Abstract:

Title: METHOD OF PREVENTING GRAFT VERSUS HOST DISEASE

A method for preventing GvHD in a human patient, comprising administering to a patient suffering from GvHD or at risk for GvHD, a humanized antibody having binding specificity for human αβ7 integrin, wherein the human patient has or is going to have an allogeneic stem cell transplantation, and wherein the dosing regimen prevents, improves or eliminates GvHD.
METHOD OF PREVENTING GRAFT VERSUS HOST DISEASE

RELATED APPLICATIONS
This application claims the benefit of U.S. Provisional Application No. 62/307,896 filed on March 14, 2016. The entire contents of the foregoing application are hereby incorporated by reference.

BACKGROUND
Allogeneic hematopoietic cell transplantation, such as hematopoietic stem cell transplantation (allo-HSCT) is an important therapy that is used to treat hematological malignant disorders and hematological genetic diseases, but its use is limited by the major complication of graft-versus-host disease (GvHD). GvHD following an allo-HSCT is a major cause of morbidity and mortality. The risk of GvHD is variable and depends on patient factors, donor factors, the degree of histocompatibility between donor and recipient, the conditioning regimen, and the GvHD prophylaxis strategy employed. Conditioning the patient for allo-HSCT permits engraftment of donor hematopoietic cells and involves chemotherapy or irradiation and is given immediately prior to a transplant. The purpose of conditioning is to help eradicate the patient's disease prior to the infusion of hematopoietic stem cells (HSC) and to suppress immune reactions. The post-transplant prognosis often includes acute and chronic graft-versus-host disease that may be life-threatening. In patients receiving allogeneic hematopoietic stem cells after myeloablative conditioning, the risk of Grade 2 to 4 acute GvHD is approximately 40% to 50%. The reduction of GvHD without causing significant systemic immunosuppression may improve overall outcomes following allo-HSCT.

GvHD results from an activation of alloreactive donor lymphocytes by histocompatibility antigens on host antigen-presenting cells (APCs). It has been postulated that intestinal microflora and endotoxin exert a crucial step in APC activation, and that this process occurs in the gut-associated lymphoid tissues (GALT). Clinically, GvHD can be reduced through the use of T-cell depletion strategies and gut decontamination, highlighting the respective roles of both T cells and gastrointestinal (GI) microflora on the development of GvHD. In clinical HSCT, expression of the human lymphocyte integrin α4β7 has been shown to be significantly increased on naive and memory T cells in patients who subsequently developed intestinal acute GvHD compared with patients who developed skin acute GvHD or no GvHD. T-cell trafficking to GALT
and the interaction between α4β7 and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) has been studied in murine models of acute GvHD.

The risk of GvHD is variable and depends on patient factors, donor factors, the degree of histocompatibility between donor and recipient, the conditioning regimen, and the GvHD prophylaxis strategy. In patients receiving hematopoietic stem cells from an unrelated donor source after myeloablative conditioning, the risk of Grade 2, 3, or 4 acute GvHD is approximately 40% to 50%. Patients who develop acute GvHD have an increased risk of adverse events including infections related to immunosuppressive therapies for GvHD and the development of chronic GvHD. The combined mortality attributable to GvHD and infection is high in patients after allo-HSCT, second only to death due to primary disease. Additionally, the prognosis for patients who do not achieve a response after initial therapy for acute GvHD is poor. Thus, there remains an urgent unmet medical need for a selective anti-α4β7 antibody (e.g., vedolizumab) immunosuppressant agent that can be used for the prevention of acute GvHD.

SUMMARY OF THE INVENTION

The invention relates to the prevention of graft versus host disease (GVHD) with an antagonist of the α4β7 integrin, such as an anti-α4β7 antibody, such as a humanized anti-α4β7 antibody (e.g., vedolizumab). In some embodiments, the patient has acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML).

GvHD is a major cause of morbidity and mortality in patients undergoing allo-HSCT. The significant mortality from GvHD limits the use of HSCT as a potentially curative therapy for disease, e.g., malignant disease. Reducing nonrelapse mortality (such as from GvHD and infection) may improve overall survival after allo HSCT. Steroids and other systemic immunosuppressive agents (such as tacrolimus+short-term methotrexate) are the current standard of care (SOC) used to prevent and treat GvHD. However, this standard of care can increase the risk of infections, and is also not completely effective. Immunosuppression geared at reducing GvHD can also decrease graft-versus-tumor (GvT) effects. Therefore, reducing GvHD without systemic immunosuppression, as described in the present invention, has the potential to improve overall outcomes in allo-HSCT and possibly extend and/or save lives from this disease.

Following allo-HSCT, naive T cells in the hematopoietic stem cells (HSC) inoculum expressing low levels of α4β7 integrin circulate to host Peyer's patches (PP), or
mesenteric lymph nodes (MLN), where they encounter intestinal microbial antigens in the context of alloantigens and are activated. These activated effector T cells upregulate α4β7 integrin, next home toward the intestinal mucosa via the α4β7/MADCAM-1 pathway, and generate intestinal mucosal damage. The interaction between alloreactive effector T cells, intestinal microbes, and intestinal mucosal tissues leads to release of numerous inflammatory mediators, creating a positive feedback loop. The combination of expansion of alloreactive T cells, breakdown of intestinal barriers leading to translocation of microbes and microbial stimuli, and a systemic cytokine storm lead to diffuse systemic symptoms of GvHD.

For the prevention of GvHD, without wishing to be bound by any particular theory, it is believed that the present invention blocks the initial trafficking of T cells to secondary lymphoid organs, e.g., PP or MLN, by interfering with the α4β7/MADCAM-1 pathway. Thus, the present invention suppresses and/or prevents the evolution of acute GvHD. In some embodiments, the present invention provides for a 50% reduction in cumulative incidence & severity of acute GVHD at Day 100 and 25% reduction in 1 year mortality as compared to the current standard of care (SOC). In another embodiment, the present invention improves GvHD-free survival at 6 months and improves GvHD-free and relapse-free survival at 1 year; improved cumulative incidence and severity of acute GvHD at 6 months following HSCT; improved cumulative incidence of chronic GVHD requiring immunosuppression at 12 months; or improved GRFS (GvHD-free and relapse-free survival) compared to SOC. In some embodiments, administration of an α4β7 integrin antagonist, such as an anti-α4β7 antibody, results in a 5%, 10%, 15%, 20%, 25%, 30% reduction in the risk of mortality, e.g., from 40% to e.g., 35% or 30% or less risk of mortality from acute GvHD.

In one aspect, the invention relates to a method of preventing graft versus host disease (GvHD), wherein the method comprises the step of: administering to a human patient undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), a humanized antibody having binding specificity for human α4β7 integrin, wherein the humanized antibody is administered to the patient according to the following dosing regimen:

a. an initial dose of 75 mg, 300 mg, 450 mg or 600 mg of the humanized antibody as an intravenous infusion the day before allo-HSCT;
b. followed by a second subsequent dose of 75 mg, 300 mg, 450 mg or 600 mg of the humanized antibody as an intravenous infusion at about two weeks after the initial dose;

c. followed by a third subsequent dose of 75 mg, 300 mg, 450 mg or 600 mg of the humanized antibody as an intravenous infusion at about six weeks after the initial dose;

optionally wherein the dosing regimen results in Grade II GvHD, Grade I GvHD or no GvHD, further wherein the humanized antibody comprises an antigen binding region of nonhuman origin and at least a portion of an antibody of human origin, wherein the humanized antibody has binding specificity for the α4β7 complex, wherein the antigen-binding region comprises the Light chain CDRs of SEQ ID NO:7 (CDR1), SEQ ID NO:8 (CDR2) and SEQ ID NO:9 (CDR3); and Heavy chain CDRs: SEQ ID NO:4 (CDR1), SEQ ID NO:5 (CDR2) and SEQ ID NO:6 (CDR3).

In another aspect, the invention relates to a method of reducing the occurrence of acute graft versus host disease (GvHD), wherein the method comprises the step of: administering to a human patient undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), a humanized antibody having binding specificity for human α4β7 integrin,

wherein the humanized antibody is administered to the patient according to the following dosing regimen:

a. an initial dose of 75 mg, 300 mg, 450 mg or 600 mg of the humanized antibody as an intravenous infusion the day before allo-HSCT;

b. followed by a second subsequent dose of 300 mg of the humanized antibody as an intravenous infusion at about two weeks after the initial dose;

c. followed by a third subsequent dose of 300 mg of the humanized antibody as an intravenous infusion at about six weeks after the initial dose;

wherein the humanized antibody comprises an antigen binding region of nonhuman origin and at least a portion of an antibody of human origin, wherein the humanized antibody has binding specificity for the α4β7 complex, wherein the antigen-binding region comprises the Light chain CDRs of SEQ ID NO:7 (CDR1), SEQ ID NO:8 (CDR2) and SEQ ID NO:9 (CDR3); and Heavy chain CDRs: SEQ ID NO:4 (CDR1), SEQ ID NO:5 (CDR2) and SEQ ID NO:6 (CDR3). In some embodiments, reducing the occurrence of acute GvHD results in Grade I or Grade II GvHD, per modified Glucksberg criteria, or similar severity of GvHD per other scoring system, or no GvHD. In other embodiments, reducing the occurrence of acute GvHD is a 50% reduction in cumulative incidence and severity of Grade II-IV or Grade III-IV acute GvHD at Day 100 as compared to treatment with
methotrexate and calcineurin inhibitor alone. In other embodiments, reducing the occurrence of acute graft versus host disease (GvHD) is a reduction in 1 year mortality as compared to treatment with methotrexate and calcineurin inhibitor alone.

In another aspect, the invention relates to a method of treating a patient suffering from cancer or a nonmalignant hematological, immunological disease or autoimmune disease, comprising the steps of:

a. conditioning the immune system of the patient for hematopoietic stem cell transplant,
b. administering a humanized antibody having binding specificity for human α4β7 integrin,
c. waiting at least 12 hours,
d. administering allogeneic hematopoietic stem cells,
e. waiting thirteen days, then administering a second dose of humanized antibody having binding specificity for human α4β7 integrin, and
f. waiting four weeks, then administering a third dose of humanized antibody having binding specificity for human α4β7 integrin.

In another aspect, the invention relates to a method of suppressing an immune response in a cancer patient, wherein the method comprises the step of: administering to a human patient undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), a humanized antibody having binding specificity for human α4β7 integrin, wherein the humanized antibody is administered to the patient according to the following dosing regimen:

a. an initial dose of 75 mg, 300 mg, 450 mg or 600 mg of the humanized antibody as an intravenous infusion the day before allo-HSCT;

b. followed by a second subsequent dose of 300 mg of the humanized antibody as an intravenous infusion at about two weeks after the initial dose;

c. followed by a third subsequent dose of 300 mg of the humanized antibody as an intravenous infusion at about six weeks after the initial dose;

further wherein the humanized antibody comprises an antigen binding region of nonhuman origin and at least a portion of an antibody of human origin, wherein the humanized antibody has binding specificity for the α4β7 complex, wherein the antigen-binding region comprises the Light chain CDRs of SEQ ID NO:7 (CDR1), SEQ ID NO:8 (CDR2) and SEQ ID NO:9 (CDR3); and Heavy chain CDRs: SEQ ID NO:4 (CDR1), SEQ ID NO:5 (CDR2) and SEQ ID NO:6 (CDR3).
The humanized antibody may have a heavy chain variable region sequence of amino acids 20 to 140 of SEQ ID NO:1.
The humanized antibody may have a light chain variable region sequence of amino acids 20 to 131 of SEQ ID NO:2.

