the expanded hematopoietic stem cell composition of the disclosure is deemed to have achieved a therapeutic benefit if it alleviates a sign or a symptom of the disease. The sign or symptom of the disease may comprise one or more biomarkers associated with the disease, or one or more clinical symptoms of the disease.

For example, administration of the expanded hematopoietic stem cell composition may result in the reduction of a biomarker that is elevated in individuals suffering from the disease, or elevate the level of a biomarker that is reduced in individuals suffering from the disease.

For example, administering the expanded hematopoietic stem cell composition of the disclosure may elevate the level of an enzyme that is reduced in an individual suffering from a metabolic disorder. This change in biomarker level may be partial, or the level of the biomarker may return to levels normally seen in healthy individuals.

In one embodiment, when the disease is, for example, an inherited metabolic disorder with a neurological component, the expanded hematopoietic stem cell composition may partly or fully reduce one or more clinical symptoms of the inherited metabolic disorder. Exemplary but non-limiting symptoms that may be affected by administration of the expanded hematopoietic stem cell composition of the disclosure comprise ataxias, dystonia, movement, disorders, epilepsies, and peripheral neuropathy.

In some cases, the sign or symptom of the inherited metabolic disorder with a neurological component comprises psychological signs or symptoms. For example, the sign or symptom of the disorder may comprise acute psychotic disorder, hallucinations, depressive syndrome, other symptoms or combinations of symptoms. Methods of evaluating psychological signs or symptoms associated with metabolic disorders with a neurological component will be known to one of ordinary skill in the art.

The onset of the inherited metabolic disorder may be adult or pediatric.

The inherited metabolic disorder may lead to degeneration of the nervous system.

Alleviating a sign or a symptom of the disorder may comprise slowing the rate of neurodegeneration or the rate of the progression of the disease.

Alleviating a sign or a symptom of the disorder may comprise reversing neurodegeneration or reversing the progression of the disease. Exemplary symptoms of neurodegeneration comprise memory

loss, apathy, anxiety, agitation, loss of inhibition and mood changes. Methods of evaluating neurodegeneration, and the progression thereof, will be known to one of ordinary skill in the art.

For example, in a patient suffering from Hurler syndrome, heparan and dermatan sulfate accumulation follows from  $\alpha$ -L-iduronidase deficiency. Treatments that better clear these accumulated  
5 substrates will better correct the underlying disorder.

Additionally or alternatively, hematopoietic stem and progenitor cells can be used to treat an immunodeficiency, such as a congenital immunodeficiency. Additionally or alternatively, the compositions and methods described herein can be used to treat an acquired immunodeficiency (e.g., an acquired immunodeficiency selected from the group consisting of HIV and AIDS). In these cases, for example, a  
10 CXCR4 antagonist and/or a CXCR2 agonist may be administered to a donor, such as a donor identified as likely to exhibit release of a population of hematopoietic stem and progenitor cells from a stem cell niche, such as the bone marrow, into circulating peripheral blood in response to such treatment. The hematopoietic stem and progenitor cells thus mobilized may then be withdrawn from the donor and administered to a patient, where the cells may home to a hematopoietic stem cell niche and re-constitute  
15 a population of immune cells (e.g., T lymphocytes, B lymphocytes, NK cells, or other immune cells) that are damaged or deficient in the patient.

Hematopoietic stem and progenitor cells can also be used to treat a metabolic disorder (e.g., a metabolic disorder selected from the group consisting of glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurler syndrome or Hurler's Disease, sphingolipidoses, Sly  
20 Syndrome, alpha-Mannosidosis, X-ALD, Aspartylglucosaminuria, Wolman Disease, late infantile metachromatic leukodystrophy, Niemann Pick Type C disease, Niemann Pick Type B disease, Juvenile Tay Sachs, Infantile Tay Sachs, Juvenile Sandhoff, Infantile Sandhoff, GM1 gangliosidosis, MPSIV (Morquio), Presymptomatic or milder forms of globoid cell leukodystrophy, infantile Krabbe when newborn and asymptomatic, early diagnosis fucosidosis, Fabry, MPSIS, MPSIH/S, MPSII, MPSVI in conjunction  
25 with ERT or where alloantibodies attenuate efficacy of ERT, Pompe where alloantibodies attenuate efficacy of ERT, Mucopolipidosis II, and metachromatic leukodystrophy). In these cases, for example, a CXCR4 antagonist and/or a CXCR2 agonist may be administered to a donor, such as a donor identified as likely to exhibit release of a population of hematopoietic stem and progenitor cells from a stem cell niche, such as the bone marrow, into circulating peripheral blood in response to such treatment. The  
30 hematopoietic stem and progenitor cells thus mobilized may then be withdrawn from the donor and administered to a patient, where the cells may home to a hematopoietic stem cell niche and re-constitute a population of hematopoietic cells that are damaged or deficient in the patient.

Additionally or alternatively, hematopoietic stem or progenitor cells can be used to treat a malignancy or proliferative disorder, such as a hematologic cancer or myeloproliferative disease. In the  
35 case of cancer treatment, for example, a CXCR4 antagonist and/or a CXCR2 agonist may be administered to a donor, such as a donor identified as likely to exhibit release of a population of hematopoietic stem and progenitor cells from a stem cell niche, such as the bone marrow, into circulating peripheral blood in response to such treatment. The hematopoietic stem and progenitor cells thus mobilized may then be withdrawn from the donor and administered to a patient, where the cells may

home to a hematopoietic stem cell niche and re-constitute a population of cells that are damaged or deficient in the patient, such as a population of hematopoietic cells that is damaged or deficient due to the administration of one or more chemotherapeutic agents to the patient. In some embodiments, hematopoietic stem or progenitor cells may be infused into a patient in order to repopulate a population of cells depleted during cancer cell eradication, such as during systemic chemotherapy. Exemplary hematological cancers that can be treated by way of administration of hematopoietic stem and progenitor cells in accordance with the compositions and methods described herein are acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, and non-Hodgkin's lymphoma, as well as other cancerous conditions, including neuroblastoma.

Additional diseases that can be treated by the administration of hematopoietic stem and progenitor cells to a patient include, without limitation, adenosine deaminase deficiency and severe combined immunodeficiency, hyper immunoglobulin M syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, systemic sclerosis, systemic lupus erythematosus, multiple sclerosis, and juvenile rheumatoid arthritis.

In addition, administration of hematopoietic stem and progenitor cells can be used to treat autoimmune disorders. In some embodiments, upon infusion into a patient, transplanted hematopoietic stem and progenitor cells may home to a stem cell niche, such as the bone marrow, and establish productive hematopoiesis. This, in turn, can re-constitute a population of cells depleted during autoimmune cell eradication, which may occur due to the activity of self-reactive lymphocytes (e.g., self-reactive T lymphocytes and/or self-reactive B lymphocytes). Autoimmune diseases that can be treated by way of administering hematopoietic stem and progenitor cells to a patient include, without limitation, psoriasis, psoriatic arthritis, Type 1 diabetes mellitus (Type 1 diabetes), rheumatoid arthritis (RA), human systemic lupus (SLE), multiple sclerosis (MS), inflammatory bowel disease (IBD), lymphocytic colitis, acute disseminated encephalomyelitis (ADEM), Addison's disease, alopecia universalis, ankylosing spondylitis, antiphospholipid antibody syndrome (APS), aplastic anemia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease (AIED), autoimmune lymphoproliferative syndrome (ALPS), autoimmune oophoritis, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Chagas' disease, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Crohn's disease, cicatricial pemphigoid, coeliac sprue-dermatitis herpetiformis, cold agglutinin disease, CREST syndrome, Degos disease, discoid lupus, dysautonomia, endometriosis, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Goodpasture's syndrome, Grave's disease, Guillain-Barre syndrome (GBS), Hashimoto's thyroiditis, Hidradenitis suppurativa, idiopathic and/or acute thrombocytopenic purpura, idiopathic pulmonary fibrosis, IgA neuropathy, interstitial cystitis, juvenile arthritis, Kawasaki's disease, lichen planus, Lyme disease, Meniere disease, mixed connective tissue disease (MCTD), myasthenia gravis, neuromyotonia, opsoclonus myoclonus syndrome (OMS), optic neuritis, Ord's thyroiditis, pemphigus vulgaris, pernicious anemia, polychondritis, polymyositis and dermatomyositis, primary biliary cirrhosis, polyarteritis nodosa, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, Raynaud phenomenon, Reiter's

syndrome, rheumatic fever, sarcoidosis, scleroderma, Sjögren's syndrome, stiff person syndrome, Takayasu's arteritis, temporal arteritis (also known as "giant cell arteritis"), ulcerative colitis, collagenous colitis, uveitis, vasculitis, vitiligo, vulvodynia ("vulvar vestibulitis"), and Wegener's granulomatosis.

Hematopoietic stem cell transplant therapy may additionally be used to treat neurological disorders, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, Amyotrophic lateral sclerosis, Huntington's disease, mild cognitive impairment, amyloidosis, AIDS-related dementia, encephalitis, stroke, head trauma, epilepsy, mood disorders, and dementia. As described herein, upon transplantation into a patient, hematopoietic stem cells may migrate to the central nervous system and differentiate into, for example, microglial cells, thereby re-constituting a population of cells that may be damaged or deficient in a patient suffering from a neurological disorder. In these cases, for example, a population of hematopoietic stem cells may be administered to a patient suffering from a neurological disorder, where the cells may home to the central nervous system, such as the brain of the patient, and re-constitute a population of hematopoietic cells (e.g., microglial cells) that are damaged or deficient in the patient.

#### **Methods of Treating Inherited Metabolic Disorders - Administration of Expanded CD90+ Stem Cells for Microglial Engraftment in the Brain**

As described herein, hematopoietic stem cell transplant therapy can be administered to a subject in need of treatment so as to populate or repopulate one or more blood cell types, such as a blood cell lineage that is deficient or defective in a patient suffering from a stem cell disorder. Hematopoietic stem and progenitor cells exhibit multi-potency, and can thus differentiate into multiple different blood lineages including, in one embodiment, microglia.

In one embodiment, hematopoietic stem cell transplant therapy or hematopoietic stem cell transplantation of inherited metabolic disorders may be accomplished using cross-correction. (Wynn, R. "Stem Cell Transplantation in Inherited Metabolic Disorders" *Hematology* **2011**, pp. 285-291.) Cross correction involves engraftment of expanded HSCs in the patient or host tissue, where the implanted cells secrete the deficient enzyme and said deficient enzyme is then taken up by cells in the patient which are deficient in that enzyme.

In one embodiment, the inherited metabolic disorder to be treated is selected from Hurler syndrome (Hurler's Disease), mucopolysaccharide disorders (e.g., Maroteaux Lamy syndrome), lysosomal storage disorders, and peroxisomal disorders (e.g., X-linked adrenoleukodystrophy), glycogen storage diseases, mucopolysaccharidoses, Mucopolipidosis II, Gaucher's Disease, sphingolipidoses, and metachromatic leukodystrophy.

In certain embodiments, HSCs in the patient or in a healthy donor are mobilized using a CXCR2 agonist and/or CXCR4 antagonist of the disclosure. The CXCR4 antagonist may be plerixafor or a variant thereof, and a CXCR2 agonist may be Gro- $\beta$  or a variant thereof, such as a truncation of Gro- $\beta$ , for instance, Gro- $\beta$  T. Mobilized HSCs are then isolated from a peripheral blood sample of the subject. Methods of isolating HSCs will be readily apparent to one of ordinary skill in the art. If the HSCs were isolated from the subject with the inherited metabolic disorder, the HSCs can then be genetically modified

to correct the genetic defect leading to the disorder, expanded using the methods of the disclosure, and the corrected, expanded cells then transplanted back into the patient (an autologous transplantation). Optionally, HSCs may be expanded prior to genetic modification. Alternatively, HSCs may be mobilized using a CXCR2 agonist and/or CXCR4 antagonist of the disclosure in a healthy individual who (1) does not suffer from an inherited metabolic disorder and (2) is a compatible donor for the subject who does suffer from the inherited metabolic disorder. HSCs can be isolated from a blood sample taken from this healthy individual collected following mobilization, the HSCs can then be expanded using the expansion methods of the disclosure, and the expanded cells transplanted into the subject with the inherited metabolic disorder.

It has been found that HSCs prepared with the methods of the disclosure lead to more microglia engraftment than fresh cells or cells cultured in the presence of cytokines. This is due to the presence of more CD90+ cells in expanded cell populations.

The methods disclosed herein for treating inherited metabolic disorders in a subject in need thereof comprise the administration of an expanded population of hematopoietic stem cells to a subject in need thereof. In one embodiment, the number of expanded hematopoietic stem cells administered to the subject is equal to or greater than the amount of hematopoietic stem cells needed to achieve a therapeutic benefit. In one embodiment, the number of expanded hematopoietic stem cells administered to the subject is greater than the amount of hematopoietic stem cells needed to achieve a therapeutic benefit. In one embodiment, the therapeutic benefit achieved is proportional to the number of expanded hematopoietic stem cells that are administered.

A dose of the expanded hematopoietic stem cell composition of the disclosure is deemed to have achieved a therapeutic benefit if it alleviates a sign or a symptom of the disease. The sign or symptom of the disease may comprise one or more biomarkers associated with the disease, or one or more clinical symptoms of the disease.

For example, administration of the expanded hematopoietic stem cell composition may result in the reduction of a biomarker that is elevated in individuals suffering from the disease, or elevate the level of a biomarker that is reduced in individuals suffering from the disease.

For example, administering the expanded hematopoietic stem cell composition of the disclosure may elevate the level of an enzyme that is reduced in an individual suffering from a metabolic disorder. This change in biomarker level may be partial, or the level of the biomarker may return to levels normally seen in healthy individuals.

In one embodiment, when the disease is, for example, an inherited metabolic disorder with a neurological component, the expanded hematopoietic stem cell composition may partly or fully reduce one or more clinical symptoms of the inherited metabolic disorder. Exemplary but non-limiting symptoms that may be affected by administration of the expanded hematopoietic stem cell composition of the disclosure comprise ataxias, dystonia, movement disorders, epilepsies, and peripheral neuropathy.

In some cases, the sign or symptom of the inherited metabolic disorder with a neurological component comprises psychological signs or symptoms. For example, the sign or symptom of the disorder may comprise acute psychotic disorder, hallucinations, depressive syndrome, other symptoms or

combinations of symptoms. Methods of evaluating psychological signs or symptoms associated with metabolic disorders with a neurological component will be known to one of ordinary skill in the art.

The onset of the inherited metabolic disorder may be adult or pediatric.

The inherited metabolic disorder may lead to degeneration of the nervous system.

5 Alleviating a sign or a symptom of the disorder may comprise slowing the rate of neurodegeneration or the rate of the progression of the disease.

Alleviating a sign or a symptom of the disorder may comprise reversing neurodegeneration or reversing the progression of the disease. Exemplary symptoms of neurodegeneration comprise memory loss, apathy, anxiety, agitation, loss of inhibition and mood changes. Methods of evaluating  
10 neurodegeneration, and the progression thereof, will be known to one of ordinary skill in the art.

For example, in a patient suffering from Hurler syndrome, heparan and dermatan sulfate accumulation follows from  $\alpha$ -L-iduronidase deficiency. Treatments that better clear these accumulated substrates will better correct the underlying disorder.

#### 15 *Selection of donors and patients*

In some embodiments, the patient is the donor. In such cases, withdrawn hematopoietic stem or progenitor cells may be re-infused into the patient, such that the cells may subsequently home hematopoietic tissue and establish productive hematopoiesis, thereby populating or repopulating a line of cells that is defective or deficient in the patient (e.g., a population of megakaryocytes, thrombocytes,  
20 platelets, erythrocytes, mast cells, myeloblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T-lymphocytes, and B-lymphocytes). In this scenario, the transplanted hematopoietic stem or progenitor cells are least likely to undergo graft rejection, as the infused cells are derived from the patient and express the same HLA class I and class II antigens as expressed by the patient.

25 Alternatively, the patient and the donor may be distinct. In some embodiments, the patient and the donor are related, and may, for example, be HLA-matched. As described herein, HLA-matched donor-recipient pairs have a decreased risk of graft rejection, as endogenous T cells and NK cells within the transplant recipient are less likely to recognize the incoming hematopoietic stem or progenitor cell graft as foreign, and are thus less likely to mount an immune response against the transplant. Exemplary  
30 HLA-matched donor-recipient pairs are donors and recipients that are genetically related, such as familial donor-recipient pairs (e.g., sibling donor-recipient pairs).

In some embodiments, the patient and the donor are HLA-mismatched, which occurs when at least one HLA antigen, in particular with respect to HLA-A, HLA-B and HLA-DR, is mismatched between the donor and recipient. To reduce the likelihood of graft rejection, for example, one haplotype may be  
35 matched between the donor and recipient, and the other may be mismatched.

#### **Administration and Dosing of Hematopoietic Stem or Progenitor Cells**

Hematopoietic stem and progenitor cells described herein may be administered to a subject, such as a mammalian subject (e.g., a human subject) suffering from a disease, condition, or disorder described



herein, by one or more routes of administration. For instance, hematopoietic stem cells described herein may be administered to a subject by intravenous infusion. Hematopoietic stem cells may be administered at any suitable dosage. Non-limiting examples of dosages include about  $1 \times 10^5$  CD34+ cells/kg of recipient to about  $1 \times 10^7$  CD34+ cells/kg (e.g., from about  $2 \times 10^5$  CD34+ cells/kg to about  $9 \times 10^6$  CD34+ cells/kg, from about  $3 \times 10^5$  CD34+ cells/kg to about  $8 \times 10^6$  CD34+ cells/kg, from about  $4 \times 10^5$  CD34+ cells/kg to about  $7 \times 10^6$  CD34+ cells/kg, from about  $5 \times 10^5$  CD34+ cells/kg to about  $6 \times 10^6$  CD34+ cells/kg, from about  $5 \times 10^5$  CD34+ cells/kg to about  $1 \times 10^7$  CD34+ cells/kg, from about  $6 \times 10^5$  CD34+ cells/kg to about  $1 \times 10^7$  CD34+ cells/kg, from about  $7 \times 10^5$  CD34+ cells/kg to about  $1 \times 10^7$  CD34+ cells/kg, from about  $8 \times 10^5$  CD34+ cells/kg to about  $1 \times 10^7$  CD34+ cells/kg, from about  $9 \times 10^5$  CD34+ cells/kg to about  $1 \times 10^7$  CD34+ cells/kg, or from about  $1 \times 10^6$  CD34+ cells/kg to about  $1 \times 10^7$  CD34+ cells/kg, among others).

Hematopoietic stem or progenitor cells and pharmaceutical compositions described herein may be administered to a subject in one or more doses. When multiple doses are administered, subsequent doses may be provided one or more days, weeks, months, or years following the initial dose.

15

### Examples

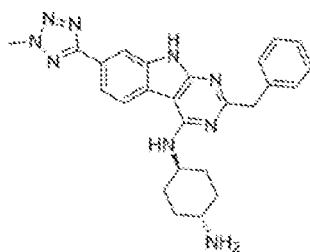
The following examples are put forth so as to provide those of ordinary skill in the art with a description of how the compositions and methods described herein may be used, made, and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention.

20

#### **Example 1. Expansion of gene-modified hematopoietic stem or progenitor cells by treatment with an aryl hydrocarbon receptor antagonist**

*Methods.* A series of aryl hydrocarbon receptor antagonists, including SR1, along with histone deacetylase (HDAC) inhibitors, and UM171 [Formula (VI)] were evaluated in the presence of cytokines to expand primary human CD34+ cells *ex vivo*. Cell number and immunophenotype were assessed by flow cytometry, and HSC function was evaluated by cell and molecular assays *in vitro*. The expanded cells were transplanted into sub-lethally irradiated NSG mice to evaluate engraftment potential *in vivo*. For editing studies, mPB and BM CD34+ cells were electroporated with CRISPR/Cas9 RNPs targeting the beta-2 microglobulin (B2M) cell surface protein. Editing rates were evaluated by flow cytometry based on loss of protein expression and TIDE analysis. Edited cells were expanded in the presence of AHR antagonist or vehicle and transplanted into NSG mice. The AHR antagonist used in the experiments described in this Example is Compound 26 herein. Engraftment and editing rates were evaluated by flow cytometry of the peripheral blood and bone marrow.

30



Formula (VI), UM171

*Results.* Based on the results of these experiments, cultures expanded with an AHR antagonist showed the largest improvement in NSG engraftment levels compared to unmanipulated cells. Culture of CD34+ cells with SR1 or another AHR antagonist, Compound 26, led to a 6-fold increase in CD34+ number and a significant increase in engraftment in NSG mice relative to vehicle-cultured CB derived CD34+ cells. The aryl hydrocarbon receptor antagonist, A, displayed complete AHR antagonism in the dioxin response element luciferase reporter assay and was a more potent antagonist compared to SR1 (a 12-fold increase in potency). To evaluate the ability of the AHR antagonist compound to effectively expand gene edited cells, mPB and BM derived CD34+ cells were treated with either vehicle or AHR antagonist and the following day edited with CRISPR/Cas9 RNPs targeting B2M. Following 7 days of expansion, the vehicle or AHR antagonist-treated cells showed 87% and 84% loss of the target protein, respectively. The expanded culture contained 3.4-fold more CD34+CD90+ cells than the vehicle-treated cells. Upon transplant, mice receiving the expanded cells showed greater than 2-fold increase in engraftment compared to those receiving vehicle-treated cells. Importantly, the editing rates of the expanded cells are maintained *in vivo* with an average of >75% of the human cells in the periphery of the mice showing loss of target protein.

*Conclusions.* These studies demonstrate that AHR antagonism is an effective strategy to expand functional HSCs and that small molecules inhibiting AHR can expand gene modified HSC from mPB and BM.

## **Example 2. Expansion and transplantation of hematopoietic stem or progenitor cells modified by CRISPR/Cas9-mediated gene silencing and lentivirus-mediated gene expression**

Achieving a high dosage of hematopoietic stem cells, such as genetically modified hematopoietic stem cells, is important for successful gene therapy. Ex vivo expansion of hematopoietic stem cells represents a method by which elevated quantities of cells may be obtained for therapeutic applications. A clinical trial in which patients received cord blood (CB)-derived hematopoietic stem cells that had been expanded ex vivo by culturing the cells in the presence of an AHR antagonist demonstrated an improvement in time to engraftment, as shown in FIG. 1. This example demonstrates the ability of AHR antagonists to expand genetically modified hematopoietic stem cells ex vivo, and to promote the engraftment and retention of genetic modification of such cells in vivo.

To investigate these activities, a series of experiments was conducted in which hematopoietic stem and progenitor cells were genetically altered, either by lentiviral transduction or CRISPR/Cas9-mediated gene editing, followed by expansion ex vivo by treatment with an AHR antagonist. The AHR

antagonist used in the experiments described in this Example is Compound 26 herein. The cells were then infused into NSG mice, and engraftment rates and retention of genetic modifications were assessed. The sections that follow explain the methods utilized for these studies and detail various findings from these experiments.

