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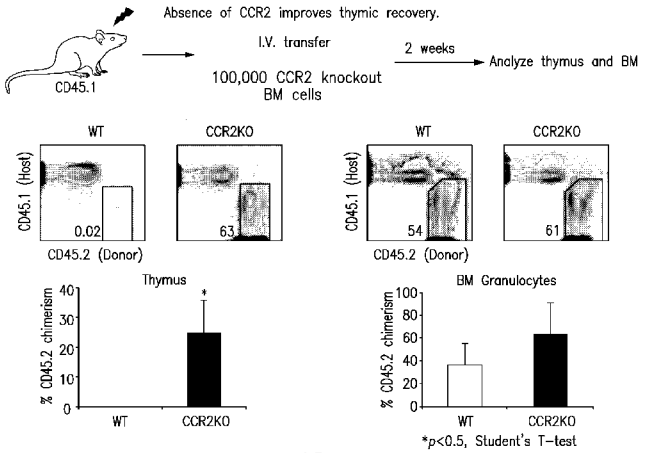


FIG. 1

(57) Abstract: The presently disclosed subject matter provides that blockade of CCR2 alone or CCR2 and CCR5 together improves thymic recovery and thymic immune function and treats or prevents graft versus host disease ("GVHD") and organ transplant rejection. Accordingly, the disclosed subject matter provides methods for increasing thymic recovery or thymic function in a subject, e.g., a human subject, by administering to the subject an effective amount of a CCR2 antagonist, a CCR5 antagonist and a CCR2 antagonist, or a dual CCR2/CCR5 antagonist. The presently disclosed subject matter also provides methods for treating, inhibiting, or preventing GVHD or organ transplant rejection in a subject, e.g., a human subject, by administering to the subject an effective amount of a CCR2 antagonist alone, a CCR5 antagonist and a CCR2 antagonist, or a dual CCR2/CCR5 antagonist.

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METHODS FOR IMPROVING THYMIC RECOVERY AND PREVENTING AND TREATING GRAFT VERSUS HOST DISEASE USING CCR2 AND CCR5 ANTAGONISTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to United States Provisional Application No. 61/731,943, filed November 30, 2012, which is hereby incorporated by reference in its entirety.

GRANT INFORMATION

This invention was made with government support under R01 HL110741 awarded by the National Heart Lung and Blood Institute. The government has certain rights in the invention.

BACKGROUND

Allogeneic stem cell transplantation ("SCT") is performed in 28,000 patients annually as a curative procedure in a variety of blood cancers. Allogeneic SCT is primarily indicated in chemotherapy-resistant cancers of the blood or bone marrow, including multiple myeloma, lymphoma and leukemia, and is effective in part due to the graft-versus-tumor (GvT) potential of the donor graft. Transplantation of donor stem cells after ablation of the recipient's native blood and bone marrow progenitors can restore essential immune and hematopoietic tissue, and donor leukocytes also aid in destruction of cancerous cells or tumors in the recipient. However, the success of allogeneic SCT can be limited by poor immune recovery and graft versus host disease ("GVHD"), a serious complication in which the transplanted cells migrate to and are activated against the recipient's tissues, damaging in particular the gut, liver and skin. 20-50% of matched related transplant recipients and 50-70% of unrelated donor transplant recipients develop clinically significant GVHD in spite of standard prophylactic immunosuppression. Certain estimates show that 15% of deaths following allogeneic SCT are caused by GVHD, and another 15% of deaths following allogeneic SCT are the result of infections related to poor immune recovery (Pasquini M., Wang Z. Current use and outcome of hematopoietic stem cell

transplantation: CIBMTR summary slides, available at: http://www.cibmtr.org). The combination of GVHD and poor immune recovery contributes to the morbidity and mortality in SCT recipients.

Autologous SCT is performed in 10,000 patients in the US alone each year. It is considered standard-of-care in certain malignancies such as myeloma, lymphoma and testicular cancer. Poor immune recovery contributes to morbidity and mortality after autologous SCT and faster and better recovery of the immune system can improve the outcome of patients after autologous SCT, including improved survival.

Migration of donor lymphocytes and progenitor cells plays a role in GVHD and immune recovery, as well as in the destruction of cancerous cells.

With respect to immune recovery, T-cell development in the thymus is dependent on a continuous supply of bone marrow-derived T-lineage progenitor cells. It has been shown that thymic recovery following SCT is highly dependent on progenitor T-cell chemotaxis. (Zlotoff D. *et al. Blood* 2010;115:1897-905; Zlotoff D., *et al. Blood* 2011;118:1962-1970). This migration is governed by sets of chemokines and chemokine receptors, each of which modulates discrete elements of immune cell function and activation.

Two C-C Chemokine Receptors, C-C Chemokine Receptor Type 2 ("CCR2") and C-C Chemokine Receptor Type 2 ("CCR5") are players in the trafficking of monocytes/macrophages and in the functions of other cell types relevant to disease pathogenesis. CCR2 and CCR5 modulate functions of monocytes/macrophages and Th1 cells as well as non-immune cells and are both involved in the pathogenesis of animal models of rheumatoid arthritis (RA), Crohn's disease, transplant rejection, atherosclerosis, and accelerated intimal hyperplasia. (Zhao, Q. (2010) *Journal of Leukocyte Biology*, 88(41).

It has been demonstrated that inhibition of CCR5 impairs chemotaxis of T-cells without affecting other T-cell functions, and significantly decreases visceral GVHD in humans (Reshef R., *et al. N. Engl. J. Med.* 2012;367:135-145). The role of CCR2 in GVHD has also been studied in a GVHD mouse model (Terwey, *et al. Blood* 2005; 106:3322-30). Modalities currently in use for both prevention and treatment of GvHD in humans can be immunosuppressive, impair immune recovery

and can limit the GvT effect. Methods to prevent GvHD and accelerate immune recovery after SCT are needed.

SUMMARY

The present disclosed subject matter provides that blockade of either CCR2 alone or both CCR5 and CCR2 together improves thymic recovery and thymic immune function, and prevents graft versus host disease (GVHD) and organ transplant rejection.

In one aspect, the present subject matter provides an exemplary method for increasing thymic recovery or thymic function in a subject, *e.g.,* a human, by administering to the subject an effective amount of a CCR2 antagonist alone, a CCR5 antagonist and a CCR2 antagonist, or a dual CCR2/CCR5 antagonist. In certain embodiments, the CCR2 antagonist and the CCR5 antagonist are administered concurrently.

In certain embodiments, the CCR2 and/or CCR5 antagonists are antibodies. In certain embodiments, CCR2 and/or CCR5 antagonists are small molecules. In certain embodiments, the dual CCR2/CCR5 antagonist is a small molecule or peptide inhibitor.

