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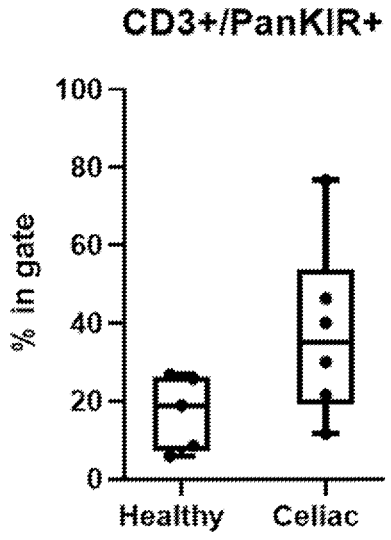


FIG. 8A

(57) Abstract: The present invention provides binding agents that specifically bind to CD8+KIR+ T regulatory cells and their use in the treatment of diseases or disorders, such as an inflammatory disease, an autoimmune disease, cancer, or an infectious disease.

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[Continued on next page]

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BINDING AGENTS AND METHODS OF USING THE SAME

STATEMENT REGARDING SEQUENCE LISTING

[0001] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 670151_403WO_Sequence_Listing.txt. The text file is 85 KB, was created on January 20, 2022, and is being submitted electronically via EFS-Web.

BACKGROUND

[0002] The immune system includes the innate immune and the adaptive immune system. The adaptive immune system has a number of cell subtypes, including T cells subsets and B cell subsets. T cell subsets include a variety of types of T cells, including naïve T lymphocytes and effector T lymphocytes, such as cytotoxic T cells and helper T cells, and regulatory T cells. The activity of these T cells types is achieved by a balance between the activity of effector T cells and regulation by regulatory T cells. While effector T cells promote inflammation, regulatory T cells are generally thought to control it. Therefore, Tregs play an important role in autoimmune pathogenesis by maintaining self-tolerance, limiting autoimmunity and by controlling expansion and activation of autoreactive CD4+ T effector cells. Disruption of the balance between effector and regulatory T cells can lead to an inappropriate activation or suppression of an immune response, the loss of self-tolerance, autoimmune disorders, and cancer. The mechanisms and the regulation of regulatory T cells to maintain balance of the immune system is only beginning to be understood.

BRIEF SUMMARY

[0003] Provided herein are binding agents and their methods of use to modulate the activity of CD8+KIR+ regulatory T cells (Tregs). The binding agents are bispecific or multi-specific and specifically bind to antigens expressed on the surface of the CD8+KIR+ Tregs. In some embodiments, the

CD8+KIR+ Tregs are MHC class I restricted. In some embodiments, the CD8+KIR+ Tregs are not MHC Qa-1 restricted. Also provided are methods of using the binding agents for the treatment of autoimmune disease, infectious disease, and cancer.

[0004] In some embodiments, a binding agent is provided that comprises a first binding domain that specifically binds to a first antigen, the first antigen selected from antigens expressed on CD8+KIR+ T regulatory cells (Tregs) other than a KIR protein; and a second binding domain that specifically binds to an inhibitory KIR protein expressed on the surface of the CD8+KIR+ Tregs, wherein the binding agent binds to CD8+KIR+ Tregs.

[0005] In some embodiments, the first antigen is selected from the group consisting of CD3, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB. In some embodiments, the first antigen is selected from the group consisting of CD3, CD5, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB. In some embodiments, the first antigen is selected from CD3, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD122, ICOS, OX-40, 2B4, 41BB, and HLA-DR. In some embodiments, the first antigen is selected from CD3, CD5, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD122, ICOS, OX-40, 2B4, 41BB, and HLA-DR. In some embodiments, the first antigen is selected from LAG-3/CD223, TIM-3, PD-1, S1000A8/9, and TLT2. In some embodiments, the first antigen is selected from CD103 (ITGAE), CD166, CD177, CXCR3, and S1000A8/9. In some embodiments, the first antigen is selected from CCR7, CXCR3, and CXCR5. In some embodiments, the first antigen is selected from PD-1, CXCR3, and ICOS. In some embodiments, the first antigen is selected

from CD3, CD5, and CD8. In some embodiments, the first antigen is selected from CD3 and CD8.

[0006] In some embodiments, the binding agent is a bispecific antibody, a diabody, an antibody Fc fusion, an scFv1-ScFv2, an $ScFv1_2$-Fc-$scFv2_2$, an IgG-scFv, a DVD-Ig, a triomab/quadroma, a two-in-one IgG, a $scFv_2$-Fc, a TandAb, an scFv-HSA-scFv, an scFv-VHH, a Fab-scFv-Fc, a Fab-VHH-Fc, a dAb-IgG, an IgG-VHH, a Tandem scFv-Fc, a $(scFv1)_2$-Fc-$(VHH)_2$, a BiTe, a DART, a crossmab, an anticalin, an affibody, an avimer, a DARPin, an adnectin, a scFv-Fc, a one-armed tandem scFv-Fc, or a DART-Fc . In some embodiments, either the first or second binding domain of the binding agent is selected from an antibody or antigen binding portion thereof, and the other binding domain is an antibody fragment. In some embodiments, the antigen binding portion is a Fab, Fab', $F(ab')_2$, Fv, scFv, or a single domain antibody (also referred to as a VHH, VNAR, sdAb, or nanobody). In some embodiments, the first binding domain comprises a heavy chain variable region and a light chain variable region. In some embodiments, the second binding domain comprises a heavy chain variable region and a light chain variable region.

[0007] In some embodiments, the first binding domain specifically binds to CD3 or a subunit of CD3, optionally CD3epsilon. In some embodiments, the first binding domain comprises a heavy chain variable region (VH) and a light chain variable region(VL), the VH and VL having amino acid sequences selected from the pairs of amino acids sequences set forth in the group consisting of:

a. SEQ ID NO:1 and SEQ ID NO:2, respectively;
b. SEQ ID NO:9 and SEQ ID NO:10, respectively;
c. SEQ ID NO:17 and SEQ ID NO:18, respectively;
d. SEQ ID NO:25 and SEQ ID NO:26, respectively;
e. SEQ ID NO:33 and SEQ ID NO:34, respectively;
f. SEQ ID NO:41 and SEQ ID NO:34, respectively;
g. SEQ ID NO:45 and SEQ ID NO:34, respectively;

h. SEQ ID NO:49 and SEQ ID NO:50, respectively;

i. SEQ ID NO:57 and SEQ ID NO:58, respectively;

j. SEQ ID NO:65 and SEQ ID NO:66, respectively; and

k. SEQ ID NO:65 and SEQ ID NO:166, respectively.

[0008] In some embodiments, the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3, and lCDR1, lCDR2, and lCDR3, respectively, the CDRs having amino acid sequences selected from the sets of amino acid sequences set forth in the group consisting:

a. SEQ ID NO:3 to SEQ ID NO:8, respectively;

b. SEQ ID NO:11 to SEQ ID NO:16, respectively;

c. SEQ ID NO:19 to SEQ ID NO:24, respectively;

d. SEQ ID NO:27 to SEQ ID NO:32, respectively;

e. SEQ ID NO:35 to SEQ ID NO:40, respectively;

f. SEQ ID NO:42 to SEQ ID NO:44 and SEQ ID NO:38 to SEQ ID NO:40, respectively;

g. SEQ ID NO:46 to SEQ ID NO:48 and SEQ ID NO:38 to SEQ ID NO:40, respectively;

h. SEQ ID NO:51 to SEQ ID NO:56, respectively;

i. SEQ ID NO:59 to SEQ ID NO:64, respectively;

j. SEQ ID NO:67 to SEQ ID NO:72, respectively; and

k. SEQ ID NOs: 67-69 and 167-169, respectively.

[0009] In some embodiments, the first binding domain specifically binds to CD8 or a subunit of CD8, optionally CD8alpha. In some embodiments, the first binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL), the VH and VL regions having the amino acid sequences selected from the pairs of amino acid sequences set forth in group consisting of:

a. SEQ ID NO:73 and SEQ ID NO:74, respectively; and

b. SEQ ID NO:81 and SEQ ID NO:82, respectively;

or the first binding domain comprises a VHH chain, the VHH chain having the amino acid sequence selected from the amino acid sequences set forth in the group consisting of the following:

c. SEQ ID NO:89;

d. SEQ ID NO:93; and

e. SEQ ID NO:97.

[0010] In some embodiments, the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3, and lCDR1, lCDR2, and lCDR3, respectively, the amino acid sequences of the CDRs selected from the amino acid sequences set forth in the group consisting of the following:

a. SEQ ID NO:75 to SEQ ID NO:80, respectively; or

b. SEQ ID NO:83 to SEQ ID NO:88, respectively;

or the first binding domain includes a VHH chain having hCDR1, hCDR2 and hCDR3, the amino acid sequences of the VHH CDRs selected from the amino acid sequences set forth in the group consisting of the following:

c. SEQ ID NO:90 to SEQ ID NO:92, respectively;

d. SEQ ID NO:94 to SEQ ID NO:96, respectively; and

e. SEQ ID NO:98 to SEQ ID NO:100, respectively.

[0011] In some embodiments, the first binding domain specifically binds to ICOS or a subunit of ICOS. In some embodiments, the first binding domain comprises a heavy chain variable region (VH) and a light chain variable region(VL), the VH and VL having amino acid sequences of SEQ ID NO:170 and SEQ ID NO:171, respectively.

[0012] In some embodiments, the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 having amino acid sequences of SEQ ID NOs:172-174, respectively, and lCDR1,

lCDR2, and lCDR3 having amino acid sequences of SEQ ID NOs:175-177, respectively.

[0013] In some embodiments, the first binding domain specifically binds to PD-1 or a subunit of PD-1. In some embodiments, the first binding domain comprises a heavy chain variable region (VH) and a light chain variable region(VL), the VH and VL having amino acid sequences of SEQ ID NO:178 and SEQ ID NO:179, respectively.

[0014] In some embodiments, the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 having amino acid sequences of SEQ ID NOs:180-182, respectively, and lCDR1, lCDR2, and lCDR3 having amino acid sequences of SEQ ID NOs:183-185, respectively.

[0015] In some embodiments, the first binding domain specifically binds to CXCR3 or a subunit of CXCR3. In some embodiments, the first binding domain comprises a heavy chain variable region (VH) and a light chain variable region(VL), the VH and VL having amino acid sequences of SEQ ID NO:186 and SEQ ID NO:187, respectively.

[0016] In some embodiments, the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 having amino acid sequences of SEQ ID NOs:188-190, respectively, and lCDR1, lCDR2, and lCDR3 having amino acid sequences of SEQ ID NOs:191-193, respectively.

[0017] In some embodiments, the first binding domain specifically binds to CD5 or a subunit of CD5. In some embodiments, the first binding domain comprises a heavy chain variable region (VH) and a light chain variable region(VL), the VH and VL having amino acid sequences of SEQ ID NO:194 and SEQ ID NO:195, respectively.

[0018] In some embodiments, the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 having amino acid sequences of SEQ ID NOs:196-198, respectively, and lCDR1, lCDR2, and lCDR3 having amino acid sequences of SEQ ID NOs:199-201, respectively.

[0019] In some embodiments, the second binding domain specifically binds to an inhibitory KIR protein selected from KIR3DL1, KIR3DL2, KIR2DL1, KIR2DL2, and KIR2DL3 or a combination thereof. In some embodiments, the second binding domain specifically binds to KIR2DL1/2/3 or KIR2DL1/2. In some embodiments, the second binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL), the VH and VL having amino acid sequences selected from the pairs of amino acid sequences set forth in the group consisting of:

a. SEQ ID NO:101 and SEQ ID NO:102, respectively;
b. SEQ ID NO:109 and SEQ ID NO:110, respectively;
c. SEQ ID NO:117 and SEQ ID NO:118, respectively;
d. SEQ ID NO:125 and SEQ ID NO:126, respectively;
e. SEQ ID NO:133 and SEQ ID NO:134, respectively;
f. SEQ ID NO:141 and SEQ ID NO:142, respectively;
g. SEQ ID NO:149 and SEQ ID NO:150, respectively; and
h. SEQ ID NO:157 and SEQ ID NO:158, respectively.

[0020] In some embodiments, the second binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3, and lCDR1, lCDR2, and lCDR3, respectively, the CDRs having amino acid sequences selected from the sets of amino acid sequences set forth in from the group consisting of:

a. SEQ ID NO:103 to SEQ ID NO:108, respectively;
b. SEQ ID NO:111 to SEQ ID NO:116, respectively;

c. SEQ ID NO:119 to SEQ ID NO:124, respectively;
d. SEQ ID NO:127 to SEQ ID NO:132, respectively;
e. SEQ ID NO:135 to SEQ ID NO:140, respectively;
f. SEQ ID NO:143 to SEQ ID NO:148, respectively;
g. SEQ ID NO:151 to SEQ ID NO:156, respectively; and
h. SEQ ID NO:159 and SEQ ID NO:164, respectively.

[0021] In some embodiments, the binding agent does not contain an Fc domain. In some embodiments, the binding agent includes an Fc domain. In some embodiments, the Fc domain is selected from an IgG1 and an IgG4 Fc domain. In some embodiments, the binding agent has substantially no effector function activity. In some embodiments, the Fc domain is an IgG1 Fc domain. In some embodiments, the Fc domain is an IgG1 Fc null.

[0022] In some embodiments, the binding agent is bivalent or tetravalent. In some embodiments, the binding agent is bispecific.

[0023] Also provided is a pharmaceutical composition comprising the binding agent of any of the embodiments described herein and a pharmaceutically acceptable carrier.

[0024] Also provided are nucleic acids encoding the binding agent of any of the embodiments described herein. Further provided is a vector comprising any of the embodiments of nucleic acids described herein. Further also provided are cell lines comprising any of the embodiments of nucleic acids or vectors described herein.

[0025] In some embodiments, provided is a method of treating an autoimmune disease, comprising administering any of the embodiments of binding agents or pharmaceutical compositions described herein to a subject in need thereof in an amount effective to decrease the number or activity of pathogenic immune cells in the subject and thereby ameliorate a symptom of the autoimmune disease.

[0026] In some embodiments, provided is a method of suppressing an immune response mediated by pathogenic immune cells, comprising contacting

CD8+KIR+ T regulatory cells (Tregs) with any of the embodiments of binding agents or pharmaceutical compositions described herein in an amount effective to activate or stimulate the CD8+KIR+ Tregs (activated Tregs), whereby the number or activity of pathogenic immune cells is decreased.

[0027] In some embodiments, provided is a method of suppressing an immune response to an autoantigen, comprising administering to a subject in need thereof any of the embodiments of binding agents or pharmaceutical compositions described herein in an amount effective to activate or stimulate the CD8+KIR+ Tregs, whereby the number or activity of pathogenic immune cells that are responsive to the autoantigen is decreased.

[0028] In some embodiments, provided is a method of suppressing an immune response to an antigen, comprising administering to a subject in need thereof any of the embodiments of binding agents or pharmaceutical compositions described herein in an amount effective to activate or stimulate the CD8+KIR+ Tregs, whereby the number or activity of pathogenic immune cells that are responsive to the antigen is decreased.

[0029] In some embodiments of these methods of treating autoimmune disease or suppressing an immune response, the CD8+KIR+ Tregs are contacted with the binding agent in vivo. In some embodiments, the CD8+KIR+ Tregs are contacted with the binding agent ex vivo. In some embodiments, the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof. In some embodiments, the pathogenic immune cells are autoreactive CD4 T cells, autoantibody producing B cells or self antigen presenting dendritic cells. In some embodiments, the pathogenic immune cells are self antigen presenting cells. In some embodiments, the titer of autoantibodies is decreased in the subject.

[0030] In some embodiments, the subject has an autoimmune disease. In some embodiments, the autoimmune disease is selected from the group consisting of celiac disease, Crohn's disease, juvenile idiopathic arthritis, inflammatory bowel disease (IBD), insulin-dependent diabetes mellitus (IDDM

or type 1 diabetes), lupus nephritis, myasthenia gravis, myocarditis, multiple sclerosis (MS), pemphigus/pemphigoid, rheumatoid arthritis (RA), scleroderma/systemic sclerosis, Sjögren's syndrome (SjS), systemic lupus erythematosus (SLE), and ulcerative colitis.

[0031] In some embodiments of the methods of treating autoimmune disease or suppressing an immune response, the binding agent specifically binds to CD8 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CD3 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CD5 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to PD-1 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to ICOS and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CXCR3 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the CD8+KIR+ Tregs are MHC class I restricted. In some embodiments, the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

[0032] In some embodiments of the methods of treating autoimmune disease or suppressing an immune response, the methods further include administering an immunosuppressive agent to the subject. In some embodiments, the administration of the binding agent to the subject results in an improved treatment outcome in the subject. In some embodiments, the improved treatment outcome is a reduced frequency or severity disease flares, reduced systemic inflammatory cytokines, or reduced self reporting of symptoms associated with the autoimmune disease.

[0033] In some embodiments of the methods of treating autoimmune disease or suppressing an immune response, the binding agent is administered intravenously. In some embodiments, the binding agent is administered subcutaneously. In some embodiments, the binding agent is administered in a

dose of about 0.01 mg/kg to about 20 mg/kg. In some embodiments, the binding agent has substantially no effector function activity.

[0034] In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein for the treatment of autoimmune disease in a subject by activating or stimulating CD8+KIR+ Tregs. In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein for the reduction of an immune response by pathogenic immune cells by activating or stimulating CD8+KIR+ Tregs. In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein for the reduction of autoantibody titer in a subject by activating or stimulating CD8+KIR+ Tregs.

[0035] In some embodiments, provided is a method of treating cancer, comprising administering any of the embodiments of the binding agents or the pharmaceutical compositions described herein, wherein the binding agent has substantially no effector function activity, to a subject in need thereof in an amount effective to activate or stimulate the CD8+KIR+ T regulatory cells (Tregs) and thereby ameliorate a symptom of the cancer.