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#### *Methods*

Lentiviral Transduction: Cryopreserved CD34+ cells from mobilized peripheral blood (mPB) were thawed and cultured overnight in media with cytokines and either vehicle (DMSO) or the AHR antagonist (expanded). The following day, cells were plated on retronectin-coated plates and transduced with a  
10 lentiviral vector, containing a green fluorescent protein (GFP) transgene under the control of the MND promoter, at an MOI of 50 in the presence of vehicle or the AHR antagonist. 24 hours later, cells were harvested, washed, and resuspended in media with cytokines and the appropriate compound.

CRISPR/Cas9 Editing and Expansion: Cryopreserved CD34+ cells from mPB and bone marrow (BM) were thawed and cultured overnight in media with cytokines and either vehicle or the AHR  
15 antagonist. Cells were electroporated with Cas9 protein (Aldevron) and synthetic, chemically-modified gRNA (Synthego) targeting beta-2-microglobulin (B2M) as a ribonucleotide protein (RNP).

Transplant into NSG mice: All of the progeny from cultured cells were transplanted into sub-lethally irradiated, female, 6-8 week old NSG mice. Engraftment and transduction/editing rates were monitored monthly by flow cytometry. "Minimally manipulated" controls were transplanted one day after  
20 editing for a total culture time of 2 days.

#### *Results*

Following mobilization and cryopreservation of CD34+ cells from peripheral blood, the cells were transduced with a lentiviral GFP-MND vector or subjected to CRISPR/Cas9-mediated silencing of the  
25 B2M cell surface protein. The cells were then cultured in the presence of an AHR antagonist for 7 days, at which point the cells were quantified and retention of genetic modification was assessed. As shown in FIGS. 2A-2D, treatment with the AHR antagonist resulted in an increase in the total quantity of cells relative to vehicle-treated cells. Further, treatment with the AHR antagonist promoted a substantial increase in the quantity of CD34+ CD90+ cells relative to vehicle-treated cells and untreated cells.  
30 Expansion with the AHR antagonist also increased the quantity of GFP+ CD34+ CD90+ cells to a greater extent than vehicle treatment or lack of treatment altogether. Turning to cells that were subject to lentiviral transduction, treatment with the AHR antagonist resulted in substantially higher quantities of total cells, CD34+ cells, and CD34+ CD90+ cells relative to vehicle-treated cells and untreated cells, as shown in FIGS. 3A – 3E. Turning to cells that were subject to CRISPR/Cas9-mediated B2M editing,  
35 treatment with the AHR antagonist resulted in substantially higher quantities of total cells, CD34+ cells, and CD34+ CD90+ cells relative to vehicle-treated cells and untreated cells, as shown in FIGS. 5A-5E. These figures also demonstrate that transduced/edited, expanded mPB cells provide higher engraftment rates than vehicle-cultured cells.

Following genetic modification and expansion, CD34+ cells were transplanted into NSG mice, and engraftment of the cells was assessed monthly post-transplant. As shown in FIGS. 4A-4C and FIGS. 6A – 6I, cells treated with the AHR antagonist for ex vivo expansion prior to transplantation generally exhibited higher engraftment rates relative to vehicle-treated cells. Further, AHR antagonist-treated cells exhibited greater retention of B2M- phenotype relative to vehicle-treated cells following transplantation. Expanded, edited mPB and BM cells additionally were found to exhibit higher engraftment rates than minimally-manipulated cells, as shown in FIGS. 7A-7N.

### Conclusions

Taken together, the data gathered from these experiments demonstrate that expansion of mPB and BM hematopoietic stem cells with an AHR antagonist results in increased engraftment compared to minimally manipulated and vehicle controls following transplantation into NSG mice. Editing rates of 80% were achieved in both mPB and BM CD34+ cells and maintained following transplantation into NSG mice. Additionally, transplantation of edited, expanded cells resulted in a greater than 2-fold improvement in engraftment compared to vehicle-cultured cells in both the PB and BM of mice after 16 weeks. Thus, expansion of gene-modified cells by treatment with an AHR antagonist simultaneously allows for increased engraftment rates while maintaining high gene editing and transduction efficiencies.

### Example 3. Treatment of a hematologic disorder by administration of a hematopoietic stem or progenitor cell graft

Using the compositions and methods described herein, a stem cell disorder, such as a hematologic pathology described herein, can be treated by administering to a patient a hematopoietic stem or progenitor cell graft. For example, a population of hematopoietic stem or progenitor cells can be isolated from a donor. Following the isolation process, a patient may then receive an infusion (e.g., an intravenous infusion) of the mobilized and isolated hematopoietic stem or progenitor cells. The patient may be the donor, or may be a patient that is HLA-matched with respect to the donor, thereby reducing the likelihood of graft rejection. The patient may be one that is suffering, for instance, from a cancer, such as a hematologic cancer described herein. Additionally or alternatively, the patient may be one that is suffering from an autoimmune disease or metabolic disorder described herein.

The engraftment of the hematopoietic stem cell transplant can be monitored, for example, by withdrawing a blood sample from the patient and determining the increase in concentration of hematopoietic stem cells or cells of the hematopoietic lineage (such as megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeloblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T-lymphocytes, and B-lymphocytes) following administration of the transplant. This analysis may be conducted, for example, from 1 hour to 6 months, or more, following hematopoietic stem cell transplant therapy (e.g., 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 3

weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 21 weeks, 22 weeks, 23 weeks, 24 weeks, or more). A finding that the concentration of hematopoietic stem cells or cells of the hematopoietic lineage has increased (e.g., by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, or more) following the transplant therapy relative to the concentration of the corresponding cell type prior to transplant therapy provides one indication that the hematopoietic stem or progenitor cell transplant therapy is efficacious in treating the stem cell disorder.

**Example 4. Engraftment of microglial cells in the brains of NSG mice following hematopoietic stem cell transplantation**

Approximately 1,000 allogeneic hematopoietic cell transplantations (HSCTs) have been performed over the last three decades for treatment of different inherited metabolic disorders to prevent symptom onset, suppress disease progression, and improve patient outcomes. The goal of HSCT in these diseases is to provide cells that produce functional enzymes otherwise deficient in patients with inherited metabolic disorders. Mechanistically, this is accomplished through repopulation of the myeloid compartment, including brain microglia, by donor derived cells. Microglia catabolize storage material in tissues; replacement of defective microglia by normal cells reestablishes an important scavenging function defective in patients with inherited metabolic disorders. Further, these normal cells secrete lysosomal enzymes, which can be taken up by neighboring cells, and thereby cross correct the metabolic disorder. Although HSCT effectively halts disease progression, central nervous system stabilization takes 6-12 months post-HSCT, perhaps reflecting the slow kinetics of microglia replacement by donor-derived cells.

We compared the ability of unmanipulated cord blood or cord blood expanded *ex vivo* using an aryl hydrocarbon receptor (AHR) antagonist to engraft the brain microglia compartment, MGTA-456. The design of the experiments described in this Example is shown in FIG. 8. The AHR antagonist used in the experiments described in this Example is Compound 2, represented by Formula (2), herein. AHR antagonism is an effective strategy to expand cord blood-derived CD34+ cells that reduces graft failure, accelerates neutrophil recovery and provides stable long-term engraftment (Wagner et al, Cell Stem Cell, 2016).

In this study, mice transplanted with MGTA-456 showed 2.8-fold higher human CD45 engraftment in the peripheral blood at week 13 compared to mice transplanted with non-expanded fresh cord blood or vehicle treated CD34+ cells (FIGS. 9A and 9B). As shown in FIG. 10, we observed an approximately 10-fold increase in human CD45+CD11b+ myeloid cells in the brains of transplanted NSG mice with MGTA-456 (n=15, p<0.0001). To confirm microglia engraftment in the brain, we also assessed the presence of Ku80+Iba1+ microglia in brain sections by morphological assessment and immunohistochemistry following transplantation, the results of which are shown, e.g., in FIG. 11.

These data demonstrate that *ex vivo* expanded human cord blood CD34+ cells, MGTA-456, significantly improves engraftment of human microglia in the brain of NSG mice. These findings

demonstrate that hematopoietic stem cells expanded ex vivo with an aryl hydrocarbon receptor antagonist, e.g., MGTA-456 expanded with Compound 2, are an effective method to accelerate recovery in patients with neurologic and inherited metabolic disorders.

## 5 Materials and Methods

### *Cord Blood Expansion and Transplantation*

Approximately 60,000 cord blood CD34+ cells were seeded in T25 flasks at a final volume of 12 mL in HSC growth media (SFEM supplemented with Pen/Strep, 50 ng/mL FLT3L, TPO, SCF, and IL-6). Flasks were incubated for 10 days at 37°C/5% CO<sub>2</sub>. Cells were cultured in the presence of 500 nM of  
10 AHR antagonist, where indicated. Cells were transferred to a larger flask when needed to maintain cells at a density less than 1x10<sup>6</sup> cells/mL throughout the culture period.

At the time of thaw, an equal number of cells to the starting cell cultures were injected into NSG mice, sublethally irradiated (200 cGy) 24 hours prior to injection. After 10 days of culture, the entire progeny of the cultures was injected into NSG mice. Peripheral blood was harvested by retro-orbital  
15 bleeding at approximately weeks 4 and 8 or by cardiac puncture at week 12 and chimerism was assessed by flow cytometry using antibodies against hCD45, mCD45, hCD33, hCD19, hCD3 and a viability dye.

### *Brain Harvesting and Processing*

At 3 months, brains were harvested. 1 hemisphere was fixed in formalin, embedded, and used for  
20 immunohistochemistry. The other hemisphere was crushed in Dounce buffer (15 mM HEPES/0.5% glucose in phenol red-free HBSS) and filtered through a 40 µM filter to create a single cell suspension and resuspended in 900 µL 0.5% BSA/PBS. Myelin was depleted from brain samples, per manufacturer's instructions, by incubating with 100 µL myelin removal beads (Miltenyi Biotec), incubating for 15 minutes at 4°C, washing with PBS, and resuspending in 1 mL MACS Buffer prior to deletion on an AutoMACs Pro.

25

### *Flow Cytometric Detection of Microglia*

Myelin-depleted samples were resuspended in 100 µL PBS and stained with antibodies against hCD45, mCD45, CD11b, CD19, CD3, and 7-AAD viability dye. Cells were washed once in PBS and resuspended in 300 µL final volume. The entire sample was acquired by flow cytometry (BD Celesta) to  
30 quantitate the number of microglia per brain hemisphere.

### *Immunohistochemical Detection of Microglia*

Embedded brains were sectioned at approximately 5 microns and stained with Ku80 (brown) and Iba-1 (red) primary antibodies. Mouse brains were analyzed from each transplanted mouse and five  
35 levels were analyzed each. Glass slides were scanned at 20X using an Aperio AT2 whole slide scanner. Image analysis was performed on the digital slide images using Visiopharm software.

## Example 5. Expansion of Gene Corrected, Engraftable Cells

### *Results*

FIG. 12A shows the proportion of CD34+CD90+ in mobilized peripheral blood cells in the G0 phase, G1 phase, of S-G2-M phase as a function of days in culture in the presence of cytokines, with or without the aryl hydrocarbon antagonist (AHR antagonist) Compound 26. The data demonstrate that substantially all CD34+CD90+ cells in mobilized peripheral blood exit the G0 phase and enter the replicative cell cycle after about 3 days in culture both in the presence or absence of an aryl hydrocarbon receptor antagonist.

FIG. 12B shows the proportion of CD34+CD90+ in cord blood cells in the G0 phase, G1 phase, of S-G2-M phase as a function of days in culture in the presence of cytokines, with or without the aryl hydrocarbon antagonist (AHR antagonist) Compound 26. The data demonstrate that substantially all CD34+CD90+ cells in cord blood exit the G0 phase and enter the replicative cell cycle after about 3 days in culture both in the presence or absence of an aryl hydrocarbon receptor antagonist.

FIG. 13A shows that higher rates of gene correction were obtained when mobilized peripheral blood cells were prestimulated (Pre-stim) (i.e., grown in culture) for 4 days (4-day) in the presence of Compound 26 prior to electroporation with gene editing reagents in comparison to mobilized peripheral blood cells prestimulated for 1 day (1-day) prior to electroporation with gene editing reagents. Comparing the data in FIG. 13A with the data in FIG. 12, these results suggest that higher rates of gene correction can be obtained with actively cycling cells.

FIG. 13B shows that similar rates of gene correction were obtained when umbilical cord blood cells were prestimulated for 4 days prior to electroporation with gene editing reagents in comparison to umbilical cord blood cells prestimulated for 1 day prior to electroporation with gene editing reagents. Comparing the data in FIGs. 13A and 13B, a higher rate of gene correction was observed after 1 day of prestimulation of umbilical cord blood cells as compared to 1 day of prestimulation of mobilized peripheral blood cells. These data suggest that as compared to mobilized peripheral blood cells, a higher proportion of umbilical cord blood cells are actively cycling after 1 day of prestimulation.

In FIGs. 14A and 14B, comparing the data for 2+2 (Pre-stim days + post EP culture days) to 4+4, a significant increase in the total number of gene-corrected cells was observed for both corrected mobilized peripheral blood cells and corrected umbilical cord blood cells.

#### *Materials and Methods*

Mobilized peripheral blood (mPB) CD34+ or cord blood (CB) CD34+ cells were thawed and pre-stimulated, i.e., cultured, in serum-free media (SFEM media supplemented with cytokines SCF, IL6, TPO, and FLT3L) in the presence or absence of Compound 26 (500 nM). Cells were pre-stimulated 1, 2, 3, or 4 days prior to electroporation with gRNA/Cas9 and oligonucleotide donor. Cells were cultured for an additional 8 days after electroporation and profiled at 2, 4, 6, or 8 days post-electroporation using a Trucount-based method of HSC quantification of CD34, CD90, and CD45RA. Cells were subcultured to maintain cell density at less than  $1 \times 10^6$  cells/mL. On day 8, genomic DNA was extracted from bulk cell cultures, and correction rate was assessed by qPCR. Number of corrected cells was determined by number of total cells at the indicated timepoint multiplied by the correction rate for that pre-stimulation condition.

### Other Embodiments

All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the invention that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are within the claims.



## CLAIMS

What is claimed is:

1. A method of producing an expanded population comprising genetically modified hematopoietic stem or progenitor cells ex vivo, the method comprising:
  - a. disrupting an endogenous gene in a plurality of hematopoietic stem or progenitor cells, thereby producing a population comprising genetically modified hematopoietic stem or progenitor cells; and
  - b. contacting the population comprising genetically modified hematopoietic stem or progenitor cells with an expanding amount of an aryl hydrocarbon receptor antagonist.
2. The method of claim 1, wherein prior to (a), the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist.
3. A method of expanding a population comprising genetically modified hematopoietic stem or progenitor cells ex vivo, wherein the cells have previously been genetically modified to disrupt an endogenous gene, the method comprising contacting a population of genetically modified hematopoietic stem or progenitor cells with an expanding amount of an aryl hydrocarbon receptor antagonist.
4. A method of producing a population comprising genetically modified hematopoietic stem or progenitor cells, wherein the cells have previously been expanded ex vivo by contacting the population with an expanding amount of an aryl hydrocarbon receptor antagonist, the method including disrupt an endogenous gene in the expanded population of hematopoietic stem or progenitor cells.
5. The method of any one of claims 1-4, wherein step (a) comprises contacting the hematopoietic stem or progenitor cells with a nuclease that catalyzes cleavage of an endogenous nucleic acid in the hematopoietic stem or progenitor cell.
6. The method of claim 5, wherein the nuclease is a CRISPR-associated protein.
7. The method of claim 6, wherein the nuclease is caspase 9.
8. The method of claim 5, wherein the nuclease is a transcription activator-like effector nuclease, a meganuclease, or a zinc finger nuclease.

9. The method of any one of claims 1-8, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, maintain disruption of the endogenous gene for at least 2 days.

10. The method of claim 9, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, maintain disruption of the endogenous gene for at least 3 days.

11. The method of claim 10, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, maintain disruption of the endogenous gene for at least 4 days.

12. The method of claim 11, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, maintain disruption of the endogenous gene for at least 5 days.

13. The method of claim 12, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, maintain disruption of the endogenous gene for at least 6 days.

14. The method of claim 13, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, maintain disruption of the endogenous gene for at least 7 days.

15. The method of claim 14, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, maintain disruption of the endogenous gene for at least 14 days.

16. The method of claim 15, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the

hematopoietic stem or progenitor cells, or progeny thereof, maintain disruption of the endogenous gene for at least 16 days.

17. A method of producing an expanded population comprising genetically modified hematopoietic stem or progenitor cells ex vivo, the method comprising:

- a. introducing a polynucleotide into a plurality of hematopoietic stem or progenitor cells, thereby producing a population comprising genetically modified hematopoietic stem or progenitor cells that express the polynucleotide; and
- b. contacting the population comprising genetically modified hematopoietic stem or progenitor cells with an expanding amount of an aryl hydrocarbon receptor antagonist.

18. The method of claim 17, wherein prior to (a), the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist.

19. A method of expanding a population comprising genetically modified hematopoietic stem or progenitor cells ex vivo, wherein the cells have previously been genetically modified by introducing a polynucleotide into the cells, the method comprising contacting a population comprising the genetically modified hematopoietic stem or progenitor cells with an expanding amount of an aryl hydrocarbon receptor antagonist.

20. A method of producing a population comprising genetically modified hematopoietic stem or progenitor cells, wherein the cells have previously been expanded ex vivo by contacting the population with an expanding amount of an aryl hydrocarbon receptor antagonist, the method comprising introducing a polynucleotide into the expanded population of hematopoietic stem or progenitor cells.

21. The method of any one of claims 1-4, wherein the introducing comprises contacting the hematopoietic stem or progenitor cells with a vector containing the polynucleotide.

22. The method of claim 21, wherein the vector is a viral vector.

23. The method of claim 22, wherein the viral vector is selected from the group consisting of adenovirus (Ad), retrovirus, poxvirus, adeno-associated virus, baculovirus, herpes simplex virus, and a vaccinia virus.

24. The method of claim 23, wherein the retrovirus is a lentivirus or a  $\gamma$ -retrovirus.
25. The method of claim 21, wherein the vector is a transposable element.
26. The method of claim 25, wherein the transposable element is a piggybac transposon or sleeping beauty transposon.
27. The method of any one of claims 17-26, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, continue to express the polynucleotide for at least 2 days.
28. The method of claim 27, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, continue to express the polynucleotide for at least 3 days.
29. The method of claim 28, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, continue to express the polynucleotide for at least 4 days.
30. The method of claim 29, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, continue to express the polynucleotide for at least 5 days.
31. The method of claim 30, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, continue to express the polynucleotide for at least 6 days.
32. The method of claim 31, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, continue to express the polynucleotide for at least 7 days.

33. The method of claim 32, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, continue to express the polynucleotide for at least 14 days.

34. The method of claim 33, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells to a patient, the hematopoietic stem or progenitor cells, or progeny thereof, continue to express the polynucleotide for at least 16 days.

35. The method of any one of claims 1-34, wherein the population comprising genetically modified hematopoietic stem or progenitor cells further comprises hematopoietic stem or progenitor cells that are not genetically modified.

36. The method of claim 35, wherein the genetically modified hematopoietic stem or progenitor cells are expanded at a rate that is proportional to the relative quantity of genetically modified hematopoietic stem or progenitor cells present in the population prior to treatment with the aryl hydrocarbon receptor antagonist.

37. The method of claim 35, wherein the hematopoietic stem or progenitor cells that are not genetically modified do not out-compete the genetically modified hematopoietic stem or progenitor cells for expansion by the aryl hydrocarbon receptor antagonist.

38. The method of claim 35, wherein the hematopoietic stem or progenitor cells that are genetically modified are expanded more rapidly than the hematopoietic stem or progenitor cells that are not genetically modified.

39. The method of any one of claims 1-38, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells into a patient, the ratio of the hematopoietic stem or progenitor cells, or progeny thereof, that are genetically modified to the total quantity of hematopoietic stem cells in a sample bone marrow or peripheral blood sample isolated from the patient is at least 75% of the ratio of genetically modified hematopoietic stem or progenitor cells to the total quantity of hematopoietic stem or progenitor cells present in the population at the time of administration of the cells to the patient.

40. The method of claim 39, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells into a patient, the ratio of the hematopoietic stem or progenitor cells, or progeny thereof, that are genetically modified to the total quantity of hematopoietic stem cells in a sample bone marrow or peripheral blood sample isolated from the patient is at least 80% of the ratio of genetically modified hematopoietic stem or progenitor cells to the total quantity of hematopoietic stem or progenitor cells present in the population at the time of administration of the cells to the patient.

41. The method of claim 40, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells into a patient, the ratio of the hematopoietic stem or progenitor cells, or progeny thereof, that are genetically modified to the total quantity of hematopoietic stem cells in a sample bone marrow or peripheral blood sample isolated from the patient is at least 85% of the ratio of genetically modified hematopoietic stem or progenitor cells to the total quantity of hematopoietic stem or progenitor cells present in the population at the time of administration of the cells to the patient.

42. The method of claim 41, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells into a patient, the ratio of the hematopoietic stem or progenitor cells, or progeny thereof, that are genetically modified to the total quantity of hematopoietic stem cells in a sample bone marrow or peripheral blood sample isolated from the patient is at least 90% of the ratio of genetically modified hematopoietic stem or progenitor cells to the total quantity of hematopoietic stem or progenitor cells present in the population at the time of administration of the cells to the patient.

43. The method of claim 42, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells into a patient, the ratio of the hematopoietic stem or progenitor cells, or progeny thereof, that are genetically modified to the total quantity of hematopoietic stem cells in a sample bone marrow or peripheral blood sample isolated from the patient is at least 95% of the ratio of genetically modified hematopoietic stem or progenitor cells to the total quantity of hematopoietic stem or progenitor cells present in the population at the time of administration of the cells to the patient.

44. The method of claim 43, wherein upon transplantation of the population comprising genetically modified hematopoietic stem or progenitor cells into a patient, the ratio of the hematopoietic stem or progenitor cells, or progeny thereof, that are genetically modified to the total quantity of hematopoietic stem cells in a sample bone marrow or peripheral blood sample isolated from the patient is the same as the ratio of genetically modified hematopoietic stem or progenitor cells to the total quantity of hematopoietic stem or progenitor cells present in the population at the time of administration of the cells to the patient.

45. The method of any one of claims 1-44, wherein the population comprising genetically modified hematopoietic stem or progenitor cells exhibits a higher engraftment potential relative to a population of hematopoietic stem or progenitor cells not treated with the aryl hydrocarbon receptor antagonist.

46. The method of any one of claims 1-45, wherein prior to expansion, the hematopoietic stem or progenitor cells are mobilized and isolated from a donor.

47. The method of claim 46, wherein the donor is a human.

48. The method of claim 46 or 47, wherein the hematopoietic stem or progenitor cells are mobilized by contacting the hematopoietic stem or progenitor cells with a mobilizing amount of a CXCR4 antagonist and/or a CXCR2 agonist.