In another aspect, the present subject matter provides method for treating or preventing graft versus host disease (GVHD) in a subject, *e.g.,* a human, by administering to the subject an effective amount of a CCR2 antagonist alone, a CCR5 antagonist and a CCR2 antagonist, or a dual CCR2/CCR5 antagonist. In still another aspect, the present subject matter provides method for treating or preventing and organ transplant rejection in a subject, *e.g.,* a human, by administering to the subject an effective amount of a CCR2 antagonist alone, a CCR5 antagonist and a CCR2 antagonist, or a dual CCR2/CCR5 antagonist. In certain embodiments, the subject has undergone a solid organ transplant or a bone marrow transplant. In certain embodiments, the CCR2 antagonist and the CCR5 antagonist are administered concurrently. In certain embodiments, the subject is has undergone hematopoietic stem cell transplantation.

In certain embodiments, the CCR2 and/or CCR5 antagonists are antibodies. In certain embodiments, CCR2 and/or CCR5 antagonists are small

molecules. In certain embodiments, the dual CCR2/CCR5 antagonist is a small molecule or peptide inhibitor.

In certain embodiments, the dual CCR2/CCR5 antagonist is a small molecule, *e.g.,* cenicriviroc.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1. Absence of CCR2 or CCR5 improves thymic recovery. Mice conditioned with irradiation received bone marrow from congenic $CCR2^{-/-}$ mice or wild type mice. After two weeks the mice were sacrificed and their thymi and bone marrows analyzed. Mice transplanted with $CCR2^{-/-}$ bone marrow demonstrated better repopulation of the thymus with hematopoietic cells as opposed to mice transplanted with wild type bone marrow. There was no significant impact on bone marrow engraftment. Similar results were obtained with $CCR5^{-/-}$ bone marrow.

DETAILED DESCRIPTION

The presently disclosed subject matter provides that blockade of CCR2 alone or CCR2 and CCR5 together improve thymic recovery and thymic immune function and treats or prevents GVHD and organ transplant rejection. In one aspect, the present disclosed subject matter is based, at least in part, on the identification that antagonism of certain chemokine receptors, *i.e.,* CCR2 alone or CCR2 and CCR5 (either separately or as a dual antagonist), can increase trafficking of progenitor cells to the thymus to thereby increase thymic recovery and immune function. As described herein, studies in knockout mouse models indicate that CCR5 and CCR2 signaling impairs migration of progenitor T-cells to the thymus, thereby contributing to impaired T-cell recovery in conditioned mice. Accordingly, the disclosed subject matter provides methods for increasing thymic recovery or thymic function in a subject, *e.g.,* a human subject, by administering to the subject an effective amount of a CCR2 antagonist, a CCR5 antagonist and a CCR2 antagonist, or a dual CCR2/CCR5 antagonist. Administration of a CCR5 antagonist alone for increasing thymic recovery or thymic function is not encompassed in the present disclosure.

In another aspect, the present disclosed subject matter is also based, at least in part, on the determination that antagonism of certain chemokine receptors,

i.e., CCR2 alone or CCR2 and CCR5 together (either separately or as a dual antagonist), can decrease chemotaxis of T-cells to host organs following stem cell transplantation (or organ transplantation) and thereby prevent graft versus host disease (GVHD) or organ transplant rejection. Accordingly, the disclosed subject matter provides methods for treating, inhibiting, or preventing GVHD or organ transplant rejection in a subject, *e.g.,* a human subject, by administering to the subject an effective amount of a CCR2 antagonist alone, a CCR5 antagonist and a CCR2 antagonist, or a dual CCR2/CCR5 antagonist. Administration of a CCR5 antagonist alone for treating, inhibiting, or preventing GVHD or organ transplant rejection is not encompassed in the present disclosure.

In certain embodiments, the antagonism of CCR2 and CCR5 at the same time has an additive effect on thymic recovery or thymic function and/or on treating, inhibiting, or preventing GVHD or organ transplant rejection. In certain embodiments, the antagonism of CCR2 and CCR5 at the same time has a synergistic effect on thymic recovery or thymic function and/or on treating, inhibiting, or preventing GVHD or organ transplant rejection.

In certain embodiments, the antagonists of the disclosed subject matter can be administered to a subject in need of improved thymic function, thymic recovery, or immune recovery for any reason (*e.g.,* as a consequence of HIV or AIDS, during treatment for cancer, or as a result of suppression of the immune system related to immune-suppressing drugs or disease), to improve, increase or enhance thymic recovery and/or immune function. It is known that patients with HIV infection, AIDS, congenital immunodeficiency or acquired deficiency of T-cell numbers or function (for example, as a result of cancer therapy or other drugs that suppress the immune system), are at risk for infections due to poor immune function. Improvement in T-cell number or function can be achieved by dual blockade of CCR5 and CCR2, or by blockade of CCR2, by improving the trafficking of progenitor T-cells to the thymus, leading to clinical benefit.

In certain embodiments, the methods of the disclosed subject matter can be used to treat a subject at risk for GVHD following hematopoietic stem cell transplantation (HSCT), and following destruction of the immune system by irradiation, *e.g.,* total body irradiation, and/or chemotherapy. Likewise, the methods

of the disclosed subject matter can be used to treat a subject at risk for GVHD following allogeneic HSCT.

HSCT can be autologous (using haematopoietic stem cells (HSC) from the patient), or allogeneic (using HSC from a donor). Conditions treated with HSCT include, for example, multiple myeloma, leukemia, pediatric cases where the subject has an inborn defect such as severe combined immunodeficiency or congenital neutropenia with defective stem cells, and also children or adults with aplastic anemia who have lost their stem cells after birth. Other conditions treated with stem cell transplants include sickle-cell disease, myelodysplastic syndrome, neuroblastoma, lymphoma, Ewing's Sarcoma, Desmoplastic small round cell tumor, testicular cancer, germ cell tumors, chronic granulomatous disease and Hodgkin's disease.

In certain embodiments, the antagonists of the disclosed subject matter can be administered to a subject following organ transplant, *e.g.,* solid organ transplant or bone marrow transplant, to treat or prevent rejection of the transplanted organ or cells in the subject.

Definitions

CCR2 is a G protein-coupled receptor that binds multiple ligands, known as macrophage chemoattractant proteins, including CCL2 (MCP-1), CCL8 (MCP-2), CCL7 (MCP-3), and CCL13 (MCP-4). CCR2 is considered to be the exclusive receptor for MCP-1. CCR2 is expressed on the so-called "inflammatory" subset of blood monocytes. It is also expressed on, and functions in, other immune/inflammatory cell types such as dendritic cells and memory Th1 cells (Zhao, Q. (2010) *Journal of Leukocyte Biology*, 88(41).