[0036] In some embodiments, provided is a method of stimulating an immune response against an antigen associated with a cancer (cancer antigen), comprising contacting CD8+KIR+ T regulatory cells (Tregs) with any of the embodiments of the binding agents or the pharmaceutical compositions described herein, wherein the binding agent has substantially no effector function activity, in an amount effective to activate or stimulate the CD8+KIR+ Tregs (activated Tregs), whereby the immune response to the cancer antigen is increased.

[0037] In some embodiments, provided is a method of treating cancer, comprising administering any of the embodiments of the binding agents or the pharmaceutical compositions described herein, wherein the binding agent has

effector function activity comprising at least ADCC, to a subject in need thereof in an amount effective to deplete the CD8+KIR+ T regulatory cells (Tregs) and thereby ameliorate a symptom of the cancer.

[0038] In some embodiments, provided is a method of stimulating an immune response against an antigen associated with a cancer (cancer antigen), comprising contacting CD8+KIR+ T regulatory cells (Tregs) with any of the embodiments of the binding agents or the pharmaceutical compositions described herein, wherein the binding agent has effector function activity comprising at least ADCC, in an amount effective to deplete the CD8+KIR+ Tregs, whereby the immune response to the cancer antigen is increased.

[0039] In some embodiments of the methods of treating cancer or stimulating an immune response to an antigen associated with cancer, the CD8+KIR+ Tregs are contacted with the binding agent in vivo. In some embodiments, the CD8+KIR+ Tregs are contacted with the binding agent ex vivo. In some embodiments, the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof. In some embodiments, the increased immune response comprises a reduction in cancer cells or depletion of immune suppressive immune cells. In some embodiments, the number of cancer cells in the subject are decreased.

[0040] In some embodiments, the cancer is selected from the group consisting of carcinomas, lymphomas, blastomas, sarcomas, and leukemias. In some embodiments, the cancer is selected from the group consisting of solid tumors such as breast, cervical, ovary, lung, colorectal (CRC) (and other cancers of the bowel), skin, esophageal, adenocarcinoma, bladder, and prostate cancers; and lymphomas.

[0041] In some embodiments, the binding agent specifically binds to CD8 and an inhibitory KIR protein on the CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CD3 and an inhibitory KIR protein on the CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CD5 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some

embodiments, the binding agent specifically binds to PD-1 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CXCR3 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to ICOS and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the CD8+KIR+ Tregs are MHC class I restricted. In some embodiments, the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

[0042] In some embodiments, the methods further include administering a chemotherapeutic agent to the subject. In some embodiments, the methods further include administering an immunotherapy to the subject. In some embodiments, the methods further include administering an immunotherapy, such as a checkpoint inhibitor, to the subject. In some embodiments, the administration of the binding agent to the subject results in an improved treatment outcome in the subject. In some embodiments, the improved treatment outcome is a partial response or complete response. In some embodiments, the improved treatment outcome is remission.

[0043] In some embodiments, the binding agent is administered intravenously. In some embodiments, the binding agent is administered subcutaneously. In some embodiments, the binding agent is administered in a dose of about 0.01 mg/kg to about 20 mg/kg.

[0044] In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein, wherein the binding agent has substantially no effector function activity, for the treatment of cancer in a subject by activating or stimulating the CD8+KIR+ Tregs.

[0045] In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein, wherein the binding agent has substantially no effector function activity, for the reduction of immune suppression by immune suppressive immune cells by activating or stimulating the CD8+KIR+ Tregs.

[0046] In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein, wherein the binding agent has substantially no effector function activity, for the reduction of tumor burden in a subject by activating or stimulating CD8+KIR+ Tregs.

[0047] In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein, wherein the binding agent has effector function activity comprising at least ADCC, for the treatment of cancer in a subject by depleting CD8+KIR+ Tregs.

[0048] In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein, wherein the binding agent has effector function activity comprising at least ADCC, for the depletion of CD8+KIR+ Tregs.

[0049] In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein, wherein the binding agent has effector function activity comprising at least ADCC, for the reduction of tumor burden in a subject by depleting CD8+KIR+ Tregs.

[0050] In some embodiments, provided are methods of treating an infection, comprising administering any of the embodiments of the binding agents or the pharmaceutical compositions described herein to a subject in need thereof in an amount effective to activate or stimulate CD8+KIR+ Tregs and thereby ameliorate a symptom of the infection.

[0051] In some embodiments, provided are methods of stimulating an immune response against infected cells caused by an infection, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with any of the embodiments of the binding agents or the pharmaceutical compositions described herein in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby the immune response against the infected cells is increased.

[0052] In some embodiments, the CD8+KIR+ Tregs are contacted with the binding agent in vivo. In some embodiments, the CD8+KIR+ Tregs are contacted with the binding agent ex vivo. In some embodiments, the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof.

[0053] In some of the embodiments of treating an infection or stimulating an immune response to infected cells, the immune response comprises a reduction in infected cells or immune suppressive immune cells selected from CD4 T regulatory cells and tolerizing DCs. In some embodiments, the number of infected cells in the subject is decreased. In some embodiments, the infection is selected from a bacterial disease, a systemic fungal disease, a rickettsial disease, a parasitic disease and a viral disease. In some embodiments, the infection is selected from the group consisting of an HIV infection, hepatitis C virus, (HCV) infection, human papillomavirus (HPV) infection, Epstein Bar Virus (EBV) infection, a coronavirus infection such as a SARS-COV2 infection (Covid-19), a cytomegalovirus (CMV) infection, and a flu virus infection.

[0054] In some embodiments, the binding agent specifically binds to CD8 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CD3 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CD5 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to PD-1 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CXCR3 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to ICOS and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the CD8+KIR+ Tregs are MHC class I restricted. In some embodiments, the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

[0055] In some embodiments, the methods further include administering an anti-microbial or an anti-viral agent to the subject. In some embodiments, the administration of the binding agent to the subject results in an improved treatment outcome in the subject. In some embodiments, the improved treatment outcome is a reduction in infection. In some embodiments, the improved treatment outcome is a reduction in infected cells. In some embodiments, the binding agent is administered intravenously. In some embodiments, the binding agent is administered subcutaneously. In some embodiments, the binding agent is administered in a dose of about 0.01 mg/kg to about 20 mg/kg. In some embodiments, the binding agent has substantially no effector function activity.

[0056] In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein for the treatment of an infection in a subject by activating or stimulating CD8+KIR+ Tregs.

[0057] In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein for the stimulation of an immune response by activating or stimulating CD8+KIR+ Tregs and thereby suppressing immune suppressive immune cells.

[0058] In some embodiments, provided is the use of any of the embodiments of the binding agents or the pharmaceutical compositions described herein for the reduction of infection in a subject by activating or stimulating CD8+KIR+ Tregs.

[0059] In some embodiments, provided is a method of reducing or preventing onset of graft versus host disease (GVHD) following a transplant, comprising administering any of the binding agents or pharmaceutical compositions described herein to a subject in need thereof in an amount effective to activate or stimulate CD8+KIR+ Tregs and thereby reduce or

ameliorate at least one symptom of GVHD. In some embodiments, the binding agent has substantially no effector function activity.

[0060] In some embodiments, provided herein is a method of treating a subject who has received a transplant, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with any of the binding agents or the pharmaceutical compositions described herein in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby GVHD is reduced or suppressed. In some embodiments, the binding agent has substantially no effector function activity.

[0061] In some embodiments, provided is a method of treating a subject who has received a transplant, comprising administering any of the binding agents or the pharmaceutical compositions described herein to a subject in need thereof in an amount effective to deplete CD8+KIR+ Tregs and thereby ameliorate a symptom of GVHD. In some embodiments, the binding agent has effector function activity comprising at least ADCC.

[0062] In some embodiments, provided is a method of suppressing GVHD against a transplant, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with any of the binding agents or the pharmaceutical compositions described herein in an amount effective to deplete CD8+KIR+ Tregs, whereby GVHD or a symptom thereof is decreased. In some embodiments, the binding agent has effector function activity comprising at least ADCC.

[0063] In some embodiments, the CD8+KIR+ Tregs are contacted with the binding agent in vivo. In some embodiments, the CD8+KIR+ Tregs are contacted with the binding agent ex vivo. In some embodiments, the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof.

[0064] In some embodiments, decreased GVHD comprises a reduction in CD4+ T cells active in GVHD. In some embodiments, the transplant is selected from the group consisting of an organ transplant, a hematopoietic stem cell transplant, an umbilical cord blood stem cell transplant, an inducible

pluripotent stem cell-derived progenitor or differentiated cell transplant and a bone marrow transplant. In some embodiments, the transplant is a hematopoietic stem cell transplant, an umbilical cord blood stem cell transplant, an inducible pluripotent stem cell-derived progenitor or differentiated cell transplant or a bone marrow transplant. In some embodiments, the transplant is allogeneic.

[0065] In some embodiments, the binding agent specifically binds to CD8 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CD3 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CD5 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to PD-1 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to CXCR3 and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the binding agent specifically binds to ICOS and an inhibitory KIR protein on CD8+KIR+ Tregs. In some embodiments, the CD8+KIR+ Tregs are MHC class I restricted. In some embodiments, the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

[0066] In some embodiments, an immunosuppressive agent is also administered to the subject.

[0067] In some embodiments, the administration of the binding agent to the subject results in an improved treatment outcome in the subject. In some embodiments, the improved treatment outcome is a reduction in a symptom associated with GVHD, reduced systemic inflammatory cytokines, reduced pathology in tissues impacted by GVHD, reduced self reporting of symptoms associated with an immune response associated with adverse effects on host tissues, improved or extended transplant engraftment, alleviation of one or more symptom(s), and/or prevention, delay, or slowing of onset or progression of rejection of the transplant, or extended transplant engraftment with

decreased use of broad spectrum immunosuppressive agents, such as corticosteroids.

[0068] In some embodiments, the binding agent is administered intravenously. In some embodiments, the binding agent is administered subcutaneously.

[0069] In some embodiments, provided in the use of any of the binding agents or the pharmaceutical compositions described herein for the treatment of GVHD associated with transplant in a subject. In some embodiments, the binding agent has substantially no effector function activity.

[0070] In some embodiments, provided is the use of any of the binding agents or the pharmaceutical compositions described herein for the treatment of GVHD associated with transplant in a subject by activating or stimulating CD8+KIR+ Tregs. In some embodiments, the binding agent has substantially no effector function activity.

[0071] In some embodiments, provided is the use of any of the binding agents or the pharmaceutical compositions described herein for the reduction of GVHD associated with a transplant by activating or stimulating CD8+KIR+ Tregs. In some embodiments, the binding agent has substantially no effector function activity.

[0072] In some embodiments, provided herein is the use of any of the binding agents or the pharmaceutical compositions described herein for the reduction of GVHD to a transplant. In some embodiments, the binding agent has substantially no effector function activity.

[0073] In some embodiments, provided is the use of any of the binding agents of or the pharmaceutical compositions described herein for the treatment of GVHD associated with a transplant in a subject by depleting CD8+KIR+ Tregs. In some embodiments, the binding agent has effector function activity comprising at least ADCC.

[0074] In some embodiments, provided is the use of any of the binding agents or the pharmaceutical compositions described herein for the depletion of

CD8+KIR+ Tregs. In some embodiments, the binding agent has effector function activity comprising at least ADCC.

[0075] In some embodiments, provided is the use of any of the binding agents or the pharmaceutical compositions described herein for the depletion of CD8+KIR+ Tregs in a subject who has received a transplant to reduce GVHD. In some embodiments, the binding agent has effector function activity comprising at least ADCC.

[0076] These and other aspects of the present invention may be more fully understood by reference to the following detailed description, non-limiting examples of specific embodiments and the appended drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0077] Figure 1 shows various formats of IgG-scFv bispecific antibodies.

[0078] Figure 2 shows various formats of certain bispecific antibodies.

[0079] Figure 3 shows various formats of additional bispecific antibodies.

[0080] Figures 4A to 4D show the effects of Ly49 blockade on CD8+KIR+ T regulatory cells.

[0081] Figure 5 shows the experimental design for assessing the effects of Ly49 blockade in a murine experimental autoimmune encephalomyelitis (EAE) model.

[0082] Figure 6 shows disease severity (as measured by clinical score) at 7 to 27 days following immunization with MOG only, MOG + SP, MOG+Ly49 blockade.

[0083] Figures 7A to 7C show characteristics of T cells prevalent in Celiac patients. Celiac patients have an increased prevalence of CD8+KIR+ T cells (Figure 7A); have an increase in percentage of CD8+ T cells with intracellular IFNgamma and perforin (Figure 7B); and have an increase in percentage of CD8+ T cells with intracellular Granzyme B (Figure 7C).

[0084] Figures 8A to 8B show that Celiac patients have more CD8+KIR+ T cells (Figure 8A) and CD8+KIR+ICOS+ T cells (Figure 8B) compared to healthy controls.

[0085] Figures 9A to 9B show that gluten peptide restimulation of CD8+KIR+ T cells from Celiac patients increases degranulation (Figure 9A, left) and Granzyme B levels (Figure 9A, right), as compared to unstimulated cells or those stimulated with control flu peptides. Gluten peptide restimulation also leads to a reduction in reactive CD4+ T cells, as compared to unstimulated cells or those restimulated with control flu peptides (Figure 9B).

[0086] Figures 10A to 10B show that KIR blockade ("KIR block") of CD8+ Tregs results in increased intracellular Granzyme B levels (Figure 10A) and increased degranulation (CD107) (Figure 10B).

[0087] Figure 11 shows an increase in cytolytic activity of CD8+ T cells, a decrease in CD4+ T cell activation, and an increase in CD4+ T cell death in PBMCs from celiac patients treated with KIR blockade and gluten restimulation.

[0088] Figure 12 shows that KIR blockade of CD8+CD16+ T cells reduced CD4 T cell activation and proliferation (CD69) in samples from three Celiac patients.

[0089] Figure 13 shows the presence of CD8+KIR+ Treg cells (upper panel) and CD8+ CD39+ Treg cells (lower panel) in samples from patients having celiac disease, Crohn's disease, multiple sclerosis (MS), systemic lupus erythematosus (SLE), ulcerative colitis (UC), or type 1 diabetes (T1D), or in healthy subjects.

[0090] Figure 14 shows expression of surface markers CD39, KLRG1, NKG2D, NKG2C, KLRB, CXCR3, and CD122 on CD8+KIR+ Treg cells isolated from celiac patient PBMC samples.

[0091] Figure 15 shows granzyme B, perforin, and IL-10 production by CD8+ Treg cells isolated from celiac patient PBMC samples.

[0092] Figure 16 shows production of RANTES and TNFβ by CD8+KIR+ Treg cells isolated from celiac patient PBMC samples, following stimulation with anti-CD3 antibodies.

[0093] Figure 17 shows a decrease in IL-17A and IL-23 production by CD4+ T cells co-cultured with CD8+KIR+ Treg cells following gliadin stimulation.

[0094] Figure 18 shows changes in activation and IFNγ by CD4+ T cells co-cultured with CD8+KIR+ Treg cells and stimulated with gluten.

[0095] Figure 19 shows an increase in anti-inflammatory cytokines in CD4+ T cells co-cultured with CD8+KIR+ Treg cells and stimulated with gliadin. IL-10 produced by CD4+ T cells is indicated using intracellular cytokine staining.

[0096] Figure 20 shows an increased CD8+ Treg cell response upon repeated antigenic exposure.

[0097] Figure 21 shows selective expression of inhibitory KIR proteins KIR2DL1/2/3 and KIR3DL1 by CD8+ Treg cells from celiac patient PBMCs.

[0098] Figure 22 shows increases in CD8+KIR+ T cells in celiac patients.

[0099] Figure 23 shows the presence of CD8+KIR+ T cells in the gut tissue of a celiac patient.

[0100] Figure 24 shows the interaction of granzyme-positive CD8+ T cells with CD4+ T cells in the gut tissue of a celiac patient. Granzyme B is shown as white, CD8+ T cells are shown as green, CD4+ T cells are shown as ochre, and the interaction between CD8+ T cells and CD4+ T cells is shown as yellow.

[0101] Figure 25 shows an increase in CD8+ Treg cells in peripheral blood 6 days (6D) after challenge with gluten.

[0102] Figure 26 shows an increase in CD8+ Treg cells in tissue biopsies 14 days (14D) following challenge with gluten.

[0103] Figure 27 shows CD4+ T cell number, costimulatory molecule expression, and proliferation, and KIR2DL expressing T cells, in celiac patient

tissue samples fourteen days after gluten challenge (relative to matched patient tissues pre-gluten challenge).

[0104] Figure 28 shows examples of bi-specific KIR binders and their expected impacts on activation signal strength.

[0105] Figure 29A shows a bi-specific antibody having a CD8 binding domain and a binding domain that targets KIR2DL1/2/3. Figure 29B shows degranulation and Figure 29C shows granzyme B levels following administration of anti-CD8 scFv/KIR FAB-Fc (at doses of 10 µg/mL, 1 µg/mL, or 0.1 µg/mL) or a monoclonal KIR blockade (at 20 µg).

[0106] Figure 30 shows dose-dependent reductions in proinflammatory cytokines following administration of anti-CD8 scFv/KIR FAB-Fc (at doses of 10 µg/mL or 1 µg/mL).

[0107] Figure 31 shows CD4+ T cell death following administration of anti-CD8 scFv/KIR FAB-Fc (at doses of 10 µg/mL, 1 µg/mL, or 0.1 µg/mL) or a monoclonal KIR blockade (at 20 µg).

[0108] Figure 32 shows CD4+ T cell death following administration of anti-CD8 scFv/KIR FAB-Fc (at doses of 10 µg/mL, 1 µg/mL, or 0.1 µg/mL).

[0109] Figure 33 shows preferential binding of the bi-specific blocker (anti-KIR2DL1/2/3 and anti-CD8) to CD8+ T cells relative to NK cells.

[0110] Figure 34 shows preferential binding of the bi-specific blocker (anti-KIR2DL1/2/3 and anti-CD8) to CD8+ T cells relative to NK cells and CD4+ T cells.