49. The method of claim 48, wherein the CXCR4 antagonist is plerixafor or a pharmaceutically acceptable salt thereof.

50. The method of claim 48 or 49, wherein the CXCR2 agonist is Gro- $\beta$ , Gro- $\beta$  T, or a variant thereof.

51. The method of claim 50, wherein the Gro- $\beta$ , Gro- $\beta$  T, or variant thereof has a purity of at least about 95% relative to deamidated versions of these peptides.

52. The method of any one of claims 1-51, wherein prior to disrupting an endogenous gene in a plurality of hematopoietic stem or progenitor cells, thereby producing a population comprising genetically modified hematopoietic stem or progenitor cells, the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist of any one of the preceding claims during a period of time sufficient to induce cell cycling.

53. The method of any one of claims 1-51, wherein prior to introducing a polynucleotide into a plurality of hematopoietic stem or progenitor cells, thereby producing a population comprising genetically modified hematopoietic stem or progenitor cells that express the polynucleotide, the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist of any one of the preceding claims during a period of time sufficient to induce cell cycling.

54. The method of claim 52 wherein prior to disrupting an endogenous gene in a plurality of hematopoietic stem or progenitor cells, thereby producing a population comprising genetically modified hematopoietic stem or progenitor cells, the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist of any one of the preceding claims for at least 1 day, preferably at least 2 days, preferably at least 3 days, preferably at least 4 days, preferably at least 5 days.

55. The method of claim 53, wherein prior to introducing a polynucleotide into a plurality of hematopoietic stem or progenitor cells, thereby producing a population comprising genetically modified hematopoietic stem or progenitor cells that express the polynucleotide, the plurality of hematopoietic stem or progenitor cells is contacted with an aryl hydrocarbon receptor antagonist of any one of the preceding claims for at least 1 day, preferably at least 2 days, preferably at least 3 days, preferably at least 4 days, preferably at least 5 days.

56. The method of any one of claims 52-55, wherein the plurality of hematopoietic stem or progenitor cells are contacted with an agent in an amount sufficient to induce cell cycling during the period of time.

57. The method of claim 56, wherein the agent is one or more cytokines.

58. The method of claim 57, wherein the cytokines are selected from the group consisting of SCF, IL6, TPO, FLT3L, and combinations thereof.

59. The method of any one of claims 52-58, wherein the period of time is sufficient to induce cell cycling in substantially all of the plurality of hematopoietic stem or progenitor cells.

60. A human blood cell preparation comprising hematopoietic stem or progenitor cells, or progeny thereof, prepared according to the method of any one of claims 1-59.



61. A method of treating a disorder in a patient, the method comprising producing an expanded population of hematopoietic stem or progenitor cells in accordance with the method of any one of claims 1-59 and infusing the resulting cells into the patient.

62. A method of treating a disorder in a patient, the method comprising infusing an expanded population of hematopoietic stem or progenitor cells produced in accordance with the method of any one of claims 1-59 into the patient.

63. A method of treating a disorder in a patient, the method comprising infusing the human blood cell preparation of claim 60 into the patient.

64. A method of treating a disorder in a patient, the method comprising contacting a population of hematopoietic stem or progenitor cells with an expanding amount of an aryl hydrocarbon receptor antagonist and infusing the resulting cells into the patient.

65. A method of treating a disorder in a patient, the method comprising infusing into the patient an expanded population of hematopoietic stem or progenitor cells produced by contacting a population of hematopoietic stem or progenitor cells with an expanding amount of an aryl hydrocarbon receptor antagonist.

66. A method of treating a disorder in a patient in need thereof, comprising administering an expanded population of hematopoietic stem cells to the patient, wherein the expanded population of hematopoietic stem cells is prepared by contacting a first population of hematopoietic stem cells with an aryl hydrocarbon receptor antagonist for a time sufficient to produce the expanded population of hematopoietic stem cells.

67. The method of any one of claims 61-66, wherein the patient is a human.

68. The method of any one of claims 61-67, wherein the disorder is a hemoglobinopathy disorder.

69. The method of claim 68, wherein the hemoglobinopathy disorder is selected from the group consisting of sickle cell anemia, thalassemia, Fanconi anemia, aplastic anemia, and Wiskott-Aldrich syndrome.

70. The method of any one of claims 61-67, wherein the disorder is a myelodysplastic disorder.

71. The method of any one of claims 61-67, wherein the disorder is an immunodeficiency disorder.

72. The method of claim 71, wherein the immunodeficiency disorder is a congenital immunodeficiency.

73. The method of claim 71, wherein the immunodeficiency disorder is an acquired immunodeficiency.

74. The method of claim 73, wherein the acquired immunodeficiency is human immunodeficiency virus or acquired immune deficiency syndrome.

75. The method of any one of claims 61-67, wherein the disorder is a metabolic disorder.

76. The method of claim 75, wherein the metabolic disorder is selected from the group consisting of glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurlers Disease, sphingolipidoses, and metachromatic leukodystrophy.

77. The method of claim 76, wherein the disorder is cancer.

78. The method of claim 77, wherein the cancer is a hematological cancer.

79. The method of claim 77, wherein the cancer is selected from the group consisting of leukemia, lymphoma, multiple myeloma, and neuroblastoma.

80. The method of claim 77, wherein the cancer is acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, or non-Hodgkin's lymphoma.

81. The method of any one of claims 61-67, wherein the disorder is a disorder selected from the group consisting of adenosine deaminase deficiency and severe combined immunodeficiency, hyper immunoglobulin M syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia

major, systemic sclerosis, systemic lupus erythematosus, multiple sclerosis, and juvenile rheumatoid arthritis.

82. The method of any one of claims 61-67, wherein the disorder is an autoimmune disorder.

83. The method of claim 82, wherein the autoimmune disorder is selected from the group consisting of multiple sclerosis, human systemic lupus, rheumatoid arthritis, inflammatory bowel disease, treating psoriasis, Type 1 diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, alopecia universalis, ankylosing spondylitis, antiphospholipid antibody syndrome, aplastic anemia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome, autoimmune oophoritis, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Chagas' disease, chronic fatigue immune dysfunction syndrome, chronic inflammatory demyelinating polyneuropathy, Crohn's disease, cicatricial pemphigoid, coeliac sprue-dermatitis herpetiformis, cold agglutinin disease, CREST syndrome, Degos disease, discoid lupus, dysautonomia, endometriosis, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Goodpasture's syndrome, Grave's disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, Hidradenitis suppurativa, idiopathic and/or acute thrombocytopenic purpura, idiopathic pulmonary fibrosis, IgA neuropathy, interstitial cystitis, juvenile arthritis, Kawasaki's disease, lichen planus, Lyme disease, Meniere disease, mixed connective tissue disease, myasthenia gravis, neuromyotonia, opsoclonus myoclonus syndrome, optic neuritis, Ord's thyroiditis, pemphigus vulgaris, pernicious anemia, polychondritis, polymyositis and dermatomyositis, primary biliary cirrhosis, polyarteritis nodosa, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, Raynaud phenomenon, Reiter's syndrome, rheumatic fever, sarcoidosis, scleroderma, Sjögren's syndrome, stiff person syndrome, Takayasu's arteritis, temporal arteritis, ulcerative colitis, uveitis, vasculitis, vitiligo, vulvodynia, and Wegener's granulomatosis.

84. The method of any one of claims 61-67, wherein the disorder is a neurological disorder.

85. The method of claim 84, wherein the neurological disorder is selected from the group consisting of Parkinson's disease, Alzheimer's disease, multiple sclerosis, Amyotrophic lateral sclerosis, Huntington's disease, mild cognitive impairment, amyloidosis, AIDS-related dementia, encephalitis, stroke, head trauma, epilepsy, mood disorders, and dementia.

86. The method of any one of claims 61-85, wherein the hematopoietic stem or progenitor cells are autologous with respect to the patient.

87. The method of any one of claims 61-85, wherein the hematopoietic stem or progenitor cells are allogeneic with respect to the patient.

88. The method of claim 87, wherein the hematopoietic stem or progenitor cells are HLA-matched with respect to the patient.

89. The method of any one of claims 61-88, wherein the hematopoietic stem or progenitor cells, or progeny thereof, maintain hematopoietic stem cell functional potential after two or more days following infusion of the hematopoietic stem or progenitor cells into the patient.

90. The method of any one of claims 61-89, wherein the hematopoietic stem or progenitor cells, or progeny thereof, localize to hematopoietic tissue and/or reestablish hematopoiesis following infusion of the hematopoietic stem or progenitor cells into the patient.

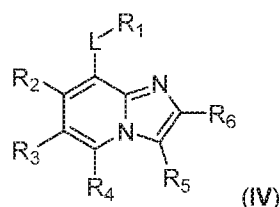
91. The method of any one of claims 61-90, wherein upon infusion into the patient, the hematopoietic stem or progenitor cells give rise to recovery of a population of cells selected from the group consisting of megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeloblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T-lymphocytes, and B-lymphocytes.

92. A method of producing microglia in the central nervous system of a human patient in need thereof, comprising administering an expanded population of hematopoietic stem cells to the patient, wherein the expanded population of hematopoietic stem cells is prepared by contacting a first population of hematopoietic stem cells with an aryl hydrocarbon receptor antagonist for a time sufficient to produce the expanded population of hematopoietic stem cells, and wherein administration of the expanded population of hematopoietic stem cells results in formation of microglia in the central nervous system of the patient.

93. A kit comprising a plurality of hematopoietic stem or progenitor cells and a package insert, wherein the package insert instructs a user to perform the method of any one of claims 1-59.

94. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is SR-1 or Compound 2.

95. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is a compound represented by formula (IV)



wherein L is selected from the group consisting of  $-NR_{7a}(CR_{8a}R_{8b})_n$ ,  $-O(CR_{8a}R_{8b})_n$ ,  $-C(O)(CR_{8a}R_{8b})_n$ ,  $-C(S)(CR_{8a}R_{8b})_n$ ,  $-S(O)_{0-2}(CR_{8a}R_{8b})_n$ ,  $-(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}C(O)(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}C(S)(CR_{8a}R_{8b})_n$ ,  $-OC(O)(CR_{8a}R_{8b})_n$ ,  $-OC(S)(CR_{8a}R_{8b})_n$ ,  $-C(O)NR_{7a}(CR_{8a}R_{8b})_n$ ,  $-C(S)NR_{7a}(CR_{8a}R_{8b})_n$ ,  $-C(O)O(CR_{8a}R_{8b})_n$ ,  $-C(S)O(CR_{8a}R_{8b})_n$ ,  $-S(O)_2NR_{7a}(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}S(O)_2(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}C(O)NR_{7b}(CR_{8a}R_{8b})_n$ , and  $-NR_{7a}C(O)O(CR_{8a}R_{8b})_n$ , wherein  $R_{7a}$ ,  $R_{7b}$ ,  $R_{8a}$ , and  $R_{8b}$  are each independently selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl, and each  $n$  is independently an integer from 2 to 6;

$R_1$  is selected from the group consisting of  $-S(O)_2NR_{9a}R_{9b}$ ,  $-NR_{9a}C(O)R_{9b}$ ,  $-NR_{9a}C(S)R_{9b}$ ,  $-NR_{9a}C(O)NR_{9b}R_{9c}$ ,  $-C(O)R_{9a}$ ,  $-C(S)R_{9a}$ ,  $-S(O)_{0-2}R_{9a}$ ,  $-C(O)OR_{9a}$ ,  $-C(S)OR_{9a}$ ,  $-C(O)NR_{9a}R_{9b}$ ,  $-C(S)NR_{9a}R_{9b}$ ,  $-NR_{9a}S(O)_2R_{9b}$ ,  $-NR_{9a}C(O)OR_{9b}$ ,  $-OC(O)CR_{9a}R_{9b}R_{9c}$ ,  $-OC(S)CR_{9a}R_{9b}R_{9c}$ , optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl, wherein  $R_{9a}$ ,  $R_{9b}$ , and  $R_{9c}$  are each independently selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

$R_2$  is selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl;

$R_3$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

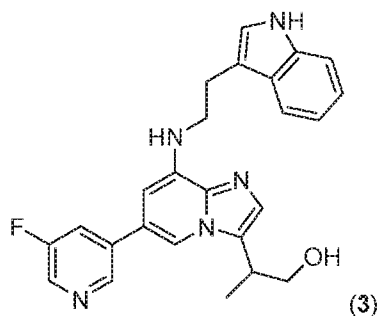
$R_4$  is selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl;

$R_5$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

$R_6$  is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; or a salt thereof.

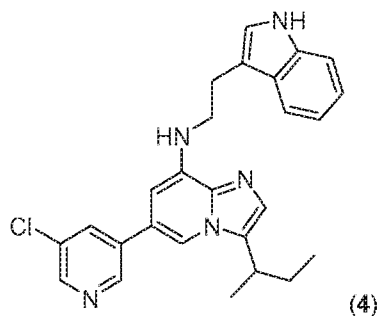
5

96. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (3)



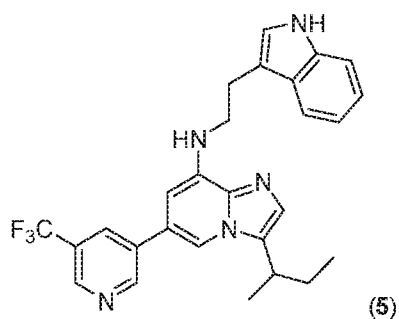
or a salt thereof.

97. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (4)



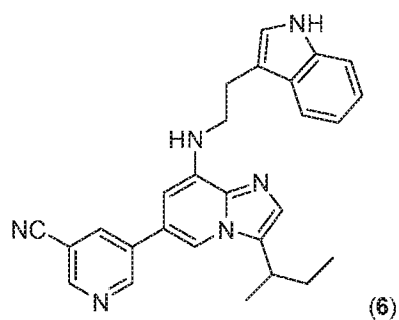
or a salt thereof.

98. The method or the kit of any one of claims 1-93 wherein the aryl hydrocarbon receptor antagonist is compound (5)



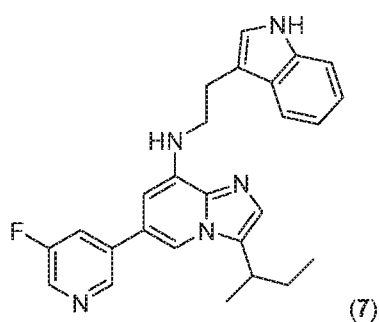
or a salt thereof.

99. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (6)



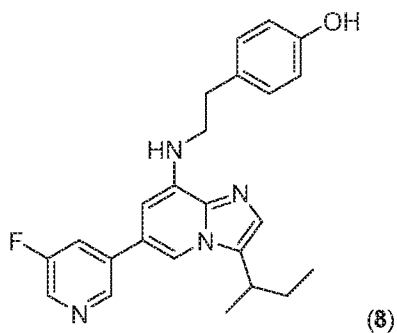
or a salt thereof.

100. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (7)



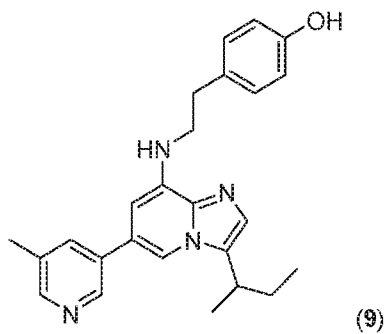
or a salt thereof.

101. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (8)



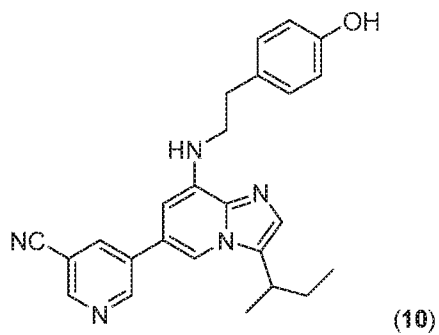
or a salt thereof.

102. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (9)



or a salt thereof.

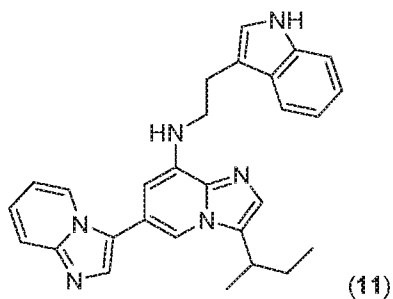
103. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (10)



or a salt thereof.

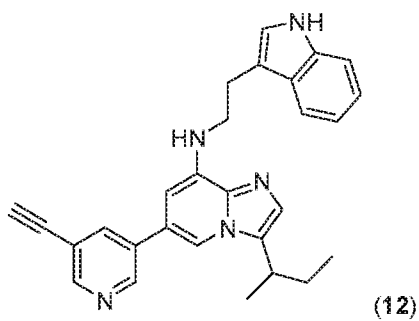


104. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (11)



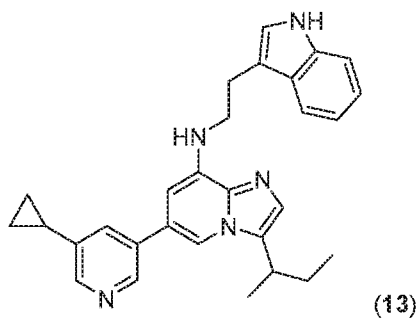
or a salt thereof.

105. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (12)



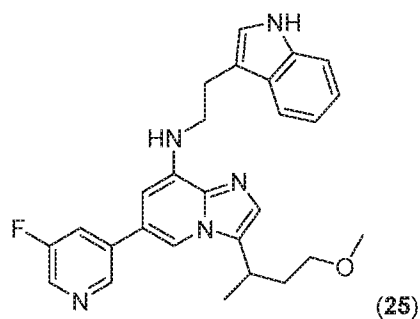
or a salt thereof.

106. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (13)



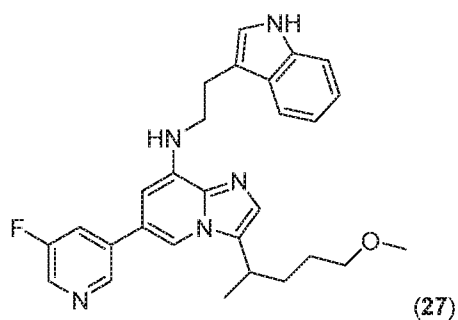
or a salt thereof.

107. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (25)



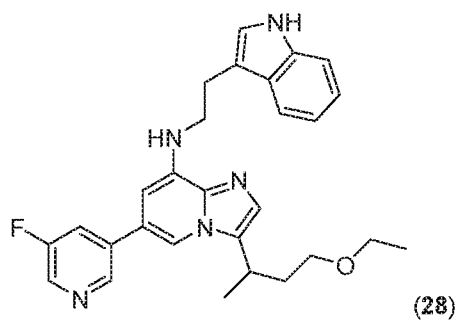
or a salt thereof.

108. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (27)



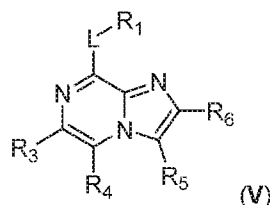
or a salt thereof.

109. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (28)



or a salt thereof.

110. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is a compound represented by formula (V)



wherein L is selected from the group consisting of  $-NR_{7a}(CR_{8a}R_{8b})_n$ ,  $-O(CR_{8a}R_{8b})_n$ ,  $-C(O)(CR_{8a}R_{8b})_n$ ,  $-C(S)(CR_{8a}R_{8b})_n$ ,  $-S(O)_{0-2}(CR_{8a}R_{8b})_n$ ,  $-(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}C(O)(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}C(S)(CR_{8a}R_{8b})_n$ ,  $-OC(O)(CR_{8a}R_{8b})_n$ ,  $-OC(S)(CR_{8a}R_{8b})_n$ ,  $-C(O)NR_{7a}(CR_{8a}R_{8b})_n$ ,  
 5  $-C(S)NR_{7a}(CR_{8a}R_{8b})_n$ ,  $-C(O)O(CR_{8a}R_{8b})_n$ ,  $-C(S)O(CR_{8a}R_{8b})_n$ ,  $-S(O)_2NR_{7a}(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}S(O)_2(CR_{8a}R_{8b})_n$ ,  $-NR_{7a}C(O)NR_{7b}(CR_{8a}R_{8b})_n$ , and  $-NR_{7a}C(O)O(CR_{8a}R_{8b})_n$ , wherein  $R_{7a}$ ,  $R_{7b}$ ,  $R_{8a}$ , and  $R_{8b}$  are each independently selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl, and each n is independently an integer from 2 to 6;

$R_1$  is selected from the group consisting of  $-S(O)_2NR_{9a}R_{9b}$ ,  $-NR_{9a}C(O)R_{9b}$ ,  $-NR_{9a}C(S)R_{9b}$ ,  $-NR_{9a}C(O)NR_{9b}R_{9c}$ ,  $-C(O)R_{9a}$ ,  $-C(S)R_{9a}$ ,  $-S(O)_{0-2}R_{9a}$ ,  $-C(O)OR_{9a}$ ,  $-C(S)OR_{9a}$ ,  $-C(O)NR_{9a}R_{9b}$ ,  $-C(S)NR_{9a}R_{9b}$ ,  $-NR_{9a}S(O)_2R_{9b}$ ,  $-NR_{9a}C(O)OR_{9b}$ ,  $-OC(O)CR_{9a}R_{9b}R_{9c}$ ,  $-OC(S)CR_{9a}R_{9b}R_{9c}$ , optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl, wherein  $R_{9a}$ ,  $R_{9b}$ , and  $R_{9c}$  are each independently selected from the group consisting of hydrogen, optionally substituted  
 10 aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

$R_3$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

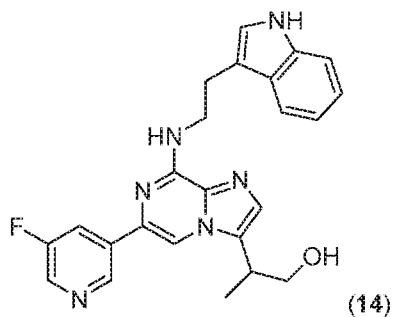
20  $R_4$  is selected from the group consisting of hydrogen and optionally substituted C1-4 alkyl;

$R_5$  is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl; and

25  $R_6$  is selected from the group consisting of hydrogen, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted cycloalkyl, and optionally substituted heterocycloalkyl;

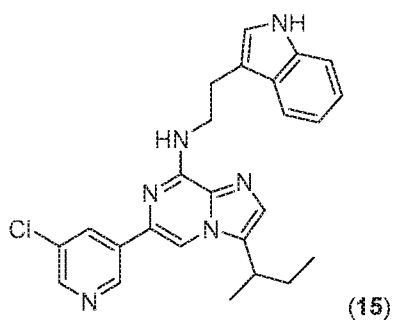
or a salt thereof.

111. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (14)



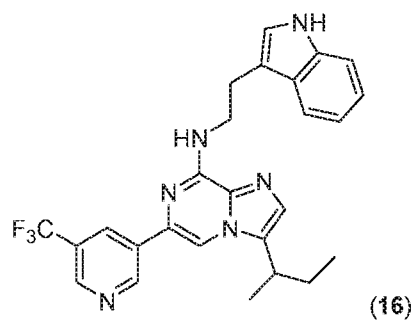
or a salt thereof.

112. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (15)



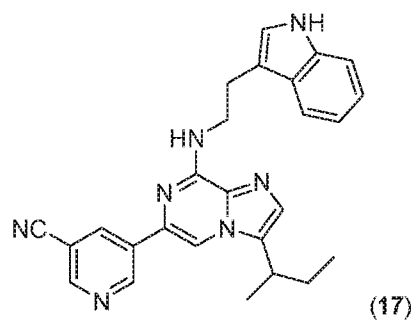
or a salt thereof.

113. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (16)



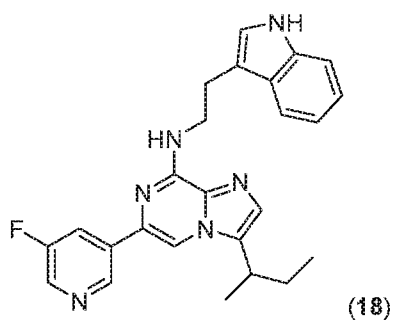
or a salt thereof.

114. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (17)



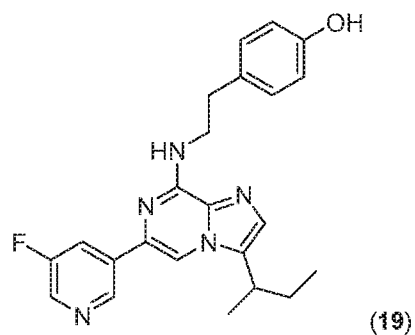
or a salt thereof.

115. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (18)



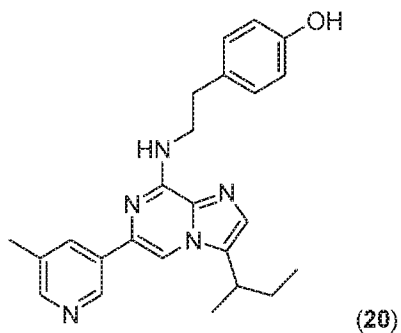
or a salt thereof.

116. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (19)



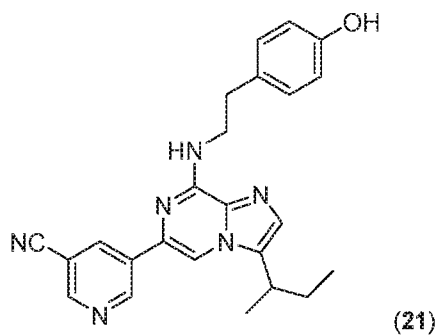
or a salt thereof.

117. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (20)



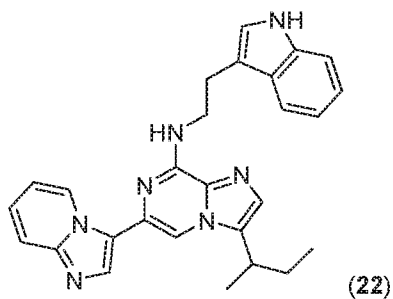
or a salt thereof.

118. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (21)



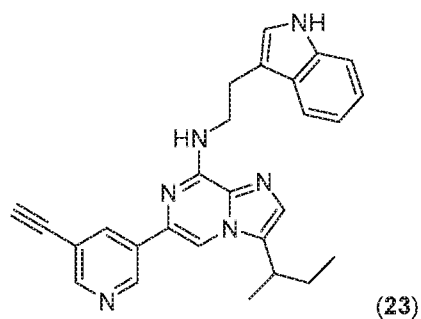
or a salt thereof.

119. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (22)



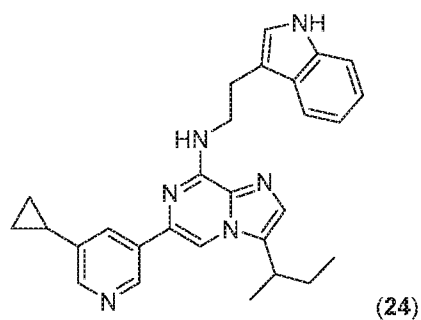
or a salt thereof.

120. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (23)



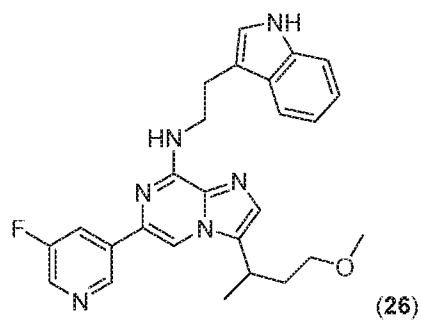
or a salt thereof.

121. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (24)



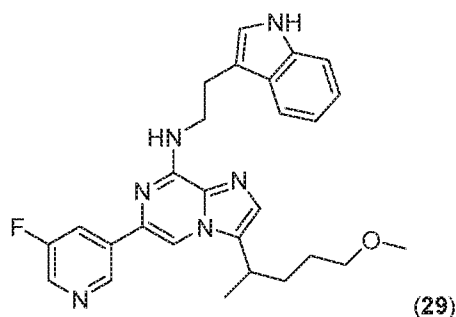
or a salt thereof.

122. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (26)



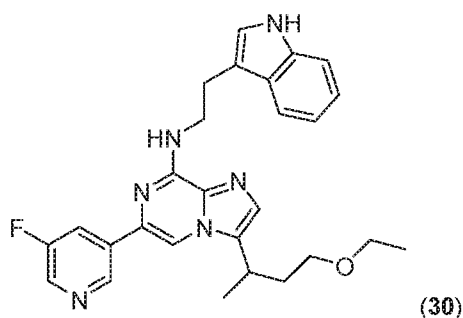
or a salt thereof.

123. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (29)



or a salt thereof.

124. The method or the kit of any one of claims 1-93, wherein the aryl hydrocarbon receptor antagonist is compound (30)



or a salt thereof.

125. A composition for use in treating a disorder in a patient, said composition comprising hematopoietic stem or progenitor cells, or progeny thereof, prepared according to the method of any one of the preceding claims.

126. Use of a composition comprising hematopoietic stem or progenitor cells, or progeny thereof, prepared according to the method of any one of the preceding claims in preparing a medicament for treating a disorder in a patient.

127. The composition of claim 125 or use of claim 126, wherein the patient is a human.

128. The composition of claim 125 or use of claim 126, wherein the disorder is a hemoglobinopathy disorder.



129. The composition or use of claim 128 wherein the hemoglobinopathy disorder is selected from the group consisting of sickle cell anemia, thalassemia, Fanconi anemia, aplastic anemia, and Wiskott-Aldrich syndrome.

130. The composition of claim 125 or use of claim 126, wherein the disorder is an immunodeficiency disorder.

131. The composition or use of claim 130 wherein the immunodeficiency disorder is a congenital immunodeficiency.

132. The composition or use of claim 130, wherein the immunodeficiency disorder is an acquired immunodeficiency.

133. The composition of use of claim 132, wherein the acquired immunodeficiency is human immunodeficiency virus or acquired immune deficiency syndrome.

134. The composition of claim 125 or use of claim 126, wherein the disorder is a metabolic disorder.

135. The composition or use of claim 134, wherein the metabolic disorder is selected from the group consisting of glycogen storage diseases, mucopolysaccharidoses, Gaucher's Disease, Hurlers Disease, sphingolipidoses, and metachromatic leukodystrophy.

136. The composition of claim 125 or use of claim 126, wherein the disorder is cancer.

137. The composition or use of claim 136, wherein the cancer is a hematological cancer.

138. The composition or use of claim 136, wherein the cancer is selected from the group consisting of leukemia, lymphoma, multiple myeloma, and neuroblastoma.

139. The composition or use of claim 136, wherein the cancer is acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, or non-Hodgkin's lymphoma.

140. The composition of claim 125 or use of claim 126, wherein the disorder is a disorder selected from the group consisting of adenosine deaminase deficiency and severe combined immunodeficiency, hyper immunoglobulin M syndrome, Chediak-Higashi disease, hereditary lymphohistiocytosis, osteopetrosis, osteogenesis imperfecta, storage diseases, thalassemia major, systemic sclerosis, systemic lupus erythematosus, multiple sclerosis, and juvenile rheumatoid arthritis.

141. The composition of claim 125 or use of claim 126, wherein the disorder is an autoimmune disorder.

142. The composition or use of claim 141, wherein the autoimmune disorder is selected from the group consisting of multiple sclerosis, human systemic lupus, rheumatoid arthritis, inflammatory bowel disease, treating psoriasis, Type 1 diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, alopecia universalis, ankylosing spondylitis, antiphospholipid antibody syndrome, aplastic anemia, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome, autoimmune oophoritis, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Chagas' disease, chronic fatigue immune dysfunction syndrome, chronic inflammatory demyelinating polyneuropathy, Crohn's disease, cicatricial pemphigoid, coeliac sprue-dermatitis herpetiformis, cold agglutinin disease, CREST syndrome, Degos disease, discoid lupus, dysautonomia, endometriosis, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Goodpasture's syndrome, Grave's disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, Hidradenitis suppurativa, idiopathic and/or acute thrombocytopenic purpura, idiopathic pulmonary fibrosis, IgA neuropathy, interstitial cystitis, juvenile arthritis, Kawasaki's disease, lichen planus, Lyme disease, Meniere disease, mixed connective tissue disease, myasthenia gravis, neuromyotonia, opsoclonus myoclonus syndrome, optic neuritis, Ord's thyroiditis, pemphigus vulgaris, pernicious anemia, polychondritis, polymyositis and dermatomyositis, primary biliary cirrhosis, polyarteritis nodosa, polyglandular syndromes, polymyalgia rheumatica, primary agammaglobulinemia, Raynaud phenomenon, Reiter's syndrome, rheumatic fever, sarcoidosis, scleroderma, Sjögren's syndrome, stiff person syndrome, Takayasu's arteritis, temporal arteritis, ulcerative colitis, uveitis, vasculitis, vitiligo, vulvodynia, and Wegener's granulomatosis.

143. The composition of claim 125 or use of claim 126, wherein the disorder is a neurological disorder.

144. The composition or use of claim 143, wherein the neurological disorder is selected from the group consisting of Parkinson's disease, Alzheimer's disease, multiple sclerosis, Amyotrophic lateral sclerosis, Huntington's disease, mild cognitive impairment, amyloidosis, AIDS-related dementia, encephalitis, stroke, head trauma, epilepsy, mood disorders, and dementia.

145. The composition of claim 125 or use of claim 126, wherein the hematopoietic stem or progenitor cells are autologous with respect to the patient.

146. The composition of claim 125 or use of claim 126, wherein the hematopoietic stem or progenitor cells are allogenic with respect to the patient.

147. The composition or use of claim 146, wherein the hematopoietic stem or progenitor cells are HLA-matched with respect to the patient.

148. The composition or use of any one of the preceding claims, wherein the hematopoietic stem or progenitor cells, or progeny thereof, maintain hematopoietic stem cell functional potential after two or more days following infusion of the hematopoietic stem or progenitor cells into the patient.

149. The composition or use of any one of the preceding claims, wherein the hematopoietic stem or progenitor cells, or progeny thereof, localize to hematopoietic tissue and/or reestablish hematopoiesis following infusion of the hematopoietic stem or progenitor cells into the patient.

150. The composition or use of any one of the preceding claims, wherein upon infusion into the patient, the hematopoietic stem or progenitor cells give rise to recovery of a population of cells selected from the group consisting of megakaryocytes, thrombocytes, platelets, erythrocytes, mast cells, myeloblasts, basophils, neutrophils, eosinophils, microglia, granulocytes, monocytes, osteoclasts, antigen-presenting cells, macrophages, dendritic cells, natural killer cells, T-lymphocytes, and B-lymphocytes.