The humanized antibody may have a heavy chain comprising amino acids 20 to 470 of SEQ ID NO:1 and a light chain comprising amino acids 20 to 238 of SEQ ID NO:2. In some embodiments, the humanized antibody is vedolizumab.

In a further aspect, the invention relates to a method of treating a transplant patient, wherein the transplant patient is a recipient of an infusion of allogeneic hematopoietic cells, comprising administering an anti-α4β7 antagonist. In some embodiments, the α4β7 integrin antagonist is an anti-α4β7 antibody. In some embodiments, the anti-α4β7 antibody is a humanized antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic illustrating an overview of the study design from days -1 to +50. Allo-HSCT occurs on day 0. Vedolizumab is administered the day before the allo-HSCT (day -1), and on days +13 and +42 after allo-HSCT.

FIG. 2 illustrates how blocking the a4p7/MADCAM-1 interaction in GALT and MLNs may reduce the generation of allo-reactive memory T cells and their subsequent entry into the gut, thereby reducing the occurrence of GvHD.

FIG. 3 is a graph showing simulated and observed PK data from three patients. The PK simulated data is shown by the region between the jagged lines (2.5 and 97.5 percentiles of simulated data), the dashed black line without dots represents the median of simulated data, the points and lines are individual observed data plotted using nominal times, and the horizontal dashed line represents the LLOQ of 0.2 mcg/mL.

DETAILED DESCRIPTION

The present invention relates to a method of treating disease through preventing GvHD. The method comprises administering an α4β7 integrin antagonist, such as an anti-α4β7 antibody, to a patient undergoing allogeneic hematopoietic cell transplant, such as allogeneic hematopoietic stem cell transplant (allo-HSCT). In some embodiments, the disease suffered by the patient is cancer, e.g., hematological cancer (such as leukemia, lymphoma, myeloma or myelodysplastic syndrome). In other embodiments, the disease
suffered by the patient is characterized by a nonmalignant hematological or immunological defect (such as a bone marrow failure syndrome, hemoglobinopathy, or SCID). In one aspect, the transplant patient is conditioned, e.g., undergoes a process to prepare the body to receive the transplant. In some embodiments, the conditioning is myeloablative conditioning (“myelo conditioning”) or reduced-intensity conditioning (RIC), e.g., less, such as 10%, 20%, 30%, 40%, 20-40%, 30-50% or 50% less, of the agents used in myeloablative conditioning. In some embodiments, the conditioning is chemically-induced, e.g. by cyclophosphamide and/or busulfan and/or fludarabine, radiation-induced, e.g., by total body irradiation, or induced by a combination of chemical treatment and radiation, such as cyclophosphamide and total body irradiation.

In one aspect, the patient, e.g., transplant patient, is administered allogeneic hematopoietic cells, e.g., as an infusion. In some embodiments, the allogeneic hematopoietic cells are allogeneic hematopoietic stem cells, i.e., the patient receives an allogeneic hematopoietic stem cell transplant (allo-HSCT). In some embodiments, the allogeneic hematopoietic cells are allogeneic leukocytic cells. In some embodiments, the allogeneic leukocytic cells comprise lymphocytes, e.g., T-lymphocytes. In some embodiments, the allogeneic leukocytic cells comprise lymphocytes expressing a chimeric antigen receptor. In some embodiments, the allogeneic leukocytic cells comprise natural killer cells. In some embodiments, the allogeneic leukocytic cells comprise cytotoxic T-lymphocytes, e.g., T-cells expressing CD8. In some embodiments, the allogeneic leukocytic cells are selected to consist of at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% lymphocytes. In some embodiments, the allogeneic leukocytic cells are selected to consist of at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% T-lymphocytes. In some embodiments, the allogeneic hematopoietic cells have one or more recombinant modifications known in the art to control their behavior in the patient.

In some embodiments, the α4β7 antagonist, such as an anti-α4β7 antibody, prevents graft versus host disease (GVHD). In some embodiments, the α4β7 antagonist, such as an anti-α4β7 antibody, does not prevent graft versus tumor activity. In some embodiments, the transplanted cells engraft with tolerance to the patient’s tissues. In some embodiments, the invention relates to methods of preventing graft versus host disease (GvHD) by administering an anti-α4β7 antibody to a patient undergoing allo-HSCT. In some embodiments, the α4β7 antagonist is administered to a patient prior to receiving hematopoietic cells, such as allogeneic hematopoietic stem cells, and further is provided
during hematopoietic cell engraftment, and thereby prevents GVHD. In other embodiments, the α4β7 antagonist is administered to a patient shortly after, such as up to seven days after, receiving the hematopoietic cells. In some embodiments, the anti-α4β7 antibody is a humanized antibody, e.g., a humanized antibody with the epitopic specificity of Act-1 mouse monoclonal antibody. In some embodiments, the anti-α4β7 antibody is vedolizumab.

The hematopoietic cells, e.g., stem cells, may be derived from bone marrow or from blood (e.g., peripheral blood or umbilical cord blood) of a non-self donor, i.e., allogeneic. In some embodiments, the hematopoietic cells, e.g., stem cells, may be manipulated before infusion, e.g., enriched for or depleted of certain cells by antibody-selection or other mechanism, expanded in vitro, or subjected to gene editing or gene therapy. Examples of compositions of hematopoietic cells which are enriched or depleted for infusion include cells, which can be collected by e.g., negative selection, e.g., separation of leukocytes from red blood cells (e.g., differential centrifugation through a dense sugar or polymer solution (e.g., FICOLL® solution (Amersham Biosciences division of GE healthcare, Piscataway, NJ) or HISTOPAQUE®-1077 solution, Sigma-Aldrich Biotechnology LP and Sigma-Aldrich Co., St. Louis, MO)) and/or positive selection by binding cells to a selection agent (e.g., a reagent which binds to a B-cell marker, such as CD19 or CD20, a myeloid progenitor marker, such as CD34, CD38, CD117, CD138, CD133, or ZAP70, or to a T-cell marker, such as CD2, CD3, CD4, CD5 or CD8 for direct isolation (e.g., the application of a magnetic field to solutions of cells comprising magnetic beads (e.g., from Miltenyi Biotec, Auburn, CA) or other beads, e.g., in a column (R&D Systems, Minneapolis, MN) which bind to the cell markers) or fluorescent-activated cell sorting). In one embodiment, the differential centrifugation concentrates a cell layer comprising leukocytes.

In some embodiments, the patient is suffering from a disease, such as cancer or a non-malignant disease. In some embodiments, the patient has leukemia, for example, acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML). In some embodiments, the patient has a myelodysplastic or myeloproliferative disease. In some embodiments, the patient has lymphoma, such as non-Hodgkin’s lymphoma or Hodgkin's lymphoma. In some embodiments, the patient has a nonmalignant hematological disorder, such as a hemoglobinopathy, e.g., sickle cell disease or thalassemia, bone marrow failure syndrome, e.g., aplastic anemia, Fanconi’s anemia, or other marrow failure syndromes, an immune disease, such as severe combined immunodeficiency (SCID) or autoimmune
disease, such as diabetes. In some embodiments, the patient has a disorder treatable with an organ transplant, such as sclerosing cholangitis, cirrhosis, or hemochromatosis (e.g., for a liver transplant); congestive heart disease, dilated coronary myopathy, or severe coronary artery disease (e.g., for a heart transplant); cystic fibrosis, chronic obstructive pulmonary disease, or pulmonary fibrosis (e.g., for a lung transplant); or diabetes, polycystic kidney disease, systemic lupus erythematosus, or focal segmental glomerulosclerosis (e.g., for a kidney transplant). In some embodiments, the patient is receiving two transplants, for example a hematopoietic cell transplant, e.g., for the purpose of tolerance induction, and a solid organ transplant, e.g., transplant of a liver, a heart, a lung or a kidney. In another example, the patient is receiving two transplants, first an allo-HSCT and second, allogeneic T cells via donor leukocyte infusion (DLI). In this example, there is potential for development of acute GvHD in both transplant procedures and thus administration of an $\alpha 4\beta 7$ integrin antagonist, such as an anti-$\alpha 4\beta 7$ antibody, to a patient may be useful for both transplants.

Acute graft-versus-host-disease is characterized by damage to tissues such as the liver, skin (rash), gastrointestinal tract, and other mucosa caused by alloreactive immune cells such as T-cells. In some embodiments, autoreactive immune cells, may cause acute graft-versus-host disease. Immune cells may become reactive from the hematopoietic cell infusion, or activated upon recognition of signals in tissues of the patient, e.g., the transplant patient. Signals recognized by alloreactive hematopoietic cells or autoreactive immune cells may be induced from the conditioning regimen or from tumor lysis syndrome, e.g., as a result of GVT activity. Prevention of GvHD may result from sustained $\alpha 4\beta 7$ blockade beginning at the time of hematopoietic cell, e.g., hematopoietic stem cell infusion. Prophylactic administration of vedolizumab to patients undergoing allo-HSCT may prevent trafficking of alloreactive T-cells to GALT, (e.g., Peyer's patches) or mesenteric lymph nodes, and GI mucosa, thereby preventing the development of acute GvHD. Sustained $\alpha 4\beta 7$ blockade may further prevent GvHD during hematopoietic cell engraftment, e.g., to block autoreactive immune cells. The anti-$\alpha 4\beta 7$ antibody is provided at a dose sufficient to achieve sustained receptor saturation throughout the first 100 days following allo-HSCT, the time period in which the vast majority of acute GvHD occurs. Grade III-IV or index C-D acute GvHD is a risk factor for the development of chronic GvHD, so therapies that can prevent acute GvHD may reduce the risk of the development of chronic GvHD (Flowers M.E.D. et al. Blood 2011 Mar 17 117(11):3214-19).
One aspect of the invention comprises an $\alpha 4\beta 7$ integrin antagonist (e.g., vedolizumab) for use in the prevention of GvHD. Unlike healthy subjects, patients undergoing a conditioning regimen, e.g., myeloablative or reduced intensity conditioning, followed by hematopoietic cell transplant, such as allo-HSCT are expected to have markedly changing T-cell populations with variable $\alpha 4\beta 7$ integrin expression during the post-transplant period. For example, engraftment of HSCs comprises homing of the engrafting HSCs to the bone marrow and maturation and homing of donor lymphocytes to secondary lymphoid organs and other tissues causing high susceptibility of the patient to infection while the engraftment occurs. Systemic treatments, e.g., administration of immunosuppressive agents (such as corticosteroids, cyclosporine, methotrexate and mycophenolate mofetil, and antibody therapies like alemtuzumab, anti-thymocyte globulin, or rituximab, and anti-TNF therapies) used to control aberrant activation of lymphocytes may affect the engraftment and the response to the graft or disease, e.g., cancer or nonmalignant hematological disorder. Gut selective therapies (such as anti-$\alpha 4\beta 7$ antibody) offer the potential to decrease the generation and homing of allo-reactive gut specific lymphocytes in this setting while potentially preserving the GVT effect of the graft.