DETAILED DESCRIPTION

Definitions

[0111] For convenience, certain terms in the specification, examples, and claims are defined here. Unless stated otherwise, or implicit from context, the following terms and phrases have the meanings provided below. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed invention, because the scope of the invention is

limited only by the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0112] As used herein and unless otherwise indicated, the terms “a” and “an” are taken to mean “one”, “at least one”, or “one or more”. Unless otherwise required by context, singular terms used herein shall include pluralities and plural terms shall include the singular.

[0113] Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

[0114] The terms “decrease,” “reduce,” “reduced”, “reduction”, “decrease”, and “inhibit” are all used herein generally to mean a decrease by a statistically significant amount relative to a reference.

[0115] The terms “increased”, “increase”, “enhance”, or “activate” are all used herein to generally mean an increase by a statically significant amount relative to a reference.

[0116] The terms “isolated” or “partially purified” as used herein refer in the case of a nucleic acid, polypeptide or protein, to a nucleic acid, polypeptide or protein separated from at least one other component (*e.g.*, nucleic acid or polypeptide or protein) that is present with the nucleic acid, polypeptide or protein as found in its natural source and/or that would be present with the nucleic acid, polypeptide or protein when expressed by a cell, or secreted in the case of secreted polypeptides and proteins. A chemically synthesized nucleic acid, polypeptide or protein, or one synthesized using in vitro transcription/translation, is considered “isolated.” The terms “purified” or “substantially purified” refer to an isolated nucleic acid, polypeptide or protein that is at least 95% by weight the subject nucleic acid, polypeptide or protein, including, for example, at least 96%, at least 97%, at least 98%, at least 99%, or more.

[0117] As used herein, the terms "protein" and "polypeptide" are used interchangeably herein to designate a series of amino acid residues each connected to each other by peptide bonds between the alpha-amino and carboxyl groups of adjacent residues. The terms "protein" and "polypeptide" also refer to a polymer of protein amino acids, including modified amino acids (*e.g.*, phosphorylated, glycated, glycosylated, etc.) and amino acid analogs, regardless of its size or function. "Protein" and "polypeptide" are often used in reference to relatively large polypeptides, whereas the term "peptide" is often used in reference to small polypeptides, but usage of these terms in the art overlaps. The terms "protein" and "polypeptide" are used interchangeably herein when referring to an encoded gene product and fragments thereof. Thus, exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, orthologs, paralogs, fragments and other equivalents, variants, fragments, and analogs of the foregoing.

[0118] CD3epsilon is a protein that is expressed on T cells, including regulatory T cells. CD3epsilon polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP000724.1; this sequence is incorporated by reference herein.

[0119] CD5 is a protein expressed on T cells and B cells. CD5 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_055022.2 and NP_001333385.1; these sequences are incorporated by reference herein.

[0120] CD8alpha is a protein that is expressed on T cells, including regulatory T cells. CD8alpha polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_001759.3, NP001139345.1, NP_741969.1, NP_001369627.1, NP_757362.1, NP_001171571.1, NP_742100.1, NP_742099.1, and NP_004922; these sequences are incorporated by reference herein.

[0121] KIR3DL1 is a protein expressed on NK cells and on some T cells. It is also known as CD158E1, KIR, KIR2DL5B, KIR3DL1/S1, NKAT-3, NKAT3,

NKB1, and NKB1B. KIR3DL1 polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_037421.2 and NP_001309097.1; these sequences are incorporated by reference herein.

[0122] KIR3DL2 is a protein expressed on NK cells and on some T cells. It is also known as 3DL2, CD158K, KIR-3DL2, NKAT-4, NKAT4, NKAT4B, and p140. KIR3DL2 polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_006728.2 and NP_001229796.1; these sequences are incorporated by reference herein.

[0123] KIR2DL1 is a protein expressed on NK cells and on some T cells. It is also known as CD158A, KIR-K64, KIR221, KIR2DL3, NKAT, NKAT-1, NKAT1, and p58.1. KIR2DL1 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_055033.2; this sequence is incorporated by reference herein.

[0124] KIR2DL2 is a protein expressed on NK cells and on some T cells. It is also known as CD158B1, CD158b, NKAT-6, NKAT6, and p58.2. KIR2DL2 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_055034.2; this sequence is incorporated by reference herein.

[0125] KIR2DL3 is a protein expressed on NK cells and on some T cells. It is also known as CD158B2, CD158b, GL183, KIR-023GB, KIR-K7b, KIR-K7c, KIR2DL, KIR2DS5, KIRCL23, NKAT, NKAT2, NKAT2A, NKAT2B, and p58. KIR2DL3 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_056952.2; this sequence is incorporated by reference herein.

[0126] CD27 is also referred to as TNF receptor superfamily member 7, S152, LPFS2, T14, TNFRSF7, and Tp55. CD27 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_001233.2; this sequence is incorporated by reference herein.

[0127] CD38 is also referred to as ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1, ADPRC1, and ADPRC 1. CD38 polypeptides include, but are not

limited to, those having the amino acid sequence set forth in NP_001766.2; this amino acid sequence is incorporated by reference herein.

[0128] CD39 is also known as ectonucleoside triphosphate diphosphohydrolase 1, SPG64 ATPDase, and NTPDase-1. It encodes plasma membrane protein that hydrolyzes extracellular ATP and ADP to AMP. CD39 polypeptides include, but are not limited to, those having the amino acid sequences disclosed in NP_001307845.1, NP_001157651.1, NP_001157650.1, NP_001091645.1, NP_001767.3, NP_001299583.1, NP_001157655.1, NP_001157654.1, and NP_001157653.1; these amino acid sequences are incorporated by reference herein.

[0129] CD40L, or CD40 ligand, is also referred to as CD154, HIGM1, IGM, IMD3, T-BAM, TNFSF5, TRAP, gp39, and hCD40L. It is expressed on the surface of T cells. CD40L polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_000065.1; this sequence is incorporated by reference herein.

[0130] CD45 is referred to as protein tyrosine phosphatase receptor type C, B220, CD45R, GP180, L-CA, LCA, LY5, and T200. It has many isoforms, including CD45RA, CD45Rb, and CD45RO. CD45 RA and CD45Rb are expressed on naïve T cells. CD45RO is expressed on memory T cells. CD45R0 polypeptides include, but are not limited to, those having the amino acid sequence disclosed in P08575-4. CD45RA polypeptides include, but are not limited to, those having the amino acid sequence disclosed in P08575-8. CD45RB polypeptides include, but are not limited to, those having the amino acid sequence disclosed in P08575-9. *See* UniProtKB database. These sequences are incorporated by reference herein.

[0131] CD73 is also referred to 5' nucleotidase ecto, CALJA, CD73, E5NT, NT, NT5, NTE, eN, and eNT. CD73 polypeptides include, but are not limited to, those disclosed in NP_001191742.1 and NP_002517.1; these amino acid sequences are incorporated by reference herein.

[0132] CD103, or integrin subunit alpha E (ITGAE), is also referred to as HUMINAE. CD103 polypeptides include, but are not limited to, those having the amino acid sequence disclosed in NP_002199.3; this amino acid sequence is incorporated by reference herein.

[0133] CD122, or interleukin 2 receptor subunit beta, is also referred to as IL15RB, IMD63, and P70-75. CD122 polypeptides include, but are not limited to, those having the amino acid sequences disclosed in NP_001333152.1, NP_001333151.1, and NP_000869.1; these amino acid sequences are incorporated by reference herein.

[0134] CD166, or activated leukocyte cell adhesion molecule (ALCAM), is also referred to as MEMD. CD166 polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_001618.2, NP_001230209.1, NP_001230210.1, and NP_001230212.1; these amino acid sequences are incorporated by reference herein.

[0135] CD177 is also referred to as HNA-2a, HNA2A, NB1, NB1 GP, PRV-1, and PRV1. CD177 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_065139.2; this amino acid sequence is incorporated by reference herein.

[0136] CCR7, or C-C motif chemokine receptor 7, is also referred to as BLR2, CC-CKR-7, CCR-7, CD197, CDw197, CMKBR7, and EBI1. CCR7 polypeptides includes, but are not limited to, those having the amino acid sequences set forth in NP_001829.1, NP_001288643.1, NP_001288645.1, NP_001288646.1, and NP_001288647.1; these amino acid sequences are incorporated by reference herein.

[0137] CXCR3, or C-X-C motif chemokine receptor 3, is also referred to as GPR9, MigR, CD182, CD183, Mig-R, CKR-L2, CMKAR3, and IP10-R. CXCR3 polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_001495.1 and NP_001136269.1; these amino acid sequences are incorporated by reference herein.

[0138] CXCR5, or C-X-C motif chemokine 5, is also referred to as BLR1, CD185, and MDR15. CXCR5 polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_001707.1 and NP_116743.1; these amino acid sequences are incorporated by reference herein.

[0139] HLA-DR is a class II histocompatibility antigen composed of two chains. HLA-DR alpha chain polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_061984.2. HLA-DR beta chain polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_002116.2, NP_072049.2, NP_001346123.1, and NP_001346122.1. These amino acid sequences are incorporated by reference herein.

[0140] ICOS, or inducible T cell costimulatory, is also referred to as AILIM, CD278, and CVID1. ICOS polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_036224.1; this amino acid sequence is incorporated by reference herein.

[0141] LAG-3, or CD223, is also referred to as lymphocyte activating 3. LAG-3 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_002277.4; this amino acid sequence is incorporated by reference herein.

[0142] OX-40 is also referred to as TNF receptor superfamily member 4 or TNFRSF4, ACT35, CD134, IMD16, and TXGP1L. OX-40 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_003318.1; this amino acid sequence is incorporated by reference herein.

[0143] PD-1 is also referred to as programmed cell death protein 1. PD-1 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_005009.2; this amino acid sequence is incorporated by reference herein.

[0144] S1000A8/9, or S100A8 and S100A9, respectively, are Ca^{2+} binding proteins belonging to the S100 family. S100A8 or S100-A8 is also referred to as 60B8AG, CAGA, CFAG, CGLA, CP-10, L1Ag, MA387, MIF,

MRP8, NIF, and P8. S100A8 polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_001306125.1, NP_001306126.1, NP_001306127.1, NP_001306130.1, and NP_002955.2. S100A9, or S100-A9, is also referred to as 60B8AG, CAGB, CFAG, CGLB, L1AG, LIAG, MAC387, MIF, MRP14, NIF, and P14. S100A9 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_002956.1. These amino acid sequences are incorporated by reference herein.

[0145] TIM-3, also referred to as Hepatitis A virus cellular receptor 2 (HAVCR2), is also known as CD366, HAVcr-2, KIM-3, SPTCL, TIM3, TIMD-3, and TIMD3. TIM-3 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_116171.3; this amino acid sequence is incorporated by reference herein.

[0146] TLT-2, or triggering receptor expressed on myeloid cells like 2 (TREML2), is also referred to as C6orf76 or dJ238O23.1. TLT-2 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_079083.2; this amino acid sequence is incorporated by reference herein.

[0147] 2B4, or CD244, is also referred to as NAIL, NKR2B4, Nmrk, and SLAMF4. 2B4 polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_057466.1, NP_001160135.1, or NP_001160136.1; these amino acid sequences are incorporated by reference herein.

[0148] 41BB, or TNF receptor superfamily member 9 (TNFSF9), is also referred to as ILA, 4-1BB, CD137, and CDw137. 41BB polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_001552.2; this amino acid sequence is incorporated by reference herein.

[0149] As used herein, an “epitope” refers to the amino acids conventionally bound by an immunoglobulin VH/VL pair, such as the antibodies and other binding agents described herein. An epitope can be formed on a polypeptide from contiguous amino acids or noncontiguous amino acids

juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5, about 9, or about 8-10 amino acids in a unique spatial conformation. An epitope defines the minimum binding site for an antibody or other binding agent, and thus represent the target of specificity of an antibody, antigen binding portion thereof or other immunoglobulin-based binding agent. In the case of a single domain antibody, an epitope represents the unit of structure bound by a variable domain in isolation.

[0150] As used herein, "specifically binds" refers to the ability of a binding agent (*e.g.*, an antibody or antigen binding portion thereof) described herein to bind to a target with a KD 10^{-5} M (10000 nM) or less, *e.g.*, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less. Specific binding can be influenced by, for example, the affinity and avidity of the antibody or other binding agent and the concentration of target polypeptide. A person of ordinary skill in the art can determine appropriate conditions under which the antibodies and other binding agents described herein selectively bind to a target antigen using any suitable methods, such as titration of a binding agent in a suitable cell binding assay. A binding agent specifically bound to a target is not displaced by a non-similar competitor. In certain embodiments, a binding agent, such as an antibody or antigen-binding portion thereof is said to specifically bind to its target when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules.

[0151] In some embodiments, a binding agent, such as an antibody or antigen-binding portion thereof or other binding agent as described herein specifically binds to a target polypeptide with a dissociation constant (KD) of 10^{-5} M (10000 nM) or less, *e.g.*, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less. In some embodiments, an antibody or antigen-binding portion thereof or other binding agent as described herein specifically binds to a target

polypeptide with a dissociation constant (KD) of from about 10^{-5} M to 10^{-6} M. In some embodiments, an antibody or antigen-binding portion thereof or other binding agent as described herein specifically binds to a target polypeptide with a dissociation constant (KD) of from about 10^{-6} M to 10^{-7} M. In some embodiments, an antibody or antigen-binding portion thereof or other binding agent as described herein specifically binds to a target polypeptide with a dissociation constant (KD) of from about 10^{-7} M to 10^{-8} M. In some embodiments, an antibody or antigen-binding portion thereof or other binding agent as described herein specifically binds to a target polypeptide with a dissociation constant (KD) of from about 10^{-8} M to 10^{-9} M. In some embodiments, an antibody or antigen-binding portion thereof or other binding agent as described herein specifically binds to a target polypeptide with a dissociation constant (KD) of from about 10^{-9} M to 10^{-10} M. In some embodiments, an antibody or antigen-binding portion thereof or other binding agent as described herein specifically binds to a target polypeptide with a dissociation constant (KD) of from about 10^{-10} M to 10^{-11} M. In some embodiments, an antibody or antigen-binding portion thereof or other binding agent as described herein specifically binds to a target polypeptide with a dissociation constant (KD) of from about 10^{-11} M to 10^{-12} M. In some embodiments, an antibody or antigen-binding portion thereof or other binding agent as described herein specifically binds to a target polypeptide with a dissociation constant (KD) of less than 10^{-12} M.

[0152] As used herein, the term “consisting essentially of” refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment.

[0153] The term “consisting of” refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[0154] Other than in the examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about". The term "about" when used in connection with percentages can mean +/-1%.

[0155] The term "statistically significant" or "significantly" refers to statistical significance and generally means a two standard deviation (2SD) difference, above or below a reference value.

[0156] Other terms are defined herein within the description of the various aspects of the invention.

Modulation of CD8+KIR+ Regulatory T cells

[0157] Provided herein are binding agents comprising binding domains that specifically bind to antigens expressed on CD8+KIR+ regulatory T cells (Tregs). In some embodiments, the CD8+KIR+ Tregs are MHC class I restricted. In some embodiments, the CD8+KIR+ Tregs are not MHC Qa-1 (HLA-E) restricted. Also provided are methods of using the binding agents for the treatment of autoimmune disease, infectious disease, and cancer.

[0158] The binding agents include a first binding domain that specifically binds to a T cell antigen expressed on the CD8+KIR+ Tregs, other than a KIR protein, and a second binding domain that specifically binds to an inhibitory KIR protein expressed on the CD8+KIR+ Tregs. In some embodiments, the first binding domain specifically binds to an antigen selected from CD3, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, CD5, and 41BB. In some embodiments, the first binding domain specifically binds to an antigen selected from CD3, CD5, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB. In some embodiments, the first binding domain

specifically binds to a subunit of an antigen selected from CD3, CD8, CD40L, CD122, HLA-DR, OX-40, S1000A8/9, and 41BB/CD137.

[0159] In some embodiments, the first antigen is selected from a functional agonist that can activate the CD8 KIR+ Tregs. In some embodiments, such an antigen is, for example, CD3, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD122, ICOS, OX-40, 2B4, 41BB, and HLA-DR. In some embodiments, such an antigen is, for example, CD3, CD5, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD122, ICOS, OX-40, 2B4, 41BB, and HLA-DR. In some embodiments, the first binding domain has agonist activity when bound to such an antigen.

[0160] In some embodiments, the first antigen is selected from a functional antagonist to reduce functional inhibition of CD8 KIR+ Tregs. In some embodiments, such as antigen is, for example, LAG-3/CD223, TIM-3, PD-1, S1000A8/9, and TLT2. In some embodiments, the first binding domain has antagonist activity (*e.g.*, blocking activity) when bound to such an antigen.

[0161] In some embodiments, the first antigen is a tethering moiety to enhance specificity of binding agent to CD8 KIR+ Tregs. In some embodiments, such an antigen is, for example, CD3, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB. In some embodiments, such an antigen is, for example, CD3, CD5, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB. In some embodiments, the first binding domain specifically binds to such an antigen.

[0162] In some embodiments, the first antigen is a tethering moiety to enhance tissue specificity. In some embodiments, such an antigen is, for example, CD103 (ITGAE), CD166, CD177, CXCR3, and S1000A8/9. In some embodiments, the first binding domain specifically binds to such an antigen.

[0163] In some embodiments, the first antigen is an agonist to enhance CD8 KIR+ Treg cell migration. In some embodiments, such an antigen is, for example, CCR7, CXCR3, or CXCR5. In some embodiments, the first binding domain specifically binds to such an antigen.

[0164] In some embodiments, the first antigen is selected from PD-1, ICOS, and CXCR3. In some embodiments, the first binding domain specifically binds to such an antigen.