FIG. 1

Clinical evidence of AHR antagonist-mediated expansion of CB HSCs

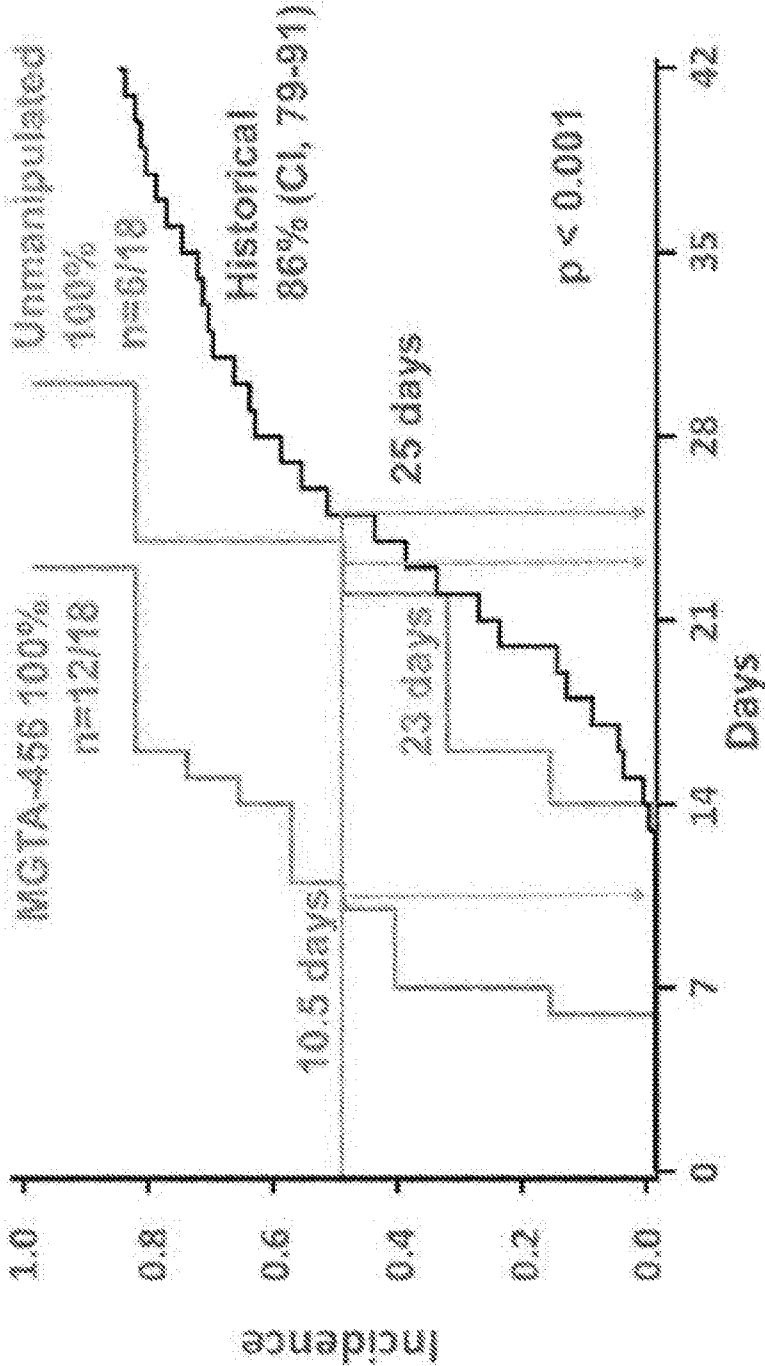


FIG. 2A

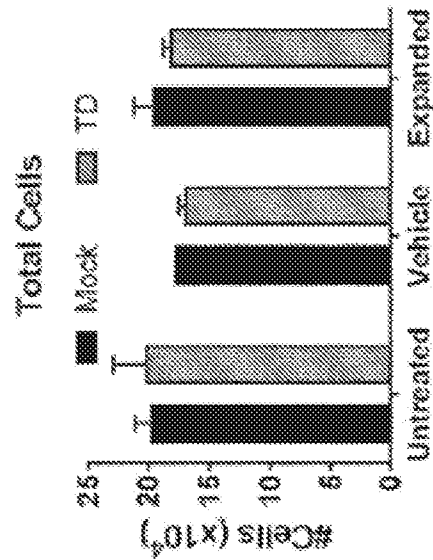


FIG. 2B

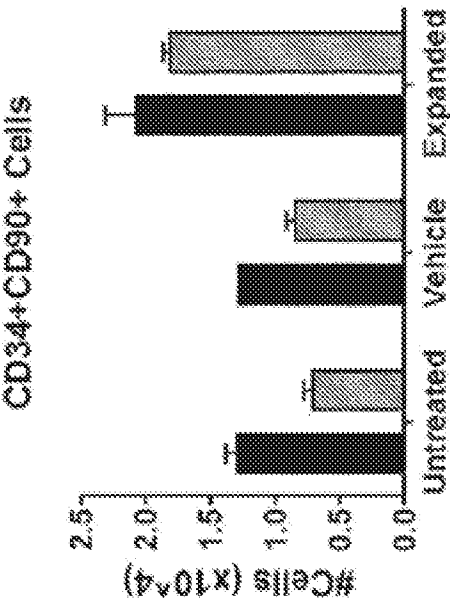


FIG. 2C

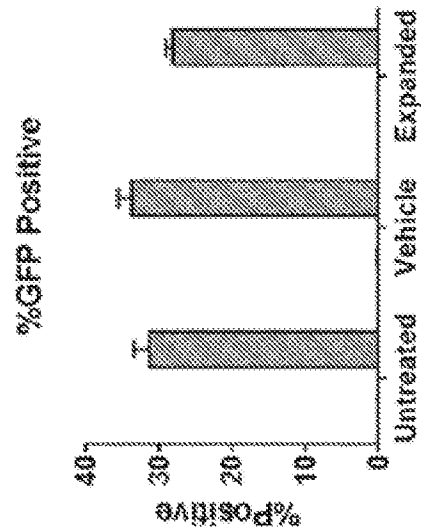


FIG. 2D

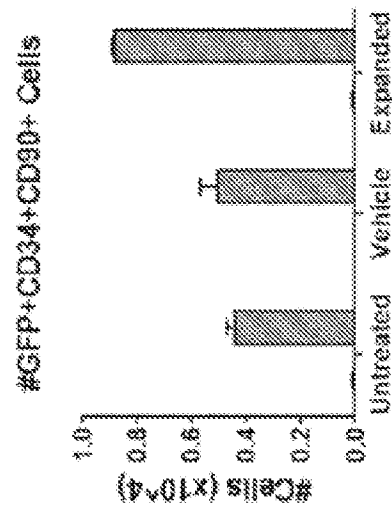


FIG. 3A

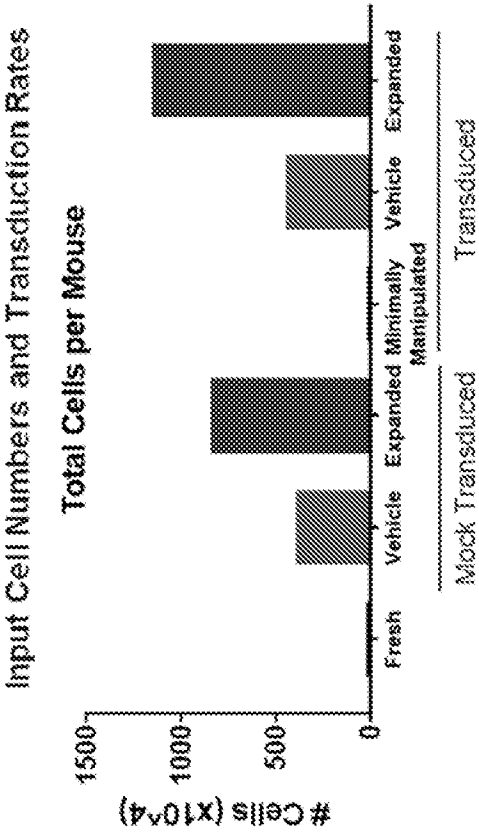


FIG. 3B

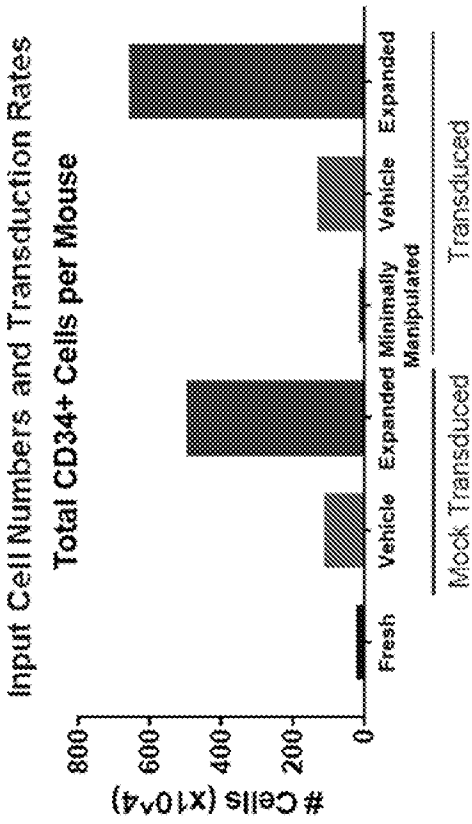


FIG. 3C

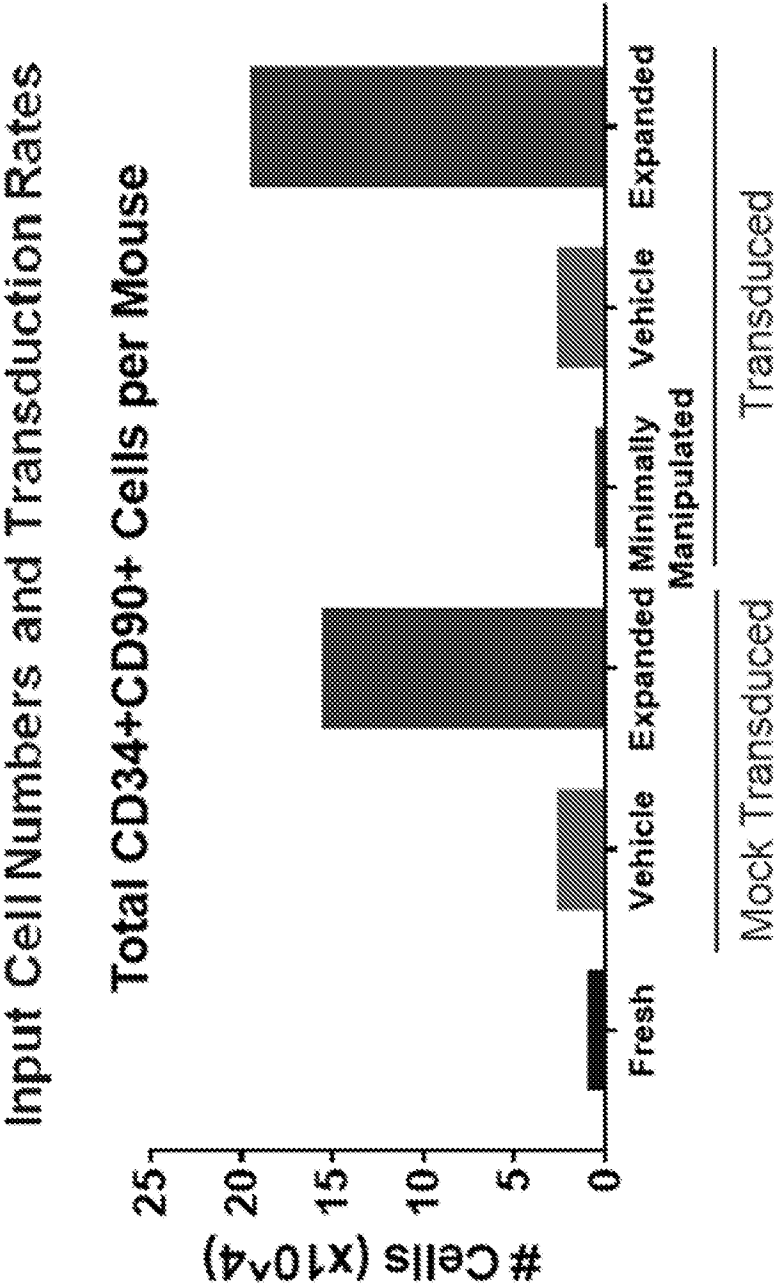




FIG. 3D

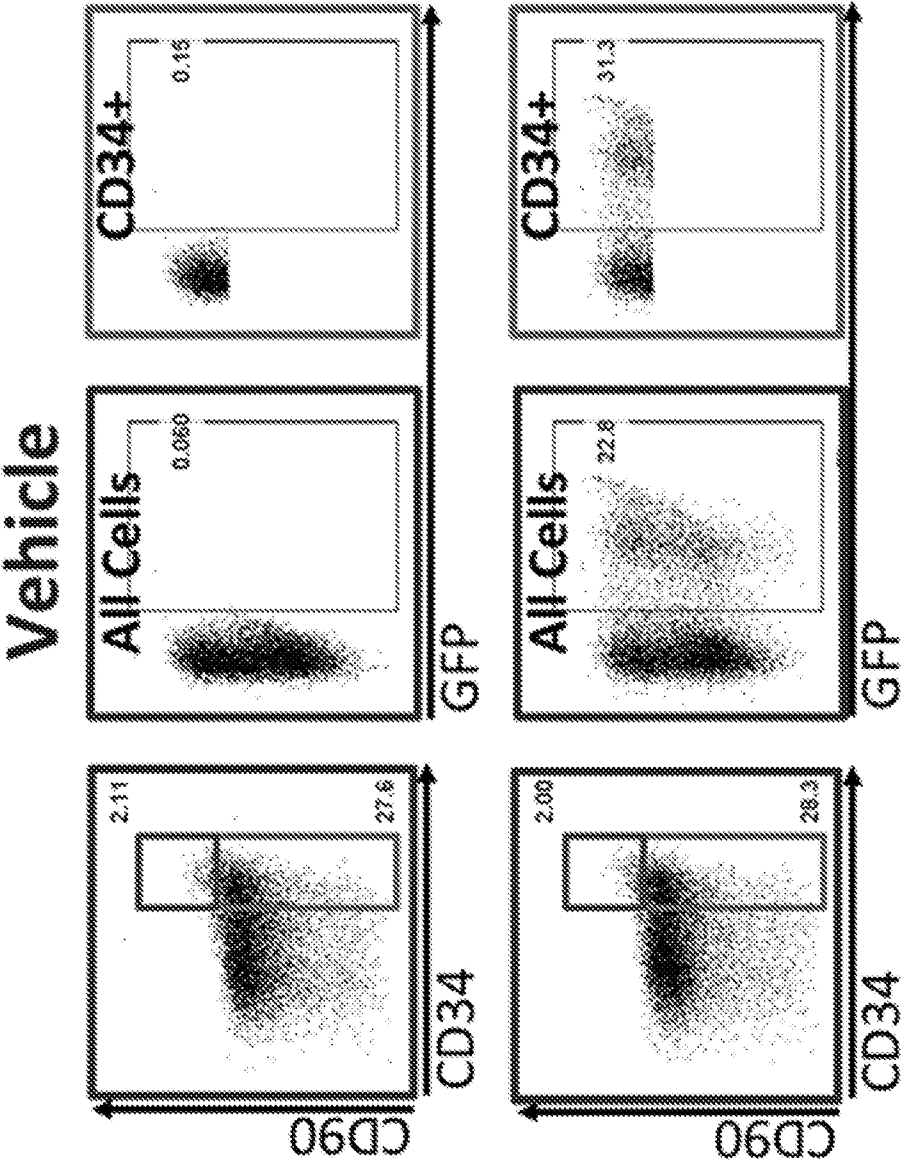
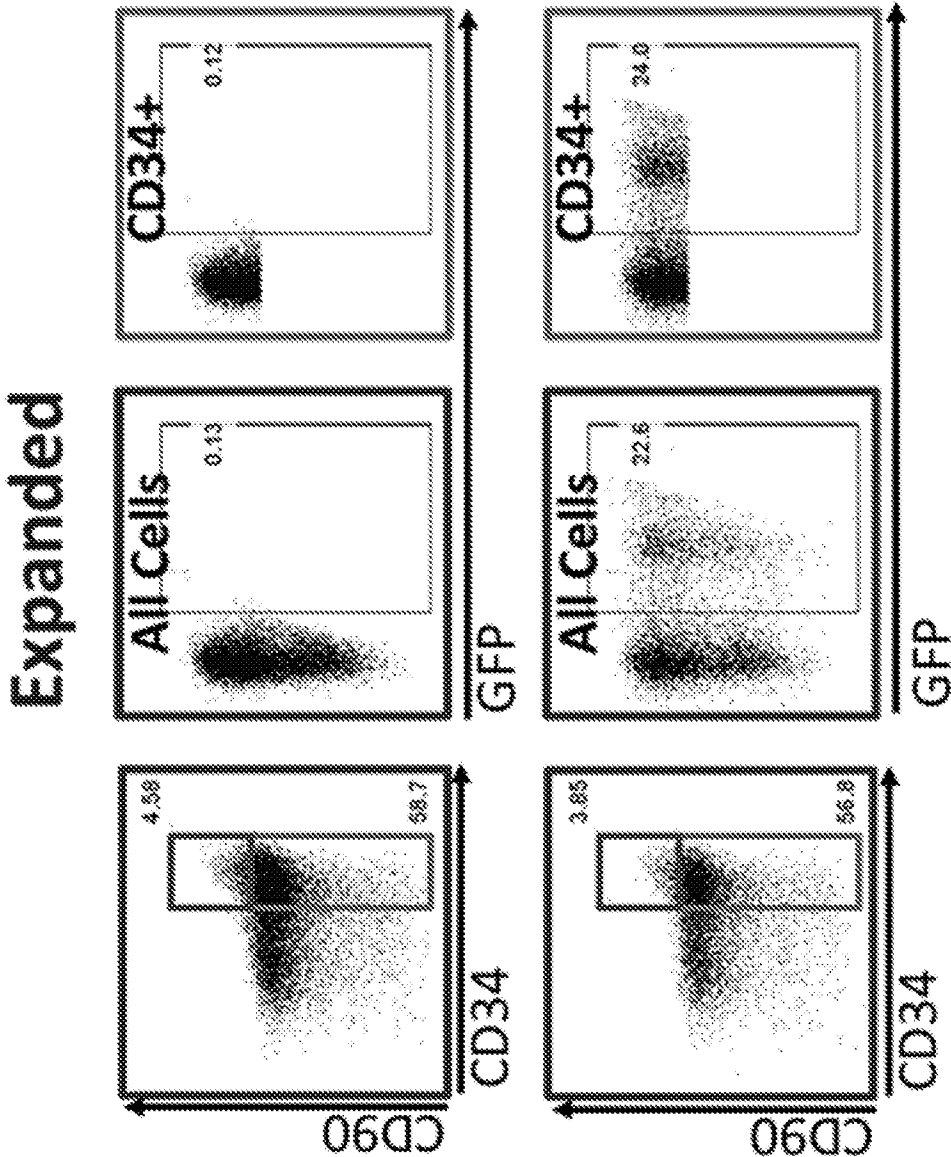
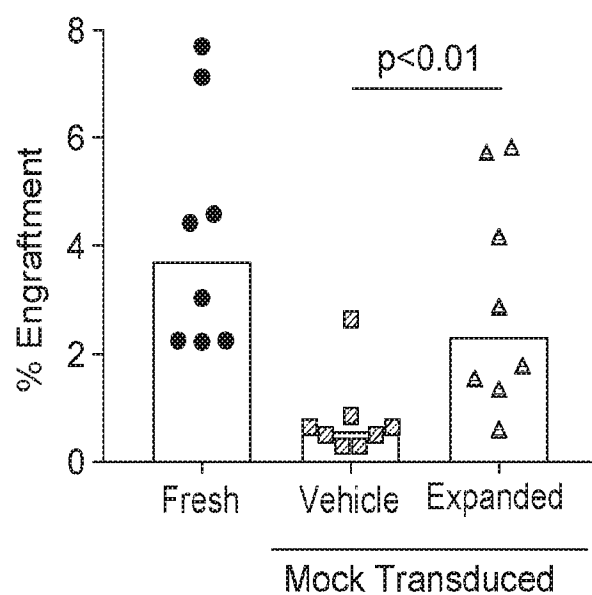


FIG. 3E



**FIG. 4A**

*In Vivo* Engraftment and Transduction Rates  
Peripheral Blood

**FIG. 4B**

*In Vivo* Engraftment and Transduction Rates  
Peripheral Blood

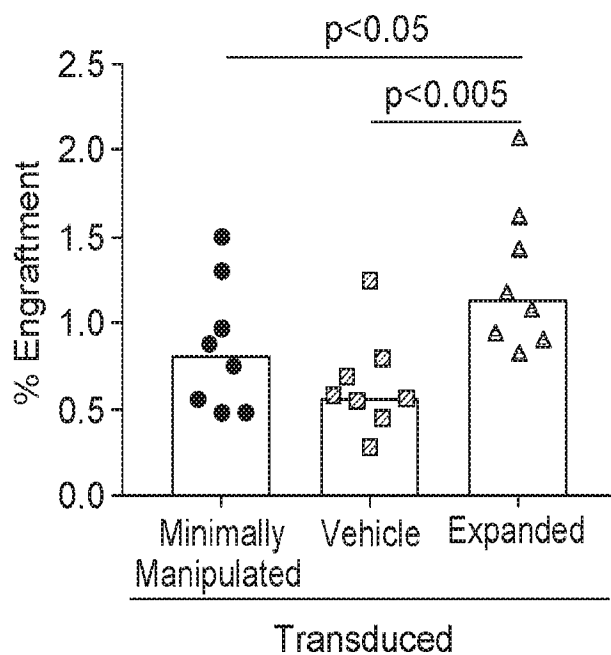
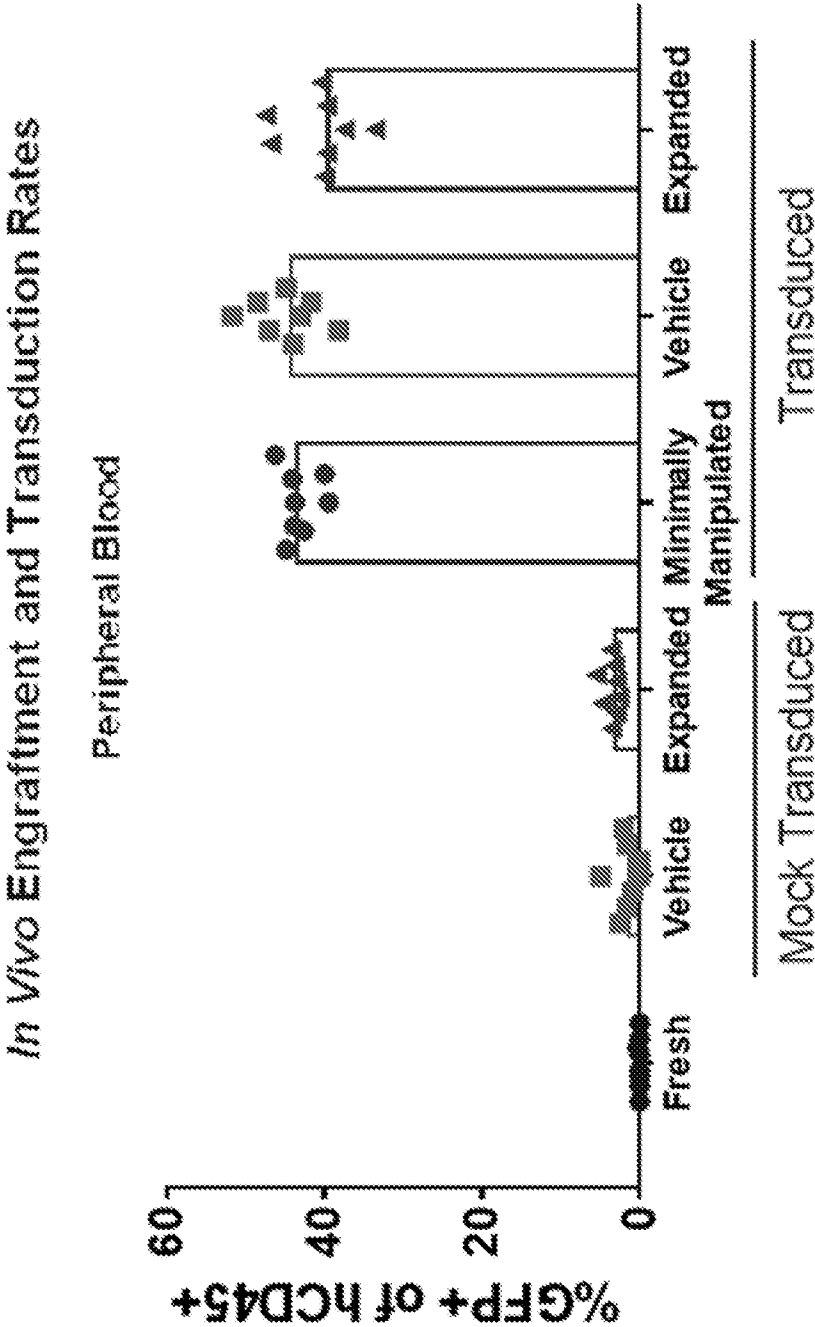
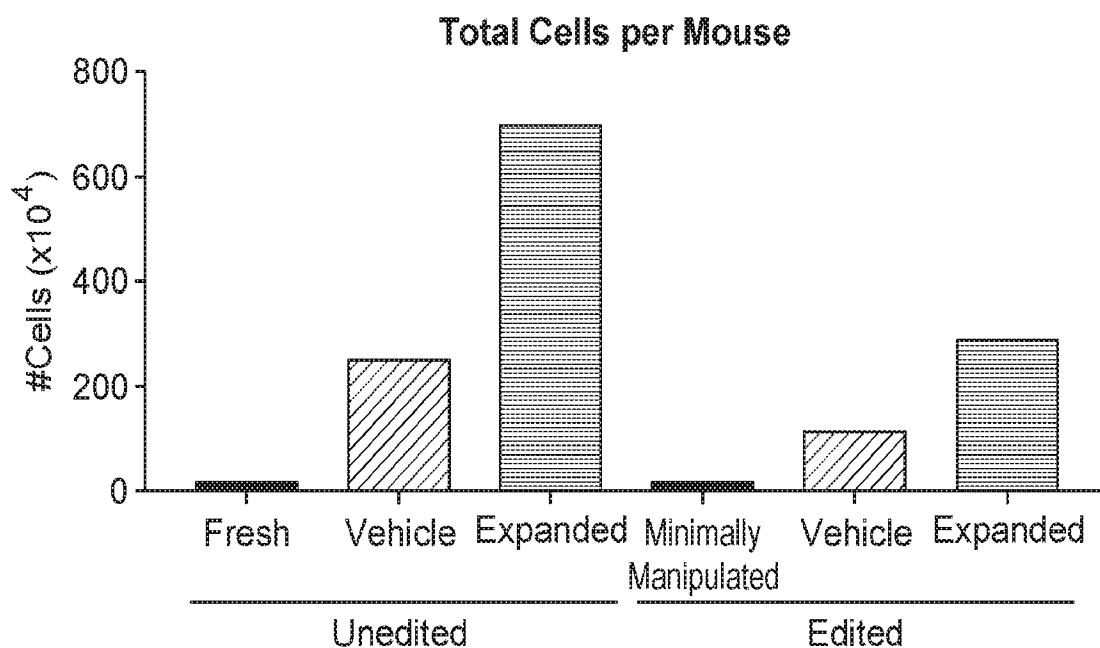