CCR5 is also a G protein-coupled receptor that binds multiple ligands, including CCL4 (MIP-1β), CCL5 (RANTES), CCL3 (MIP-1α), CCL8 (MCP-2), and CCL3L1 (MIP-1α /LD78β). CCR5 is expressed predominantly on macrophages differentiated from blood monocytes and Th1 cells activated in response to inflammatory stimuli. It is also expressed on non-immune cells such as osteoclasts and VSMCs (Zhao, Q. (2010) *Journal of Leukocyte Biology*, 88(41).

An "antagonist" to CCR2 or CCR5, refers to any agent that blocks, suppresses or reduces CCR2 or CCR5 biological activity. A "CCR2/CCR5 dual

antagonist" is an antagonist agent that blocks, suppresses or reduces both CCR2 and CCR5 biological activity.

The term "antagonist" does not imply a specific mechanism of biological action and is deemed to expressly include and encompass all pharmacological, physiological, and biochemical interactions with CCR2 and/or CCR5 whether direct or indirect, or whether interacting with CCR2 and/or CCR5, or through another mechanism, and its consequences which can be achieved by a variety of different, and chemically divergent, compositions. Exemplary CCR2 and/or CCR5 antagonists include, but are not limited to, anti-CCR2 or anti-CCR5 antibodies, an anti-sense molecule directed to CCR2 or CCR5 (including an anti-sense molecule directed to a nucleic acid encoding CCR2 or CCR5), a CCR2 and/or CCR5 inhibitory compound, *e.g.,* a small molecule, a CCR2 and/or CCR5 peptide antagonist, a CCR2 or CCR5 structural analog, and a mutation resulting in a decrease or inhibition of CCR2 or CCR5 activity. In certain embodiments, a dual CCR2/CCR5 antagonist that can be used in the methods of the present subject matter is cenicriviroc (CVC or TBR-652, formerly known as TAK-652; Tobira Therapeutics).

For purpose of the present subject matter, it will be explicitly understood that the term "antagonist" encompass all the previously identified terms, titles, and functional states and characteristics whereby the CCR2 and/or CCR5 itself, a CCR2 and/or CCR5 biological activity, or the consequences of the biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree. In certain embodiments, a CCR2 and/or CCR5 antagonist binds (physically interacts with) CCR2 or CCR5 (*e.g.*, an antibody), and/or reduces (impedes and/or blocks) CCR2 and/or CCR5 receptor signaling. Examples of types of CCR2 and CCR5 antagonists are provided herein.

An "antibody" (interchangeably used in plural form) as used herein is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')2, Fv), single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion, humanized antibodies, chimeric antibodies, diabodies linear antibodies, single chain antibodies,

multispecific antibodies (*e.g.*, bispecific antibodies) and any other modified configuration of the immunoglobulin molecule that includes an antigen recognition site of the required specificity. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these can be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

A "monoclonal antibody" refers herein to a homogeneous antibody population wherein the monoclonal antibody includes amino acids (naturally occurring and non-naturally occurring) that are involved in the selective binding of an antigen. Monoclonal antibodies are highly specific, being directed against a single antigenic site. The term "monoclonal antibody" encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')2, Fv), single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that includes an antigen recognition site of the required specificity and the ability to bind to an antigen. It is not intended to be limited as regards to the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, etc.).

"Humanized" antibodies refer herein to a molecule having an antigen binding site that is substantially derived from an immunoglobulin from a non-human species and the remaining immunoglobulin structure of the molecule based upon the structure and/or sequence of a human immunoglobulin. The antigen binding site can include either complete variable domains fused onto constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate framework regions in the variable domains. Antigen binding sites can be wild type or modified by one or more amino acid substitutions, *e.g.*, modified to resemble human

immunoglobulin more closely. Some forms of humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody. In some instances, framework region (FR) residues or other residues of the human immunoglobulin replaced by corresponding non-human residues. Furthermore, humanized antibodies can include residues which are not found in the recipient antibody or in the donor antibody.

An "individual" or "subject" herein is a vertebrate, such as a human or a non-human animal. Non-limiting examples of non-human animals include primates, farm animals, sport animals, rodents and pets.

An "effective amount" of a substance as that term is used herein is that amount sufficient to effect beneficial or desired results, including clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. In the context of administering a composition that increases thymic recovery or immune function, an effective amount of an agent which is an antagonist to CCR2 alone, or CCR5 and CCR2, is an amount sufficient to achieve such a modulation as compared to the thymic recovery obtained when there is no antagonist administered. In the context of administering a composition that prevents or treats GVHD, an effective amount of an agent which is an antagonist to CCR2 alone, or CCR5 and CCR2, is an amount sufficient to achieve such a modulation as compared to the GVHD that occurs when there is no antagonist administered. An effective amount can be administered in one or more administrations. In certain embodiments, a CCR2 antagonist and a CCR5 antagonist can be administered at the same time (*e.g.*, concurrently), or at separate times. In certain embodiments, one or more CCR2 antagonists, one or more dual CCR2/CCR5 antagonists or one or more CCR2 and CCR5 antagonists can be administered at the same time (*e.g.*, concurrently), or at separate times. In certain embodiments, a dual CCR2/CCR5 antagonist is administered. Administration of a CCR5 antagonist alone is not encompassed in the present disclosure.

As used herein, and as well-understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. For purposes of this subject matter, beneficial or desired clinical results include, but are not limited to, alleviation or amelioration of one or more symptoms, diminishment of

extent of disease, stabilized (i.e., not worsening) state of disease, prevention of disease, delay or slowing of disease progression, and/or amelioration or palliation of the disease state. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

A "pharmaceutical composition" as used herein is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for therapeutic use in vitro, in vivo or ex vivo.

As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For non-limiting examples of carriers, stabilizers and adjuvants, see Martin, REMINGTON'S PHARM. SCI., 15th Ed. (Mack Publ. Co., Easton (1975)).

CCR2 and CCR5 Antagonists

The methods for increasing thymic recovery and immune function and treating or preventing GVHD or organ transplant rejection include administering a CCR2 antagonist, a CCR5 antagonist and a CCR2 antagonist, or a dual CCR2/CCR5 antagonist. Examples of such agents include, but are not limited to, antibodies and fragments thereof, small molecules, peptide inhibitors, and antisense RNA and RNA interference agents. In certain embodiments, the methods include administering one or more antagonist that is capable of reducing or partially inhibiting or completely inhibiting the activity of CCR2 alone or CCR2 and CCR5.