[0165] In some embodiments, the first antigen is selected from CD3 or CD8. In some embodiments, the first antigen is selected from CD3, CD5, or CD8. In some embodiments, the first antigen is selected from a subunit of CD3 or CD8. In some embodiments, the first antigen is CD3epsilon. In some embodiments, the first antigen is CD8alpha.

[0166] The second binding domain of the binding agent specifically binds to an inhibitory KIR protein (killer cell immunoglobulin like receptor protein). The inhibitory KIR protein can be, for example, KIR3DL1, KIR3DL2, KIR2DL1, KIR2DL2, or KIR2DL3 or a combination thereof, such as specifically binding to KIR2DL1/2/3 or KIR2DL1/2 proteins. In some embodiments, the KIR protein is selected from KIR3DL1, KIR3DL2, KIR2DL1, KIR2DL2, or KIR2DL3 or a combination thereof, such as KIR2DL1/2/3 or KIR2DL1/2 proteins. In some embodiments, the second binding domain is a KIR protein antagonist that blocks KIR protein interaction with its binding partner.

[0167] A binding agent can be any suitable agent that includes binding domains for both antigens. In some embodiments, a binding agent is bispecific (*i.e.*, having binding domains for two different antigens). In some embodiments, a binding agent is bivalent (*i.e.*, having two binding domains). In some embodiments, the binding agent is tetravalent (*i.e.*, having four binding domains).

[0168] The binding domains of the binding agents can be derived from antibodies or from non-antibody formats. In some embodiments, a binding domain is derived from an antibody or antigen binding portions thereof (*i.e.*, an

antibody fragment). In some embodiments, the antibody fragment is a Fab, Fab', F(ab')2, Fv, scFv, or a single domain antibody (also referred to as a VHH, VNAR, sdAb, or nanobody). In some embodiments, a binding domain is or is derived from an anticalin, affibody, avimer, DARPin, or adnectin.

[0169] In some embodiments, the binding agent is a bispecific antibody, a diabody, an antibody Fc fusion, scFv1-ScFv2, an $ScFv1_2$-Fc-$scFv2_2$, an IgG-scFv, a DVD-Ig, a triomab/quadroma, a two-in-one IgG, a $scFv_2$-Fc, a TandAb, an scFv-HSA-scFv, an scFv-VHH, a Fab-scFv-Fc, a Fab-VHH-Fc, a dAb-IgG, an IgG-VHH, a Tandem scFv-Fc, a $(scFv1)_2$-Fc-$(VHH)_2$, a BiTe, a DART, a crossmab, an anticalin, an affibody, an avimer, a DARPin, an adnectin, a scFv-Fc, a one-armed tandem scFv-Fc, or a DART-Fc (*see, e.g.*, Figures 2 and 3). In some embodiments, the IgG-scFv is an IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, 2scFV-IgG, or IgG-2scFv (as shown in Figure 1).

[0170] In some embodiments, the binding agent comprises a first binding domain comprising a heavy chain variable region and a light chain variable region. In some embodiments, the heavy and light chain variable regions of the first binding domain specifically bind to an antigen expressed on a CD8+KIR+ Treg, such as CD3, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB. In some embodiments, the heavy and light chain variable regions of the first binding domain specifically bind to an antigen expressed on a CD8+KIR+ Treg, such as CD3, CD5, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB. In some embodiments, the heavy and light chain variable regions of the first binding domain specifically bind to a subunit of an antigen expressed on a CD8+KIR+ Treg, such as CD3, CD8, CD40L, CD122, HLA-DR, OX-40, S1000A8/9, and 41BB/CD137. In some embodiments, the heavy and light chain variable regions of the first binding

domain specifically bind to a subunit of an antigen expressed on a CD8+KIR+ Treg, such as CD3, CD5, CD8, CD40L, CD122, HLA-DR, OX-40, S1000A8/9, and 41BB/CD137.

[0171] In some embodiments, the first antigen is selected from a functional agonist that can activate the CD8 KIR+ Tregs. In some embodiments, such an antigen is, for example, CD3, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD122, ICOS, OX-40, 2B4, 41BB, and HLA-DR. In some embodiments, such an antigen is, for example, CD3, CD5, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD122, ICOS, OX-40, 2B4, 41BB, and HLA-DR. In some embodiments, the first binding domain has agonist activity when bound to such an antigen.

[0172] In some embodiments, the first antigen is selected from a functional antagonist to reduce functional inhibition of CD8 KIR+ Tregs. In some embodiments, such as antigen is, for example, LAG-3/CD223, TIM-3, PD-1, S1000A8/9, and TLT2. In some embodiments, the first binding domain has antagonist activity (*e.g.*, blocking activity) when bound to such an antigen.

[0173] In some embodiments, the first antigen is a tethering moiety to enhance specificity of binding agent to CD8 KIR+ Tregs. In some embodiments, such an antigen is, for example, CD3, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB. In some embodiments, such an antigen is, for example, CD3, CD5, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB. In some embodiments, the first binding domain specifically binds to such an antigen.

[0174] In some embodiments, the first antigen is a tethering moiety to enhance tissue specificity. In some embodiments, such an antigen is, for

example, CD103 (ITGAE), CD166, CD177, CXCR3, and S1000A8/9. In some embodiments, the first binding domain specifically binds to such an antigen.

[0175] In some embodiments, the first antigen is an agonist to enhance CD8 KIR+ Tregs cell migration. In some embodiments, such an antigen is, for example, CCR7, CXCR3, or CXCR5. In some embodiments, the first binding domain specifically binds to such an antigen.

[0176] In some embodiments, the first antigen is selected from PD-1, ICOS, and CXCR3. In some embodiments, the first binding domain specifically binds to such an antigen.

[0177] In some embodiments, the first antigen is selected from CD3 or CD8. In some embodiments, the first antigen is selected from CD3, CD5, or CD8. In some embodiments, the first antigen is selected from a subunit of CD3 or CD8. In some embodiments, the first antigen is CD3epsilon. In some embodiments, the first antigen is CD8alpha.

[0178] Antibodies for use in the binding domains described herein are known in the art.

[0179] Antibodies to CD3 have been described in, for example, US Patent Nos. 5,929,212; 5,885,573; and 8,551,478 and in International Patent Publication WO2018223004.

[0180] Antibodies to CD8 have been described in, for example, Published US Patent Application Nos. 20190382488 and 20190071500 and International Patent Publication WO2014164553 and WO2017134306.

[0181] Antibodies to CD5 have been described in, for example, Published US Patent Application Nos. 2018/0104308, 2011/0250203, and 2008/0254027.

[0182] Antibodies to CD27 have been described in, for example, Published US Patent Application Nos. 20210009706, 20200247898, and 20200131272.

[0183] Antibodies to CD38 have been described in, for example, Published US Patent Application Nos. 20200408765, 20200399391, 20090304710, and 20050158305.

[0184] Antibodies to CD39 have been described in, for example, Published US Patent Application Nos. 20190062448, 20130273062, and 20100303828.

[0185] Antibodies to CD40L have been described in, for example, Published US Patent Application Nos. 20190092868, 20100092482, 20030031668, and 20010018041.

[0186] Antibodies to CD45RA, CD45RB, and CD45RO have been described in, for example, Published US Patent Application Nos. 20030232009 and 20020168362 and are available from commercial sources.

[0187] Antibodies to CD73 have been described in, for example, Published US Patent Application Nos. 20200148781, 20200071404, 20190256598, and 20160145350.

[0188] Antibodies to CD103 (ITGAE) have been described in, for example, Published US Patent Application No. 20050266001.

[0189] Antibodies to CD122 have been described in, for example, Published US Patent Application Nos. 20180362655 and 20110250213.

[0190] Antibodies to CD166 have been described in, for example, Published US Patent Application Nos. 20160355587 and 20090269787.

[0191] Antibodies to CD177 have been described in, for example, Published US Patent Application No. 20190125832.

[0192] Antibodies to CCR7 have been described in, for example, Published US Patent Application Nos. 20200216548, 20180237529, and 20150344580.

[0193] Antibodies to CXCR3 have been described in, for example, Published US Patent Application Nos. 20190119391, 20190008955, and 20130251733.

[0194] Antibodies to CXCR5 have been described in, for example, Published US Patent Application Nos. 20190169283, 20160053014, and 20130236476.

[0195] Antibodies to HLA-DR have been described in, for example, Published US Patent Application Nos. 20180355043 and 20190071503.

[0196] Antibodies to ICOS have been described in, for example, Published US Patent Application Nos. 20160304610 and 20110243929.

[0197] Antibodies to LAG-3/CD223 have been described in, for example, Published US Patent Application Nos. 20210009687, 20200277372, 20200071403, and 20190276538.

[0198] Antibodies to OX40 have been described in, for example, Published US Patent Application Nos. 20140377284, 20140308276, and 20100196359.

[0199] Antibodies to PD-1 have been described in, for example, Published US Patent Application Nos. 20190322749, 20190309069, 20170313774, and 20110171215.

[0200] Antibodies to S1000A8/9 have been described in, for example, Published US Patent Application Nos. 20180256710 and 20200023045.

[0201] Antibodies to TIM-3 have been described in, for example, Published US Patent Application Nos. 20180072804, 20170306016, and 20150086574.

[0202] Antibodies to TLT-2 have been described in, for example, Published US Patent Application No. 20130216540.

[0203] Antibodies to 2B4 are available, for example, from commercial vendors.

[0204] Antibodies to 41BB have been described in, for example, Published US Patent Application Nos. 20170198050 and 20200347144.

[0205] In some embodiments, the first binding domain specifically binds to CD3epsilon and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively; SEQ

ID NO:9 and SEQ ID NO:10, respectively; SEQ ID NO:17 and SEQ ID NO:18, respectively; SEQ ID NO:25 and SEQ ID NO:26, respectively; SEQ ID NO:33 and SEQ ID NO:34, respectively; SEQ ID NO:41 and SEQ ID NO:34, respectively; SEQ ID NO:45 and SEQ ID NO:34, respectively; SEQ ID NO:49 and SEQ ID NO:50, respectively; SEQ ID NO:57 and SEQ ID NO:58, respectively; SEQ ID NO:65 and SEQ ID NO:66, respectively; or SEQ ID NO:65 and SEQ ID NO:166, respectively.

[0206] In some embodiments, the first binding domain specifically binds to CD3epsilon and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively; SEQ ID NO:9 and SEQ ID NO:10, respectively; SEQ ID NO:17 and SEQ ID NO:18, respectively; SEQ ID NO:25 and SEQ ID NO:26, respectively; SEQ ID NO:33 and SEQ ID NO:34, respectively; SEQ ID NO:41 and SEQ ID NO:34, respectively; SEQ ID NO:45 and SEQ ID NO:34, respectively; SEQ ID NO:49 and SEQ ID NO:50, respectively; SEQ ID NO:57 and SEQ ID NO:58, respectively; or SEQ ID NO:65 and SEQ ID NO:66, respectively; or SEQ ID NO:65 and SEQ ID NO:166, respectively; wherein the framework regions of the heavy and light chain variable regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4, or 1 to 2 amino acid substitutions, deletions, or insertions, and wherein the CDRs of the heavy or light chain variable regions are not modified.

[0207] In some embodiments, the first binding domain comprises an amino acid sequence (*e.g.*, a VH, VL, hCDR1, hCDR1, hCDR3, lCDR1, lCDR2, and/or lCDR3) according to any one or more of SEQ ID NOs:1-72 and 166-169.

[0208] In some embodiments, the first binding domain specifically binds to CD8alpha and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:73 and SEQ ID NO:74, respectively; or SEQ ID NO:81 and SEQ ID NO:82, respectively; or the binding domain comprises a VHH chain having the amino acid sequence set forth in SEQ ID NO:89, SEQ ID NO:93, or SEQ ID NO:97.

[0209] In some embodiments, the first binding domain specifically binds to CD8alpha and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:73 and SEQ ID NO:74, respectively; or SEQ ID NO:81 and SEQ ID NO:82, respectively; or the binding domain comprises a VHH chain having the amino acid sequence set forth in SEQ ID NO:89, SEQ ID NO:93, or SEQ ID NO:97, respectively; wherein the framework regions of the heavy and light chain variable regions or VHH chain are optionally modified with from 1 to 8, 1 to 6, 1 to 4, or 1 to 2 amino acid substitutions, deletions, or insertions, and wherein the CDRs of the heavy or light chain variable regions or the VHH chain are not modified.

[0210] In some embodiments, the first binding domain comprises an amino acid sequence (*e.g.*, a VH, VL, hCDR1, hCDR1, hCDR3, lCDR1, lCDR2, and/or lCDR3) according to any one or more of SEQ ID NOs:73-100.

[0211] In some embodiments, the first binding domain specifically binds to CD3epsilon and the heavy chain variable regions has complementarity determining regions hCDR1, hCDR2, and hCDR3, the light chain variable region lCDR1, lCDR2, and lCDR3, and the amino acid sequences of the heavy and light chain variable region CDRs are set forth in SEQ ID NO:3 to SEQ ID NO:8, respectively; SEQ ID NO:11 to SEQ ID NO:16, respectively; SEQ ID NO:19 to SEQ ID NO:24, respectively; SEQ ID NO:27 to SEQ ID NO:32, respectively; SEQ ID NO:35 to SEQ ID NO:40, respectively; SEQ ID NO:42 to SEQ ID NO:44 and SEQ ID NO:38 to SEQ ID NO:40, respectively; SEQ ID NO:46 to SEQ ID NO:48 and SEQ ID NO:38 to SEQ ID NO:40, respectively; SEQ ID NO:51 to SEQ ID NO:56, respectively; SEQ ID NO:59 to SEQ ID NO:64, respectively; or SEQ ID NO:67 to SEQ ID NO:72, respectively. In some embodiments, the first binding domain specifically binds to CD3epsilon and comprises light chain variable region lCDR1, lCDR2, and lCDR3 having the amino acid sequences set forth in SEQ ID NOs:167, 168, and 169, respectively.

[0212] In some embodiments, the first binding domain specifically binds to CD8alpha and has heavy chain variable regions having complementarity

determining regions hCDR1, hCDR2, and hCDR3 and the light chain variable region has lCDR1, lCDR2, and lCDR3, the amino acid sequences of the heavy and light chain variable region CDRs are set forth in SEQ ID NO:75 to SEQ ID NO:80, respectively; or SEQ ID NO:83 to SEQ ID NO:88, respectively; or the first binding domain includes a VHH chain having hCDR1, hCDR2, and hCDR3, and the amino acid sequences of the VHH CDRs are set forth in SEQ ID NO:90 to SEQ ID NO:92, respectively; SEQ ID NO:94 to SEQ ID NO:96, respectively; or SEQ ID NO:98 to SEQ ID NO:100, respectively.

[0213] In some embodiments, the first binding domain specifically binds to ICOS and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:170 and SEQ ID NO:171, respectively.

[0214] In some embodiments, the first binding domain specifically binds to ICOS and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:170 and SEQ ID NO:171, respectively; wherein the framework regions of the heavy and light chain variable regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4, or 1 to 2 amino acid substitutions, deletions, or insertions, and wherein the CDRs of the heavy or light chain variable regions are not modified.

[0215] In some embodiments, the first binding domain comprises an amino acid sequence (*e.g.*, a VH, VL, hCDR1, hCDR1, hCDR3, lCDR1, lCDR2, and/or lCDR3) according to any one or more of SEQ ID NOs:170-177.

[0216] In some embodiments, the first binding domain specifically binds to PD-1 and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:178 and SEQ ID NO:179, respectively.

[0217] In some embodiments, the first binding domain specifically binds to PD-1 and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:178 and SEQ ID NO:179, respectively; wherein the framework regions of the heavy and light chain variable regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4, or 1 to 2 amino acid

substitutions, deletions, or insertions, and wherein the CDRs of the heavy or light chain variable regions are not modified.

[0218] In some embodiments, the first binding domain comprises an amino acid sequence (*e.g.*, a VH, VL, hCDR1, hCDR1, hCDR3, lCDR1, lCDR2, and/or lCDR3) according to any one or more of SEQ ID NOs:178-185.

[0219] In some embodiments, the first binding domain specifically binds to CXCR3 and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:186 and SEQ ID NO:187, respectively.

[0220] In some embodiments, the first binding domain specifically binds to CXCR3 and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:186 and SEQ ID NO:187, respectively; wherein the framework regions of the heavy and light chain variable regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4, or 1 to 2 amino acid substitutions, deletions, or insertions, and wherein the CDRs of the heavy or light chain variable regions are not modified.

[0221] In some embodiments, the first binding domain comprises an amino acid sequence (*e.g.*, a VH, VL, hCDR1, hCDR1, hCDR3, lCDR1, lCDR2, and/or lCDR3) according to any one or more of SEQ ID NOs:186-193.

[0222] In some embodiments, the first binding domain specifically binds to CD5 and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:194 and SEQ ID NO:195, respectively.

[0223] In some embodiments, the first binding domain specifically binds to CD5 and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:194 and SEQ ID NO:195, respectively; wherein the framework regions of the heavy and light chain variable regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4, or 1 to 2 amino acid substitutions, deletions, or insertions, and wherein the CDRs of the heavy or light chain variable regions are not modified.

[0224] In some embodiments, the first binding domain comprises an amino acid sequence (*e.g.*, a VH, VL, hCDR1, hCDR1, hCDR3, lCDR1, lCDR2, and/or lCDR3) according to any one or more of SEQ ID NOs:194-201.

[0225] In some embodiments, the binding agent comprises a second binding domain comprising a heavy chain variable region and a light chain variable region. The second binding domain of the binding agent specifically binds to an inhibitory KIR protein (killer cell immunoglobulin like receptor protein). The inhibitory KIR protein can be KIR3DL1, KIR3DL2, KIR2DL1, KIR2DL2, or KIR2DL3 or a combination thereof, such as specifically binding to KIR2DL1/2/3 or KIR2DL1/2 proteins.

[0226] Antibodies to inhibitory KIR proteins are known in the art.

[0227] Antibodies to KIR3DL1 have been described in, for example, US Patent No. 5,770,387 and International Patent Publication WO2018148223.