FIG. 4C



**FIG. 5A**

Input Cell Numbers and Editing Rates

**FIG. 5B**

Input Cell Numbers and Editing Rates

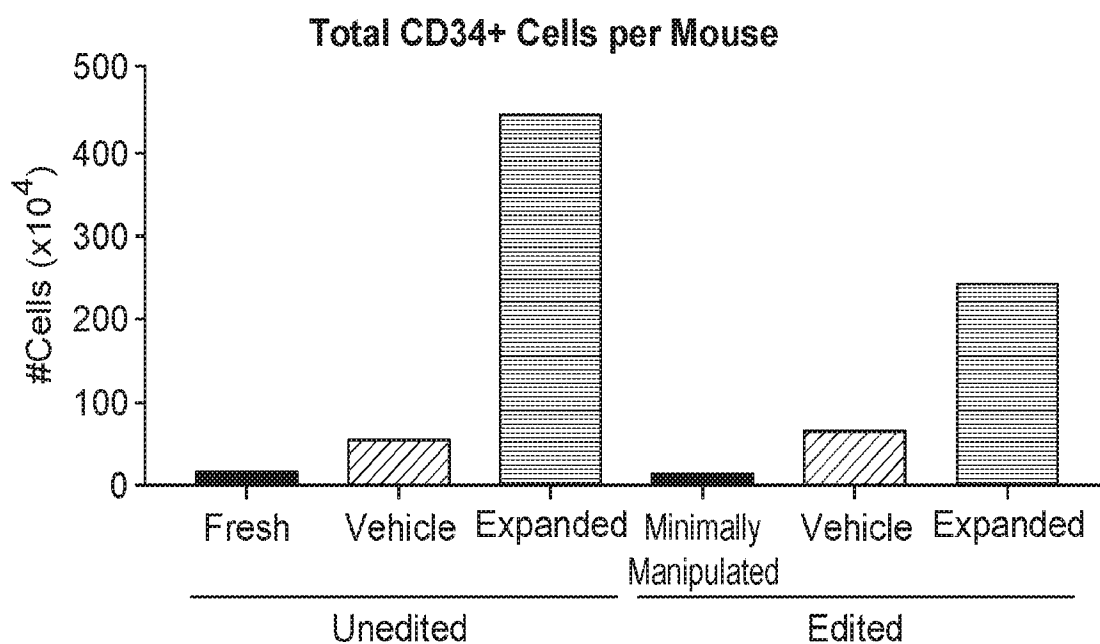


FIG. 5C

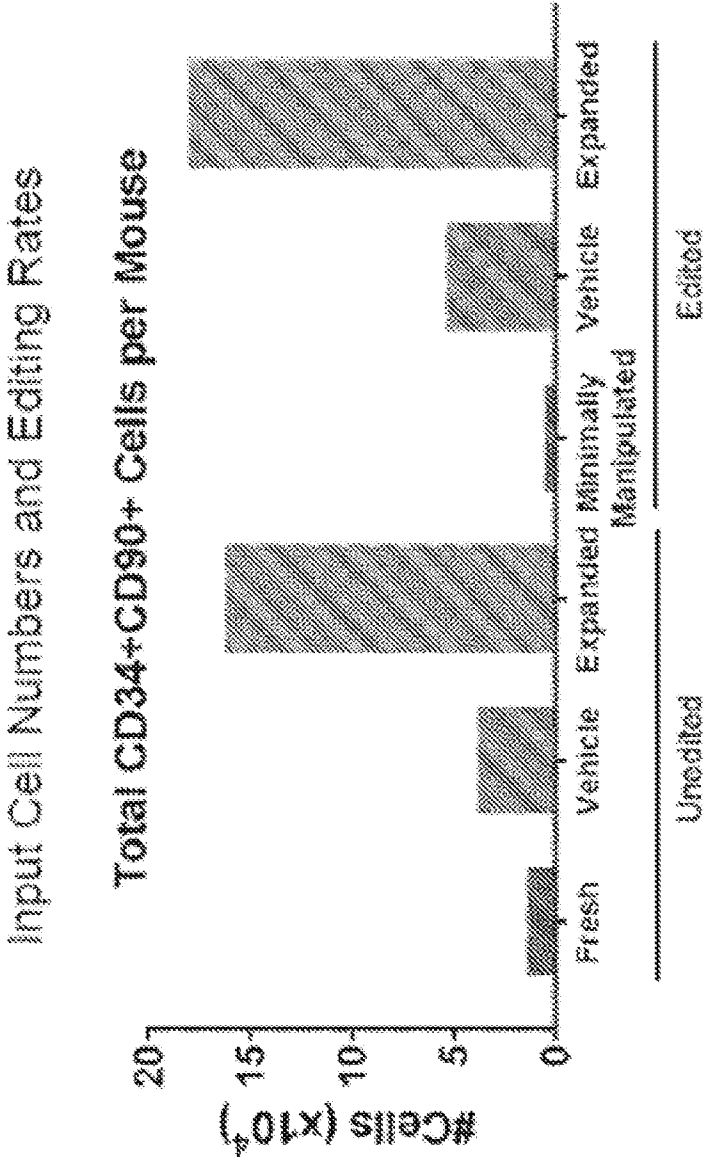


FIG. 5D

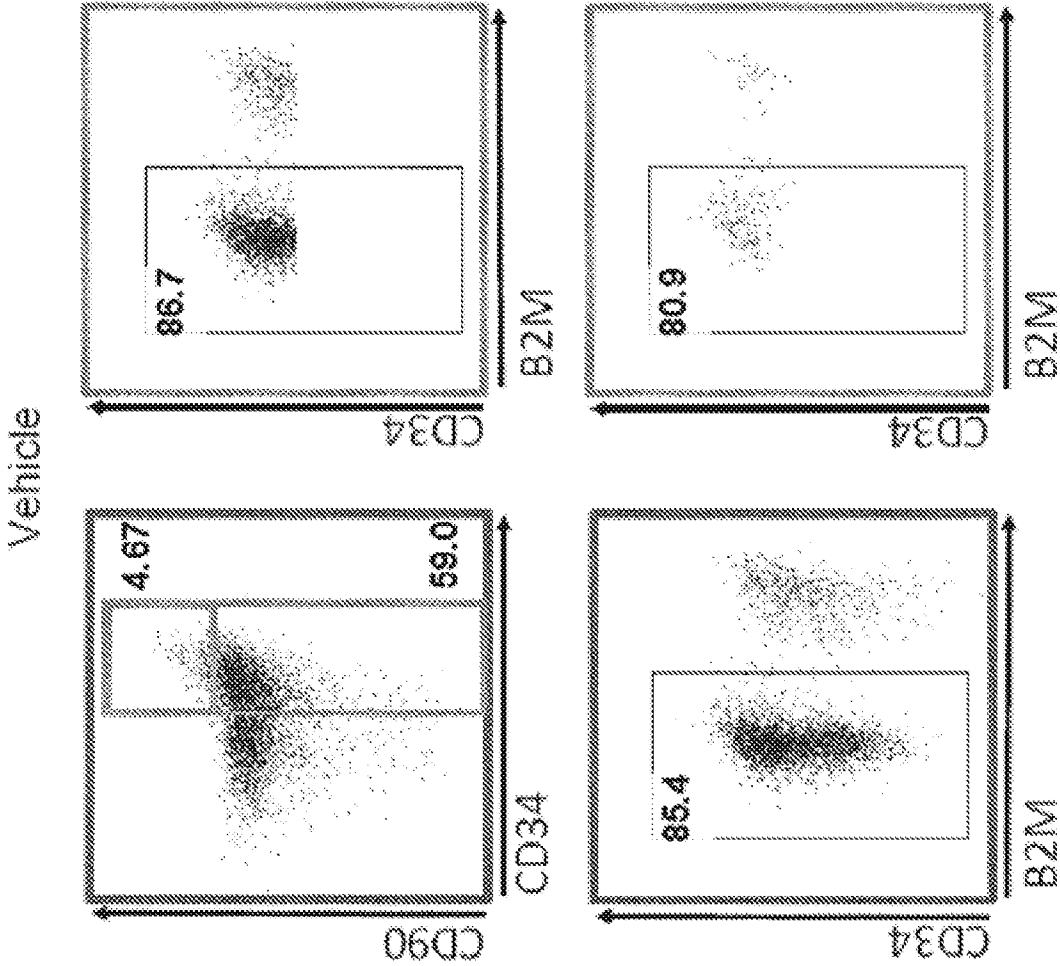
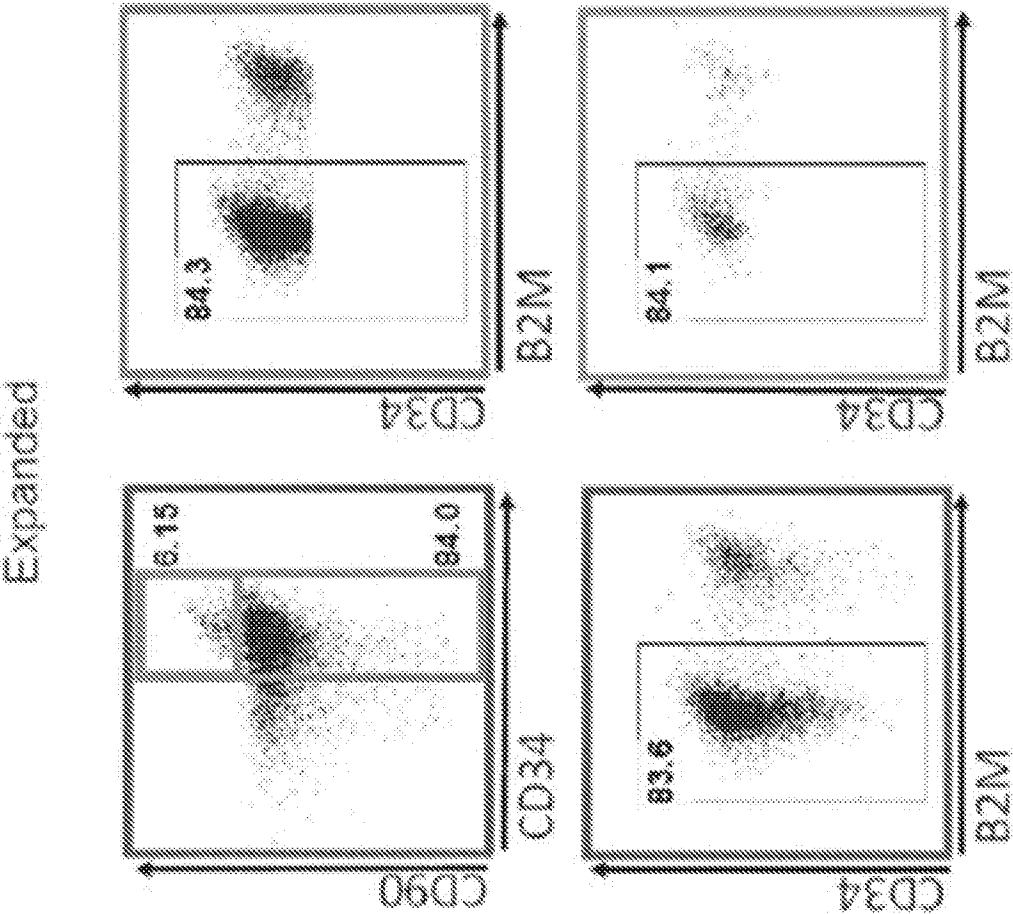
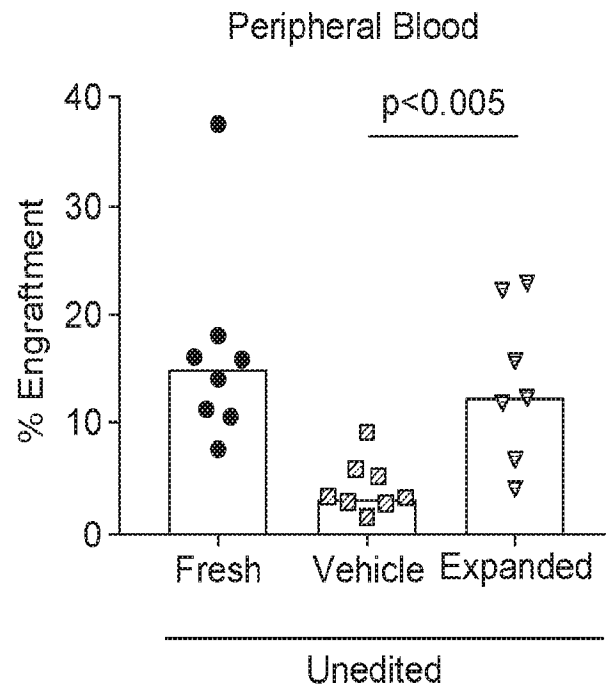
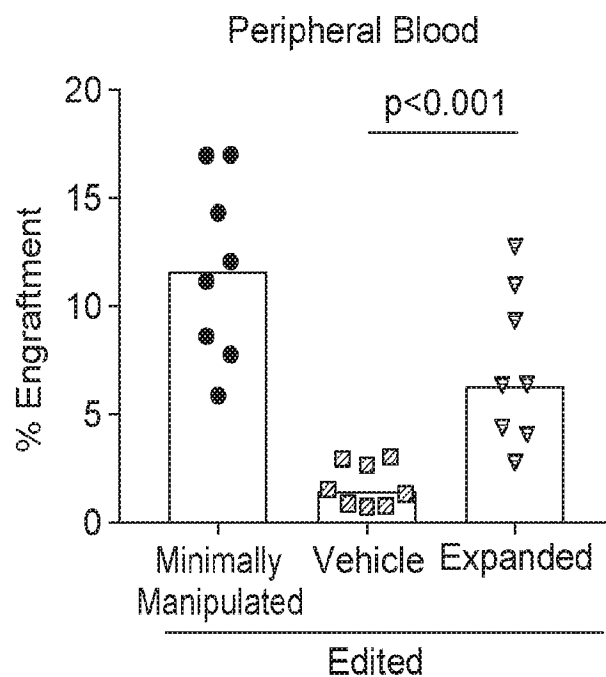


FIG. 5E





**FIG. 6A****FIG. 6B**

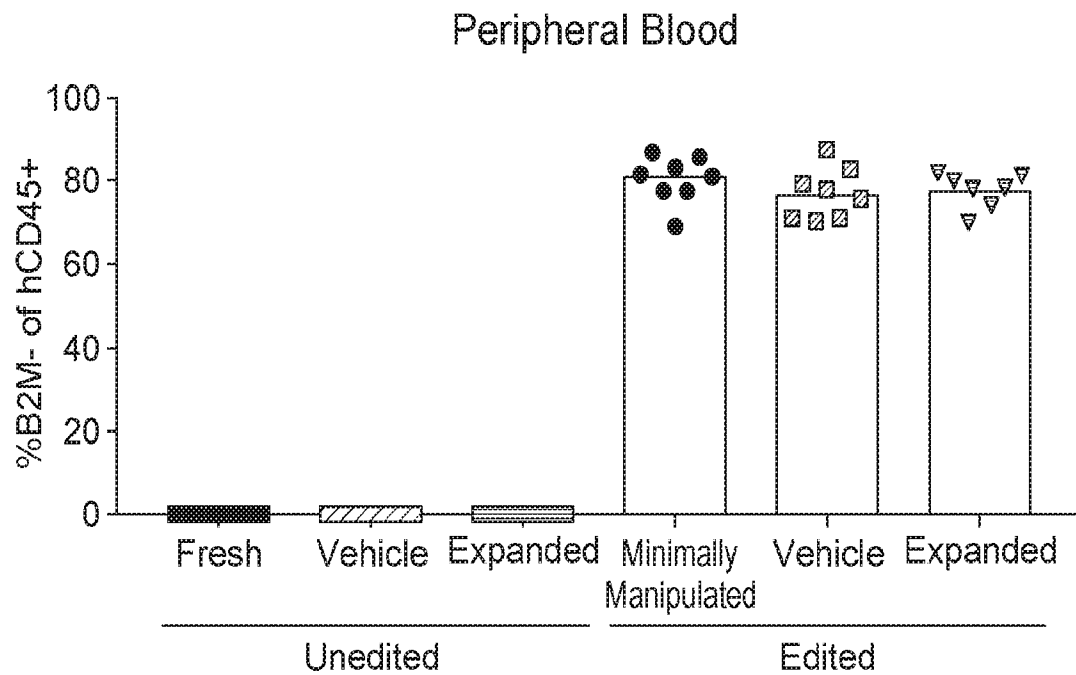
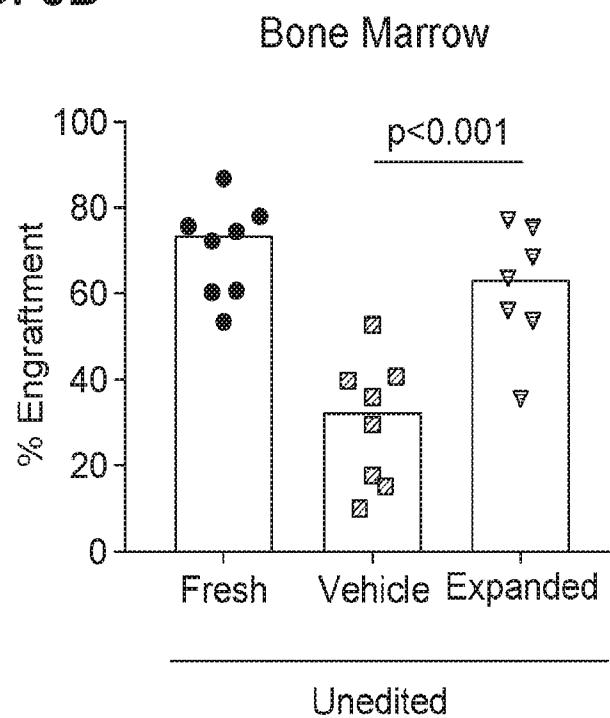
**FIG. 6C****FIG. 6D**

FIG. 6E

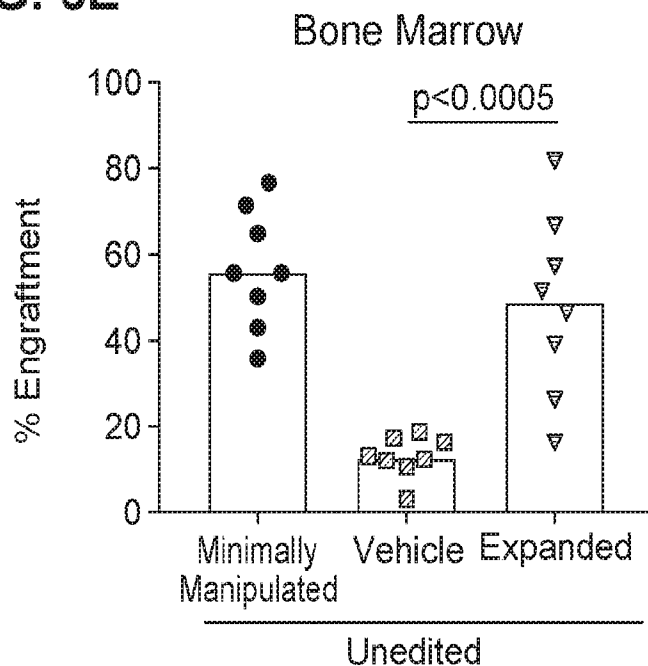
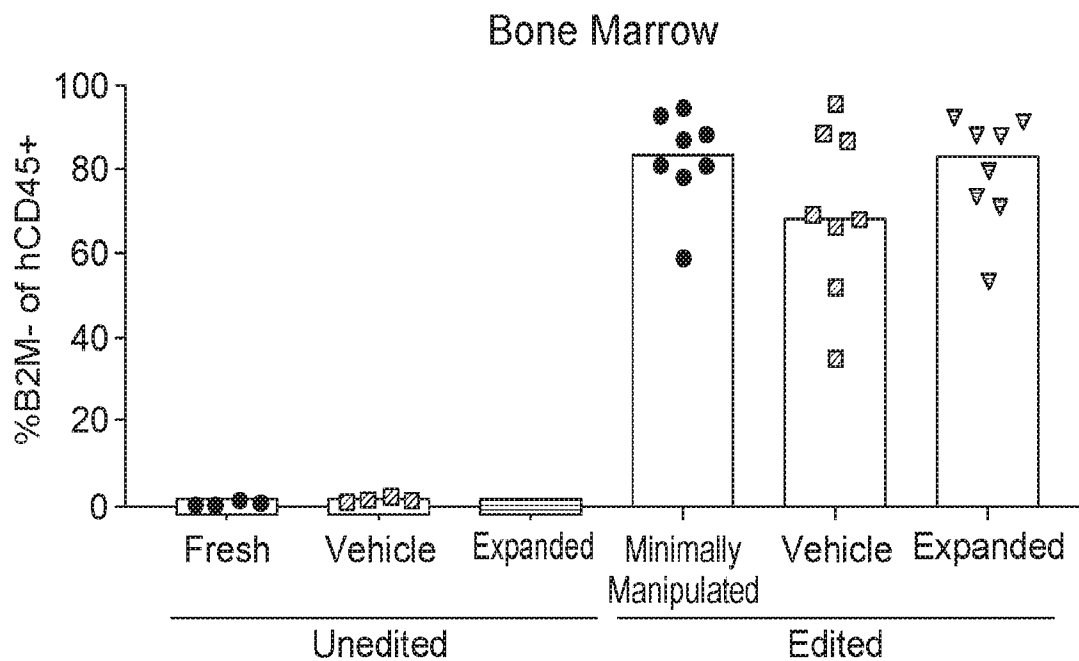


FIG. 6F



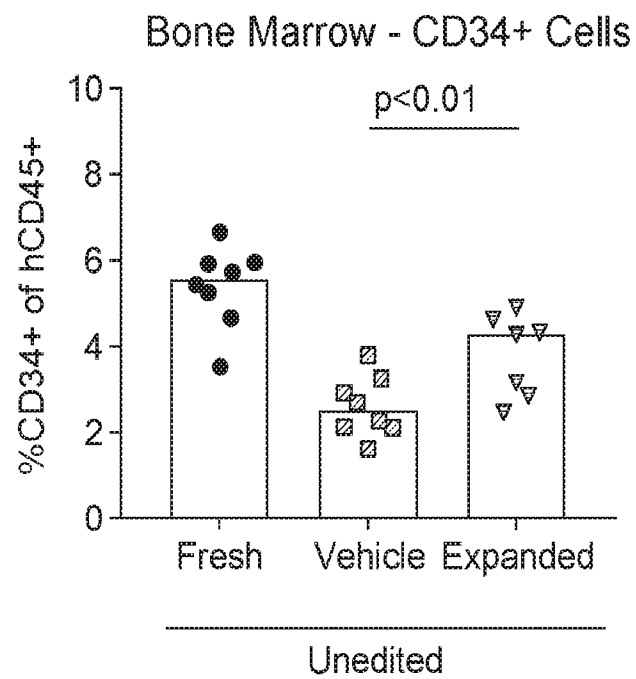
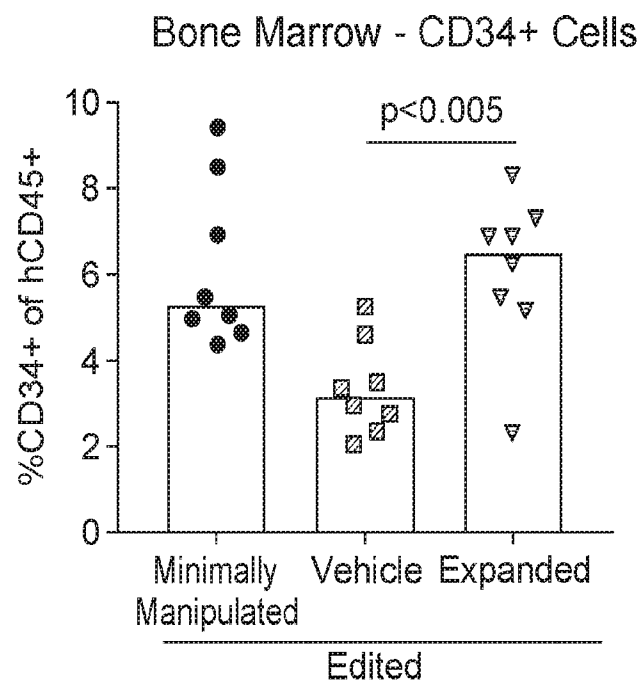
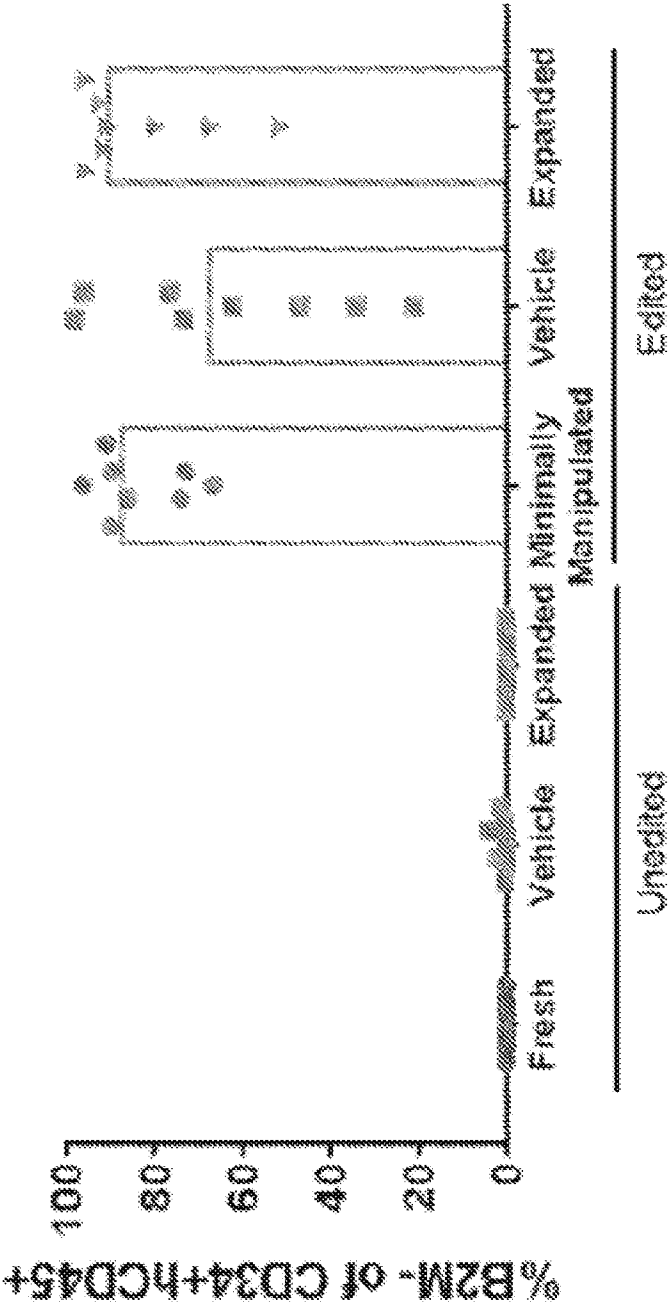
**FIG. 6G****FIG. 6H**