**Definitions**

The term "pharmaceutical formulation" refers to a preparation that contains an $\alpha 4\beta 7$ antagonist, such as an anti-$\alpha\beta^7$ antibody, in such form as to permit the biological activity of the antibody to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

The cell surface molecule, "$\alpha 4\beta 7$ integrin," or "$\alpha 4\beta 7$," is a heterodimer of an $\alpha$ chain (CD49D, ITGA4) and a $\beta_7$ chain (ITGB7). Each chain can form a heterodimer with an alternative integrin chain, to form $\alpha 4\beta 1$ or $\alpha 6\beta 1$. Human $\alpha$ and $\beta_7$ genes (GenBank (National Center for Biotechnology Information, Bethesda, MD) RefSeq Accession numbers NM_000885 and NM_000889, respectively) are expressed by B and T lymphocytes, particularly memory CD4+ lymphocytes. Typical of many integrins, $\alpha 4\beta 7$ can exist in either a resting or activated state. Ligands for $\alpha 4\beta 7$ include vascular cell adhesion molecule (VCAM), fibronectin and mucosal addressin (MAdCAM (e.g., MAdCAM-1)).

An "$\alpha 4\beta 7$ antagonist" is a molecule which antagonizes, reduces or inhibits the function of $\alpha 4\beta 7$ integrin. Such antagonist may antagonize the interaction of $\alpha 4\beta 7$...
integrin with one or more of its ligands. An α4β7 antagonist may bind either chain of the heterodimer or a complex requiring both chains of the α4β7 integrin, or it may bind a ligand, such as MAdCAM. An α4β7 antagonist may be an antibody which performs such binding function, such as an anti-α^7-integrin antibody or "anti-α^7 antibody". In some embodiments, an α4β7 antagonist, such as an anti-α^7 antibody, has "binding specificity for the α4β7 complex" and binds to α4β7, but not to α4β1 or αβ7.

The term "antibody" or "antibodies" herein used in the broadest sense and specifically covers full length antibody, antibody peptide(s) or immunoglobulin(s), monoclonal antibodies, chimeric antibodies (including primatized antibodies), polyclonal antibodies, human antibodies, humanized antibodies and antibodies from non-human species, including human antibodies derived from a human germline immunoglobulin sequence transduced into the non-human species, e.g., mouse, sheep, chicken or goat, recombinant antigen binding forms such as monobodies and diabodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two full length antibodies (e.g., each portion comprising the antigen binding region of an antibody to a different antigen or epitope), and individual antigen binding fragments of any of the foregoing, e.g., of an antibody or the antibody from which it is derived, including dAbs, Fv, scFv, Fab, F(ab')2, Fab'.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

"Antigen binding fragments" of an antibody preferably comprise at least the variable regions of the heavy and/or light chains of an anti-α^7 antibody. For example, an antigen binding fragment of vedolizumab can comprise amino acid residues 20-131 of the humanized light chain sequence of SEQ ID NO:2 and amino acid residues 20-140 of the humanized heavy chain sequence of SEQ ID NO:1. Examples of such antigen binding fragments include Fab fragments, Fab' fragments, Fv fragments, scFv and F(ab')2 fragments. Antigen binding fragments of an antibody can be produced by enzymatic cleavage or by recombinant techniques. For instance, papain or pepsin cleavage can be used to generate Fab or F(ab')2 fragments, respectively. Antibodies can also be produced
in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a recombinant construct encoding the heavy chain of an F(ab′)₂ fragment can be designed to include DNA sequences encoding the CH₁ domain and hinge region of the heavy chain. In one aspect, antigen binding fragments inhibit binding of α4β7 integrin to one or more of its ligands (e.g. the mucosal addresin MAdCAM (e.g., MAdCAM-1), fibronectin).

A "therapeutic monoclonal antibody" is an antibody used for therapy of a human subject. Therapeutic monoclonal antibodies disclosed herein include anti-α4β7 antibodies.

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include Clq binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor; BCR), and the like. To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as those described in U.S. Pat. Nos. 5,500,362 or 5,821,337 may be performed.

Depending on the amino acid sequence of the constant domain of their heavy chains, full length antibodies can be assigned to different "classes". There are five major classes of full length antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of antibodies are well known.

The "light chains" of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. residues 24-34 (LI), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (HI), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (e.g. residues 26-32 (LI), 50-52 (L2) and 91-96 (L3) in the
light chain variable domain and 26-32 (HI), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk J. Mol. Biol. 796:901-917 (1987). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined. The hypervariable region or the CDRs thereof can be transferred from one antibody chain to another or to another protein to confer antigen binding specificity to the resulting (composite) antibody or binding protein.

"Humanized" forms of non-human (e.g., rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human antibody are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992).


An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. In certain embodiments, the antibody will be purified (1) to greater than 95% by weight of protein as determined by the Lowry method, and alternatively, more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or non-
reducing conditions using Coomassie blue or silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

"Cancer" or "tumor" is intended to include any malignant or neoplastic growth in a patient, including an initial tumor and any metastases. The cancer can be of the hematological or solid tumor type. Hematological tumors include tumors of hematological origin, including, e.g., myelomas (e.g., multiple myeloma), leukemias (e.g., Waldenstrom's syndrome, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, granulocytic leukemia, monocytic leukemia, acute lymphocytic leukemia, other leukemias), lymphomas (e.g., B-cell lymphomas, such as diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, plasmocytoma, or reticulum cell sarcoma), and myeloproliferative neoplasms, such as myelodysplastic syndrome, thrombocytemia, polycytemia vera, or myelofibrosis. Solid tumors can originate in organs, and include cancers such as in skin, lung, brain, breast, prostate, ovary, colon, kidney, pancreas, liver, esophagus, stomach, intestine, bladder, uterus, cervix, testis, adrenal gland, etc. As used herein, cancer cells, including tumor cells, refer to cells that divide at an abnormal (increased) rate or whose control of growth or survival is different than for cells in the same tissue where the cancer cell arises or lives. Cancer cells include, but are not limited to, cells in carcinomas, sarcomas, myelomas, leukemias, lymphomas, and tumors of the nervous system including glioma, meningoma, medulloblastoma, schwannoma or epidymoma.

"Treatment" refers to therapeutic treatment. Those in need of treatment include those already with disease. Hence, the patient, e.g., human, to be treated herein may have been diagnosed as suffering from a disease, such as cancer or a nonmalignant hematological disease or suffering from the conditioning regimen. Alternatively, the patient may not have GvHD, but is a transplant patient, e.g., a patient undergoing conditioning for an allogeneic hematopoietic cell transplant, a candidate for or patient who is undergoing allogeneic hematopoietic cell transplant, e.g., allo-HSCT, or who underwent allogeneic hematopoietic cell transplant, e.g., allo-HSCT, recently, e.g., within the previous five months. Further, or alternatively, the patient may be planned to receive allogeneic T cells via donor leukocyte infusion (DLI) e.g., following allo-HSCT. The terms "patient" and "subject" are used interchangeably herein.
"Prevention" refers to a treatment that results in the absence or reduction in the severity of an adverse event. In a population of patients, when treatment typically results in a certain percentage of adverse events, or a certain percentage of adverse events that are severe, but a treatment administered for prevention purposes instead results in a lower percentage of adverse events (i.e., a lower or reduced risk of adverse events) or a lower percentage of adverse events that are severe (i.e., a lower or reduced risk that the adverse event is severe).

In the context of allogeneic hematopoietic stem cell transplant patients, such as patients who undergo myeloablative or reduced-intensity conditioning and receive allogeneic hematopoietic stem cell transplants, the adverse event of graft-versus-host disease has at least a 25% risk, a 30% to 60% risk, a 35% to 55% risk, a 40% to 50% risk, or a 45% to 65% risk, and may result in 30% to 50% of the severe treatment related mortality that results from all adverse events. Prevention of the adverse GVHD, or prevention of high grade, e.g. grade III or IV or index C or D, GVHD may reduce the percent risk of the adverse event or may reduce the percent risk that GVHD leads to treatment related mortality of transplant patients. In some embodiments, the administration of an α4β7 antagonist, such as an anti-α4β7 antibody, prevents GVHD in a patient. In other embodiments, the administration of an α4β7 antagonist, such as an anti-α4β7 antibody, prevents the intestinal manifestation of GVHD in a patient. In some embodiments, the administration of an α4β7 antagonist, such as an anti-α4β7 antibody, prevents the intestinal manifestation of GVHD in a patient, but does not prevent one or more manifestations of GVHD in skin or liver. In some embodiments, the administration of an α4β7 antagonist, such as an anti-α4β7 antibody, reduces the use of immunosuppressive therapy in the patient. In some embodiments, the administration of an α4β7 antagonist, such as an anti-α4β7 antibody, to a patient undergoing allo-HSCT results in engraftment of the stem cells. In some embodiments, the administration of an α4β7 antagonist, such as an anti-α4β7 antibody, to a patient undergoing allo-HSCT results in engraftment of the stem cells and a graft-versus-tumor (GVT) effect.

The anti-α4β7 antibody is substantially pure and desirably substantially homogeneous (i.e. free from contaminating proteins etc.). "Substantially pure" antibody means a composition comprising at least about 90% antibody by weight, based on total weight of the protein in the composition, at least about 95% or 97% by weight. "Substantially homogeneous" antibody means a composition comprising protein wherein
at least about 99% by weight of protein is specific antibody, e.g., anti-α4β7 antibody, based on total weight of the protein.

An anti-α4β7 antibody, vedolizumab, a humanized monoclonal antibody that has binding specificity for the α4β7 integrin, is already indicated for the treatment of patients with moderately to severely active ulcerative colitis (UC) and Crohn’s disease (CD). Vedolizumab may also be used in the prevention of GvHD. Vedolizumab has a novel gut-selective mechanism of action. By binding to cell surface-expressed α4β7, vedolizumab is an α4β7 antagonist and blocks a subset of memory gut-homing T lymphocytes from interacting with mucosal addressin cell adhesion molecule-1 (MAdCAM-1) expressed on endothelial cells.