[0228] Antibodies to KIR3DL2 have been described in, for example, Published US Application No. 20200199228 and 20150232556.

[0229] Antibodies to KIR2DL1, KIR2DL2, KIR2DL3, and combinations thereof have been describes in, for example, US Patent Nos. 10,668,180 and 10,253,095, International Patent Publication WO2006003179, Published US Application Nos. 20150290316 and 20130251711 and European Patent No. 3072522.

[0230] In some embodiments, the second binding domain specifically binds to KIR3DL1 and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:133 and SEQ ID NO:134, respectively; SEQ ID NO:141 and SEQ ID NO:142, respectively; or SEQ ID NO:149 and SEQ ID NO:150, respectively.

[0231] In some embodiments, the first binding domain specifically binds to KIR3DL1 and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:133 and SEQ ID NO:134, respectively; SEQ ID NO:141 and SEQ ID NO:142, respectively; or SEQ ID NO:149 and SEQ ID NO:150, respectively; wherein the framework regions of the heavy and light

chain variable regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4, or 1 to 2 amino acid substitutions, deletions, or insertions, and wherein the CDRs of the heavy or light chain variable regions are not modified.

[0232] In some embodiments, the first binding domain specifically binds to KIR3DL2 and the heavy and light chain variable regions have the amino acid sequences set forth in the amino acid sequences set forth in SEQ ID NO:157 and SEQ ID NO:158, respectively.

[0233] In some embodiments, the first binding domain specifically binds to KIR3DL2 and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:157 and SEQ ID NO:158, respectively; wherein the framework regions of the heavy and light chain variable regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4, or 1 to 2 amino acid substitutions, deletions, or insertions, and wherein the CDRs of the heavy or light chain variable regions are not modified.

[0234] In some embodiments, the first binding domain specifically binds to KIR2DL1/2/3 and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:101 and SEQ ID NO:102, respectively; SEQ ID NO:109 and SEQ ID NO:110, respectively; SEQ ID NO:117 and SEQ ID NO:118, respectively; or SEQ ID NO:125 and SEQ ID NO:126, respectively.

[0235] In some embodiments, the first binding domain specifically binds to KIR2DL1/2/3 and the heavy and light chain variable regions have the amino acid sequences set forth in SEQ ID NO:101 and SEQ ID NO:102, respectively; SEQ ID NO:109 and SEQ ID NO:110, respectively; SEQ ID NO:117 and SEQ ID NO:118, respectively; or SEQ ID NO:125 and SEQ ID NO:126, respectively; wherein the framework regions of the heavy and light chain variable regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4, or 1 to 2 amino acid substitutions, deletions, or insertions, and wherein the CDRs of the heavy or light chain variable regions are not modified.

[0236] In some embodiments, the first binding domain specifically binds to KIR3DL1 and the heavy chain variable region has complementarity

determining regions (CDR) hCDR1, hCDR2, and hCDR3, the light chain variable region has lCDR1, lCDR2, and lCDR3,and the amino acid sequences of the heavy and light chain variable region CDRs have the amino acid sequences set forth in SEQ ID NO:135 to SEQ ID NO:140, respectively; SEQ ID NO:143 to SEQ ID NO:148, respectively; or SEQ ID NO:151 to SEQ ID NO:156, respectively.

[0237] In some embodiments, the first binding domain specifically binds to KIR3DL2 and the heavy chain variable region has complementarity determining regions hCDR1, hCDR2, and hCDR3, the light chain variable region has lCDR1, lCDR2, and lCDR3, and the amino acid sequences of the heavy and light chain variable region CDRs are set forth in the amino acid sequences of SEQ ID NO:159 to SEQ ID NO:164, respectively.

[0238] In some embodiments, the first binding domain specifically binds to KIR2DL1/2/3 and the heavy chain variable region has complementarity determining regions hCDR1, hCDR2, and hCDR3, the light chain variable region has lCDR1, lCDR2, and lCDR3, the amino acid sequences of the heavy and light chain variable region CDRs are set forth in SEQ ID NO:103 to SEQ ID NO:108, respectively; SEQ ID NO:111 to SEQ ID NO:116, respectively; SEQ ID NO:119 to SEQ ID NO:124, respectively; or SEQ ID NO:127 to SEQ ID NO:132, respectively.

Binding Agents

[0239] The binding agent can be any suitable agent that includes at least a first binding domain and a second binding domain, wherein the first binding domain that specifically binds to a first antigen that is selected from antigens expressed on CD8+KIR+ T regulatory cells (Tregs), other than a KIR protein; and a second binding domain that specifically binds to an inhibitory KIR protein, wherein the binding agent binds to CD8+KIR+ Tregs.

[0240] In some embodiments, a binding agent is bispecific (*i.e.*, having binding domains for two different antigens). In some embodiments, a binding agent is bivalent (*i.e.*, having two binding domains). In some embodiments, the

binding agent is tetravalent (*i.e.*, having four binding domains). In some embodiments, the binding agent is trivalent, hexavalent, or octavalent.

[0241] The binding domains of the binding agents can be derived from antibodies or from non-antibody formats. In some embodiments, a binding domain is derived from an antibody or antigen binding portion thereof (*i.e.*, an antigen binding antibody fragment). In some embodiments, the antibody fragment is a Fab, Fab', F(ab')2, Fv, scFv, or a single domain antibody (also referred to as a VHH, VNAR, sdAb, or nanobody). In some embodiments, a binding domain is derived from an anticalin, affibody, avimer, DARPin, adnectin, or a receptor ectodomain Fc fusion protein.

[0242] In some embodiments, the binding agent is a bispecific antibody, a diabody, an antibody Fc fusion, scFv1-ScFv2, an $ScFv1_2$-Fc-$scFv2_2$, an IgG-scFv, a DVD-Ig, a triomab/quadroma, a two-in-one IgG, a $scFv_2$-Fc, a TandAb, an scFv-HSA-scFv, an scFv-VHH, a Fab-scFv-Fc, a Fab-VHH-Fc, a dAb-IgG, an IgG-VHH, a Tandem scFv-Fc, a $(scFv1)_2$-Fc-$(VHH)_2$, a BiTe, a DART, a crossmab, an anticalin, an affibody, an avimer, a DARPin, an adnectin, a scFv-Fc, a one-armed tandem scFv-Fc, or a DART-Fc . In some embodiments, the IgG-scFv is an IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, 2scFV-IgG, or IgG-2scFv (as shown in Figure 1).

[0243] As used herein, the term “antibody” refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site (antigen binding portion) that specifically binds to a target antigen. The term generally refers to antibodies comprised of two immunoglobulin heavy chain variable regions and two immunoglobulin light chain variable regions including full length antibodies (having heavy and light chain constant regions) and antigen-binding portions thereof; including, for example, an intact monoclonal antibody, a Fab, a Fab', a $F(ab')_2$, a Fv, a disulfide linked Fv, a scFv, a single domain antibody (dAb), a diabody, a multi-specific antibody, a dual specific antibody, a bispecific antibody, and single chain antibodies (*see, e.g.*, Huston et al., Proc. Natl. Acad.

Sci. U.S.A., 85, 5879-5883 (1988) and Bird et al., Science 242, 423-426 (1988), which are incorporated herein by reference).

[0244] In an antibody, each heavy chain is composed of a variable region (abbreviated as VH) and a constant region. The heavy chain constant region may include three domains CH1, CH2, and CH3 and optionally a fourth domain, CH4. Each of these domains is referred to as an "Fc domain". As used herein, when a binding agent includes an Fc domain, it can include one or more Fc domains, or an entire Fc region, unless otherwise specified by context. Each light chain is composed of a variable region (abbreviated as VL) and a constant region or constant domain. The light chain constant region is a CL domain. The VH and VL regions may be further divided into hypervariable regions referred to as complementarity-determining regions (CDRs) and interspersed with conserved regions referred to as framework regions (FR). Each VH and VL region thus consists of three CDRs and four FRs that are arranged from the N terminus to the C terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. This structure is well known to those skilled in the art.

[0245] As used herein, an "antigen-binding portion" of an antibody refers to the portions of an antibody as described herein having the VH and VL sequences or the heavy and light chain variable region CDRs. In accordance with the term "antigen-binding portion" of an antibody, examples of antigen binding portions include a Fab, a Fab', a $F(ab')_2$, a Fv, a disulfide linked Fv, a scFv, a single domain antibody (dAb), a diabody, and single chain antibodies. As used herein, the terms Fab, $F(ab')_2$ and Fv refer to the following: (i) an Fab fragment, *i.e.* a monovalent fragment composed of the VL, VH, CL and CH1 domains; (ii) an $F(ab')_2$ fragment, *i.e.* a bivalent fragment comprising two Fab fragments linked to one another in the hinge region via a disulfide bridge; and (iii) an Fv fragment composed of the VL and VH domains of an antibody. Although the two domains of the Fv fragment, namely VL and VH, are encoded by separate coding regions, they may further be linked to one another using a synthetic linker, *e.g.* a poly-G4S amino acid sequence (`(G4S)n` disclosed as

SEQ ID NO: 165, wherein n =1 to 5), making it possible to prepare them as a single protein chain in which the VL and VH regions combine in order to form monovalent molecules (known as single chain Fv (ScFv)). The term "antigen-binding portion" of an antibody is also intended to include such single chain antibodies.

[0246] Other forms of single chain antibodies such as "diabodies" are likewise included here. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker connecting the VH and VL domains that is too short for the two domains to be able to combine on the same chain, thereby forcing the VH and VL domains to pair with complementary domains of a different chain (VL and VH, respectively), and to form two antigen-binding sites (*see*, for example, Holliger, R, et al. (1993) Proc. Natl. Acad. Sci. USA 90:64446448; Poljak, R. J, et al. (1994) Structure 2:1121-1123).

[0247] An immunoglobulin constant region, or Fc region, refers to a heavy or light chain constant region. Human heavy chain and light chain constant region amino acid sequences are known in the art. A constant region can be of any suitable type, which can be selected from the classes of immunoglobulins, IgA, IgD, IgE, IgG, and IgM. Several immunoglobulin classes can be further divided into isotypes, *e.g.*, IgGl, IgG2, IgG3, IgG4, or IgAl, and IgA2. The heavy-chain constant regions (Fc) that corresponds to the different classes of immunoglobulins can be α, δ, ε, γ, and μ, respectively. The light chains can be one of either kappa (or κ) and lambda (or λ).

[0248] In some embodiments the binding agent lacks an Fc region or domains thereof. In some embodiments, the binding agent has an entire Fc region or an Fc domain thereof. In some embodiments, the binding agent has an Fc region or Fc domain of an IgG1 isotype. In some embodiments, the binding agent has an Fc region or Fc domain of an IgG2 isotype. In some embodiments, the binding agent has an Fc region or Fc domain of an IgG3 isotype. In some embodiments, the binding agent has an Fc region or Fc

domain of an IgG4 isotype. In some embodiments, an Fc domain can have a hybrid isotype comprising constant regions from two or more isotypes. In some embodiments, an Fc region or Fc domain can be an IgG1 or IgG4 constant region.

[0249] In some embodiments, the C-terminus of an Fc domain (*e.g.*, the heavy chain) can be a complete C-terminus ending with the amino acid residues PGK. In some embodiments, the C-terminus of the Fc domain also can be a shortened C-terminus in which one or two of the C terminal amino acid residues have been removed. In some embodiments, the C-terminus of the Fc domain is a shortened C-terminus ending PG. In some embodiments, a binding agent comprising a heavy chain including a C-terminal CH3 domain comprises the C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to Kabat EU index). In some embodiments, a binding agent comprising a heavy chain including a C-terminal CH3 domain comprises a C-terminal glycine residue (G446, numbering according to Kabat EU index).

[0250] The binding agents as described herein are multispecific, typically bispecific binding agents. In some embodiments, the binding agents are multispecific antibodies or antibody-like molecules, such as bispecific antibodies. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites or antigens. The binding agents described herein typically have binding specificities for different antigens. Bispecific antibodies can be prepared as full length antibodies or antibody fragments. Bispecific and multi-specific antibodies include the following: an scFv1-ScFv2, an $ScFv1_2$-Fc-$scFv2_2$, an IgG-scFv, a DVD-Ig, a triomab/quadroma, a two-in-one IgG, a $scFv_2$-Fc, a TandAb, an scFv-HSA-scFv, an scFv-VHH, a Fab-scFv-Fc, a Fab-VHH-Fc, a dAb-IgG, an IgG-VHH, a Tandem scFv-Fc, a $(scFv1)_2$-Fc-$(VHH)_2$, a scFv-Fc, a one-armed tandem scFv-Fc, and a DART-Fc In some embodiments, the IgG-scFv is an IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, svFc-(L)IgG, 2scFV-IgG, or IgG-2scFv (as shown in Figure 1).

[0251] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (*see* Milstein and Cuello, Nature 305: 537 (1983)), WO 93/08829, and Traunecker et al., EMBO J. 10: 3655 (1991)), and "knob-in-hole" engineering (*see, e.g.*, U.S. Pat. No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking of two or more antibodies or fragments (*see, e.g.*, U.S. Pat. No. 4,676,980, and Brennan et al., Science, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (*see, e.g.*, Kostelny et al., J. Immunol., 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (*see, e.g.*, Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)); and using single-chain Fv (scFv) dimers (see, *e.g.* Gruber et al., J. Immunol., 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt et al. J. Immunol. 147: 60 (1991).

[0252] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies", are also included herein (*see, e.g.* US 2006/0025576A1).

[0253] The binding agents (*e.g.*, antibodies or antigen binding fragments) herein also include a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to two different antigens (*see, e.g.*, US 2008/0069820 and Bostrom et al., 2009, Science 323:1610-14). "Crossmab" antibodies are also included herein (*see, e.g.*, WO 2009/080251, WO 2009/080252, WO2009/080253, WO2009/080254, and WO2013/026833).

[0254] In some embodiments, the binding agents comprise different antigen-binding sites, fused to one or the other of the two subunits of the Fc domain; thus, the two subunits of the Fc domain may be comprised in two non-identical polypeptide chains. Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides. To improve the yield and purity of the bispecific molecules in

recombinant production, it will thus be advantageous to introduce in the Fc domain of the binding agent a modification promoting the association of the desired polypeptides.

[0255] Accordingly, in particular aspects relates to a binding agent comprising (a) at least a first binding domain, (b) a second binding domain, and (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the Fc domain comprises a modification promoting the association of the first and second subunit of the Fc domain. The site of most extensive protein-protein interaction between the two subunits of a human IgG Fc domain is in the CH3 domain of the Fc domain. Thus, in one aspect said modification is in the CH3 domain of the Fc domain.

[0256] In a specific aspect, the Fc modification is a so-called "knob-into-hole" modification, comprising a "knob" modification in one of the two subunits of the Fc domain and a "hole" modification in the other one of the two subunits of the Fc domain. In a particular aspect, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W (EU numbering) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S, and Y407V (numbering according to Kabat EU index).

[0257] The knob-into-hole technology is described *e.g.* in U.S. Pat. Nos. 5,731,168; 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001). Generally, the method involves introducing a protuberance ("knob") at the interface of a first polypeptide and a corresponding cavity ("hole") in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine).

[0258] Accordingly, in some embodiments, in a CH3 domain of an Fc domain an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain which is positionable in a cavity within a CH3 domain of a second Fc domain, and in the CH3 domain of the second Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second Fc domain within which the protuberance within the CH3 domain of the first Fc domain is positionable. The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, *e.g.*, by site-specific mutagenesis, or by peptide synthesis. In a specific embodiment, in the CH3 domain of the first Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the CH3 domain of the second Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V). In another embodiment, in the second Fc domain additionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A).

[0259] In yet a further aspect, in the first Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C), and in the second Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C). Introduction of these two cysteine residues results in the formation of a disulfide bridge between the two Fc domains that further stabilizes the dimer (Carter (2001), J Immunol Methods 248, 7-15). In some embodiments, the first Fc domain comprises the amino acid substitutions S354C and T366W (EU numbering) and the second Fc domain comprises the amino acid substitutions Y349C, T366S, and Y407V (numbering according to Kabat EU index).

[0260] In some embodiments, a modification promoting association of the first and the second Fc domains comprises a modification mediating electrostatic steering effects, *e.g.* as described in PCT publication WO

2009/089004. Generally, this method involves replacement of one or more amino acid residues at the interface of the two Fc domains by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable.

[0261] In some embodiments, a binding agent comprises one or more scFvs or "single-chain variable fragments". An scFv is a fusion protein of the variable regions of the heavy (VH) and light chain (VL) variable regions of an antibody, connected with a short linker peptide of ten to about 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. This protein retains the specificity of the original antibody, despite removal of the constant regions and the introduction of the linker. scFv antibodies are, *e.g.*, described in Houston, J. S., Methods in Enzymol. 203 (1991) 46-96. Methods for making scFv molecules and designing suitable peptide linkers are described in, for example, U.S. Pat. No. 4,704,692; U.S. Pat. No. 4,946,778; Raag and Whitlow, FASEB 9:73-80 (1995) and Bird and Walker, TIBTECH, 9: 132-137 (1991).

[0262] Binding agents that are scFv-Fcs have been described by Sokolowska-Wedzina et al., Mol. Cancer Res. 15(8):1040-1050, 2017.

[0263] In some embodiments, a binding agent is a "bispecific T cell engager" or BiTE (*see, e.g.*, WO2004/106381, WO2005/061547, WO2007/042261, and WO2008/119567). This approach utilizes two antibody variable domains arranged on a single polypeptide. For example, a single polypeptide chain can include two single chain Fv (scFv) fragments, each having a variable heavy chain (VH) and a variable light chain (VL) domain separated by a polypeptide linker of a length sufficient to allow intramolecular association between the two domains. This single polypeptide further includes a polypeptide spacer sequence between the two scFv fragments. Each scFv recognizes a different epitope, and these epitopes may be specific for different proteins, such that both proteins are bound by the BiTE.