FIG. 6I

Bone Marrow – CD34+ Cells



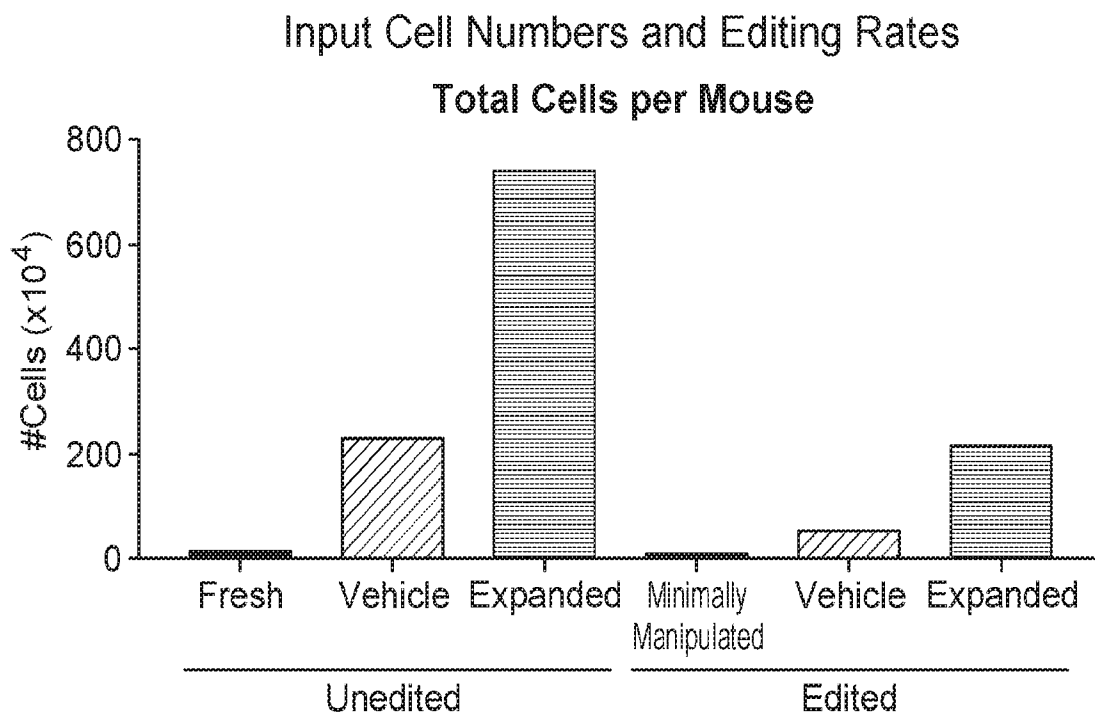
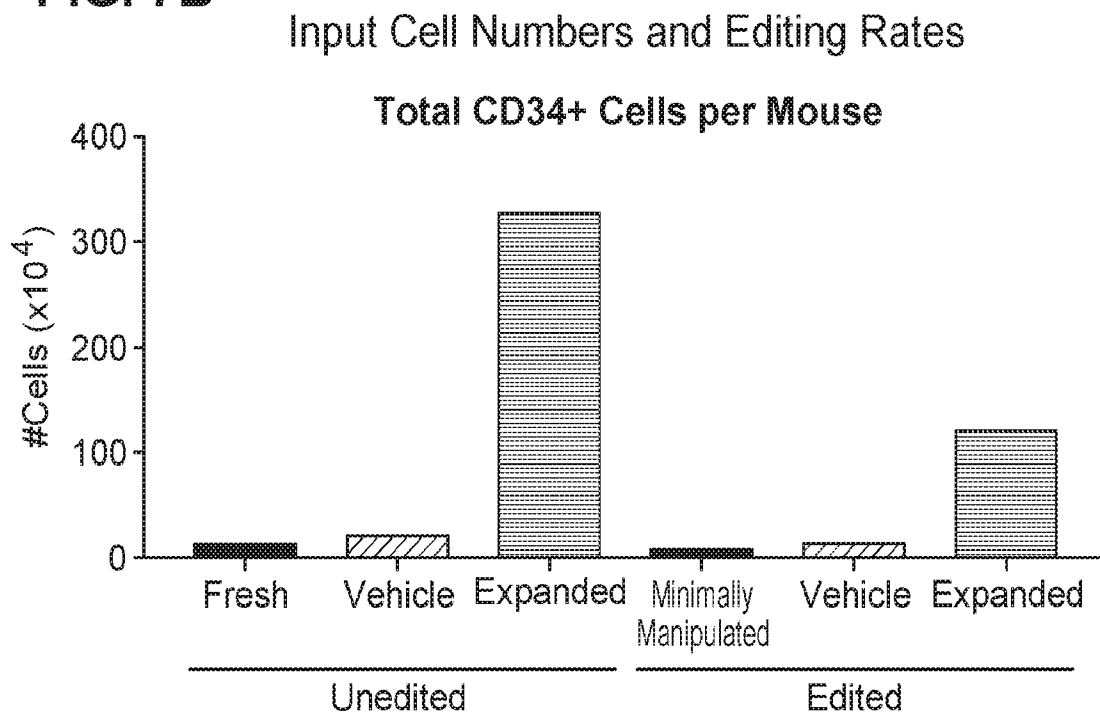
**FIG. 7A****FIG. 7B**

FIG. 7C

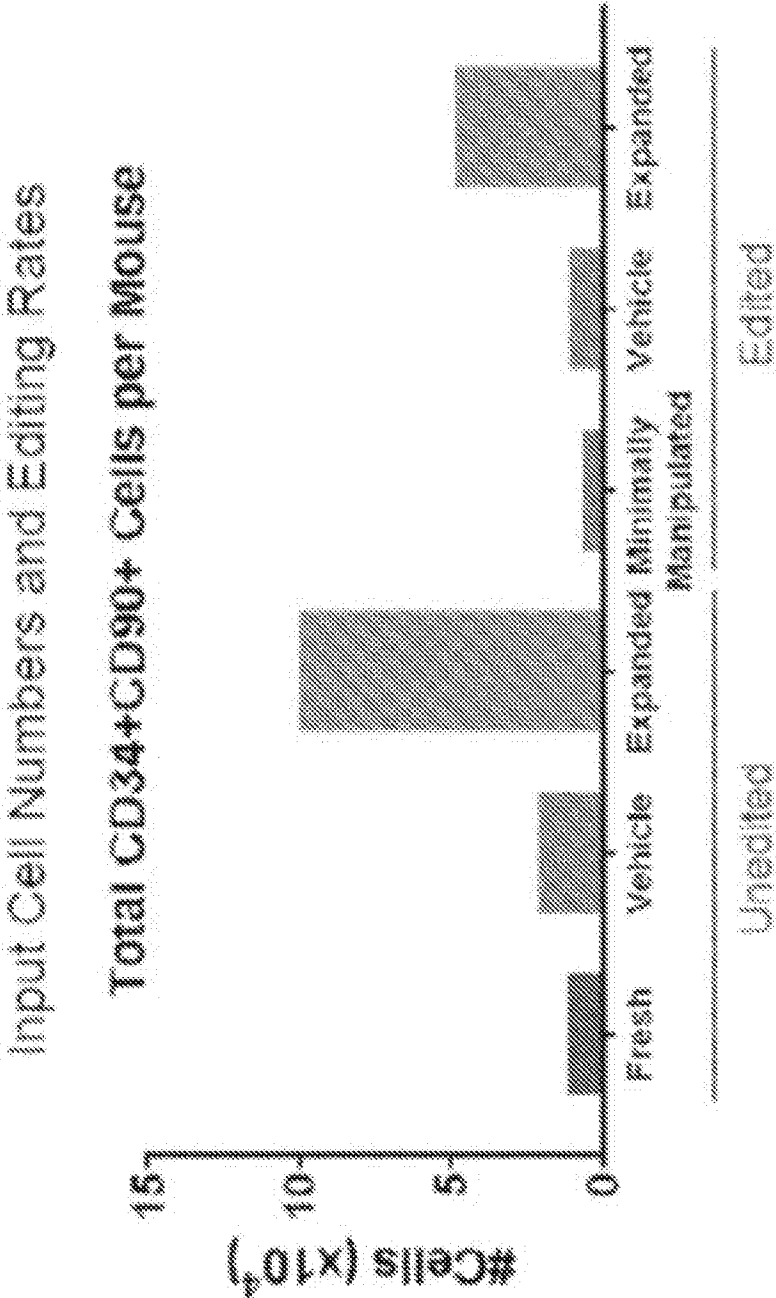


FIG. 7D

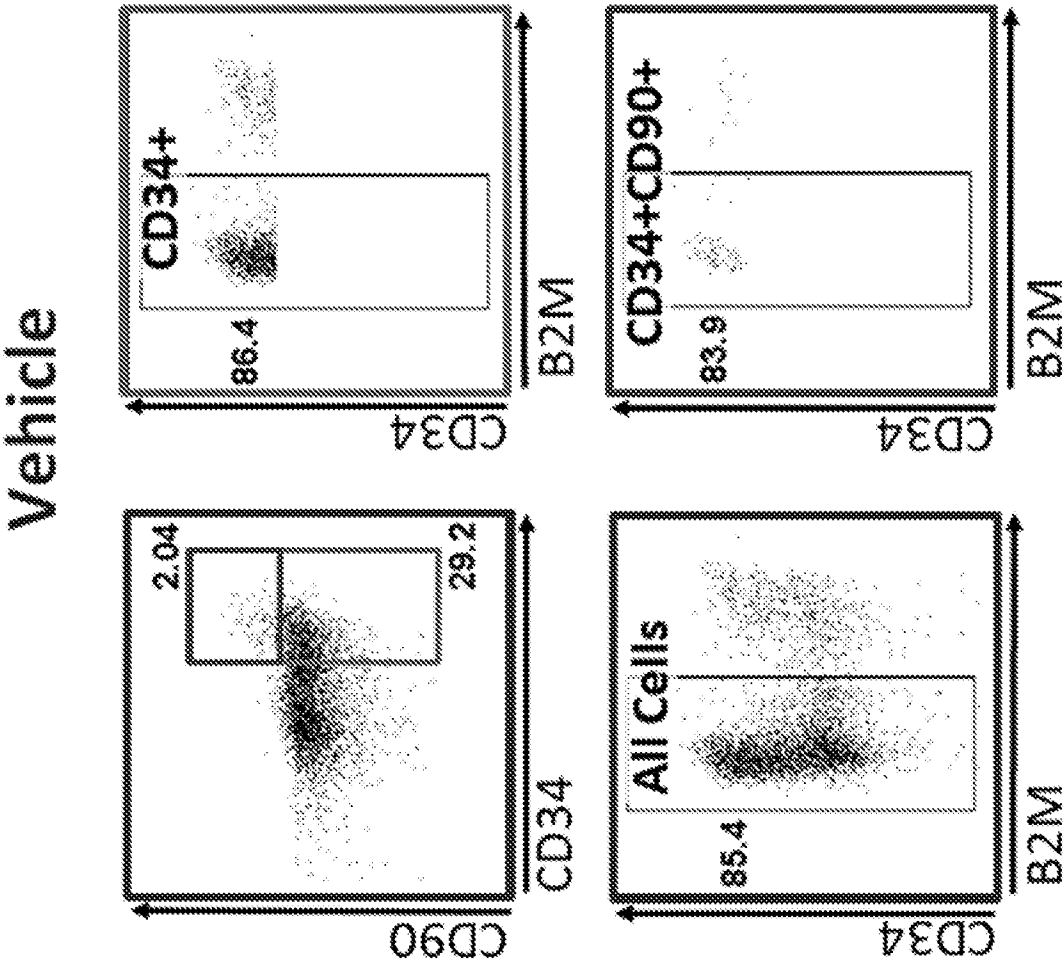
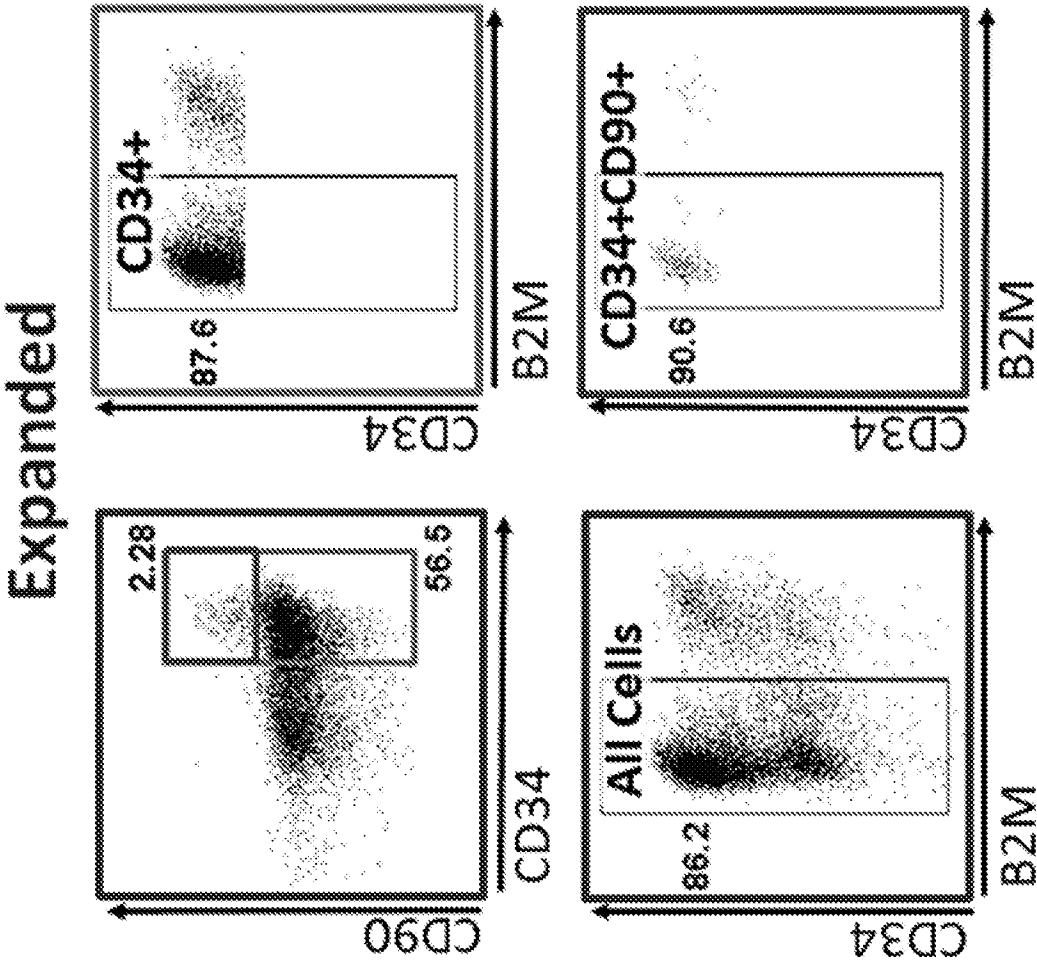
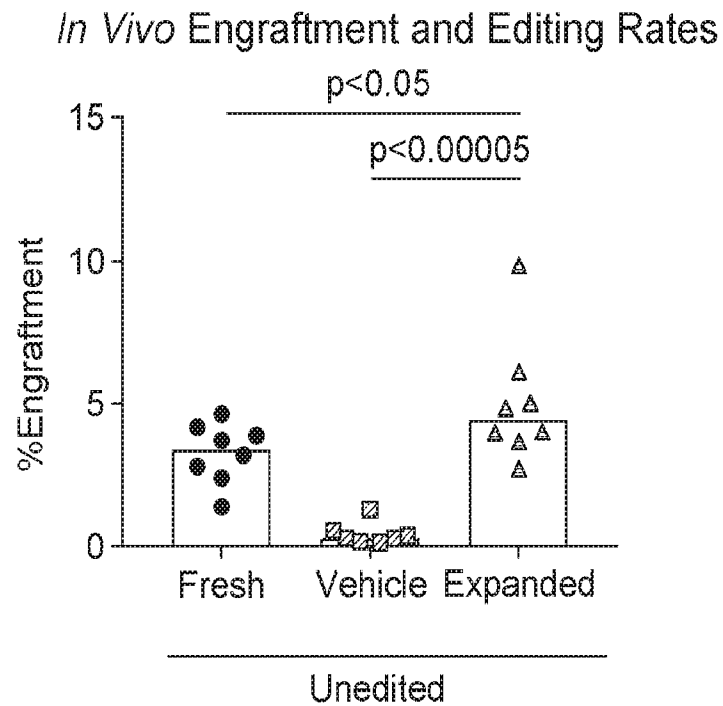
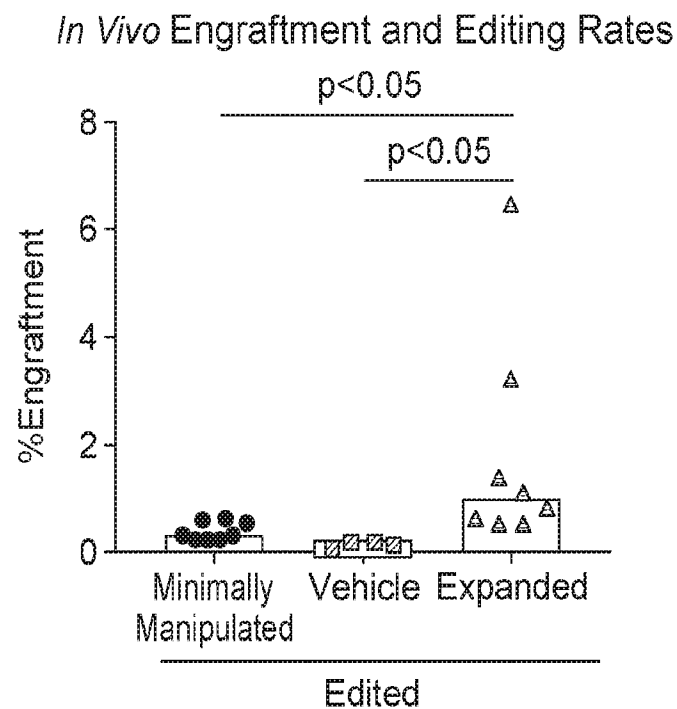




FIG. 7E

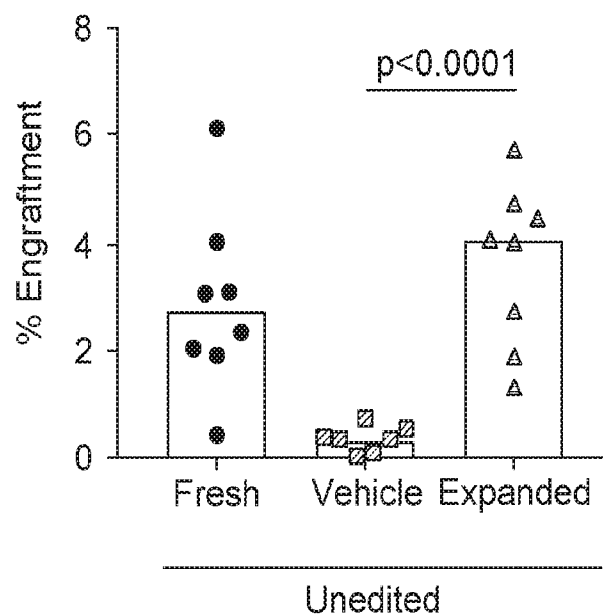


**FIG. 7F****FIG. 7G**



**FIG. 7I**

*In Vivo* Engraftment and Editing Rates  
Peripheral Blood

**FIG. 7J**

*In Vivo* Engraftment and Editing Rates  
Peripheral Blood

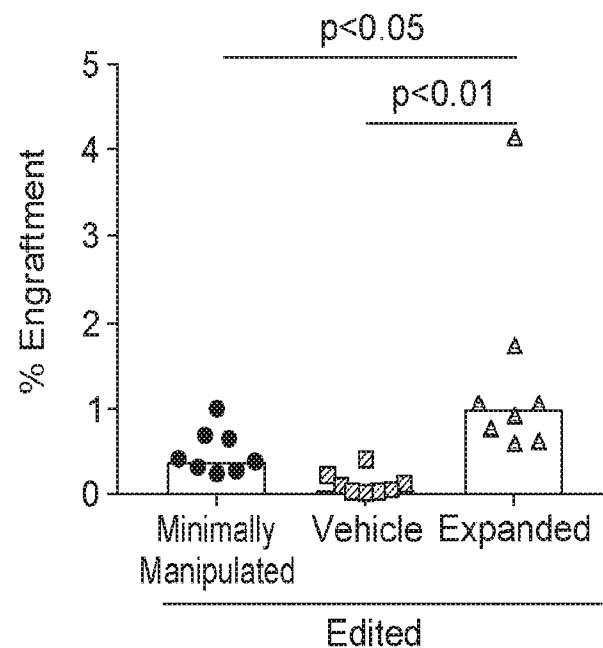
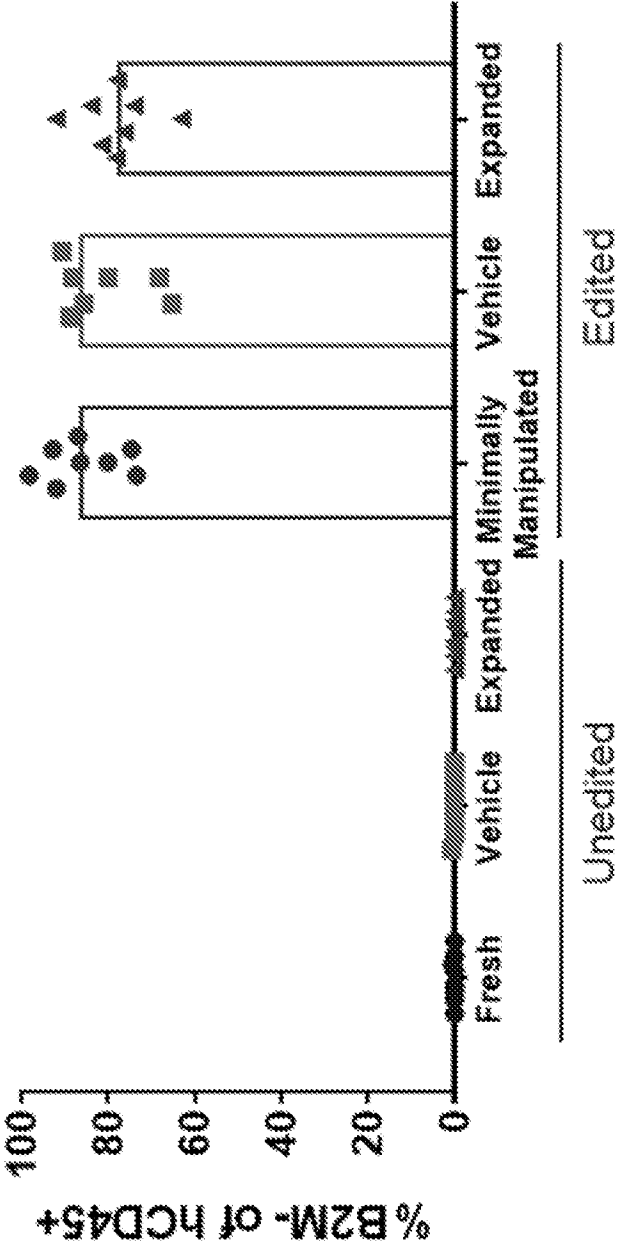