Several factors are associated with accelerated clearance of antibodies including the presence of anti-drug antibodies, sex, body size, concomitant immunosuppressant use, disease type, albumin concentration, and degree of systemic inflammation. Furthermore, a consistent relationship between efficacy and exposure, in distinction to drug dose, has been observed for many of these agents, such that higher trough drug concentrations are associated with greater efficacy. Differences in drug clearance may be an important explanation for this observation. For example, cancer patients undergo immunosuppressive treatment of the tumor and treatment for infection. Therefore, an understanding of the determinants of clearance for therapeutic antibodies in transplant patients may result in optimization of drug regimens.

In previous studies, single-dose pharmacokinetics, pharmacodynamics (α4β7 receptor saturation), safety, and tolerability of vedolizumab were investigated over a dose range of 0.2 to 10 mg/kg in healthy volunteers (intravenous [IV] infusion) (unpublished data). After reaching peak concentrations, vedolizumab serum concentrations fell in a generally biexponential fashion until concentrations reached approximately 1 to 10 ng/mL. Thereafter, concentrations appeared to fall in a nonlinear fashion. The multiple-dose pharmacokinetics and pharmacodynamics of vedolizumab have been investigated following IV infusions of 0.5 and 2 mg/kg in patients with CD and infusion of 2, 6, and 10 mg/kg in patients with UC. Vedolizumab pharmacokinetics was generally linear following an IV infusion over the dose range of 2 to 10 mg/kg in patients with UC. After multiple-dose administration, rapid and near complete α4β7 receptor saturation was achieved following the initial dose of vedolizumab.
The efficacy and safety of vedolizumab induction and maintenance therapy were demonstrated in patients with CD in the GEMINI 2 (ClinicalTrials.gov number, NCT00783692) and GEMINI 3 (ClinicalTrials.gov number, NCT01224171) trials. The exposure-response (efficacy) relationships of vedolizumab in patients with CD for induction and maintenance therapy have been presented elsewhere.

Prevention of Graft-Versus-Host Disease (GvHD) with an α4β7 antagonist

The invention relates to a method of treating disease in a patient by preventing GvHD, or a GvHD-related adverse event, in an allogeneic hematopoietic cell transplant patient, e.g., human patient, e.g., undergoing allo-HSCT. The human patient may be an adult (e.g., 18 years or older), an adolescent, or a child. A pharmaceutical composition comprising an anti-α4β7 antibody can be used as described herein for treating a transplant patient, a cancer patient, a nonmalignant hematological disease patient or preventing GvHD in a subject suffering therefrom.

The severity of acute GvHD is measured according to the modified Glucksberg criteria (Table 2) and Blood and Marrow Transplant Clinical Trials Network (BMT CTN)-modified International Bone Marrow Transplant Registry Database (IBMTR) index Table 3). The clinical stages and grades of GvHD are divided as shown in Table 1.

### Table 1: Acute Graft-versus-Host Disease Clinical Stage

<table>
<thead>
<tr>
<th>Stage</th>
<th>Skin</th>
<th>Liver</th>
<th>Intestinal tract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bilirubin: SI units</td>
<td>Diarrhea/day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(standard units)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Maculopapular rash &lt;25% of body surface (a)</td>
<td>34-50 µmol/L</td>
<td>&gt;500 mL</td>
</tr>
<tr>
<td>2</td>
<td>Maculopapular rash 25%-50% of body surface</td>
<td>51-102 µmol/L</td>
<td>&gt;1000 mL</td>
</tr>
<tr>
<td>3</td>
<td>Rash &gt;50% of body surface</td>
<td>103-225 µmol/L</td>
<td>&gt;1500 mL</td>
</tr>
<tr>
<td>4</td>
<td>Generalized erythroderma with bullous formation</td>
<td>&gt;255 µmol/L</td>
<td>Severe abdominal pain, with or without ileus</td>
</tr>
</tbody>
</table>

(a)
The allogeneic hematopoietic cells, e.g., allo-HSC, may engraft with no GvHD, only skin GvHD, only liver GvHD, only skin and liver GvHD, no intestinal GvHD and only skin or liver GvHD, no grade IV GvHD, no grade III or IV GvHD, only stage 1 or stage 2 intestinal GvHD and only stage 2-3 skin and/or liver GvHD, only Grade I to II GvHD, or no or only skin GvHD, only index A GvHD, only index A or B GvHD, no index C or D GvHD, or any of the foregoing together with GVT, after administration of the α4β7 antagonist, e.g., an anti-α4β7 antibody.

Preventing the development of acute GvHD may be the result of decreasing or blocking trafficking of alloreactive T-cells to GALT, mesenteric lymph nodes and/or GI mucosa. Prevention of GvHD, e.g., acute GVHD, may be considered successful if at about 50 days, about 75 days, about 90 days, about 100 days, about 110 days, about 120 days, about 150 days, or about 180 days, after allogeneic hematopoietic cell transplant, e.g., allo-HSCT, the patient shows no signs of acute GvHD. In some embodiments, the patient undergoing allogeneic hematopoietic cell transplant, e.g., allo-HSCT is treated with a regimen that comprises no further administration of immunosuppressive therapy,
e.g., no administration of immunosuppressive therapy after the conditioning treatment or after the initial transplant period, e.g., immediately before and/or immediately after, e.g., 0 to 1 weeks, 0 to 2 weeks, 0 to 3 weeks or 0 to 4 weeks, after the allogeneic hematopoietic cell transplant.

Remission is defined by conventional World Health Organization (WHO) criteria: <5% blast cells, count recovery, and no evidence of extramedullary disease. Remission of acute and/or chronic GvHD may last for about 4, about 5, about 6, about 9, or about 12 months after allo-HSCT.

GvHD relapse or progression-free survival (GRFS) is defined as Grade 3-4 acute GvHD, chronic GvHD requiring systemic immunosuppression, disease relapse or progression, or death due to any cause.

Engraftment is a process whereby the transplanted hematopoietic cells populate in the patient or adjust to the patient tissue environment, e.g., proliferate, differentiate, begin performing the function characteristic of the hematologic cell from which it is derived or is programmed to become with the maturation signals. Engraftment of allo-HSCT is measured by quantifying blood components, such as neutrophils and platelets. The timing of engraftment depends on the source of the hematopoietic stem cells, e.g., longer for cord blood stem cells than for peripheral blood stem cells. Neutrophil engraftment (recovery of absolute neutrophil count [ANC]) is defined by an ANC>500/mm^3 for 3 consecutive days or >2000/mm^3 for 1 day. The first day of the 3-day period is considered the day of neutrophil engraftment.

The mean expression of α4β7 on peripheral blood lymphocytes may be measured by the MadCAM-1-Fc binding inhibition assay before and after dosing with an anti-α4β7 antibody (e.g., vedolizumab) in the allogeneic hematopoietic cell transplant patient, e.g., myeloablative allo-HSCT population.

Changes in blood or serum biomarkers, including, but not limited to, interleukin-6 (IL-6), interleukin-17 (IL-17), and suppressor of tumorigenicity 2 (ST2) and/or cellular biomarkers, including, but not limited to CD8+, CD38+, CD8+ bright effector memory T cells, and CD4+ memory T cells, may be predictive of the onset or severity of acute GvHD. Detection of an increase one or more of such markers after allo-HSCT may indicate the onset of acute GVHD. Detection of the biomarkers may be accomplished from immunodetection of the biomarker, e.g., by antibody binding to cells, e.g., blood cells, expressing the biomarker and measurement of the amount of antibody binding, e.g., by flow cytometry or by antibody binding to soluble biomarkers in serum and
measurement of the amount of antibody binding, e.g., by ELISA. Comparison of the amount of the biomarker with a control or a sample obtained early in the transplant process or prior to transplant, or to a predetermined standard, e.g., the amount of the biomarker in a population of non-transplant subjects, may provide an indication of whether the amount of the biomarker is changed, e.g., increased. In some embodiments, administration of an α4β7 antagonist, such as an anti-α4β7 antibody, to a patient undergoing allogeneic hematopoietic cell transplant, e.g., allo-HSCT, prevents a change or an increase in one or more of these biomarkers.

Patients may be tested to see if they are positive for antibodies directed against the α4β7 antagonist, such as anti-α4β7 antibody, for example, positive for anti-vedolizumab antibody at various time points, for example, at baseline, day 20, and day 100 after allo-HSCT.

Patients may be tested for development of GvHD requiring systemic immunosuppression.

An α4β7 antagonist, such as an anti-α4β7 antibody, is administered in an effective amount which inhibits binding of α4β7 integrin to a ligand thereof. For therapy, an effective amount will be sufficient to achieve the desired prophylactic effect (e.g., decreasing or eliminating trafficking of alloreactive T-cells to GALT, mesenteric lymph nodes and or GI mucosa and reducing the incidence or severity of GvHD). An effective amount of an anti-α4β7 antibody, e.g., an effective titer sufficient to maintain saturation, e.g., neutralization, of α4β7 integrin, can result in sustained α4β7 blockade at the time of hematopoietic stem cell infusion. An α4β7 antagonist, such as an anti-α4β7 antibody may be administered in a unit dose or multiple doses. The dosage can be determined by methods known in the art and can be dependent, for example, upon the individual's age, sensitivity, tolerance and overall well-being. Examples of modes of administration include topical routes such as nasal or inhalational or transdermal administration, enteral routes, such as through a feeding tube or suppository, and parenteral routes, such as intravenous, intramuscular, subcutaneous, intraarterial, intraperitoneal, or intravitreal administration. Suitable dosages for antibodies can be from about 0.1 mg/kg body weight to about 10.0 mg/kg body weight per treatment, for example about 2 mg/kg to about 7 mg/kg, about 3 mg/kg to about 6 mg/kg, or about 3.5 to about 5 mg/kg. In particular embodiments, the dose administered is about 0.3 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, or about 10 mg/kg. In some embodiments,
vedolizumab is administered at a dose of 50 mg, 75 mg, 100 mg, 300 mg, 450 mg, 500 mg or 600 mg. In some embodiments, vedolizumab is administered at a dose of 108 mg, 90 to 120 mg, 216 mg, 160 mg, 165 mg, 155 to 180 mg, 170 mg or 180 mg. In some embodiments, vedolizumab is administered at a dose of 180 to 250 mg, 300 to 350 mg, or 300 to 500 mg.

In the case of an α4β7 antagonist, such as an anti-α4β7 antibody which is stored as a lyophilized solid, the antibody is reconstituted in a solution such as water for injection prior to administration. If prepared for infusion, the final dosage form, e.g., after dilution of the reconstituted antibody (e.g., in a saline, Ringer's or 5% dextrose infusion system) of the anti-α4β7 antibody can be about 0.5 mg/ml to about 5 mg/ml for administration. The final dosage form may be at a concentration of between about 0.3 mg/ml to about 3.0 mg/ml, about 1.0 mg/ml to about 1.4 mg/ml, about 1.0 mg/ml to about 1.3 mg/ml, about 1.0 mg/ml to about 1.2 mg/ml, about 1.0 to about 1.1 mg/ml, about 1.1 mg/ml to about 1.4 mg/ml, about 1.1 mg/ml to about 1.3 mg/ml, about 1.1 mg/ml to about 1.2 mg/ml, about 1.2 mg/ml to about 1.4 mg/ml, about 1.2 mg/ml to about 1.3 mg/ml, or about 1.3 mg/ml to about 1.4 mg/ml. The final dosage form may be at a concentration of about 0.6 mg/ml, 0.8 mg/ml, 1.0 mg/ml, 1.1 mg/ml, about 1.2 mg/ml, about 1.3 mg/ml, about 1.4 mg/ml, about 1.5 mg/ml, about 1.6 mg/ml, about 1.8 mg/ml or about 2.0 mg/ml. In one embodiment, the total dose is 75 mg. In one embodiment, the total dose is 150 mg, 225 mg, 375 mg or 525 mg. In another embodiment, the total dose is 300 mg. In one embodiment, the total dose is 450 mg. In one embodiment, the total dose is 600 mg. An anti-α4β7 antibody dose may be diluted into 250 ml saline, Ringer's or 5% dextrose solution for administration.