CCR2 Antagonists

Suitable CCR2 antagonists for use with the methods described herein include, but are not limited to, small molecule pharmaceutical compounds, peptide antagonists, and antibodies. Anti-CCR2 antibodies are discussed in detail below. Examples of small molecule CCR2 inhibitors include, without limitation, MK-0812, produced by Merck (Beaulieu, A., Hasler F., Mola E. M., et al. The efficacy and safety of a CCR2 receptor antagonist in the treatment of rheumatoid arthritis (RA). Ann. Rheum. Dis., 2006;65:175-176), INCB-8696, produced by Incyte (Sharrack B.,

Leach T., Jacobsen E., et al. Frequent MRI study of a novel CCR2 antagonist in relapsing-remitting multiple sclerosis. Ann. Neurol., 2007;62:S74-S75), CCX140, produced by Chemocentryx (Struthers M., Curr. Topics Med. Chem., 2010;10:1278-1298), BMS-741672, produced by Bristol Myers Squibb (Struthers M., Pasternak A. Curr. Topics Med. Chem., 2010;10:1278-1298), JNJ17166864, produced by Johnson & Johnson (Buntinx M., Hermans B., et al. J. Pharm. Exp. Therap., 2008;327(1):1-9), CNTO888, produced by Centocor (Struthers M., Curr. Topics Med. Chem., 2010;10:1278-1298), INCB3284, produced by Incyte (Struthers M., Curr. Topics Med. Chem., 2010;10:1278-1298), and PF-04136309, produced by Pfizer (Struthers M., Curr. Topics Med. Chem., 2010;10:1278-1298).

Additional small molecule inhibitors of CCR2 that are suitable for use with the subject matter disclosed herein include without limitation the compounds disclosed in US2009/0233946, spiropiperidine-based compounds such as those described in US2008/318990 and WO2009/061881, and biarylaminopiperidinyl amide compounds such as those disclosed in WO2009/043747, the contents of which are hereby expressly incorporated by reference.

Examples of peptidyl CCR2 antagonists include MCP-1 (9-76), also referred to as MCP-1 7-ND (Gong J. H., et al. J. Exp. Med., 1997;186(1): 131-137), and the engineered fusokine construct GMME1 (Rafei M., et al. J. Immunol. 2009;183:1759-1766).

CCR5 Antagonists

Suitable CCR5 antagonists for use with the methods described herein include, but are not limited to, small molecule pharmaceutical compounds, peptidyl antagonists, and antibodies. Anti-CCR5 antibodies are discussed in detail below.

Non-limiting examples of small molecule CCR5 inhibitors include INCB009471, developed by Incyte (Stellbrink H. J. Antivir Chem Chemother. 2009;19(5):189-200), vicriviroc, developed by Schering-Plough (Schurmann D.,. et al. 11th Conference on Retroviruses and Opportunistic Infections. San Francisco, USA, 8-11 February 2004. Abstract 140LB), aplaviroc, developed by GlaxoSmithKline (Maeda K., et al. Journal of Virology 78:8654–8662), TAK-220 and TAK-652, developed by Takeda (Baba M, Nishimura O, Kanzaki N. PNAS, 1999;96:5698–5703, Iizawa Y, et. al. 10th Conference on Retroviruses and

Opportunistic Infections. Boston, USA, 10–14 February 2003. Abstract 11), and SCH-351125, developed by Schering-Plough (Strizki J. M., et al. PNAS 2001;98:12718–12723).

A non-limiting example of a suitable peptide inhibitor of CCR5 for use with the disclosed subject matter includes the peptide with amino acid sequence AFDWTFVPSLIL (Wang F. Y., et al. Biosci Biotechnol Biochem. 2006 Sep;70(9):2035-41). Examples of suitable antibodies include, without limitation, PRO 140, developed by Progenics (Khatib N., Das S. Recent Pat Antiinfect Drug Discov. 2010 Jan;5(1):18-22), ROAb13 and ROAb14, developed by Roche (Schanzer J., Jekle A., Nezu J., et al. Antimicrob Agents Chemother. 2011 May; 55(5): 2369–2378, Ji C., et al. Antiviral Res. 2007 May;74(2):125-37), and HGS004, produced by Human Genome Sciences (Roschke V., et al. 44th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, Abstract 2871; Washington, D C. October 30 - November 2, 2004).

Dual CCR2/CCR5 antagonists

Examples of small molecule dual CCR2/CCR5 antagonists include, without limitation, cenicriviroc (CVC or TBR-652, formerly known as TAK-652; Tobira Therapeutics) (USPN 8,183,273 and USPN 7,371,772), BMS-813160, produced by Bristol Myers Squibb (Norman P. Expert Opin Ther Pat. 2011; 21(12):1919-24), TAK-779, produced by Takeda (Yang Y. F., et al. Eur. J. Immunol., 2002;32(8):2124-2132), LYSN 2238290, produced by Eli Lilly (Singh J. P. et al. 91st Annual Scientific Sessions of the American Heart Association.:503 abstr. 3922, 8 Nov 2008), NIBR-803 and NIBR-177, produced by Novartis (Braddock, M. 11th annual Inflammatory and Immune Diseases Drug Discovery and Development Summit 12-13 March 2007, San Francisco, USA. Expert Opin. Investig. Drugs, 2007;16(6):909-917, Horuk, R. Nat. Rev. Drug Discov., 2009;8(1):23-33), and INCB10820/PF-4178903, produced by Pfizer (Zheng C., et al. Bioorg Med Chem Lett. 2011 Mar 1;21(5):1442-6). Additional non-limiting examples of small molecule dual CCR2/CCR5 inhibitors include the molecules disclosed for that purpose in patent applications WO2008/101905, WO2009/013211, and WO2008/014360, the contents of which are hereby expressly incorporated by reference.

An example of a suitable peptide compound for use with the disclosed subject matter includes, without limitation, RAP103, produced by Rapid Pharmaceuticals (Padi S. S., et al. Pain. 2012 Jan;153(1):95-106).

Antibodies to CCR2 or CCR5

In accordance with the present subject matter, anti-CCR2 or anti-CCR5 antibodies can be polyclonal or monoclonal; can be from any of a number of human, non-human eukaryotic, cellular, fungal or bacterial sources; can be encoded by genomic or vector-borne coding sequences; and can be elicited against native or recombinant CCR2 or CCR5 or fragments thereof with or without the use of an adjuvant, all according to a variety of methods and procedures well-known in the art for generating and producing antibodies. Generally, neutralizing antibodies against CCR2 or CCR5 are suitable for therapeutic applications. Examples of such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single-chain, and various human or humanized types of antibodies, as well as various fragments thereof, such as Fab fragments and fragments produced from specialized expression systems.