[0264] As it is a single polypeptide, the bispecific T cell engager may be expressed using any prokaryotic or eukaryotic cell expression system known in the art, *e.g.*, a CHO cell line. However, specific purification techniques (*see, e.g.*, EP1691833) may be necessary to separate monomeric bispecific T cell engagers from other multimeric species, which may have biological activities other than the intended activity of the monomer. In one exemplary purification scheme, a solution containing secreted polypeptides is first subjected to a metal affinity chromatography, and polypeptides are eluted with a gradient of imidazole concentrations. This eluate is further purified using anion exchange chromatography, and polypeptides are eluted using with a gradient of sodium chloride concentrations. Finally, this eluate is subjected to size exclusion chromatography to separate monomers from multimeric species. In some embodiments, a binding agent that is a bispecific antibody is composed of a single polypeptide chain comprising two single chain FV fragments (scFV) fused to each other by a peptide linker.

[0265] A single-domain antibody is an antibody fragment consisting of a single monomeric variable antibody domain. Single domains antibodies can be derived from the variable domain of the antibody heavy chain from camelids (*e.g.*, nanobodies or VHH fragments). Furthermore, the term single-domain antibody includes an autonomous human heavy chain variable domain (aVH) or VNAR fragments derived from sharks (*see, e.g.*, Hasler et al., Mol. Immunol. 75:28-37, 2016).

[0266] Techniques for producing single domain antibodies (DABs or VHH) are known in the art, as disclosed for example in Cossins et al. (2006, Prot Express Purif 51:253-259 and Li et al., Immunol. Lett. 188:89-95, 2017). Single domain antibodies may be obtained, for example, from camels, alpacas or llamas by standard immunization techniques. (*See, e.g.*, Muyldermans et al., TIBS 26:230-235, 2001; Yau et al., J Immunol Methods 281:161-75, 2003; and Maass et al., J Immunol Methods 324:13-25, 2007.) A VHH may have potent antigen-binding capacity and can interact with novel epitopes that are

inacessible to conventional VH-VL pairs (*see, e.g.*, Muyldermans et al., 2001). Alpaca serum IgG contains about 50% camelid heavy chain only IgG antibodies (HCAbs) (*see, e.g.*, Maass et al., 2007). Alpacas may be immunized with antigens and VHHs can be isolated that bind to and neutralize the target antigen (*see, e.g.*, Maass et al., 2007). PCR primers that amplify alpaca VHH coding sequences have been identified and may be used to construct alpaca VHH phage display libraries, which can be used for antibody fragment isolation by standard biopanning techniques well known in the art (*see, e.g.*, Maass et al., 2007).

[0267] In some embodiments, a binding agent is a IgG-scFV. IgG-scFv formats include IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, svFc-(L)IgG, 2scFV-IgG, and IgG-2scFv. These and other bispecific antibody formats and methods of making them have been described in for example, Brinkmann and Kontermann, MAbs 9(2):182-212 (2017); Wang et al., Antibodies, 2019, 8, 43; Dong et al., 2011, MAbs 3:273-88; Natsume et al., J. Biochem. 140(3):359-368, 2006; Cheal et al., Mol. Cancer Ther. 13(7):1803-1812, 2014; and Bates and Power, Antibodies, 2019, 8, 28.

[0268] Igg-like dual-variable domain antibodies (DVD-Ig) have been described by Wu et al., 2007, Nat Biotechnol 25:1290-97; Hasler et al., Mol. Immunol. 75:28-37, 2016 and in WO 08/024188 and WO 07/024715. Triomabs have been described by Chelius et al., MAbs 2(3):309-319, 2010. 2-in-1-IgGs have been described by Kontermann et al., Drug Discovery Today 20(7):838-847, 2015. Tanden antibody or TandAb have been described by Kontermann et al., id. ScFv-HSA-scFv antibodies have also been described by Kontermann et al. (id.).

[0269] In some embodiments, the binding agent is a scaffold antigen binding protein, such as for example, fibronectin and designed ankyrin repeat proteins (DARPins) which have been used as alternative scaffolds for antigen-binding domains, *see, e.g.*, Gebauer and Skerra, Engineered protein scaffolds as next-generation antibody therapeutics. Curr Opin Chem Biol 13:245-255

(2009) and Stumpp et al., Darpins: A new generation of protein therapeutics. Drug Discovery Today 13: 695-701 (2008). In some embodiments, a scaffold antigen binding protein is selected from the group consisting of Lipocalins (Anticalin), a Protein A-derived molecule such as Z-domain of Protein A (Affibody), an A-domain (Avimer/Maxibody), a serum transferrin (trans-body); a designed ankyrin repeat protein (DARPin), a fibronectin (AdNectin), a C-type lectin domain (Tetranectin); a variable domain of a new antigen receptor beta-lactamase (VNAR fragments), a human gamma-crystallin or ubiquitin (Affilin molecules); a kunitz type domain of human protease inhibitors, and microbodies such as the proteins from the knottin family, peptide aptamers and fibronectin (adnectin).

[0270] Lipocalins are a family of extracellular proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids, and lipids. They have a rigid beta-sheet secondary structure with a number of loops at the open end of the conical structure which can be engineered to bind to different target antigens. Anticalins are between 160-180 amino acids in size, and are derived from lipocalins. For further details, *see* Biochim Biophys Acta 1482: 337-350 (2000), U.S. Pat. No. 7,250,297B1, and US20070224633.

[0271] Designed Ankyrin Repeat Proteins (DARPins) are derived from Ankyrin which is a family of proteins that mediate attachment of integral membrane proteins to the cytoskeleton. A single ankyrin repeat is a 33 residue motif consisting of two alpha-helices and a beta-turn. They can be engineered to bind different target antigens by randomizing residues in the first alpha-helix and a beta-turn of each repeat. Their binding interface can be increased by increasing the number of modules (a method of affinity maturation). For further details, *see* J. Mol. Biol. 332, 489-503 (2003), PNAS 100(4), 1700-1705 (2003) and J. Mol. Biol. 369, 1015-1028 (2007), and US20040132028A1.

Fc Domain Modifications to Alter Effector Function

[0272] In some embodiments, an Fc region or Fc domain has substantially no binding to at least one Fc receptor selected from FcγRI (CD64),

FcyRIIA (CD32a), FcyRIIB (CD32b), FcyRIIIA (CD16a), and FcyRIIIB (CD16b). In some embodiments, an Fc region or domain exhibits substantially no binding to any of the Fc receptors selected from FcyRI (CD64), FcyRIIA (CD32a), FcyRIIB (CD32b), FcyRIIIA (CD16a), and FcyRIIIB (CD16b). As used herein, "substantially no binding" refers to weak to no binding to a selected Fcgamma receptor or receptors. In some embodiments, "substantially no binding" refers to a reduction in binding affinity (*e.g.*, increase in Kd) to a Fc gamma receptor of at least 1000-fold. In some embodiments, an Fc domain or region is an Fc null. As used herein, an "Fc null" refers to an Fc region or Fc domain that exhibits weak to no binding to any of the Fcgamma receptors. In some embodiments, an Fc null domain or region exhibits a reduction in binding affinity (*e.g.*, increase in Kd) to Fc gamma receptors of at least 1000-fold.

[0273] In some embodiments, an Fc domain has reduced or substantially no effector function activity. As used herein, "effector function activity" refers to antibody dependent cellular cytotoxicity (ADCC), antibody dependent cellular phagocytosis (ADCP) and/or complement dependent cytotoxicity (CDC). In some embodiments, an Fc domain exhibits reduced ADCC, ADCP, or CDC activity, as compared to a wildtype Fc domain. In some embodiments, an Fc domain exhibits a reduction in ADCC, ADCP, and CDC, as compared to a wildtype Fc domain. In some embodiments, an Fc domain exhibits substantially no effector function (*i.e.*, the ability to stimulate ADCC, ADCP, or CDC). As used herein, "substantially no effector function" refers to a reduction in effector function activity of at least 1000-fold, as compared to a wildtype Fc domain.

[0274] In some embodiments, an Fc domain has reduced or no ADCC activity. As used herein reduced or no ADCC activity refers to a decrease in ADCC activity of an Fc domain by of a factor of at least 10, at least 20, at least 30, at least 50, at least 100, or at least 500.

[0275] In some embodiments, an Fc domain has reduced or no CDC activity. As used herein reduced or no CDC activity refers to a decrease in

CDC activity of an Fc domain by of a factor of at least 10, at least 20, at least 30, at least 50, at least 100, or at least 500.

[0276] In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of ADCC and/or CDC activity. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks Fcgamma receptor (hence likely lacking ADCC activity). The primary cells for mediating ADCC, NK cells, express FcgammaRIII only, whereas monocytes express FcgammaRI, FcgammaRII, and FcgammaRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-492 (1991). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest are described in U.S. Pat. No. 5,500,362 (*see, e.g.*, Hellstrom, I. et al. Proc. Nat'l Acad. Sci. USA 83:7059-7063 (1986)) and Hellstrom, I et al., Proc. Nat'l Acad. Sci. USA 82:1499-1502 (1985); U.S. Pat. No. 5,821,337 (*see* Bruggemann, M. et al., J. Exp. Med. 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see, e.g.*, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96™ non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, *e.g.*, in an animal model such as that disclosed in Clynes et al., Proc. Nat'l Acad. Sci. USA 95:652-656 (1998).

[0277] C1q binding assays may also be carried out to confirm that an antibody or Fc domain or region is unable to bind C1q and hence lacks CDC activity or has reduced CDC activity. *See, e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see*, for example, Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996); Cragg, M. S. et al., Blood 101:1045-1052 (2003); and Cragg, M. S. and M. J. Glennie, Blood 103:2738-2743 (2004)).

[0278] In some embodiments, an Fc domain has reduced or no ADCP activity. As used herein reduced or no ADCP activity refers to a decrease in ADCP activity of an Fc domain by of a factor of at least 10, at least 20, at least 30, at least 50, at least 100, or at least 500.

[0279] ADCP binding assays may also be carried out to confirm that an antibody or Fc domain or region lacks ADCP activity or has reduced ADCP activity. *See, e.g.*, US20190079077 and US20190048078 and the references disclosed therein.

[0280] Antibodies with reduced effector function activity include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327, and 329 (*see* U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297, and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (*see* U.S. Pat. No. 7,332,581). Certain antibody variants with diminished binding to FcRs are also known. (*See, e.g.*, U.S. Pat. No. 6,737,056; WO 2004/056312, and Shields et al., J. Biol. Chem. 9(2): 6591-6604 (2001).)

[0281] In certain embodiments, a binding agent comprises an Fc domain or region with one or more amino acid substitutions which diminish FcgammaR binding, *e.g.*, substitutions at positions 234 and 235 of the Fc region (EU numbering of residues). In some embodiments, the substitutions are L234A and L235A (LALA). In some embodiments, the Fc domain further comprises D265A and/or P329G in an Fc region derived from a human IgG1 Fc region. In some embodiments, the substitutions are L234A, L235A, and P329G (LALA-PG) in an Fc region derived from a human IgG1 Fc region. (*See, e.g.*, WO 2012/130831). In some embodiments, the substitutions are L234A, L235A, and D265A (LALA-DA) in an Fc region derived from a human IgG1 Fc region.

[0282] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either diminished) C1q binding and/or Complement

Dependent Cytotoxicity (CDC), *e.g.*, as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

Modification of Binding Domains

[0283] In some embodiments, a binding domain may be modified by a conservative substitution or substitutions. For conservative amino acid substitutions, a given amino acid can be replaced by a residue having similar physiochemical characteristics, *e.g.*, substituting one aliphatic residue for another (such as Ile, Val, Leu, or Ala for one another), or substitution of one polar residue for another (such as between Lys and Arg; Glu and Asp; or Gln and Asn). Other such conservative amino acid substitutions, *e.g.*, substitutions of entire regions having similar hydrophobicity characteristics, are well known. Polypeptides comprising conservative amino acid substitutions can be tested in any one of the assays described herein to confirm that a desired activity, *e.g.*, antigen-binding activity and specificity of a native or reference polypeptide is retained.

[0284] For conservative substitutions, amino acids can be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in Biochemistry, second ed., pp. 73-75, Worth Publishers, New York (1975)): (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M); (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q); (3) acidic: Asp (D), Glu (E); and (4) basic: Lys (K), Arg (R), His (H).

[0285] Alternatively, for conservative substitutions naturally occurring residues can be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions will entail exchanging a member of one of these classes or another class.

[0286] Particular conservative substitutions include, for example; Ala to Gly or to Ser; Arg to Lys; Asn to Gln or to His; Asp to Glu; Cys to Ser; Gln to

Asn; Glu to Asp; Gly to Ala or to Pro; His to Asn or to Gln; Ile to Leu or to Val; Leu to Ile or to Val; Lys to Arg, to Gln or to Glu; Met to Leu, to Tyr or to Ile; Phe to Met, to Leu or to Tyr; Ser to Thr; Thr to Ser; Trp to Tyr; Tyr to Trp; and/or Phe to Val, to Ile or to Leu.

[0287] In some embodiments, a conservatively modified variant of a binding domain preferably is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, identical to the reference VH or VL sequence, wherein the VH and VL CDRs are not modified. The degree of homology (percent identity) between the reference and modified sequence can be determined, for example, by comparing the two sequences using freely available computer programs commonly employed for this purpose on the world wide web (*e.g.*, BLASTp or BLASTn with default settings).

[0288] Modification of a native (or reference) amino acid sequence can be accomplished by any of a number of techniques known to one of skill in the art. Mutations can be introduced, for example, at particular sites by synthesizing oligonucleotides containing the desired mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes a variant having the desired amino acid insertion, substitution, or deletion. Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered nucleotide sequence having particular codons altered according to the substitution, deletion, or insertion desired. Techniques for making such alterations are very well established and include, for example, those disclosed by Walder et al. (Gene 42:133, 1986); Bauer et al. (Gene 37:73, 1985); Craik (BioTechniques, January 1985, 12-19); Smith et al. (Genetic Engineering: Principles and Methods, Plenum Press, 1981); and U.S. Pat. Nos. 4,518,584 and 4,737,462, which are herein incorporated by reference in their entireties.

CD8+KIR+ Regulatory T Cells

[0289] The regulatory T cells are characterized by the phenotype of being CD8+ KIR+ and are typically MHC Class I restricted. In humans, the CD8+Kir+ regulatory T cells express inhibitory KIR proteins. In some embodiments, the KIR proteins expressed by the cells can include one or more of the inhibitory KIR proteins, *e.g.*, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL5, KIR3DL1, and KIR3DL2; and may specifically include one or more of KIR2DL2, KIR2DL3, and KIR3DL1. In some embodiments, the CD8+KIR+ regulatory T cells are not HLA E (Qa-1b) restricted. (*See, e.g.*, Lohwasser et al., International Immunology 13:321-327 (2001) and Sarantopoulos et al., J. Clin. Invest. 114(9):1218-1221 (2004) for a general explanation of murine Qa-1b and human HLA E restriction.) In some embodiments, the CD8+KIR+ regulatory T cells can also be characterized as being CD44+, CD122+, and are not HLA E (Qa-1b) restricted. In some embodiments, the CD8+KIR+ regulatory T cells can also be characterized as being CD28-. In some embodiments, the CD8+KIR+ regulatory T cells can also be characterized as being CD28-, CD44+, and CD122+. In some embodiments, the CD8+KIR+ regulatory T cells can also be characterized as being CD28-, CD44+, and CD122+, and are not HLA E (Qa-1b) restricted.

[0290] In some embodiments, the CD8+KIR+ Tregs express the following antigens: CD3, CD8, PD-1, CD16, CD122, CD39, CXCR3, ICOS, CD103, and inhibitory KIR proteins.

[0291] In some embodiments, CD8+KIR+ Tregs express one or more of the following antigens: CD3, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB. In some embodiments, CD8+KIR+ Tregs express one or more of the following antigens: CD3, CD5, CD16, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, KLRB1, KLRG1, LAG-3/CD223,

NKG2C, NKG2D, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB. In some embodiments, CD8+KIR+ Tregs express one or more of the following antigens: CD39, KLRB1, KLRG1, NKG2C, NKG2D, CXCR3, and CD122.

Production of Binding Agents

[0292] In various embodiments, binding agents can be produced in human, murine, or other animal-derived cells lines. Recombinant DNA expression can be used to produce the binding agents. This allows the production of antibodies as well as a spectrum of antigen binding portions and other binding agents (including fusion proteins) in a host species of choice. The production of antibodies, antigen binding portions thereof and other binding agents in bacteria, yeast, transgenic animals, and chicken eggs are also alternatives for cell-based production systems. The main advantages of transgenic animals are potential high yields from renewable sources.

[0293] As used herein, the term "nucleic acid" or "nucleic acid sequence" or "polynucleotide sequence" or "nucleotide" refers to a polymeric molecule incorporating units of ribonucleic acid, deoxyribonucleic acid, or an analog thereof. The nucleic acid can be either single-stranded or double-stranded. A single-stranded nucleic acid can be one strand nucleic acid of a denatured double-stranded DNA. In some embodiments, the nucleic acid can be a cDNA, *e.g.*, a nucleic acid lacking introns.

[0294] Nucleic acid molecules encoding the amino acid sequence of an antibody, or antigen binding portion thereof, as well as other binding agents can be prepared by a variety of methods known in the art. These methods include, but are not limited to, preparation of synthetic nucleotide sequences encoding of an antibody, antigen binding portion or other binding agent(s). In addition, oligonucleotide-mediated (or site-directed) mutagenesis, PCR-mediated mutagenesis, and cassette mutagenesis can be used to prepare nucleotide sequences encoding an antibody or antigen binding portion as well as other binding agents. A nucleic acid sequence encoding at least an antibody, antigen binding portion thereof, binding agent, or a polypeptide thereof, as described

herein, can be recombined with vector DNA in accordance with conventional techniques, such as, for example, blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed, *e.g.*, by Maniatis et al., Molecular Cloning, Lab. Manual (Cold Spring Harbor Lab. Press, NY, 1982 and 1989), and Ausubel et al., Current Protocols in Molecular Biology (John Wiley & Sons), 1987-1993, and can be used to construct nucleic acid sequences and vectors that encode an antibody or antigen binding portion thereof or a VH and/or VL polypeptide thereof. Where the binding agent comprises antibodies or antigen binding portions thereof, in some embodiments, a VH polypeptide is encoded by a first nucleic acid. In some embodiments, a VL polypeptide is encoded by a second nucleic acid. In some embodiments, the VH and VL polypeptides are encoded by one nucleic acid.