The dose can be administered to the patient over about 20 minutes, about 25 minutes, about 30 minutes, about 35 minutes, or about 40 minutes.

The dosing regimen can be optimized to result in the prevention of GvHD or the reduction of the risk of severe Grade or index level, e.g., Grade III or IV, index C or index D of GvHD suffered by the patient. In some embodiments, the dosing regimen does not alter the ratio of CD4 to CD8 in cerebrospinal fluid of patients receiving treatment. For example, the anti-α4β7 antagonist does not impair immune surveillance of the nervous system, e.g., the brain or spinal cord.

In one embodiment, the dosing regimen comprises an initial dose the day before an allogeneic stem cell transplantation (allo-HSCT), a subsequent dose approximately two weeks after the initial dose, and a second subsequent dose approximately six weeks after...
the initial dose. In an embodiment, the initial dose of the anti-α4β7 antibody is at least 12 hours before the allogeneic stem cell infusion. Although this anti-α4β7 antibody dosing regimen is useful for the induction dose and schedule of vedolizumab approved for the treatment of Crohn’s Disease or ulcerative colitis, subjects undergoing an allogeneic hematopoietic cell transplant, such as being treated with a conditioning regimen followed by the transplant, e.g., allo-HSCT, are expected to have markedly changing T-cell populations with variable α4β7 integrin expression during the post-transplant period. Furthermore, if the patient contracts infections or GVHD or has other adverse effects from the transplant procedure, clearance of the anti-α4β7 antibody may be affected. For example, if kidney damage results from the agents used for conditioning, treatment with dialysis could increase the clearance of antibodies from the bloodstream. Alternatively, after myeloablative therapy, there may be other physiological conditions that may result in unexpectedly high clearance of the anti-α4β7 antibody during initial therapy.

In some embodiments, an anti-α4β7 antibody is administered prior to allogeneic hematopoietic cell transplant, e.g., allo-HSCT. In some embodiments, an α4β7 antagonist, such as an anti-α4β7 antibody, is administered to a patient prior to and after allogeneic hematopoietic cell transplant, e.g., allo-HSCT. In some embodiments, an α4β7 antagonist, such as an anti-α4β7 antibody, is administered to a patient after allogeneic hematopoietic cell transplant, e.g., allo-HSCT, e.g., within 1 day after, 1 to 2 days after, 1 to 3 days after, 2 to 3 days after or 2 to 4 days after, 2 days after, 3 days after, 4 days after, 5 days after, 6 days after or 7 days after allogeneic hematopoietic cell transplant, e.g., allo-HSCT. For example, an anti-α4β7 antibody, e.g., vedolizumab, may be administered by intravenous infusion as an initial dose the day before allogeneic hematopoietic cell transplant, e.g., allo-HSCT, and then again at two, and six weeks after the initial dose.

The α4β7 antagonist, such as anti-α4β7 antibody may be administered to an individual (e.g., a human) alone or in conjunction with another agent. The α4β7 antagonist, such as an anti-α4β7 antibody can be administered before, along with or subsequent to administration of the additional agent. In one embodiment, more than one α4β7 antagonist which inhibits the binding of α4β7 integrin to its ligands is administered. In such an embodiment, an agent, e.g., a monoclonal antibody, such as an anti-MAAdCAM (e.g., anti-MAAdCAM-1) or an anti-VCAM-1 monoclonal antibody can be administered. In another embodiment, the additional agent inhibits the binding of leukocytes to an endothelial ligand in a pathway different from the α4β7 pathway. Such an agent can inhibit the binding, e.g. of chemokine (C-C motif) receptor 9 (CCR9)-expressing
lymphocytes to thymus expressed chemokine (TECK or CCL25) or an agent which prevents the binding of LFA-1 to intercellular adhesion molecule (ICAM). For example, an anti-TECK or anti-CCR9 antibody or a small molecule CCR9 inhibitor, such as inhibitors disclosed in PCT publication WO03/099773 or WO04/046092, or anti-ICAM-1 antibody or an oligonucleotide which prevents expression of ICAM, is administered in addition to a formulation of the present invention. In yet another embodiment, one or more additional active ingredients (e.g., methotrexate or a calcineurin inhibitor, e.g., tacrolimus or cyclosporin) commonly administered for GvHD prophylaxis therapy, may be administered in conjunction with an α4β7 antagonist, such as an anti-α4β7 antibody in a method of the present invention. In an embodiment, the dose of the co-administered medication can be decreased over time during the period of treatment by the α4β7 antagonist, such as an anti-α4β7 antibody.

In some embodiments, the co-administered medication is a calcineurin inhibitor, such as tacrolimus. In some embodiments, the calcineurin inhibitor treatment is started before allogeneic hematopoietic cell transplant, e.g., allo-HSCT and continued until at least day 100. In one embodiment, tacrolimus treatment may start during conditioning for the allogeneic hematopoietic cell transplant, e.g., allo-HSCT. The tacrolimus treatment may achieve a trough concentration of about 1 ng/dL, about 2 ng/dL, about 3 ng/dL, about 4 ng/dL, about 5 ng/dL, about 6 ng/dL, about 7 ng/dL, about 8 ng/dL, about 9 ng/dL, about 10 ng/dL, or about 5-10 ng/dL. Tacrolimus treatment may be kept at therapeutic levels for about 2 weeks, about 6 weeks, about 2 months, about 3 months, about 100 days after allogeneic hematopoietic cell transplant, e.g., allo-HSCT if no signs of GvHD are observed. Tacrolimus treatment may be discontinued by about 5 months, about 6 months, about 7 months after allogeneic hematopoietic cell transplant, e.g., allo-HSCT.

In some embodiments, the co-administered medication is methotrexate. In an embodiment, methotrexate is administered to the patient at about 2, 4, 6, 8, 10, or 12 mg/m² IV after allogeneic hematopoietic cell transplant, e.g., allo-HSCT (e.g., on days 1, 3, 6, and 11). The amount of methotrexate administered to the patient may be modified, or held, based on toxicity.

In one embodiment, the method comprises administering an effective amount of an anti-α4β7 antibody to a patient. If the anti-α4β7 antibody is in a formulation which is in a solid, e.g., dry state, the process of administration can comprise a step of converting the formulation to a liquid state. In one aspect, a dry formulation can be reconstituted, e.g., by a liquid as described above, for use in injection, e.g. intravenous, intramuscular or
subcutaneous injection. In another aspect, a solid or dry formulation can be administered
topically, e.g., in a patch, cream, aerosol or suppository.

The α4β7 antagonist, which is an anti-α4β7 antibody, can bind to an epitope on the
a4 chain (e.g., humanized MAb 21.6 (Bendig et al., U.S. Pat. No. 5,840,299), on the β7
chain (e.g., FIB504 or a humanized derivative (e.g., Fong et al., U.S. Pat. No. 7,528,236)),
or to a combinatorial epitope formed by the association of the a4 chain with the β7 chain.
AMG-181 or other antibodies described in US 2010/0254975 are anti-α4β7 antibodies. In
one aspect, the antibody binds a combinatorial epitope on the α4β7 complex, but does not
bind an epitope on the a4 chain or the β7 chain unless the chains are in association with
each other. The association of α4 integrin with β7 integrin can create a combinatorial
epitope for example, by bringing into proximity residues present on both chains which
together comprise the epitope or by conformationally exposing on one chain, e.g., the α4
integrin chain or the β7 integrin chain, an epitopic binding site that is inaccessible to
antibody binding in the absence of the proper integrin partner or in the absence of integrin
activation. In another aspect, the anti-α4β7 antibody binds both the α4 integrin chain and
the β7 integrin chain, and thus, is specific for the α4β7 integrin complex. The anti-α4β7
antibody can bind α4β7 but not bind α4β1, and/or not bind α4β7, for example. In another
aspect, the anti-α4β7 antibody binds to the same or substantially the same epitope as the
Chem., 269(11): 8348-8354, 1994). Murine ACT-1 Hybridoma cell line, which produces
the murine Act-1 monoclonal antibody, was deposited under the provisions of the
Budapest Treaty on Aug. 22, 2001, on behalf Millennium Pharmaceuticals, Inc., 40
Landsdowne Street, Cambridge, Mass. 02139, U.S.A., at the American Type Culture
Collection, 10801 University Boulevard, Manassas, Va. 20110-2209, U.S.A., under
Accession No. PTA-3663. In another aspect, the anti-α4β7 antibody is a human antibody
or an α4β7 binding protein using the CDRs provided in U.S. Patent Application
Publication No. 2010/0254975.

In one aspect, the α4β7 antagonist is an anti-MAdCAM antibody (see e.g., US
Patent No. 8,277,808, PF-00547659 or antibodies described in WO2005/067620), or an
engineered form of a ligand, such as a MAdCAM-Fc chimera such as described in US
In one aspect, the anti-α4β7 antibody inhibits binding of α4β7 to one or more of its ligands (e.g. the mucosal addressin, e.g., MAdCAM (e.g., MAdCAM-1), fibronectin, and/or vascular addressin (VCAM)). Primate MAdCAMs are described in the PCT publication WO 96/24673, the entire teachings of which are incorporated herein by this reference. In another aspect, the anti-α4β7 antibody inhibits binding of α4β7 to MAdCAM (e.g., MAdCAM-1) and/or fibronectin without inhibiting the binding of VCAM.