For example, to produce a CCR2 or CCR5 antibody, various host animals can be immunized by injection with a CCR2 or CCR5 product, or a portion thereof including, but not limited to, portions of the CCR2 or CCR5 in a recombinant protein. In certain embodiments, host animals can include but are not limited to rabbits, mice, and rats. In certain embodiments, adjuvants can be used to increase the immunological response, depending on the host species. Such adjuvants include, but are not limited to, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and can include useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

Additionally, monoclonal anti-CCR2 or -CCR5 antibodies can be prepared using any technique that provides for the production of antibody molecules by continuous cell lines in culture. Such techniques, include but are not limited to, the hybridoma technique originally described by Kohler and Milstein, 1975, Nature, 256:495-497, the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today, 4:72, Cote et al, 1983, Proc. Natl. Acad. Sci., 80:2026-2030) and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer

Therapy, Alan R. Liss, Inc., pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. Alternatively or additionally, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce single chain antibodies specific to CCR2 or CCR5.

Compositions comprising antagonists (e.g., antibodies) can include more than one antibody. For example, a composition comprising an antibody that binds CCR2 and an antibody that binds to CCR5 is contemplated.

In certain embodiments, a method for assessing the neutralizing ability of an antibody to CCR2 or CCR5 is to use an in vivo model. In certain embodiments, neutralization of CCR2 or CCR5 can be determined in vitro using a chemotaxis assay, for example.

Non-limiting examples of antibodies to CCR2 include MLN1202, produced by Millennium (Davidson M., et al. *Circulation*, 2007;116(16):II-172), and ABN912, produced by Novartis (Haringman, J. et al. *Arthritis Rheum.*, 2006;54(8):2387-2392).

Non-limiting examples of antibodies to CCR5 include PRO 140, developed by Progenics (Khatib N., Das S. PRO 140--a novel CCR5 co-receptor inhibitor. Recent Pat Antiinfect Drug Discov. 2010 Jan;5(1):18-22), ROAb13 and ROAb14, developed by Roche (Schanzer J. et al. Antimicrob Agents Chemother. 2011 May; 55(5): 2369–2378; Ji C, et al. Antiviral Res. 2007 May;74(2):125-37), and HGS004, produced by Human Genome Sciences (Roschke V., et al. 44th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, Abstract 2871; Washington, D C. October 30 - November 2, 2004).

Kits

The present subject matter also contemplates kits including a composition having a CCR2 antagonist, a CCR2 antagonist and a CCR5 antagonist, or a dual CCR2/CCR5 antagonist. The composition can include more than one antagonist. More than one antibody can be included in the kit, for example, an

antibody that binds to CCR2 and an antibody that binds to CCR5. Likewise, more than one small molecule antagonist can be included in the kit (*e.g.,* a CCR5 antagonist and a CCR2 antagonist). The antagonist(s) can be in any state suitable for packing in a kit, such as lyophilized or resuspended in a pharmaceutically acceptable excipient. The kit can further include instructions for use, such as dosing regimen, and/or adjuvants that can be used with the composition(s).

Methods of Using Antagonists

The disclosed subject matter provides methods for increasing thymic recovery and/or immune function in a subject, *e.g.,* a human subject, by administering to the subject an effective amount of a CCR2 antagonist alone, a CCR5 antagonist and a CCR2 antagonist (*e.g.,* concurrently), or a dual CCR2/CCR5 antagonist. The disclosed subject matter also provides methods for treating, inhibiting, or preventing GVHD or organ transplant rejection in a subject, *e.g.,* a human subject, by administering to the subject an effective amount of a CCR2 antagonist alone, a CCR5 antagonist and a CCR2 antagonist (*e.g.,* concurrently), or a dual CCR2/CCR5 antagonist.

In certain embodiments, the CCR2 antagonist and the CCR5 antagonist have an additive effect on thymic recovery and/or immune function, and/or on treating, inhibiting, or preventing GVHD or organ transplant rejection. In certain embodiments, the CCR2 antagonist and the CCR5 antagonist have a synergistic effect on thymic recovery and/or immune function, and/or on treating, inhibiting, or preventing GVHD or organ transplant rejection.

These methods are practiced by administering to an individual a composition comprising an effective amount of a CCR2 antagonist alone, a CCR5 antagonist and a CCR2 antagonist, or a dual CCR2/CCR5 antagonist (e.g., antibody or small molecule or peptide inhibitor) to inhibit or reduce CCR2 and/or CCR5 activity. The antagonist can be, for example, but not by way of limitation, a small molecule, an antibody or antibody fragment, or an antisense RNA or RNA interference agent.

For treatment, the composition including an effective amount of a CCR2 antagonist alone, a CCR5 antagonist and a CCR2 antagonist, or a dual CCR2/CCR5 antagonist (e.g., neutralizing antibody or small molecule) can be administered as a pharmaceutical composition by using a pharmaceutically acceptable

excipient or carrier. Two separate compositions, one including a CCR2 antagonist, and one including a CCR5 antagonist, can also be administered as separate pharmaceutical compositions to the subject. The composition(s) can be administered orally or parenterally (*e.g.*, intravenous, subcutaneous, intramuscular, and intraperitoneal) alone or by formulating together with a pharmaceutically acceptable carrier in the form of a solid preparation such as tablet, capsule, granule, etc. or powder, or a liquid preparation such as syrup, injectable solution, etc. In certain embodiments, the compositions are prepared with carriers that will protect the compound(s) against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid.

Examples of a dosage form for parenteral administration include injectable solution, infusion, suppository, etc.

The administration can be in a single dose or repeatedly over a period of time or as needed as dictated by the appearance of symptoms associated with, for example, GVHD or organ transplant rejection. In certain embodiments, the administration is prior to HSCT or organ transplantation, immediately following HSCT or organ transplantation, or concurrently with HSCT or organ transplantation, and ongoing for a certain period of time to prevent the onset of GVHD or organ transplant rejection. For example, administration can be ongoing one or more days, weeks, months, or indefinitely. Likewise, in certain embodiments, administration can begin when the patient develops symptoms of GVHD or organ transplant rejection and can continue for one or more days, weeks, months, or indefinitely.

In certain embodiments, the administration can be prior to or following ablation of the immune system, or other depletion or suppression of immune function, or at the same time as ablation or other depletion or suppression of immune function, and can continue for a certain period of time. For example, administration can be ongoing one or more days, weeks, months, or indefinitely, to increase or maintain thymic recovery or immune function.

A physician or one of skill in the art can monitor the individual for progress during the course of the treatment and determine the appropriate dosage regime.