[0295] A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences that contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences that encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed (*e.g.*, an antibody or antigen binding portion thereof) are connected in such a way as to permit gene expression of a polypeptide(s) or antigen binding portions in recoverable amounts. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, as is well known in the analogous art. *See, e.g.*, Sambrook et al., 1989; Ausubel et al., 1987-1993.

[0296] Accordingly, the expression of an antibody or antigen-binding portion thereof or other binding agent as described herein can occur in either prokaryotic or eukaryotic cells. Suitable hosts include bacterial or eukaryotic hosts, including yeast, insects, fungi, bird, and mammalian cells either in vivo or

in situ, or host cells of mammalian, insect, bird, or yeast origin. The mammalian cell or tissue can be of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog, or cat origin, but any other mammalian cell may be used. Further, by use of, for example, the yeast ubiquitin hydrolase system, in vivo synthesis of ubiquitin-transmembrane polypeptide fusion proteins can be accomplished. The fusion proteins so produced can be processed in vivo or purified and processed in vitro, allowing synthesis of an antibody or antigen binding portion thereof as described herein with a specified amino terminus sequence. Moreover, problems associated with retention of initiation codon-derived methionine residues in direct yeast (or bacterial) expression may be avoided. (*See, e.g.*, Sabin et al., 7 Bio/Technol. 705 (1989); Miller et al., 7 Bio/Technol. 698 (1989).) Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeast are grown in medium rich in glucose can be utilized to obtain recombinant antibodies or antigen-binding portions thereof or other binding agents. Known glycolytic genes can also provide very efficient transcriptional control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase gene can be utilized.

[0297] Production of antibodies or antigen-binding portions thereof and other binding agents in insects can be achieved, for example, by infecting an insect host with a baculovirus engineered to express a polypeptide by methods known to those of ordinary skill in the art. *See* Ausubel et al., 1987-1993.

[0298] In some embodiments, the introduced nucleic acid sequence (encoding an antibody or antigen binding portion thereof or a polypeptide thereof or other binding agent) is incorporated into a plasmid or viral vector capable of autonomous replication in a recipient host cell. Any of a wide variety of vectors can be employed for this purpose and are known and available to those of ordinary skill in the art. *See, e.g.*, Ausubel et al., 1987-1993. Factors of importance in selecting a particular plasmid or viral vector include: the ease

with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

[0299] Exemplary prokaryotic vectors known in the art include plasmids such as those capable of replication in E. coli. Other gene expression elements useful for the expression of DNA encoding antibodies or antigen-binding portions thereof and other binding agents include, but are not limited to (a) viral transcription promoters and their enhancer elements, such as the SV40 early promoter, (Okayama et al., 3 Mol. Cell. Biol. 280 (1983)), Rous sarcoma virus LTR (Gorman et al., 79 PNAS 6777 (1982)), and Moloney murine leukemia virus LTR (Grosschedl et al., 41 Cell 885 (1985)); (b) splice regions and polyadenylation sites such as those derived from the SV40 late region (Okayarea et al., 1983), and (c) polyadenylation sites such as in SV40 (Okayama et al., 1983). Immunoglobulin-encoding DNA genes can be expressed as described by Liu et al., infra, and Weidle et al., 51 Gene 21 (1987), using as expression elements the SV40 early promoter and its enhancer, the mouse immunoglobulin H chain promoter enhancers, SV40 late region mRNA splicing, rabbit S-globin intervening sequence, immunoglobulin and rabbit S-globin polyadenylation sites, and SV40 polyadenylation elements.

[0300] For immunoglobulin encoding nucleotide sequences, the transcriptional promoter can be, for example, human cytomegalovirus, the promoter enhancers can be cytomegalovirus and mouse/human immunoglobulin.

[0301] In some embodiments, for expression of DNA coding regions in rodent cells, the transcriptional promoter can be a viral LTR sequence, the transcriptional promoter enhancers can be either or both the mouse immunoglobulin heavy chain enhancer and the viral LTR enhancer, and the polyadenylation and transcription termination regions. In other embodiments,

DNA sequences encoding other proteins are combined with the above-recited expression elements to achieve expression of the proteins in mammalian cells.

[0302] Each coding region or gene fusion is assembled in, or inserted into, an expression vector. Recipient cells capable of expressing the variable region(s) or antigen binding portions thereof are then transfected singly with nucleotides encoding an antibody or an antibody polypeptide or antigen-binding portion thereof, or are co-transfected with a polynucleotide(s) encoding VH and a VL chain coding regions. The transfected recipient cells are cultured under conditions that permit expression of the incorporated coding regions and the expressed antibody chains or intact antibodies or antigen binding portions are recovered from the culture.

[0303] In some embodiments, the nucleic acids containing the coding regions encoding an antibody or antigen-binding portion thereof are assembled in separate expression vectors that are then used to co-transfect a recipient host cell. Each vector can contain one or more selectable genes. For example, in some embodiments, two selectable genes are used, a first selectable gene designed for selection in a bacterial system and a second selectable gene designed for selection in a eukaryotic system, wherein each vector has a set of coding regions. This strategy results in vectors which first direct the production, and permit amplification, of the nucleotide sequences in a bacterial system. The DNA vectors so produced and amplified in a bacterial host are subsequently used to co-transfect a eukaryotic cell, and allow selection of a co-transfected cell carrying the desired transfected nucleic acids (*e.g.*, encoding antibody heavy and light chains). Non-limiting examples of selectable genes for use in a bacterial system are the gene that confers resistance to ampicillin and the gene that confers resistance to chloramphenicol. Selectable genes for use in eukaryotic transfectants include the xanthine guanine phosphoribosyl transferase gene (designated gpt) and the phosphotransferase gene from Tn5 (designated neo). Alternatively the fused nucleotide sequences encoding VH and VL chains can be assembled on the same expression vector.

[0304] For transfection of the expression vectors and production of the antibodies or antigen binding portions thereof or other binding agents, the recipient cell line can be a Chinese Hamster ovary cell line (*e.g.*, DG44) or a myeloma cell. Myeloma cells can synthesize, assemble and secrete immunoglobulins encoded by transfected immunoglobulin genes and possess the mechanism for glycosylation of the immunoglobulin. For example, in some embodiments, the recipient cell is the recombinant Ig-producing myeloma cell SP2/0. SP2/0 cells only produce immunoglobulins encoded by the transfected genes. Myeloma cells can be grown in culture or in the peritoneal cavity of a mouse, where secreted immunoglobulin can be obtained from ascites fluid.

[0305] An expression vector encoding an antibody or antigen-binding portion thereof or other binding agent can be introduced into an appropriate host cell by any of a variety of suitable means, including such biochemical means as transformation, transfection, protoplast fusion, calcium phosphate-precipitation, and application with polycations such as diethylaminoethyl (DEAE) dextran, and such mechanical means as electroporation, direct microinjection and microprojectile bombardment. Johnston et al., 240 Science 1538 (1988), as known to one of ordinary skill in the art.

[0306] Yeast provides certain advantages over bacteria for the production of immunoglobulin heavy and light chains. Yeasts carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies exist that utilize strong promoter sequences and high copy number plasmids which can be used for production of the desired proteins in yeast. Yeast recognizes leader sequences of cloned mammalian gene products and secretes polypeptides bearing leader sequences (*i.e.*, pre-polypeptides). *See, e.g.*, Hitzman et al., 11th Intl. Conf. Yeast, Genetics & Molec. Biol. (Montpelier, France, 1982).

[0307] Yeast gene expression systems can be routinely evaluated for the levels of production, secretion and the stability of antibodies, and assembled antibodies and antigen binding portions thereof. Various yeast gene expression

systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeasts are grown in media rich in glucose can be utilized. Known glycolytic genes can also provide very efficient transcription control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase (PGK) gene can be utilized. Another example is the translational elongation factor 1alpha promoter. A number of approaches can be taken for evaluating optimal expression plasmids for the expression of immunoglobulins in yeast. *See* II DNA Cloning 45, (Glover, ed., IRL Press, 1985) and *e.g.*, U.S. Publication No. US 2006/0270045 A1.

[0308] Bacterial strains can also be utilized as hosts for the production of the antibody molecules or antigen binding portions thereof or other binding agents described herein. E. coli K12 strains such as E. coli W3110, Bacillus species, enterobacteria such as Salmonella typhimurium or Serratia marcescens, and various Pseudomonas species can be used. Plasmid vectors containing replicon and control sequences which are derived from species compatible with a host cell are used in connection with these bacterial hosts. The vector carries a replication site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. A number of approaches can be taken for evaluating the expression plasmids for the production of antibodies and antigen binding portions thereof in bacteria (*see* Glover, 1985; Ausubel, 1987, 1993; Sambrook, 1989; Colligan, 1992-1996).

[0309] Host mammalian cells can be grown in vitro or in vivo. Mammalian cells provide post-translational modifications to immunoglobulin molecules including leader peptide removal, folding and assembly of VH and VL chains, glycosylation of the antibody molecules, and secretion of functional antibody and/or antigen binding portions thereof.

[0310] Mammalian cells which can be useful as hosts for the production of antibody proteins, in addition to the cells of lymphoid origin described above, include cells of fibroblast origin, such as Vero or CHO-K1 cells. Exemplary

eukaryotic cells that can be used to express immunoglobulin polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, CHO-K1, and DG44 cells; PERC6™ cells (Crucell); and NSO cells. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the heavy chains and/or light chains. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[0311] In some embodiments, one or more antibodies or antigen-binding portions thereof or other binding agents can be produced in vivo in an animal that has been engineered or transfected with one or more nucleic acid molecules encoding the polypeptides, according to any suitable method.

[0312] In some embodiments, an antibody or antigen-binding portion thereof is produced in a cell-free system. Non-limiting exemplary cell-free systems are described, *e.g.*, in Sitaraman et al., Methods Mol. Biol. 498: 229-44 (2009); Spirin, Trends Biotechnol. 22: 538-45 (2004); and Endo et al., Biotechnol. Adv. 21: 695-713 (2003).

[0313] Many vector systems are available for the expression of the VH and VL chains in mammalian cells (*see* Glover, 1985). Various approaches can be followed to obtain intact antibodies. As discussed above, it is possible to co-express VH and VL chains and optionally the associated constant regions in the same cells to achieve intracellular association and linkage of VH and VL chains into complete tetrameric H_2L_2 antibodies or antigen-binding portions thereof. The co-expression can occur by using either the same or different plasmids in the same host. Nucleic acids encoding the VH and VL chains or antigen binding portions thereof can be placed into the same plasmid, which is then transfected into cells, thereby selecting directly for cells that express both chains. Alternatively, cells can be transfected first with a plasmid encoding one chain, for example the VL chain, followed by transfection of the resulting cell line with a VH chain plasmid containing a second selectable marker. Cell lines

producing antibodies, antigen-binding portions thereof or other binding agents via either route could be transfected with plasmids encoding additional copies of peptides, VH, VL, or VH plus VL chains in conjunction with additional selectable markers to generate cell lines with enhanced properties, such as higher production of assembled antibodies or antigen binding portions thereof or enhanced stability of the transfected cell lines.

[0314] Additionally, plants have emerged as a convenient, safe, and economical alternative expression system for recombinant antibody production, which are based on large scale culture of microbes or animal cells. Antibodies or antigen binding portions can be expressed in plant cell culture, or plants grown conventionally. The expression in plants may be systemic, limited to sub-cellular plastids, or limited to seeds (endosperms). *See, e.g.*, U.S. Patent Pub. No. 2003/0167531; U.S. Pat. No. 6,080,560; U.S. Pat. No. 6,512,162; and WO 0129242. Several plant-derived antibodies have reached advanced stages of development, including clinical trials (*see, e.g.*, Biolex, N.C.).

[0315] For intact antibodies, the variable regions (VH and VL) of the antibodies are typically linked to at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Human constant region DNA sequences can be isolated in accordance with well-known procedures from a variety of human cells, such as immortalized B-cells (*see, e.g.*, WO 87/02671; which is incorporated by reference herein in its entirety). An antibody can contain both light chain and heavy chain constant regions. The heavy chain constant region can include CH1, hinge, CH2, CH3, and, sometimes, CH4 regions. In some embodiments, the CH2 domain can be deleted or omitted.

[0316] Alternatively, techniques described for the production of single chain antibodies (*see, e.g.*, U.S. Pat. No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989); which are incorporated by reference herein in their entireties) can be adapted to produce single chain antibodies that

specifically bind to the desired antigen. Single chain antibodies are formed by linking the heavy and light chain variable regions of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli can also be used (*see, e.g.*, Skerra et al., Science 242:1038-1041 (1988); which is incorporated by reference herein in its entirety). Method of making other binding agents are described supra.

[0317] Intact (*e.g.*, whole) antibodies, their dimers, individual light and heavy chains, or antigen binding portions thereof can be recovered and purified by known techniques, *e.g.*, immunoadsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), ammonium sulfate precipitation, gel electrophoresis, or any combination of these. *See generally*, Scopes, Protein Purification (Springer-Verlag, N.Y., 1982). Substantially pure antibodies or antigen binding portions thereof of at least about 90% to 95% homogeneity are advantageous, as are those with 98% to 99% or more homogeneity, particularly for pharmaceutical uses. Once purified, partially or to homogeneity as desired, an intact antibody or antigen binding portions thereof can then be used therapeutically or in developing and performing assay procedures, immunofluorescent staining, and the like. *See generally*, Vols. I & II Immunol. Meth. (Lefkovits & Pernis, eds., Acad. Press, NY, 1979 and 1981).

Pharmaceutical Formulations

[0318] In some aspects the binding agents relate to compositions comprising active ingredients (*i.e.*, including a binding agent as described herein or a nucleic acid encoding an antibody or antigen-binding portion thereof or other binding agent as described herein). In some embodiments, the composition is a pharmaceutical composition. As used herein, the term "pharmaceutical composition" refers to the active agent in combination with a pharmaceutically acceptable carrier accepted for use in the pharmaceutical industry. The phrase "pharmaceutically acceptable" is employed herein to refer

to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0319] The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on any particular formulation. Typically such compositions are prepared as injectable either as liquid solutions or suspensions; however, solid forms suitable for rehydration, or suspensions, in liquid prior to use can also be prepared. A preparation can also be emulsified or presented as a liposome composition. An antibody or antigen binding portion thereof or other binding agent can be mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, a pharmaceutical composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance or maintain the effectiveness of the active ingredient (*e.g.*, an antibody or antigen binding portion thereof or other binding agent). The pharmaceutical compositions as described herein can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of a polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like. Physiologically tolerable

carriers are well known in the art. Exemplary liquid carriers are sterile aqueous solutions that contain the active ingredients (*e.g.*, an antibody and/or antigen binding portions thereof or other binding agent) and water, and may contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes. Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions. The amount of an active agent that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques.

[0320] In some embodiments, a pharmaceutical composition comprising an antibody or antigen-binding portion thereof or other binding agent or a nucleic acid encoding an antibody or antigen-binding portion thereof or other binding agent as described herein can be a lyophilisate.

[0321] In some embodiments, a syringe comprising a therapeutically effective amount of a binding agent, or a pharmaceutical composition described herein is provided.

Treatment of Inflammatory and Autoimmune Disease

[0322] In some aspects, the binding agents as described herein can be used in a method(s) comprising administering a binding agent or a pharmaceutical composition as described herein to a subject having an inflammatory disease. In some aspects, the binding agents as described herein can be used in a method(s) comprising administering a binding agent or a pharmaceutical composition as described herein to a subject having an autoimmune disease or having an immune response to an autoantigen or having an immune response to an antigen that causes or results in the onset of an autoimmune disorder (which may be collectively referred to as an

autoimmune disease herein). In some embodiments, the subject is in need of treatment for an autoimmune disease. In some embodiments, provided are methods of treating an autoimmune disease, comprising administering any of the binding agents described herein or the pharmaceutical compositions described herein to a subject in need thereof in an amount effective to decrease the number or activity of pathogenic immune cells in the subject and thereby ameliorate a symptom of the autoimmune disease. In some embodiments, provided are methods for suppressing an immune response mediated by pathogenic immune cells, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with any of the binding agents as described herein or the pharmaceutical compositions as described herein in an amount effective to activate or stimulate the CD8+KIR+ Tregs (activated Tregs), whereby the number or activity of pathogenic immune cells is decreased. In some embodiments, provided are methods for suppressing an immune response to an autoantigen, comprising administering to a subject in need thereof any of the binding agents described herein or the pharmaceutical compositions described herein in an amount effective to activate or stimulate CD8+KIR+ Tregs, whereby the number or activity of pathogenic immune cells that are responsive to the autoantigen is decreased. In some embodiments, provided are methods for preventing an immune response to an autoantigen that causes or results in the onset of an autoimmune disorder, comprising administering to a subject in need thereof any of the binding agents described herein or the pharmaceutical compositions described herein in an amount effective to activate or stimulate CD8+KIR+ Tregs, whereby the number or activity of pathogenic immune cells that are responsive to the autoantigen is decreased or the titer of autoantibodies is decreased in the subject. In some embodiments, provided are methods for suppressing an immune response to an autoantigen, that causes or results in the onset of an autoimmune disorder, comprising administering to a subject in need thereof any of the binding agents described herein or the pharmaceutical compositions described herein in an amount

effective to activate or stimulate CD8+KIR+ Tregs, whereby the number or activity of pathogenic immune cells that are responsive to the autoantigen is decreased or the titer of autoantibodies is decreased in the subject. In some embodiments, provided are methods for suppressing an immune response to an antigen or autoantigen that causes or results in the onset of an autoimmune disorder, comprising administering to a subject in need thereof any of the binding agents described herein or the pharmaceutical compositions described herein in an amount effective to activate or stimulate CD8+KIR+ Tregs, whereby the immune response to the antigen or autoantigen is decreased or suppressed in the subject.