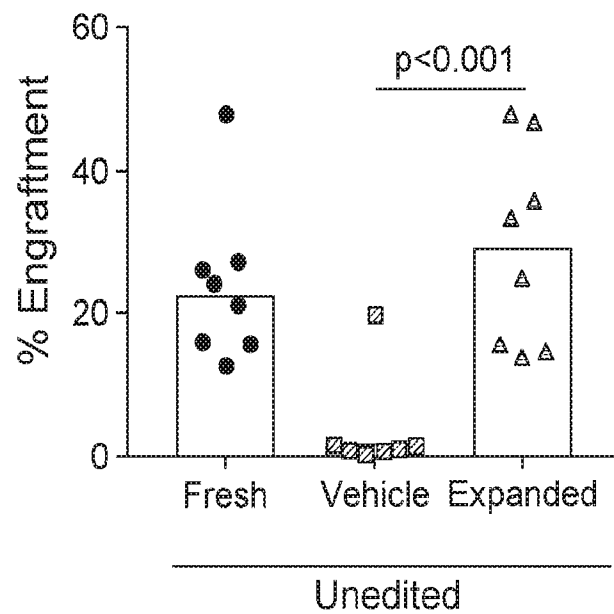
FIG. 7K

*In Vivo* Engraftment and Editing Rates  
Peripheral Blood



**FIG. 7L**

*In Vivo* Engraftment and Editing Rates  
Bone Marrow

**FIG. 7M**

*In Vivo* Engraftment and Editing Rates  
Bone Marrow

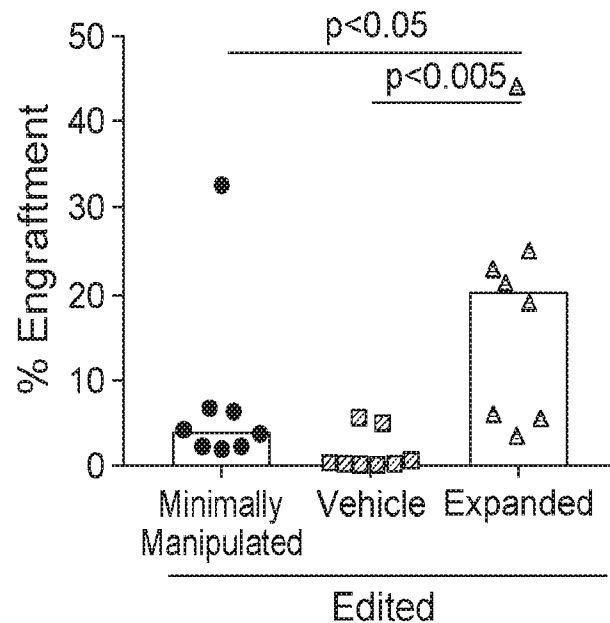


FIG. 7N

*In Vivo* Engraftment and Editing Rates  
Bone Marrow

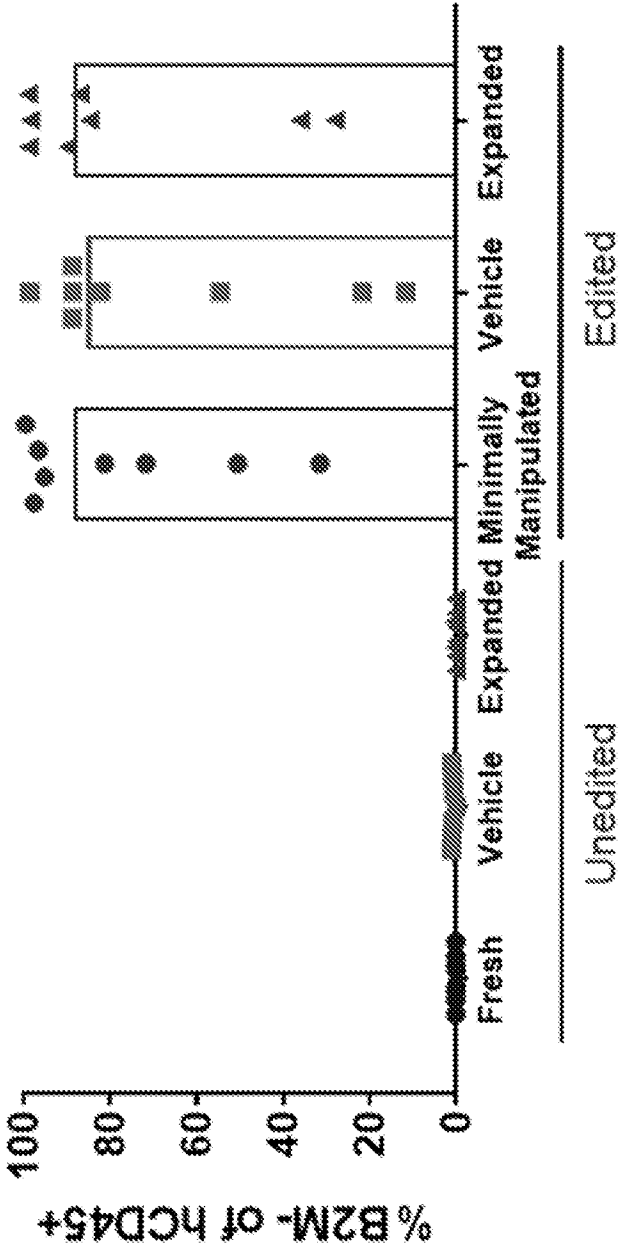


FIG. 8

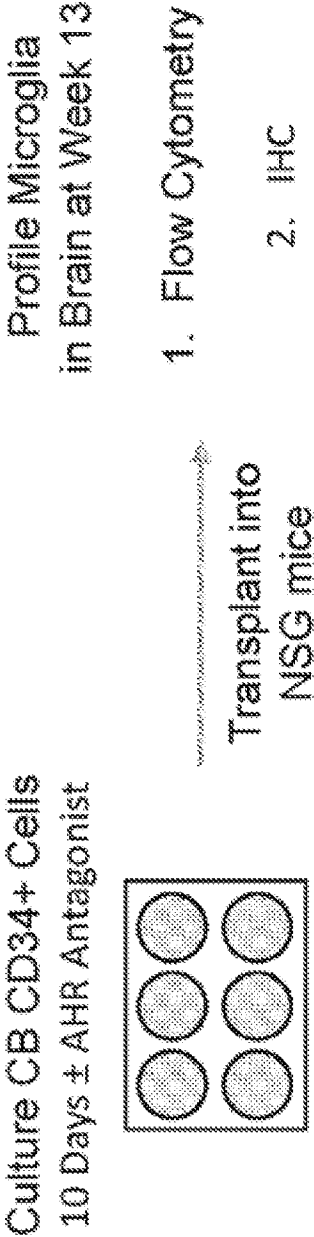




FIG. 9A

hCD45+CD11b+ Number

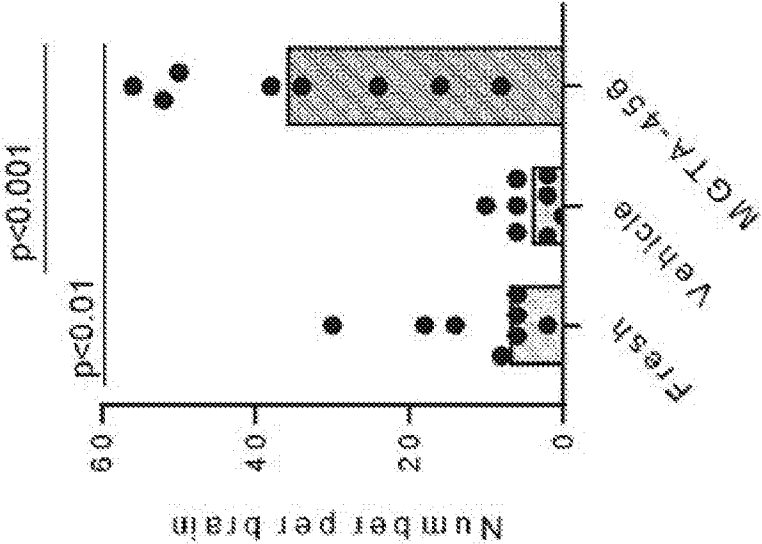
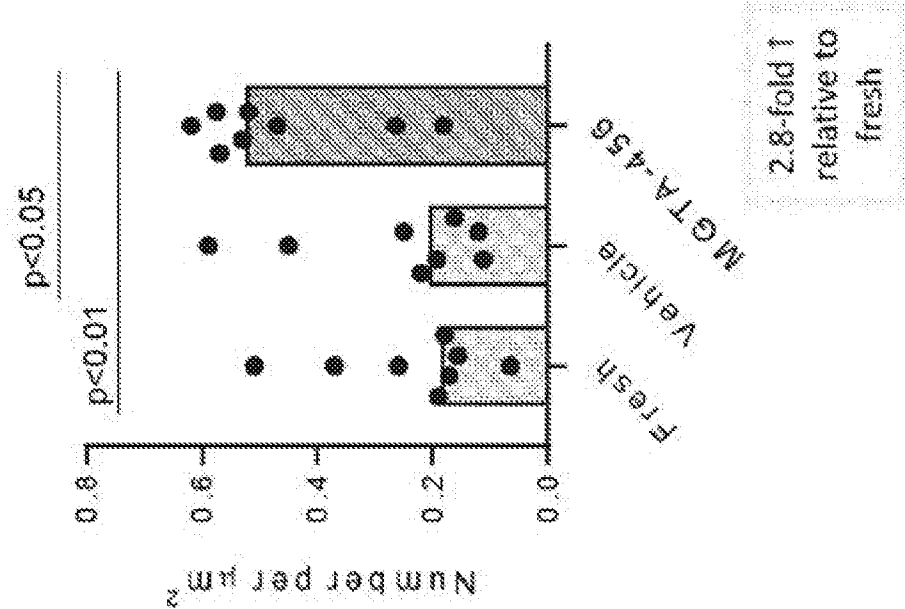


FIG. 9B

Ku80+lba-1+ Number



**FIG. 10**  
Microglia Engraftment in NSG Brain  
hCD45+CD11b+ Number

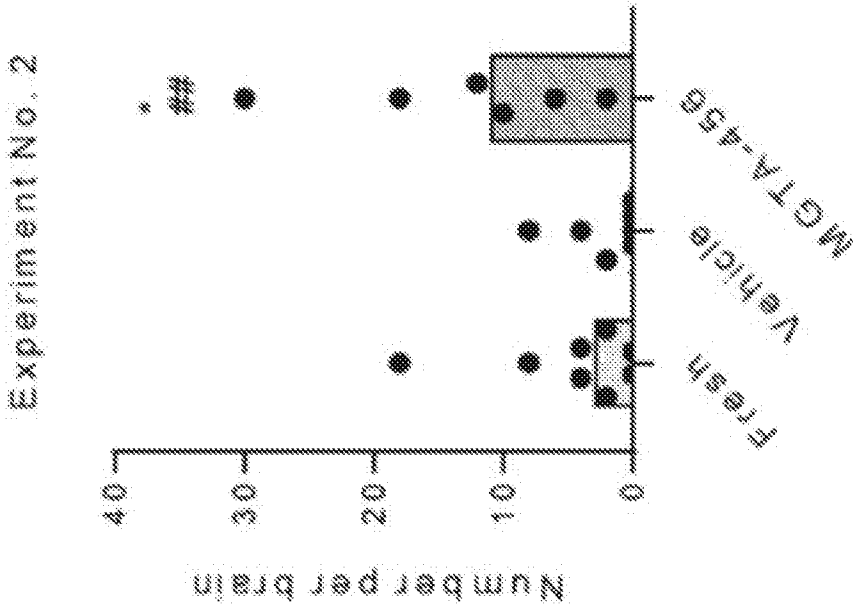
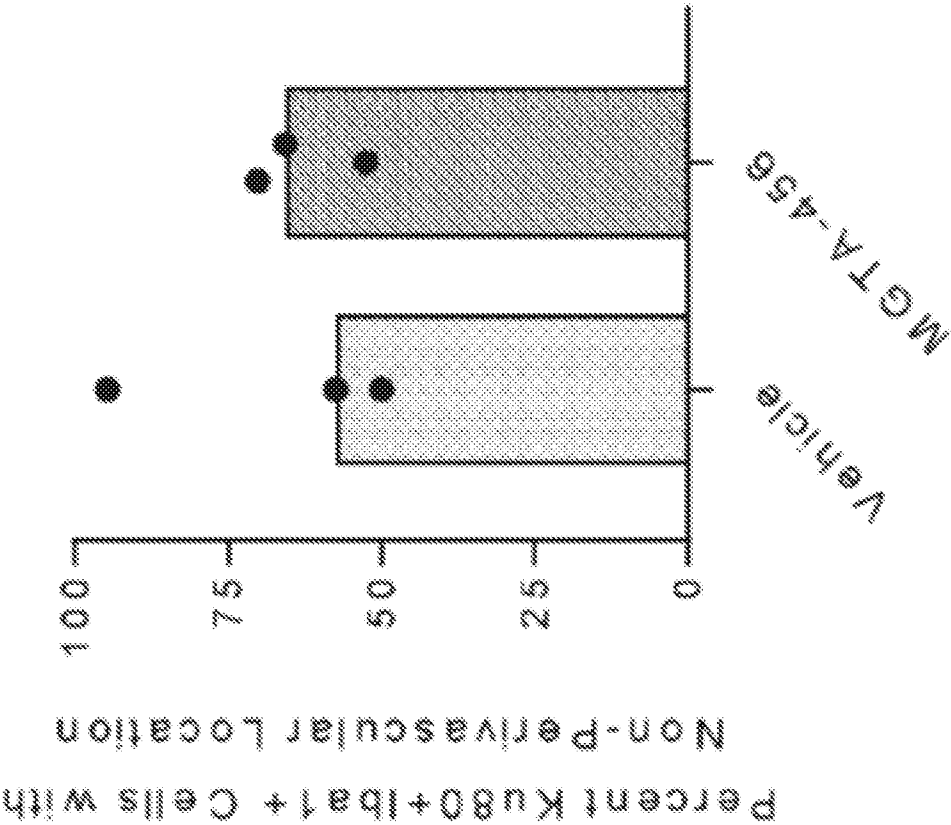
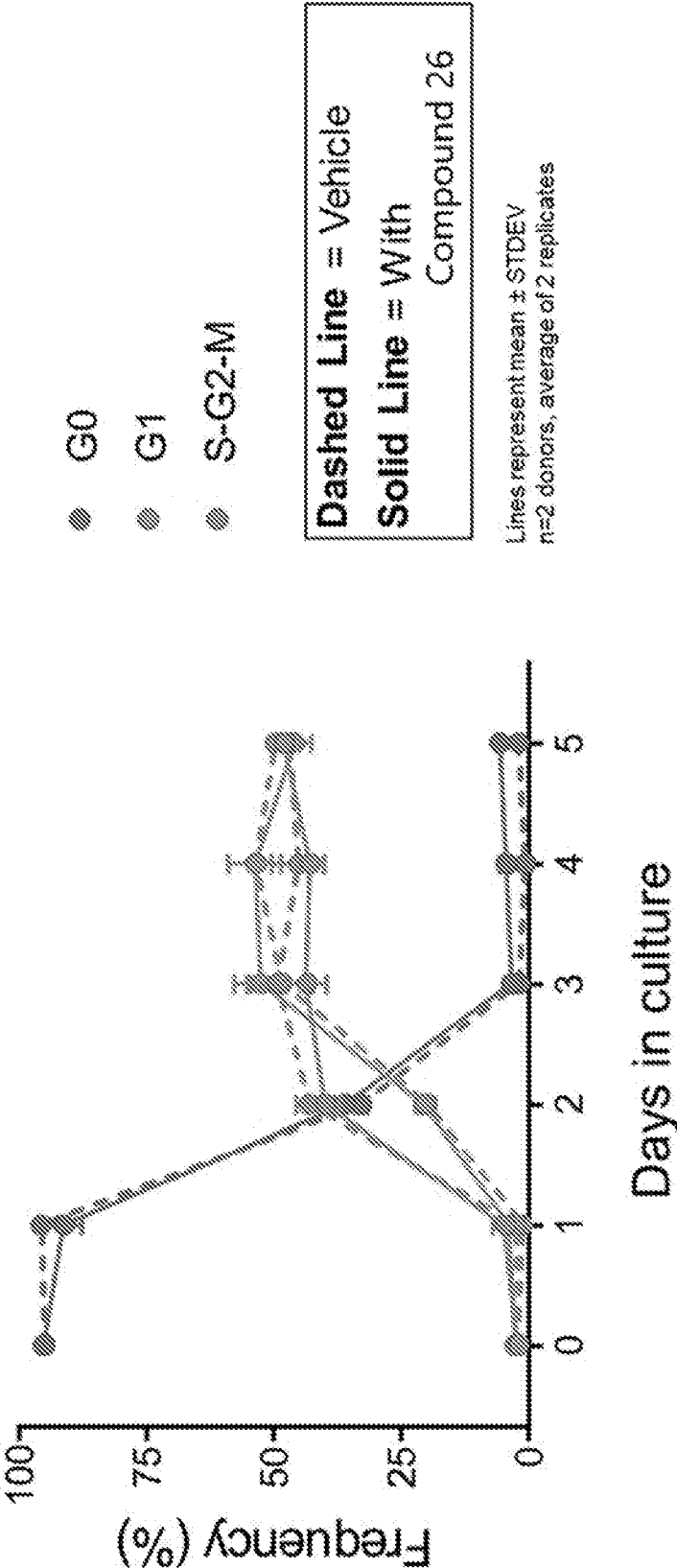


FIG. 11



**FIG. 12A**  
**Cell Cycle of**  
**CD34+CD90+ Mobilized Peripheral Blood Cells**



**FIG. 12B**  
**Cell Cycle of**  
**CD34+CD90+ Cord Blood Cells**

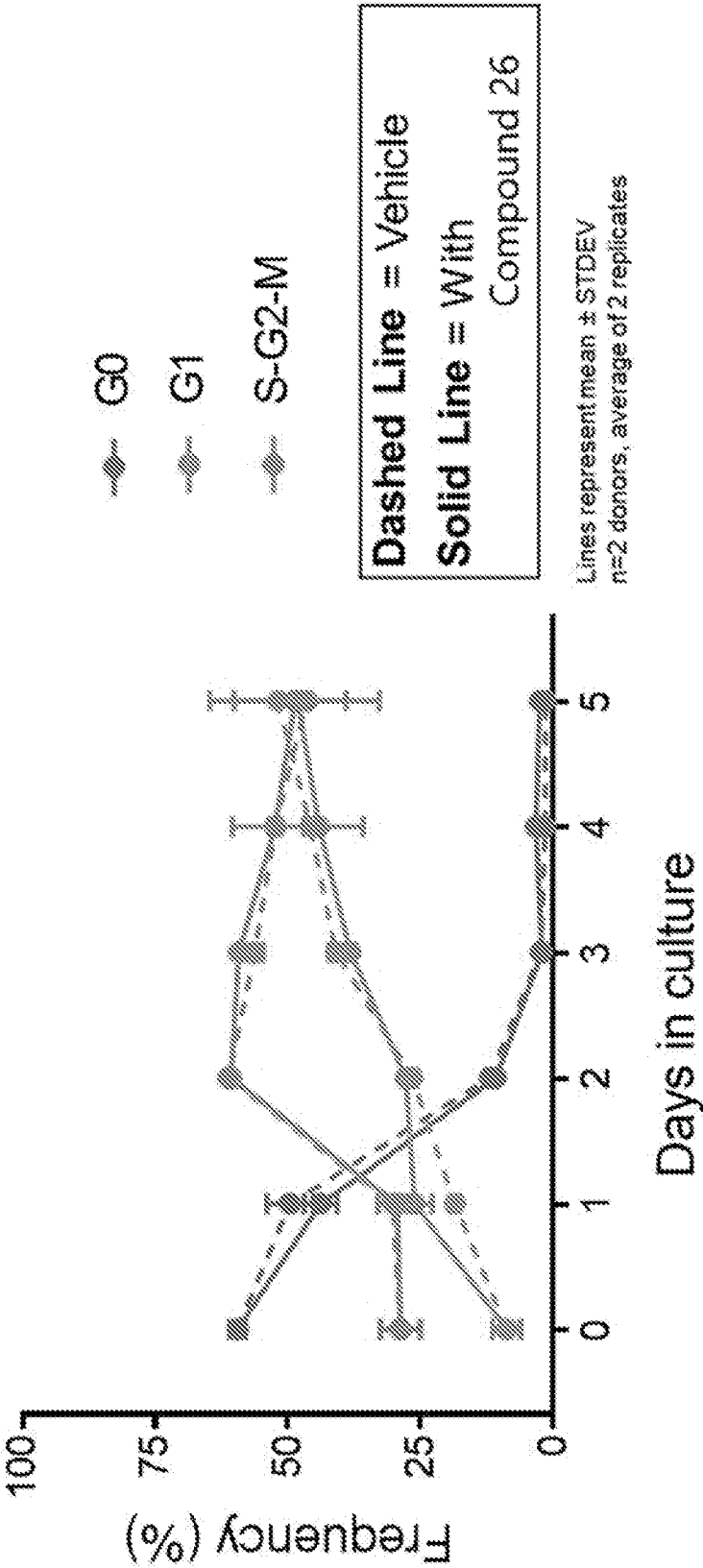


FIG. 13A

Correction Rate in  
Mobilized Peripheral Blood

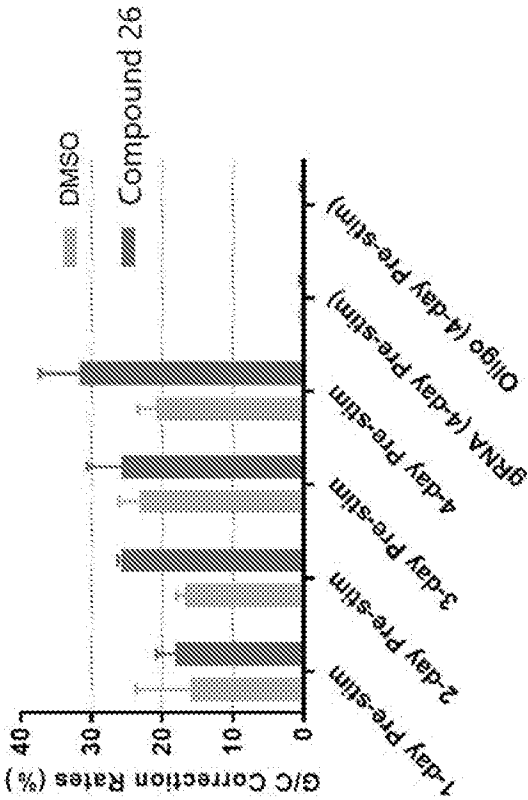
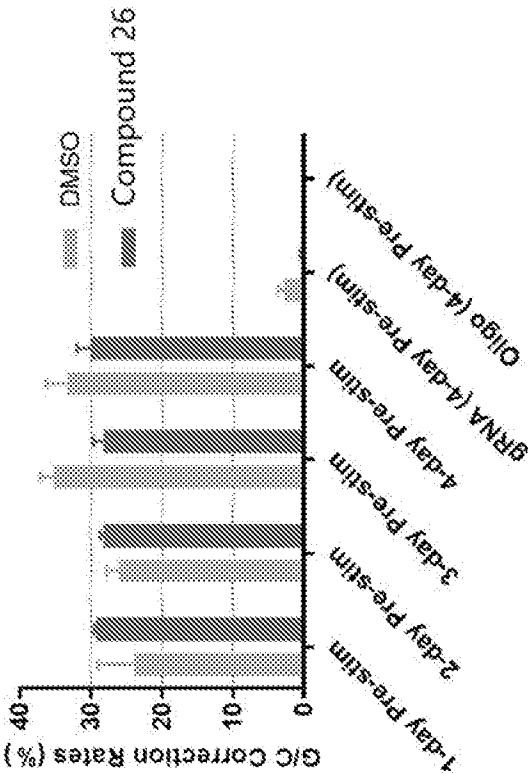


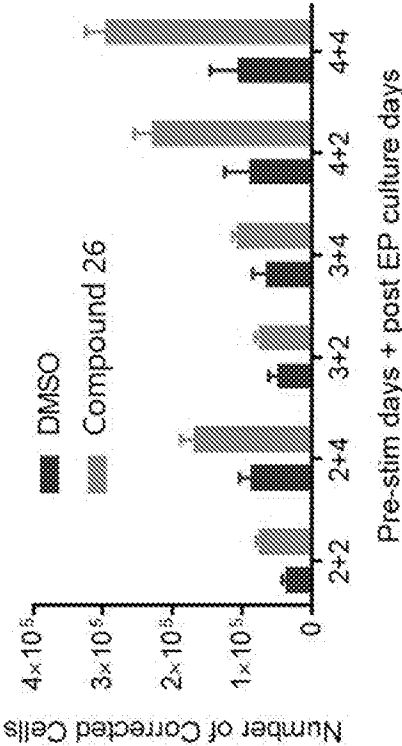
FIG. 13B

Correction Rate in  
Cord Blood



**FIG. 14A**

**Number of Corrected  
Mobilized Peripheral Blood  
Cells**



**FIG. 14B**

**Number of Corrected Cord  
Blood Cells**