A daily dosage of the pharmaceutical composition can be varied according to the patient's condition, weight, and route of administration. In certain embodiments, in the case of oral administration of, for example, small molecule antagonist(s), the dose can be about 5 to 1000 mg as an active ingredient, per adult patient of 50 kg, about 10 to 600 mg, about 10 to 300 mg, or about 15 to 150 mg. For administration with an antibody, the dosage can range from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example, dosages can be 1 mg/kg body weight, 3 mg/kg body weight, or 10 mg/kg body weight or within the range of 1-10 mg/kg.

In another aspect, the present subject matter provides for methods of delaying development of GVHD or organ transplant rejection by administration of a composition including an effective amount of a CCR2 antagonist, a CCR5 antagonist and a CCR2 antagonist, or a dual CCR2/CCR5 antagonist. In some cases, the GVHD or organ transplant rejection is prevented from occurring at all.

The CCR2 and CCR5 antagonist(s) can be co-administered in combination with other immunosuppressive or anti-inflammatory reagents as known in the art including, for example, cyclosporine, tacrolimus, rapamycin, steroids, azatguioprine, mycophenolate mophetil, mizoribine, etc.

The following Examples are offered for the purpose of illustrating the disclosed subject matter and are not to be construed as limitations.

EXAMPLES

EXAMPLE 1: Absence of CCR2 and CCR5 receptors improves thymic recovery.

Materials and Methods

As shown in Figure 1, wild-type C57Bl/6 mice, 5 mice per group, were irradiated with a Cesium source at a rate of 42.5 rads/min for 21.2 minutes, which is a total dose of 900 rads, to destroy their native hematopoietic stem cells. After irradiation, mice were rested for 2 hours. Irradiated mice were transplanted intravenously with 100,000 $CCR2^{-/-}$ CD45.2 bone marrow cells, $CCR5^{-/-}$ bone marrow cells, or with wild type bone marrow cells by retro-orbital injection using a size 27.5 gauge needle. 100,000 CD45.1 wild type bone marrow cells were injected into both

groups of recipients to provide competition. $CCR2^{-/-}$ and $CCR5^{-/-}$ bone marrow cells were obtained from commercially available mice from Jackson Laboratories.

Two weeks after transplantation, the transplanted mice were sacrificed and analyzed. Their bone marrow and thymi were analyzed for CD45 chimerism. Briefly, single cell suspensions of thymus and bone marrow were prepared, RBCs were lysed in bone marrow, counted in a hematocytometer, and 10e6 cells were stained with optimal concentrations of monoclonal antibody to CD45.1, CD45.2, CD4 and CD8 before being analyzed using a flow cytometer. The numbers of recovered subsets were then determined. Statistic significance was determined using Student's t-test in Microsoft Excel.

Results

The mice transplanted with $CCR2^{-/-}$ or $CCR5^{-/-}$ bone marrow cells demonstrated greater repopulation of the thymus with hematopoietic (CD45+) cells relative to the mice transplanted with wild-type bone marrow ($p<0.05$, Student's T-test). In particular, an average of 23% of CD4 +CD8+ DP were derived from CCR2 knockout donor cells compared to 0% of DP from wild type donor mice. An average of 3% of DP were derived from CCR5 knockout cells compared to 1% of DP from wild type donor mice.

EXAMPLE 2: The in vitro effect of dual CCR2/CCR5 inhibition on lymphocyte and monocyte chemotaxis, T-cell function and growth, and hematopoietic stem cell differentiation.

To show that dual inhibition of CCR5 and CCR2 will inhibit chemotaxis of lymphocytes, monocytes and progenitor T-cells without affecting T-cell proliferation, cytokine secretion and cytotoxicity, and without affecting differentiation of hematopoietic stem cells, several in vitro assays are performed. A series of functional assays test the in vitro effect of cenicriviroc on T-cells. In addition, the effect of cenicriviroc on the growth and differentiation of hematopoietic stem cells in colony forming assays is tested. These tests are performed using human and mouse peripheral blood mononuclear cells (PBMC).

Cenicriviroc (TBR-652) is a dual CCR2/CCR5 antagonist pharmaceutical compound developed by Tobira Therapeutics. Cenicriviroc is currently in Phase 2b trials for the treatment of HIV-1 infection.

1) Chemotaxis assays

Chemotaxis assays are conducted using either normal donor PBMC, mouse lymph node, or splenic T-cells using a chemotaxis chamber and a standard 5-um pore size polycarbonate membrane. For each experimental condition, $5x10^5$ PBMC or isolated T-cells or monocytes are placed in the top compartment of a chemotaxis chamber and allowed to migrate to the lower compartment of the chamber. Discrete experiments are conducted with each of the CCR5 and CCR2 ligands CCL3, CCL4, CCL5 and CCL2 (MCP-1), as well as cenicriviroc or a control. The controls are mariviroc (a CCR5 inhibitor), RS102895 (a CCR2 inhibitor) and control media. Preliminary experiments are first conducted with each experimental cell type and treatment chemokine to determine the optimal concentration of the chemokines and the optimal assay length. Based on the results of the preliminary experiments, further assays determine the relationship between cenicriviroc concentration and chemotaxis inhibition in lymphocytes and monocytes. After migration, the cells are counted by flow cytometry. Cenicriviroc and control experimental results are analyzed and compared.

2) Human T-cell proliferation assays

To study the in vitro effect of cenicriviroc on human T-cell proliferation, normal donor PBMC proliferation is stimulated in vitro with cognate cytomegalovirus peptide in the presence of cenicriviroc or control. Subsequently, staining for cytomegalovirus-specific T-cells is conducted using MHC-class I fluorochrome-conjugated tetramers. Cenicriviroc and control experimental results are analyzed and compared.

3) Human T-cell cytokine secretion assays

To study the capacity of human T-cells to secrete cytokines in the presence of cenicriviroc, normal donor PBMC are stimulated in vitro with cognate

cytomegalovirus peptide in the presence of cenicriviroc or control. After PBMC stimulation, intracellular staining for interferon-γ, tumor necrosis factor-α and interleukin-2 is performed and measured by flow cytometry. Cenicriviroc and control experimental results are analyzed and compared.

4) Human hematopoietic stem cell colony forming assays

Colony forming assays are performed in methylcellulose medium enriched with recombinant human (rh) GM-CSF, rhIL-3, rhSCF, and rhEpo with or without cenicriviroc in various concentrations to study the effect of cenicriviroc on human hematopoietic stem cells. CD34-positive cells are seeded in the medium and incubated for 7 or 14 days. After incubation, colonies are identified and counted. Cenicriviroc and control experimental results are analyzed and compared.

5) Human T-cell cytotoxicity assays

The cytotoxicity of human T-cells to peptide-loaded target cells with and without cenicriviroc administration is tested in a flow-cytometry based assay. T2 cells are loaded overnight with CMV peptide, labeled with CFSE and then incubated with normal donor lymphocytes that were grown in culture in the presence of CMV peptide. The proportion of T2 cells that are killed is measured using a viability stain (4-AAD) and measured by flow cytometry after normalization to standardized beads.