In one aspect, the anti-α4β7 antibodies for use in the treatments are humanized versions of the mouse Act-1 antibody. Suitable methods for preparing humanized antibodies are well-known in the art. Generally, the humanized anti-α4β7 antibody will contain a heavy chain that contains the 3 heavy chain complementarity determining regions (CDRs, CDR1, SEQ ID NO:4, CDR2, SEQ ID NO:5 and CDR3, SEQ ID NO:6) of the mouse Act-1 antibody and suitable human heavy chain framework regions; and also contain a light chain that contains the 3 light chain CDRs (CDR1, SEQ ID NO:7, CDR2, SEQ ID NO:8 and CDR3, SEQ ID NO:9) of the mouse Act-1 antibody and suitable human light chain framework regions. The humanized Act-1 antibody can contain any suitable human framework regions, including consensus framework regions, with or without amino acid substitutions. For example, one or more of the framework amino acids can be replaced with another amino acid, such as the amino acid at the corresponding position in the mouse Act-1 antibody. The human constant region or portion thereof, if present, can be derived from the κ or λ light chains, and/or the γ (e.g., γ1, γ2, γ3, γ4), μ, α (e.g., α1, α2), δ or ε heavy chains of human antibodies, including allelic variants. A particular constant region (e.g., IgG1), variant or portions thereof can be selected in order to tailor effector function. For example, a mutated constant region (variant) can be incorporated into a fusion protein to minimize binding to Fc receptors and/or ability to fix complement (see e.g., Winter et al, GB 2,209,757 B; Morrison et al, WO 89/07142; Morgan et al, WO 94/29351, Dec. 22, 1994). Humanized versions of Act-1 antibody were described in PCT publications nos. WO98/06248 and WO07/61679, the entire teachings of each of which are incorporated herein by this reference. Treatment methods using anti-α4β7 integrin antibodies are described in publication nos. U.S. 2005/0095238, U.S. 2005/0095238, WO2012151248 and WO 2012/151247.

In one aspect, the anti-α4β7 antibody is vedolizumab. Vedolizumab IV (also called MLN0002, ENTYVIO™ or KYNTELES™) is a humanized antibody (Ig) G1 mAb directed against the human lymphocyte integrin α4β7. The α4β7 integrin mediates
lymphocyte trafficking to GI mucosa, gut-associated lymphoid tissue (GALT) and mesenteric lymph nodes through adhesive interaction with mucosal addressin cell adhesion molecule-1 (MAdCAM-1), which is expressed on the endothelium of mesenteric lymph nodes and GI mucosa. Vedolizumab binds the α4β7 integrin, antagonizes its adherence to MAdCAM-1 and as such, impairs the migration of naive T cells to the GALT and mesenteric lymph nodes and gut homing leukocytes into GI mucosa.

In another aspect, the humanized anti-α4β7 antibody for use in the treatment comprises a heavy chain variable region comprising amino acids 20 to 140 of SEQ ID NO:1, and a light chain variable region comprising amino acids 20 to 131 of SEQ ID NO:2 or amino acids 1 to 112 of SEQ ID NO:3. If desired, a suitable human constant region(s) can be present. For example, the humanized anti-α4β7 antibody can comprise a heavy chain that comprises amino acids 20 to 470 of SEQ ID NO:1 and a light chain comprising amino acids 1 to 219 of SEQ ID NO:3. In another example, the humanized anti-α4β7 antibody can comprise a heavy chain that comprises amino acids 20 to 470 of SEQ ID NO:1 and a light chain comprising amino acids 20 to 238 of SEQ ID NO:2.

Vedolizumab is cataloged under Chemical Abstract Service (CAS, American Chemical Society) Registry number 943609-66-3).

Substitutions to the humanized anti-α4β7 antibody sequence can be, for example, mutations to the heavy and light chain framework regions, such as a mutation of isoleucine to valine on residue 2 of SEQ ID NO: 10; a mutation of methionine to valine on residue 4 of SEQ ID NO: 10; a mutation of alanine to glycine on residue 24 of SEQ ID NO: 11; a mutation of arginine to lysine at residue 38 of SEQ ID NO: 11; a mutation of alanine to arginine at residue 40 of SEQ ID NO: 11; a mutation of methionine to isoleucine on residue 48 of SEQ ID NO: 11; a mutation of isoleucine to leucine on residue 69 of SEQ ID NO: 11; a mutation of arginine to valine on residue 71 of SEQ ID NO: 11; a mutation of threonine to isoleucine on residue 73 of SEQ ID NO:11; or any combination thereof; and replacement of the heavy chain CDRs with the CDRs (CDR1, SEQ ID NO:4, CDR2, SEQ ID NO:5 and CDR3, SEQ ID NO:6) of the mouse Act-1 antibody; and replacement of the light chain CDRs with the light chain CDRs (CDR1, SEQ ID NO:7, CDR2, SEQ ID NO:8 and CDR3, SEQ ID NO:9) of the mouse Act-1 antibody.

The present invention provides a method for preventing GvHD in an allogeneic hematopoietic cell transplant, e.g., allogeneic hematopoietic stem cell transplant patient with vedolizumab. The method comprises the steps of administering an initial 300 mg dose of an anti-α4β7 antibody to a hematologic cancer patient, such as a person suffering
from leukemia, performing an allo-HSCT one day after the initial dose of vedolizumab, administering a subsequent 300 mg dose two weeks after the initial dose, and a second subsequent 300 mg dose six weeks after the initial dose. Alternatively, in some embodiments, the dose of the anti-α4β7 antibody is lower, e.g., 75 mg or 150 mg, or higher, e.g., 450 mg or 600 mg, than 300 mg.

The invention provides an anti-α4β7 antibody for use in preventing GVHD in a patient having an allogeneic hematopoietic cell transplant, e.g., allo-HSCT, the use comprising administering an initial dose of the anti-α4β7 antibody the day before the allo-HSCT, two weeks after the initial dose, and six weeks after the initial dose. The use in preventing may further comprise administration of tacrolimus and/or methotrexate. In some embodiments, the anti-α4β7 antibody is vedolizumab.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature and patent citations are incorporated herein by reference.

EXEMPLIFICATION

Example 1

A phase Ib, open-label, dose-finding study is designed to evaluate the safety, tolerability, and clinical activity of adding vedolizumab to standard graft-versus-host disease (GvHD) prophylaxis (tacrolimus plus short-term methotrexate) in adult patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). Vedolizumab dose finding is cohort based and follows a rule-based dose-finding study design with pharmacokinetic (PK) guidance. After a tolerated dose with acceptable PK is identified, the cohort at that dose level may be expanded to further assess the tolerability and effectiveness of vedolizumab.

Eligibility is determined during the Screening period, which may last for up to 28 days before Day - 1 (designation of the day of the first IV infusion of vedolizumab). Patients who meet all eligibility criteria and provide written informed consent are enrolled in this study. Study drug is administered initially on Day - 1 before allo-HSCT and then on Days +13 and +42 after allo-HSCT. Patients who are undergoing unrelated-donor myeloablative transplant for the treatment of hematologic malignancies and who are less than or equal to 60 years of age are eligible for enrollment. After a recommended phase 2 dose is identified, the cohort at that dose level can be expanded to include additional patients receiving myeloablative conditioning or reduced-intensity conditioning "RIC"
(less than or equal to 75 years of age) who are undergoing either related or unrelated allogeneic HSCT for the treatment of hematologic malignancies or myeloproliferative neoplasms.

Patients are excluded from the study if they have received prior allogeneic transplants or if they planned to undergo umbilical cord blood transplant, receive ex vivo T-cell-depleted hematopoietic stem cells (HSCs), receive any in vivo T-cell depleting antibodies, or RIC (in the dose-finding portion only). Patients with active cerebral/meningeal disease, active cytomegalovirus (CMV) colitis, or signs and symptoms of progressive multifocal leukoencephalopathy (PML) or any history of PML are also excluded. In addition, patients with nonmalignant hematological disorders (e.g., aplastic anemia, sickle cell anemia, thalassemias, Fanconi anemia) are excluded in both portions of the study.

For PK endpoints, an evaluable patient is one who receives vedolizumab and has at least 1 PK sample collected.

Patients who remain in remission are followed for safety and development of acute and chronic GvHD for 1 year after allo-HSCT or until the patient’s death or withdrawal of consent or termination of the study by the sponsor. All patients are followed for overall survival (OS) until death, withdrawal of consent, termination of the study by the sponsor, or for a maximum of 1 year after the last patient is enrolled in the study. Patients attend a Day +100 visit (+7 days) at which time they will enter posttreatment follow-up.

Dose escalation starts with a low-dose cohort receiving vedolizumab at 75 mg IV on Day -1 and on Days +13 and +42 after allo-HSCT. HSC infusion occurs on Day 0 (no sooner than 12 hours after completion of IV infusion of vedolizumab on Day -1). The first patient in each dosing cohort is monitored for dose-limiting toxicities (DLTs) from the start of the first IV infusion of vedolizumab on Day -1 to Day +28 after allo-HSCT (the DLT observation period) including assessment for neutrophil recovery by Day +28. If the first patient in the first cohort tolerates vedolizumab IV at 75 mg and engraftment occurs, then 2 more patients will be enrolled in the first cohort. If none of the first 3 patients experience DLTs, the next cohort receives vedolizumab 300 mg IV on Day -1 and on Days +13 and +42 after allo-HSCT. If the first patient in this cohort tolerates vedolizumab IV at 300 mg and engraftment occurs, then 2 more patients are enrolled in the second cohort. If the first 3 patients at 300 mg tolerate the treatment without experiencing DLTs, then the decision on whether to increase the vedolizumab IV dose in the next cohort is guided by the PK results. If 1 of the first 3 patients in the cohort experiences a DLT, then
3 additional patients are enrolled at the same dose level and monitored for DLTs from Day -1 until Day +28. If none of the additional patients experiences a DLT, then the decision on whether to increase the vedolizumab IV dose in the next cohort is guided by the PK results. If 2 or more patients in a cohort of either 3 or 6 patients experience a DLT, then the dose of vedolizumab IV for the next cohort of 3 patients is reduced. These patients will be monitored for DLTs in the same manner that patients in the previous cohort were monitored.

After a tolerated dose level with acceptable PK is identified in patients who are undergoing unrelated-donor myeloablative transplant for the treatment of hematologic malignancies, the cohort at that dose level may be expanded to include approximately 18 additional patients undergoing myeloablative conditioning or reduced-intensity conditioning (RIC) and are receiving either related or unrelated allo-HSCT for the treatment of hematologic malignancies or myeloproliferative neoplasms. This group of patients allows for the further assessment of the tolerability and clinical activity of vedolizumab IV.

Vital signs, physical and neurological examinations, adverse event (AE) assessments, and laboratory values (chemistry, hematology, and urinalysis) are obtained to evaluate the safety and tolerability of vedolizumab IV. To exclude patients with progressive multifocal leukoencephalopathy (PML), a Risk Assessment and Minimization for PML (RAMP) questionnaire is administered at Screening and before vedolizumab IV administration on Days -1 before allo-HSCT, and on Days +13 and +42 after allo-HSCT.

Serial blood samples for the evaluation of PK of vedolizumab are obtained at prespecified time points. PK of vedolizumab is analyzed for each of the first 3 patients at each dose level. It is expected that the concentration-time profile of vedolizumab will be influenced by the level of $\alpha_4\beta_7$ target saturation. If $\alpha_4\beta_7$ is saturated, then vedolizumab clearance would be linear; if $\alpha_4\beta_7$ is not saturated, then clearance would be nonlinear indicating rapid elimination. If the clearance of vedolizumab is nonlinear at the 300 mg dose, then subsequent dosing for all patients is increased in approximately 150 mg increments (up to a maximum of 600 mg) until linear PK clearance is achieved.

Serial blood samples for determination of the serum concentration of vedolizumab and anti-vedolizumab antibodies and serum biomarkers (including, but not limited to, interleukin-6 [IL-6], interleukin-17 [IL-17], and suppressor of tumorigenicity 2 [ST2]) are obtained at pre-specified time points. In addition, blood samples will be collected to perform flow cytometry for cell immunophenotyping to measure cell populations as
determined by levels of various cellular biomarkers (such as CD8+, CD38+, CD8+ effector memory T cells, and CD4+ memory T cells), and to perform MadCAM-1-FC binding inhibition assays at pre-specified time points.

Toxicity is evaluated according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.03, effective date 14 June 2010.

Example 2

Monte Carlo simulations were run with a population pharmacokinetic model of vedolizumab serum concentration in clinical studies. Simulations included interindividual and residual variability in addition to weight and albumin effects. All other covariates were set to their reference values. One thousand adult patients were simulated in this study. Albumin and weight were randomly sampled from a normal distribution. The simulated dosing regimen was 75 mg of vedolizumab via a 30 minute IV infusion on days -1, +13, +42 (i.e., days 0, 14 and 43 relative to first dose).

Observed data from three patients enrolled in the phase Ia, open-label, dose-finding study (Example 1) was overlaid with the simulation data (see FIG. 3). The "fuzziness" of the area between the jagged lines is due to residual variability. FIG. 3 illustrates the measured and simulated vedolizumab serum concentration over time. In this figure, the vedolizumab concentration in one patient did not reach $10 \mu g/ml$ except immediately after dosing. Another patient retained more than $10 \mu g/ml$ vedolizumab for several days after the second dose, but not the first dose. A third patient retained more than $10 \mu g/ml$ vedolizumab for several days after the first dose.
SEQ ID NO:1

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Gin Val Gin Leu Val Gin Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Gly Ser Gly Tyr Thr Phe
35 40 45

Thr Ser Tyr Trp Met His Trp Val Arg Gin Ala Pro Gly Gin Arg Leu
50 55 60

Glu Trp Ile Gly Glu Ile Asp Pro Ser Glu Ser Asn Thr Asn Tyr Asn
65 70 75 80

Gin Lys Phe Lys Gly Arg Val Thr Leu Thr Val Asp Ile Ser Ala Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Gly Tyr Asp Gly Trp Asp Tyr Ala Ile Asp
115 120 125

Tyr Trp Gly Gin Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
20 130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145 150 155 160

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
25 180 185 190

Phe Pro Ala Val Leu Gin Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gin Thr Tyr lie Cys Asn

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu

Leu Ala Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp

Thr Leu Met lie Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Val Asp

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gin Tyr Asn

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gin Asp Trp

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro

Ala Pro lie Glu Lys Thr lie Ser Lys Ala Lys Gly Gin Pro Arg Glu

Pro Gin Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn

Gin Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp lie

Ala Val Glu Trp Glu Ser Asn Gly Gin Pro Glu Asn Asn Tyr Lys Thr
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
420 425 430
Leu Thr Val Asp Lys Ser Arg Trp Gin Gin Gly Asn Val Phe Ser Cys
435 440 445
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gin Lys Ser Leu
450 455 460
Ser Leu Ser Pro Gly Lys
465 470

SEQ ID NO: 2
Met Gly Trp Ser Cys lie lie Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15
Val His Ser Asp Val Val Met Thr Gin Ser Leu Ser Leu Pro Val
20 25 30
Thr Pro Gly Glu Pro Ala Ser lie Ser Cys Arg Ser Ser Gin Ser Leu
35 40 45
Ala Lys Ser Tyr Gly Asn Thr Tyr Leu Ser Trp Tyr Leu Gin Lys Pro
50 55 60
Gly Gin Ser Pro Gin Leu Leu lie Tyr Gly lie Ser Asn Arg Phe Ser
65 70 75 80
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95
Leu Lys lie Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
25 100 105 110
Leu Gin Gly Thr His Gin Pro Tyr Thr Phe Gly Gin Gly Thr Lys Val
115 120 125
Glu lie Lys Arg Thr Val Ala Ala Pro Ser Val Phe lie Phe Pro Pro
130 135 140
Ser Asp Glu Gin Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145 150 155 160
Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gin Trp Lys Val Asp Asn
165 170 175
Ala Leu Gin Ser Gly Asn Ser Gin Glu Ser Val Thr Glu Gin Asp Ser
180 185 190
Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
195 200 205
Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gin Gly
210 215 220
Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

SEQ ID NO: 3

Asp Val Val Met Thr Gin Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15
Glu Pro Ala Ser lie Ser Cys Arg Ser Ser Gin Ser Leu Ala Lys Ser
20 25 30
Tyr Gly Asn Thr Tyr Leu Ser Trp Tyr Leu Gin Lys Pro Gly Gin Ser
35 40 45
Pro Gin Leu Leu lie Tyr Gly lie Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys lie
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Leu Gin Gly
85 90 95
Thr His Gin Pro Tyr Thr Phe Gly Gin Gly Thr Lys Val Glu lie Lys
100 105 110
Arg Ala Asp Ala Ala Pro Ser Val Phe lie Phe Pro Pro Ser Asp Glu
5 115 120 125
Gin Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140
Tyr Pro Arg Glu Ala Lys Val Gin Trp Lys Val Asp Asn Ala Leu Gin
145 150 155 160
10 Ser Gly Asn Ser Gin Glu Ser Val Thr Glu Gin Asp Ser Lys Asp Ser
165 170 175
Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190
Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gin Gly Leu Ser Ser
195 200 205
Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

SEQ ID NO: 4
20 Ser Tyr Trp Met His
1 5

SEQ ID NO: 5
Glu lie Asp Pro Ser Glu Ser Asn Thr Asn Tyr Asn Gin Lys Phe Lys
25 1 5 10 15
Gly
SEQ ID NO: 6
Gly Gly Tyr Asp Gly Trp Asp Tyr Ala lie Asp Tyr
1 5 10

SEQ ID NO: 7
Arg Ser Ser Gin Ser Leu Ala Lys Ser Tyr Gly Asn Thr Tyr Leu Ser
1 5 10 15

SEQ ID NO: 8
Gly lie Ser Asn Arg Phe Ser
1 5

SEQ ID NO: 9
Leu Gin Gly Thr His Gin Pro Tyr Thr
15 5

SEQ ID NO: 10
Asp lie Val Met Thr Gin Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 4 10 15 20

Glu Pro Ala Ser lie Ser Cys Arg Ser Ser Gin Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gin Lys Pro Gly Gin Ser
35 40 45

Pro Gin Leu Leu lie Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
25 30 35 40 45

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys lie
65  70  75  80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gin Ala
85  90  95
Leu Gin Thr Pro Gin Thr Phe Gly Gin Gly Lys Val Glu lie Lys
5 100 105 110

SEQ ID NO: 11
Gin Val Gin Leu Val Gin Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1  5 10  15
10 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25  30
Ala Met His Trp Val Arg Gin Ala Pro Gly Gin Arg Leu Gin Trp Met
35 40  45
Gly Trp lie Asn Ala Gly Asn Gly Asn Thr Lys Tyr Ser Gin Lys Phe
15 50 55 60
Gin Gly Arg Val lie Thr Arg Asp Thr Ser Ala Ser Thr Ala Tyr
65 70 75  80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
20 Ala Arg Gly Gly Tyr Tyr Gly Ser Gly Ser Asn Tyr Trp Gly Gin Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115
CLAIMS

1. A method of preventing graft versus host disease (GvHD), wherein the method comprises the step of:
   - administering to a human patient undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), a humanized antibody having binding specificity for human α4β7 integrin,

   wherein the humanized antibody is administered to the patient according to the following dosing regimen:
   - a. an initial dose of 75 mg, 300 mg, 450 mg or 600 mg of the humanized antibody as an intravenous infusion the day before allo-HSCT;
   - b. followed by a second subsequent dose of 75 mg, 300 mg, 450 mg or 600 mg of the humanized antibody as an intravenous infusion at about two weeks after the initial dose;
   - c. followed by a third subsequent dose of 75 mg, 300 mg, 450 mg or 600 mg of the humanized antibody as an intravenous infusion at about six weeks after the initial dose;

   further wherein the humanized antibody comprises an antigen binding region of nonhuman origin and at least a portion of an antibody of human origin, wherein the humanized antibody has binding specificity for the α4β7 complex, wherein the antigen-binding region comprises the CDRs:
   - Light chain: CDR1 SEQ ID NO:7
     CDR2 SEQ ID NO:8 and
     CDR3 SEQ ID NO:9; and
   - Heavy chain: CDR1 SEQ ID NO:4
     CDR2 SEQ ID NO:5 and
     CDR3 SEQ ID NO:6.

2. The method of claim 1, wherein the dosing regimen results in Grade II GvHD, Grade I GvHD or no GvHD.

3. The method of claim 1 or 2, wherein said preventing results in sustained α4β7-blockade at the time of hematopoietic stem cell infusion.
4. The method of claim 1, 2, or 3, wherein tacrolimus is co-administered to the human patient.

5. The method of any one of claims 1 to 4, wherein methotrexate is co-administered to the human patient.

6. The method of any one of claims 1 to 5, wherein the humanized antibody is administered to the patient over about 30 minutes.

7. The method of any one of claims 1 to 6, wherein the humanized antibody is reconstituted from a lyophilized formulation.

8. The method of claim 7, further wherein the humanized antibody is reconstituted to comprise a stable liquid formulation.

9. The method of any one of claims 1 to 8, wherein the humanized antibody has a heavy chain variable region sequence of amino acids 20 to 140 of SEQ ID NO:1.

10. The method of any one of claims 1 to 9, wherein the humanized antibody has a light chain variable region sequence of amino acids 20 to 131 of SEQ ID NO:2.

11. The method of claim 9 or 10, wherein the humanized antibody has a heavy chain comprising amino acids 20 to 470 of SEQ ID NO:1 and a light chain comprising amino acids 20 to 238 of SEQ ID NO:2.

12. The method of any one of claims 1 to 11, wherein the humanized antibody is vedolizumab.

13. A method of treating a patient suffering from cancer or a nonmalignant hematological, immunological disease or autoimmune disease, comprising the steps of:
   a. conditioning the immune system of the patient for hematopoietic stem cell transplant,
   b. administering a humanized antibody having binding specificity for human α4β7 integrin,
   c. waiting at least 12 hours,
d. administering allogeneic hematopoietic stem cells,

e. waiting thirteen days, then administering a second dose of humanized antibody having binding specificity for human α4β7 integrin, and

f. waiting four weeks, then administering a third dose of humanized antibody having binding specificity for human α4β7 integrin.

14. The method of claim 13, further comprising administering tacrolimus to the patient.

15. The method of claim 13 or 14, further comprising administering methotrexate to the patient.

16. The method of anyone of claims 13 to 15, wherein the conditioning of the immune system is myeloablative conditioning or reduced intensity conditioning.