EXAMPLE 3: Effect of dual CCR2/CCR5 inhibitor on GVHD in a full MHC-mismatch mouse model.

This Example shows that treatment with cenicriviroc prevents GVHD by limiting the infiltration of donor cells into secondary lymphoid organs and GVHD target tissues (*e.g.*, liver, gut, and skin).

A six-arm study is conducted using a GVHD mouse model, wherein recipient mice receive cenicriviroc or no treatment. Recipient BALB/c mice receive lethal irradiation (1000 rads) and subsequently undergo bone marrow transplantation from a C57BL/6 mouse donor. Donor mice are $CCR5^{-/-}$, $CCR2^{-/-}$ or wild type. A preliminary experiment is performed to determine the dose of donor cells required to generate severe GVHD in irradiated recipients in 2-4 weeks. All experimental groups

will consist of 6 mice. Recipient mice will be studied to determine survival after transplantation or sacrificed at 10 days for histopathological assessment.

Serum levels of MCP-1, CCL3, CCL4 and CCL5 are assessed weekly. Additionally, PBMC is assessed to determine the number of CD4-positive T-cells, regulatory T-cells and T-cell receptor excision circles to indicate recent emigrants from the thymus.

At the time of sacrifice, secondary lymphoid organs and GVHD target tissues are assessed by morphological and histological analysis. In particular, samples of the anticoagulated whole blood and sections of the livers, skin, thymi, Peyer's patches and intestines of recipient mice are fixed in zinc formalin and paraffin embedded for haematoxylin and eosin staining and immunohistochemistry and subsequent histological analysis. GVHD is graded in the fixed samples using standard optical microscopy techniques and histological criteria. Prior to fixation, the macroscopic appearance of target organs is also assessed, including the size of the thymus. Cellularity of the thymus is also measured. Lymphocyte CCR5 and CCR2 expression is also determined by isolation of lymphocytes from the organs after sacrifice followed by flow cytometric analysis. Survival curves and GVHD scoring are used to compare the outcomes of treatment with $CCR5^{-/-}$, $CCR2^{-/-}$ or wild-type donor cells.

Subsequently, this experiment is repeated in athymic BALBc nude recipient mice to determine whether the observed protection against GVHD is thymic-dependent.

EXAMPLE 4: Effect of a dual CCR2/CCR5 inhibitor on thymic recovery.

This Example shows that treatment with cenicriviroc enhances thymic recovery after lethal irradiation and bone marrow transplantation by improving the trafficking of T-cell progenitors into the thymus.

Two six-arm studies are conducted using a congenic bone marrow transplant mouse model to assess the effect of cenicriviroc on thymic recovery in recipient mice. Cenicriviroc at a dose of 100 mg/kg twice daily is administered by gavage. Recipient mice are lethally irradiated and subsequently inoculated with bone marrow from a CD45-congenic mouse that is either wild-type, $CCR2^{-/-}$ or $CCR5^{-/-}$.

In the first study, recipient mice are sacrificed after six hours, and stem cell engraftment and progenitor hematopoietic cell homing to the thymus are measured. Donor bone marrow is either incubated with cenicriviroc for 1 hour *ex vivo* or is untreated. Stem cell engraftment is assessed by measuring donor chimerism in the bone marrow by flow cytometry and plating bone marrow in colony forming assays. Thymic homing will be assessed by measuring donor chimerism in the thymus by flow cytometry.

In the second study, recipient mice are treated with cenicriviroc and long term immunologic outcomes are assessed. In particular, thymic size and cellularity are assessed and splenic and circulating numbers of CD4-positive, CD-8 positive cells will be measured at 2, 4, and 8 weeks by flow cytometry. CD4, CD8, and regulatory T-cell content of PBMC is also measured by flow cytometry, and T-cell receptor excision circles in blood are measured to assess recent thymic emigrants.

Results from both studies are analyzed and compared for cenicriviroc-treated and untreated groups.

The disclosed subject matter is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the disclosed subject matter in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Patents, patent applications, publications, product descriptions, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.

* * * * * * * * * *

Claims:

1. A method for increasing thymic recovery or thymic function in a subject by administering to the subject an effective amount of a CCR2 antagonist.

2. A method for increasing thymic recovery or thymic function in a subject by administering to the subject an effective amount of a CCR5 antagonist and a CCR2 antagonist.

3. A method for increasing thymic recovery or thymic function in a subject by administering to the subject an effective amount of a dual CCR2/CCR5 antagonist.

4. The method of claim 2, wherein the CCR2 antagonist and the CCR5 antagonist are administered concurrently.

5. The method of claim 1, 2, or 3, wherein the subject is a human.

6. The method of claim 2, wherein the CCR5 antagonist is an antibody.

7. The method of claim 1 or 2, wherein the CCR2 antagonist is an antibody.

8. The method of claim 2, wherein the CCR2 antagonist and the CCR5 antagonists are antibodies.

9. The method of claim 2, wherein the CCR5 antagonist is a small molecule.

10. The method of claim 1 or 2, wherein the CCR2 antagonist is a small molecule.

11. The method of claim 2, wherein the CCR5 antagonist and the CCR2 antagonist are small molecules.

12. The method of claim 3, wherein the dual CCR2/CCR5 antagonist is a small molecule.

13. The method of claim 12, wherein the dual CCR2/CCR5 antagonist is cenicriviroc.

14. The method of claim 1 or 2, wherein the subject has undergone hematopoietic stem cell transplantation.

15. The method of claim 14, wherein the hematopoietic stem cell transplantation is autologous.

16. The method of claim 14, wherein the hematopoietic stem cell transplantation is allogeneic.

17. A method for treating or preventing graft versus host disease (GVHD) or organ transplant rejection in a subject by administering to the subject an effective amount of a CCR5 antagonist and a CCR2 antagonist.

18. A method for treating or preventing graft versus host disease (GVHD) or organ transplant rejection in a subject by administering to the subject an effective amount of a dual CCR2/CCR5 antagonist.