17. The method of anyone of claims 13 to 16, wherein the patient has an adverse event that does not include stage 3 or stage 4 GvHD of the intestine.

18. The method of anyone of claims 13 to 16, wherein the patient has an adverse event that does not include grade III or grade IV GvHD.

19. The method of anyone of claims 13 to 16, wherein the patient has leukemia or lymphoma.

20. The method of anyone of claims 13 to 16, wherein the allogeneic hematopoietic stem cells are from peripheral blood.

21. The method of anyone of claims 13 to 16, wherein the allogeneic hematopoietic stem cells engraft without further immunosuppressive therapy.

22. The method of anyone of claims 13 to 16, wherein the humanized antibody comprises an antigen binding region of nonhuman origin and at least a portion of an antibody of human origin, wherein the humanized antibody has binding specificity for the α4β7 complex, wherein the antigen-binding region comprises the CDRs:

Light chain: CDR1 SEQ ID NO:7
CDR2  SEQ ID NO: 8 and
CDR3  SEQ ID NO: 9; and
Heavy chain:  CDR1  SEQ ID NO: 4
CDR2  SEQ ID NO: 5 and
CDR3  SEQ ID NO: 6.

23. The method of claim 22, wherein the humanized antibody is reconstituted from a lyophilized formulation.

24. The method of claim 22, wherein the humanized antibody has a heavy chain variable region sequence of amino acids 20 to 140 of SEQ ID NO: 1.

25. The method of claim 22, wherein the humanized antibody has a light chain variable region sequence of amino acids 20 to 131 of SEQ ID NO: 2.

26. The method of any one of claims 22 to 25, wherein the humanized antibody has a heavy chain comprising amino acids 20 to 470 of SEQ ID NO: 1 and a light chain comprising amino acids 20 to 238 of SEQ ID NO: 2.

27. The method of any one of claims 22 to 26, wherein the humanized antibody is vedolizumab.

28. A method reducing the occurrence of acute graft versus host disease (GvHD), wherein the method comprises the step of:
   administering to a human patient undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), a humanized antibody having binding specificity for human α4β7 integrin,
   wherein the humanized antibody is administered to the patient according to the following dosing regimen:
   a. an initial dose of 75 mg, 300 mg, 450 mg or 600 mg of the humanized antibody as an intravenous infusion the day before allo-HSCT;
      b. followed by a second subsequent dose of 300 mg of the humanized antibody as an intravenous infusion at about two weeks after the initial dose;
c. followed by a third subsequent dose of 300 mg of the humanized antibody as an intravenous infusion at about six weeks after the initial dose;

wherein the humanized antibody comprises an antigen binding region of nonhuman origin and at least a portion of an antibody of human origin, wherein the humanized antibody has binding specificity for the α4β7 complex, wherein the antigen-binding region comprises the CDRs:

Light chain:  
CDR1  SEQ ID NO:7  
CDR2  SEQ ID NO:8 and  
CDR3  SEQ ID NO:9; and  

Heavy chain:  
CDR1  SEQ ID NO:4  
CDR2  SEQ ID NO:5 and  
CDR3  SEQ ID NO:6,  

thereby reducing the occurrence of GvHD.

29. The method of claim 28, wherein reducing the occurrence of acute graft versus host disease (GvHD) results in Grade I or Grade II GvHD, per modified Glucksberg criteria, or similar severity of GvHD per other scoring system, or no GvHD.

30. The method of claim 28, wherein reducing the occurrence of acute GvHD is a 50% reduction in cumulative incidence and severity of Grade II-IV or Grade III-IV acute GvHD at Day 100 as compared to treatment with methotrexate and calcineurin inhibitor alone.

31. The method of claim 28, wherein reducing the occurrence of acute graft versus host disease (GvHD) is a reduction in 1 year mortality as compared to treatment with methotrexate and calcineurin inhibitor alone.

32. A method of suppressing an immune response in a cancer patient, wherein the method comprises the step of:

administering to a human patient undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), a humanized antibody having binding specificity for human α4β7 integrin,

wherein the humanized antibody is administered to the patient according to the following dosing regimen:
a. an initial dose of 75 mg, 300 mg, 450 mg or 600 mg of the humanized antibody as an intravenous infusion the day before allo-HSCT;

b. followed by a second subsequent dose of 300 mg of the humanized antibody as an intravenous infusion at about two weeks after the initial dose;

c. followed by a third subsequent dose of 300 mg of the humanized antibody as an intravenous infusion at about six weeks after the initial dose;

further wherein the humanized antibody comprises an antigen binding region of nonhuman origin and at least a portion of an antibody of human origin, wherein the humanized antibody has binding specificity for the α4β7 complex, wherein the antigen-binding region comprises the CDRs:

Light chain:  
CDR1  SEQ ID NO:7

CDR2  SEQ ID NO:8 and

CDR3  SEQ ID NO:9; and

Heavy chain:  
CDR1  SEQ ID NO:4

CDR2  SEQ ID NO:5 and

CDR3  SEQ ID NO:6.

33. A method of treating a transplant patient, wherein the transplant patient is a recipient of an infusion of allogeneic hematopoietic cells, comprising administering an anti-α4β7 antagonist.

34. The method of claim 33, wherein, prior to the infusion, the transplant patient is the recipient of conditioning therapy selected from myeloablative conditioning or reduced intensity conditioning.

35. The method of claim 33 or 34, wherein the anti-α4β7 antagonist is administered prior to the infusion.

36. The method of claim 33 or 34, wherein the anti-α4β7 antagonist is administered in multiple doses, with at least one dose prior to the infusion.
37. The method of claim 33 or 34, wherein the anti-α4β7 antagonist is administered in multiple doses, with the first dose on the same day as the infusion.

38. The method of claim 33 or 34, wherein the anti-α4β7 antagonist is administered in multiple doses, with the first dose on the next day after the infusion.

39. The method of claim 33 or 34, wherein the anti-α4β7 antagonist is administered as a single dose the day before, the day of, or the next day after, the infusion.

40. The method of claim 35 or 36, wherein a dose of anti-α4β7 antagonist is administered between conditioning and the infusion.

41. The method of anyone of claims 33 to 40, wherein the transplant patient is suffering from cancer.

42. The method of claim 41, wherein the cancer is a hematological cancer.

43. The method of claim 42, wherein the hematological cancer is leukemia, lymphoma, myeloma or a myeloproliferative neoplasm.

44. The method of claim 43, wherein the leukemia is acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML).

45. The method of any one of claims 33 to 40, wherein the transplant patient is suffering from a nonmalignant hematological or immune disease.

46. The method of claim 45, wherein the nonmalignant hematological or immune disease is selected from the group consisting of hemoglobinopathy, bone marrow failure syndrome, and immune disease.

47. The method of anyone of claims 33 to 46, wherein the anti-α4β7 antagonist is an anti-α4β7 antibody which has binding specificity for the α4β7 integrin complex.
48. The method of claim 46, wherein the \textit{anti-α4β7} antibody is a humanized antibody, wherein the antigen-binding region of the humanized antibody comprises the CDRs:

- **Light chain:**
  - CDR1 SEQ ID NO:7
  - CDR2 SEQ ID NO:8 and
  - CDR3 SEQ ID NO:9; and

- **Heavy chain:**
  - CDR1 SEQ ID NO:4
  - CDR2 SEQ ID NO:5 and
  - CDR3 SEQ ID NO:6.

49. The method of claim 48, wherein the humanized antibody is reconstituted from a lyophilized formulation.

50. The method of claim 47 or 48, wherein the humanized antibody is administered intravenously.

51. The method of any one of claims 48 to 50, wherein the humanized antibody has a heavy chain variable region sequence of amino acids 20 to 140 of SEQ ID NO:1.

52. The method of any one of claims 48 to 51, wherein the humanized antibody has a light chain variable region sequence of amino acids 20 to 131 of SEQ ID NO:2.

53. The method of claim 51 or 52, wherein the humanized antibody has a heavy chain comprising amino acids 20 to 470 of SEQ ID NO:1 and a light chain comprising amino acids 20 to 238 of SEQ ID NO:2.

54. The method of any one of claims 48 to 53, wherein the humanized antibody is vedolizumab.

55. The method of anyone of claims 33 to 54, further comprising treating the transplant patient with tacrolimus, tacrolimus and methotrexate or methotrexate.

56. The method of anyone of claims 33 to 55, further comprising detecting engraftment of the allo-HSCs by measuring neutrophil number.
57. The method of claim 56, further comprising measuring a biomarker selected from the group consisting of interleukin-6 (IL-6), interleukin-17 (IL-17), suppressor of tumorigenicity 2 (ST2), CD8+ cells, CD38+ cells, CD8+ bright effector memory T cells, and CD4+ memory T cells, wherein the amount of the biomarker measured before or within one week after the infusion and the amount of the biomarker measured at a time 20 to 100 days after the infusion is unchanged.

58. The method of anyone of claims 33 to 57, wherein the patient has an adverse event that does not include stage 3 or stage 4 GvHD of the intestine.

59. The method of anyone of claims 33 to 58, wherein the allogeneic hematopoietic cells are allogeneic hematopoietic stem cells.

60. The method of anyone of claims 33 to 58, wherein the allogeneic hematopoietic cells are allogeneic leukocytic cells.

61. The method of claim 60, wherein the allogeneic leukocytic cells are T-lymphocytes.
OVERVIEW OF STUDY DESIGN FROM DAYS -1 TO +50

STUDY DRUG ADMINISTRATION, PHARMACOKINETIC, AND PHARMACODYNAMIC COLLECTION: DAYS -1 TO +50
• ALLO-HSCT ON DAY 0
• VEDOLIZUMAB ADMINISTERED ON DAY -1 BEFORE ALLO-HSCT AND ON DAYS +13 AND +42 AFTER ALLO-HSCT

SAMPLES FOR AVA
SAMPLES FOR FLOW CYTOMETRY
GvHD
BIOMARKERS
PK

DOSE 1 PK SPECIMENS

DOSE 2 PK SPECIMENS

DOSE 3 PK SPECIMENS

STUDY DAY
-1 0 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 50

ALLOGENEIC HSCT
VEDOLIZUMAB ADMINISTRATION

DLT OBSERVATION PERIOD (START OF THE FIRST IV INFUSION OF VENDOLIZUMAB ON DAY -1 TO DAY +28 AFTER ALLO-HSCT)

FIG. 1
PK SAMPLING FOR PATIENTS WHO HAVE BEEN DISCHARGED FROM THE HOSPITAL WILL BE ALIGNED TO CLINIC VISITS AND THEREFORE MAY NOT BE AS FREQUENT AS REPRESENTED IN THIS FIGURE.
BLOCKING THE α4β7/MADCAM-1 INTERACTION IN GALT AND MLNs MAY REDUCE THE GENERATION OF THE ALLO-REACTIVE MEMORY T CELLS THEREBY REDUCING THE OCCURRENCE OF GVHD

FIG. 2