19. The method of claim 17, wherein the CCR2 antagonist and the CCR5 antagonist are administered concurrently.

20. The method of claim 17 or 18, wherein the subject is a human.

21. The method of claim 17, wherein the CCR5 antagonist and the CCR2 antagonist is an antibody.

22. The method of claim 17, wherein the CCR5 antagonist and the CCR2 antagonist is a small molecule.

23. The method of claim 18, wherein the dual CCR2/CCR5 antagonist is a small molecule.

24. The method of claim 17 or 18, wherein the subject has undergone hematopoietic stem cell transplantation.

25. The method of claim 24, wherein the hematopoietic stem cell transplantation is autologous.

26. The method of claim 24, wherein the hematopoietic stem cell transplantation is allogeneic.

27. The method of claim 17 or 18, wherein the organ transplant is a solid organ.

28. The method of claim 17 or 18, wherein the organ transplant is a bone marrow transplant.

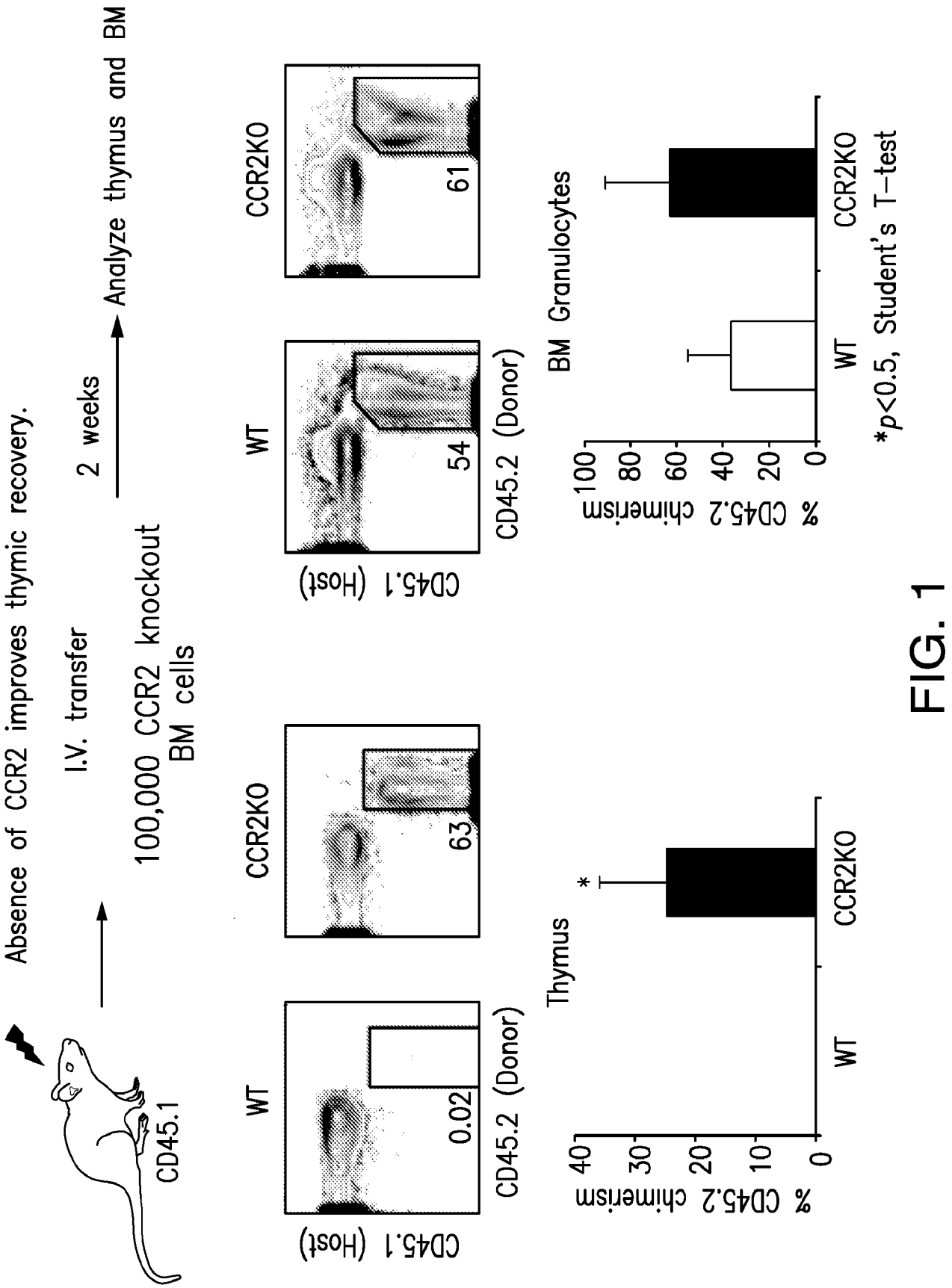

Absence of CCR2 improves thymic recovery.
I.V. transfer
2 weeks
Analyze thymus and BM
100,000 CCR2 knockout BM cells
CD45.1
WT
CCR2KO
CD45.1 (Host)
CD45.2 (Donor)
0.02
63
54
61
Thymus
BM Granulocytes
% CD45.2 chimerism
*
*p<0.5, Student's T-test

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/072613

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61P 37/06 (2014.01)
USPC - 435/69.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61P 11/00; A61P 29/00; A61P 37/00, 37/06 (2014.01)
USPC - 435/69.5, 85.1, 325, 372

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 31/00; A61K 35/26: A61K 2039/505 (2014.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	ZHAO, Dual targeting of CCR2 and CCR5: therapeutic potential for immunologic and cardiovascular diseases, J Leukoc Biol. 88:41-55. 01 April 2010 (01.04.2010). entire document	17-19, 21-23, 27 --- 7-8, 10-12, 20, 24-26, 28
Y	TERWEY et al, CCR2 is required for CD8-induced graft-versus-host disease, Blood. 106:3322-30. Epub 21 July 2005 (21.07.2005). entire document	1-16
Y	MCIVER et al, Donor lymphocyte count and thymic activity predict lymphocyte recovery and outcomes after matched-sibling hematopoietic stem cell transplant, Haematologica. 98:346-52. Epub 12 October 2012 (12.10.2012). entire document	1-16
Y	RESHEF et al, Blockade of lymphocyte chemotaxis in visceral graft-versus-host disease, N Engl J Med. 367:135-45. 12 July 2012 (12.07.2012). entire document	2-13, 20, 24-26
Y	LALEZARI et al, Safety, efficacy, and pharmacokinetics of TBR-652, a CCR5/CCR2 antagonist, in HIV-1-infected, treatment-experienced, CCR5 antagonist-naive subjects, J. Acquir Immune Defic .Syndr. 57:118-25. 01 June 2011 (01.06.2011). entire document	13
Y	GUILLAUME et al, Immune reconstitution and immunotherapy after autologous hematopoietic stem cell transplantation, Blood 92:1471-90, 01 September 1998 (01.09.1998). entire document	15, 25
Y	US 2009/0297502 A1 (LI et al) 03 December 2009 (03.12.2009) entire document	28

☐ Further documents are listed in the continuation of Box C. ☐

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

Date of the actual completion of the international search	Date of mailing of the international search report
06 February 2014	25 FEB 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774