[0323] As used herein, the term "autoantigen" refers to an antigen (*e.g.*, a cell surface protein or other antigen) that is normally recognized by the immune system (*e.g.*, a healthy human immune system) as self. An antigen or autoantigen becomes the target of a humoral or cell mediated immune response in an autoimmune disease or condition or it triggers an excessive immune response (collectively "autoimmune disease"). As used herein, to "activate or stimulate" CD8+KIR+ Tregs, or activated CD8+KIR+ Tregs, refer to an increase of the regulatory T cell functions of such cells, such as the ability to suppress an immune response, and in particular an immune response to an autoantigen or to suppressing an immune response to an autoantigen that causes or results in the onset of an autoimmune disorder. Activation or stimulation of CD8+KIR+ Tregs may include removal of a suppressive effect on such cells, so as to restore the CD8+KIR+ Tregs (*e.g.*, restore balance to the immune system or restore balanced immune activity in a subject or in the subject needing treatment prior to the onset of disease). Activation or stimulation of CD8+KIR+ Tregs may also include removal of CD4+ cells, B cells, or other cells mediating an immune response, such as by elimination, for example, cytolysis, of such cells.

[0324] As used herein, pathogenic immune cells refer to immune cells that are reactive with an autoantigen or that induce a response to an

autoantigen. Examples of such pathogenic immune cells include autoreactive CD4+ T cells, autoantibody producing B cells, self antigen presenting dendritic cells, and other self antigen presenting cells, as are known in the art.

[0325] In some embodiments, the CD8+KIR+ Tregs are contacted with the binding agent in vivo. In some embodiments, the CD8+KIR+ Tregs are contacted with the binding agent ex vivo. The activated CD8+KIR+ Tregs can then be administered in an effective amount to a subject in need thereof.

[0326] In some embodiments, the activated CD8+KIR+ Tregs exert a suppressive effect on pathogenic immune cells, such as autoreactive CD4+ T cells, autoantibody producing B cells, self antigen presenting dendritic cells, or self antigen presenting cells. In some embodiments, the activated CD8+KIR+ Tregs exert a suppressive effect on pathogenic immune cells, such as autoreactive CD4+ T cells, autoantibody producing B cells, and self antigen presenting dendritic cells. In some embodiments, the activated CD8+KIR+ Tregs deplete pathogenic immune cells, such as autoreactive CD4+ T cells, autoantibody producing B cells, and self antigen presenting dendritic cells. In some embodiments, the activated CD8+KIR+ Tregs modulate the pathogenic effect of pathogenic immune cells and decrease the titer of autoantibodies in the subject. In some embodiments, the activated CD8+KIR+ Tregs decrease the titer of autoantibodies in the subject.

[0327] In some embodiments, the binding agent is selected from any of the binding agents described herein, in each case that has reduced effector function activity or has substantially no effector function activity. In some embodiments, the reduced effector function activity is reduced or no ADCC, ADCP, or CDC effector function activity. In some embodiments, having substantially no effector function activity means having substantially no ADCC, ADCP, and CDC effector function activity. In some embodiments, a binding agent lacks an Fc domain or region and has reduced effector function or substantially no effector function. In some embodiments, a binding agent has an Fc domain or region with reduced effector function or substantially no

effector function due to amino acid substitutions in the Fc domain or region. In some embodiments, a binding agent has an Fc domain or region with reduced effector function or substantially no effector function due to amino acid substitutions in the Fc domain or region, such as Fc null substitutions. In some embodiments, a binding agent lacks an Fc domain or region or has an Fc domain or region with reduced binding to one or more Fcgamma receptors or is an Fc null domain. In some embodiments, a binding agent lacks an Fc domain or region. In some embodiments, a binding agent has an Fc domain or region with reduced binding to one or more Fcgamma receptors or is an Fc null domain. In some embodiments, a binding agent has an Fc domain or region with reduced binding to one or more Fcgamma receptors due to amino acid substitutions in the Fc domain or region.

[0328] Without intending to be bound by any particular theory, the reduction or absence of effector function activity by a binding agent may limit the interaction of the binding agent with other cell types (*i.e.*, non-CD8+KIR+ Tregs) and/or limit depletion of the CD8+KIR+ Tregs bound by the binding agent.

[0329] In some embodiments, the subject in need of treatment has an autoimmune disease. In some embodiments, the subject in need of treatment has an autoimmune disease, such as for example, autoimmune-induced hepatitis, Addison's Disease, Alopecia Areata, Alport's Syndrome, Ankylosing Spondylitis, Anti-phospholipid Syndrome, Arthritis, Ascariasis, Aspergillosis Atopic Allergy, Atopic Dermatitis, Atopic Rhinitis, Autoimmune Hemolytic Anemia, Autoimmune Hepatitis, Autoimmune Myositis, Behcet's Disease, Bird-Fancier's Lung, Bronchial Asthma, Caplan's Syndrome, Cardiomyopathy, Celiac Disease, Chagas' Disease, Chronic Glomerulonephritis, Chronic Graft versus Host Disease, Cogan's Syndrome, Cold Agglutinin Disease, CREST Syndrome, Crohn's Disease, Cryoglobulinemia, Cushing's Syndrome, Dermatomyositis, Discoid Lupus, Dressier' s Syndrome, Eaton-Lambert Syndrome, Encephalomyelitis, Endocrine ophthalmopathy, Erythematosus,

Evan's Syndrome, Felty's Syndrome, Fibromyalgia, Fuch's Cyclitis, Gastric Atrophy, Gastrointestinal Allergy, Giant Cell Arteritis, Glomerulonephritis, Goodpasture's Syndrome, Graft v. Host Disease, Graves' Disease, Guillain-Barre Disease (Syndrome), Hashimoto's Thyroiditis, Hemolytic Anemia, Henoch-Schonlein Purpura, Hyperviscosity Syndrome, Idiopathic Adrenal Atrophy, Idiopathic Pulmonary Fibrosis, Idiopathic Thrombocytopenic Purpura, IgA Nephropathy, Inflammatory Bowel Disease (Syndrome), Insulin-Dependent Diabetes Mellitus (IDDM or Type I), Juvenile Arthritis, Juvenile Idiopathic Arthritis, Juvenile Diabetes Mellitus (Type I), Lambert-Eaton Syndrome Laminitis, Lichen Planus, Lupoid Hepatitis, Lupus, Lupus Nephritis, Lymphopenia, Macroglobulinemia, Meniere's Disease, Mixed Connective Tissue Disease, Monoclonal Gammopathy of Undermined Origin, Multiple Sclerosis, Myasthenia Gravis, Myocarditis, Pemphigus/Pemphigoid, Pernicious Anemia, POEMS syndrome, Polyglandular Syndromes, Polyarteritis Nodosa, Polymyositis, Presenile Dementia, Primary Agammaglobulinemia, Primary Biliary Cirrhosis/Cholangitis, Psoriasis, Psoriatic Arthritis, Raynauds Phenomenon, Reiter's Syndrome, Rheumatic Fever, Rheumatoid Arthritis, Sampter's Syndrome, Schmidt's Syndrome, Scleroderma/Systemic Sclerosis, Shulman's Syndrome, Sjörgen's Syndrome, Stiff-Man Syndrome, Sympathetic Ophthalmia, Systemic Lupus Erythematosus, Takayasu's Arteritis, Temporal Arteritis, Thyroiditis, Thrombocytopenia, Thyrotoxicosis, Toxic Epidermal Necrolysis, Type B Insulin Resistance, Type I Diabetes Mellitus, Ulcerative Colitis, Uveitis, Vitiligo, Waldenstrom's Macroglobulinemia, and/or Wegener's Granulomatosis.

[0330] In some embodiments the autoimmune disease is autoimmune hepatitis, celiac disease, Crohn's disease, juvenile idiopathic arthritis, inflammatory bowel disease (IBD), insulin-dependent diabetes mellitus (IDDM or type 1 diabetes), lupus nephritis, myasthenia gravis, myocarditis, multiple sclerosis (MS), pemphigus/pemphigoid, primary biliary cirrhosis/cholangitis,

rheumatoid arthritis (RA), scleroderma/systemic sclerosis, Sjögren's syndrome (SjS), systemic lupus erythematosus (SLE), or ulcerative colitis.

[0331] In some embodiments, the autoimmune disease is selected from autoimmune hepatitis, celiac disease, Crohn's disease, inflammatory bowel disease (IBD), insulin-dependent diabetes mellitus (IDDM or type 1 diabetes), multiple sclerosis (MS), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or ulcerative colitis.

[0332] The methods described herein include administering a therapeutically effective amount of a binding agent to a subject having an autoimmune disease. As used herein, the phrases "therapeutically effective amount", "amount effective", "effective amount", and "effective dose" may refer to an amount of the binding agent as described herein that provides a therapeutic benefit in the treatment of, management of, prevention of relapse, delay of, or prevention of onset of an autoimmune disease, *e.g.*, an amount that provides a statistically significant decrease in at least one symptom, sign, or marker of an autoimmune disease. Determination of a therapeutically effective amount is well within the capability of those skilled in the art. Generally, a therapeutically effective amount can vary with the subject's history, age, condition, and sex, as well as the severity and type of the medical condition in the subject, and administration of other pharmaceutically active agents.

[0333] It is contemplated that the methods herein reduce symptoms, pathology, disease progression, or disease flares in a subject. As used herein, a "subject" refers to a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal, or game animal. Primates include chimpanzees, cynomolgus monkeys, spider monkeys, and macaques, *e.g.*, Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits, and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species, *e.g.*, domestic cat, canine species, *e.g.*, dog, fox, wolf, avian species, *e.g.*, chicken, emu, ostrich, and fish, *e.g.*, trout, catfish and salmon. In certain embodiments, the subject is a mammal, *e.g.*, a primate, *e.g.*,

a human. The terms, "patient", "individual", and "subject" are used interchangeably herein.

[0334] Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but is not limited to these examples. Mammals other than humans can be advantageously used, for example, as subjects that represent animal models of, for example, various autoimmune diseases. In addition, the methods described herein can be used to treat domesticated animals and/or pets. A subject can be male or female. In certain embodiments, the subject is a human.

[0335] A subject can be one who has been previously diagnosed with or identified as suffering from an autoimmune disease and in need of treatment, but need not have already undergone treatment for the autoimmune disease. Alternatively, a subject can also be one who has not been previously diagnosed as having an autoimmune disease in need of treatment. A subject can be one who exhibits one or more risk factors for a condition or one or more complications related to an autoimmune disease who does not exhibit risk factors. A "subject in need" of treatment for an autoimmune disease can be a subject having that disease or diagnosed as having that disease. In some embodiments, the subject is in need of treatment if the subject is at risk for developing an immune response to an antigen or autoantigen that may cause or result in the onset of an autoimmune disorder. In other embodiments, a subject "at risk of developing" an autoimmune disease refers to a subject diagnosed as being at risk for developing the disease or condition.

[0336] As used herein, the terms "treat", "treatment", "treating", or "amelioration" when used in reference to a disease, disorder, or medical condition (*e.g.*, autoimmune disease), refer to therapeutic treatments for a condition, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down, or stop the progression or severity of a symptom or condition. The term "treating" includes reducing or alleviating at least one adverse effect or

symptom of a condition or disease. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced. Alternatively, treatment is "effective" if the progression of a condition is reduced or halted. That is, "treatment" may include not just the improvement of symptoms or markers, but also a cessation or at least slowing of progress or worsening of symptoms that would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, reduction in one or more symptoms, reducing disease flares in the subject, alleviation of one or more symptom(s), diminishment of extent of the deficit, stabilized (*i.e.*, not worsening) state of an autoimmune disease, or a prevention, delay, or slowing of onset or progression of the autoimmune disease. As used herein, the term "administering" refers to contacting a binding agent as described herein or a nucleic acid encoding the binding agent as described herein (*e.g.*, by administration to a subject) by a method or route which results in binding of the binding agent to the CD8+KIR+ Tregs. Similarly, a pharmaceutical composition comprising a binding agent as described herein or a nucleic acid encoding the binding agent as described herein disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject.

[0337] The dosage ranges for a binding agent depend upon the potency, and encompass amounts large enough to produce the desired effect, *e.g.*, reduction in one or more symptoms, reducing disease flares in the subject, alleviation of one or more symptom(s), diminishment of extent of the deficit, stabilized (*i.e.*, not worsening) state of an autoimmune disease, or a prevention, delay, or slowing of onset or progression of the autoimmune disease. The dosage should not be so large as to cause unacceptable adverse side effects. Generally, the dosage will vary with the age, condition, and sex of the subject and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of any complication. In some embodiments, the dosage ranges from about 0.01 mg/kg body weight to about 20 mg/kg body weight. In some embodiments, the dosage ranges from about

0.5 mg/kg body weight to about 15 mg/kg body weight. In some embodiments, the dose range is from about 0.5 mg/kg body weight to about 5 mg/kg body weight. Alternatively, the dose range can be titrated to maintain serum levels between 1 ug/mL and 1000 ug/mL.

[0338] In some embodiments, a subject receives a single dose of any of the binding agents described herein, such as for treatment of an acute autoimmune disease or condition. In some embodiments, a subject receives a single dose of any of the binding agents described herein, such as for prevention of an immune response that may lead to an autoimmune disease or condition. In some embodiments, a subject receives repeated doses of any of the binding agents described herein, such as for treatment of a chronic autoimmune disease or condition. In some embodiments, a subject receives repeated doses of any of the binding agents described herein, such as for prevention of an immune response that may lead to an autoimmune disease or condition. In some embodiments, the doses are administered weekly, biweekly, every three weeks, monthly, bi-monthly, or every 6 months for several weeks, months, or years. The duration of treatment depends upon the subject's clinical progress and responsiveness to treatment.

[0339] In some embodiments, a dose can be administered intravenously. In some embodiments, an intravenous administration can be an infusion occurring over a period of from about 10 minutes to about 4 hours. In some embodiments, an intravenous administration can be an infusion occurring over a period of from about 30 minutes to about 90 minutes. In some embodiments, a dose can be administered subcutaneously.

[0340] Pharmaceutical compositions containing any of the binding agents described herein can be administered in a unit dose. The term "unit dose" when used in reference to a pharmaceutical composition refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material (*e.g.*, a binding agent), calculated to

produce the desired therapeutic effect in association with the required physiologically acceptable diluent, *i.e.*, carrier, or vehicle.

[0341] In some embodiments, administration of any of the binding agents described herein can result in an improved treatment outcome, such as reduced systemic inflammatory cytokines, reduced pathology in tissues impacted by disease, reduced flare frequency and/or severity, reduced self reporting of symptoms associated with disease, alleviation of one or more symptom(s), and/or prevention, delay, or slowing of onset or progression of the autoimmune disease.

[0342] In some embodiments, a binding agent or a pharmaceutical composition of any of the binding agents described herein, is administered with an immunotherapy. As used herein, "immunotherapy" refers to therapeutic strategies designed to modulate the subject's own immune system. Examples of an immunotherapy include, but are not limited to, antibodies such as check point inhibitors and modulators and immunosuppressive agents, such as cyclosporine, cyclosporine A, mycophenylate mofetil, sirolimus, tacrolimus, etanercept, prednisone, azathioprine, methotrexate cyclophosphamide, prednisone, aminocaproic acid, chloroquine, hydroxychloroquine, hydrocortisone, dexamethasone, chlorambucil, DHEA, danazol, bromocriptine, meloxicam, infliximab, abatacept, belatacept, and adalimumab.

Treatment of Transplant Complications

[0343] In some embodiments, provided are methods of treating complications of a transplant associated with graft versus host disease or GVHD. In some embodiments, provided are methods for reducing complications of GVHD in a subject, comprising administering a binding agent or a pharmaceutical composition as described herein to a subject who has received a transplant to suppress a host immune response to the transplant. In some embodiments, provided are methods for reducing complications of GVHD in a subject, comprising administering a binding agent or a pharmaceutical composition as described herein to a subject who has received a transplant to

suppress an immune response associated with the transplant. In some embodiments, provided are methods of reducing GVHD comprising administering a binding agent or a pharmaceutical composition as described herein to a subject who has received a transplant and is experiencing GVHD, whereby a symptom of GVHD is reduced. In some embodiments, provided are methods of suppressing GVHD comprising administering a binding agent or a pharmaceutical composition as described herein to a subject who has received a transplant and is experiencing GVHD, whereby GVHD is suppressed or reduced. In some embodiments, provided are methods of suppressing or reducing GVHD comprising contacting a binding agent or a pharmaceutical composition as described herein with CD8+KIR+ T regulatory cells (Tregs) from a subject who has received a transplant, whereby the CD8+KIR+ T regulatory cells (Tregs) are activated. In some embodiments, provided are methods of suppressing or reducing GVHD comprising contacting a binding agent or a pharmaceutical composition as described herein with CD8+KIR+ T regulatory cells (Tregs) from a subject who has received a transplant, whereby the CD8+KIR+ T regulatory cells (Tregs) of the transplant are depleted. In some embodiments, provided are methods of suppressing or reducing GVHD comprising contacting a binding agent or a pharmaceutical composition as described herein with CD8+KIR+ T regulatory cells (Tregs) from a subject who has received a transplant, whereby the CD8+KIR+ T regulatory cells (Tregs) are depleted.

[0344] In some embodiments, the subject has received a hematopoietic stem cell transplant, an umbilical cord blood stem cell transplant, an inducible pluripotent stem cell-derived progenitor or differentiated cell transplant, a bone marrow transplant or a solid organ transplant. The transplant is typically an allogeneic transplant. In some embodiments, the subject has received a hematopoietic stem cell transplant, an umbilical cord blood stem cell transplant, an inducible pluripotent stem cell-derived progenitor or differentiated cell transplant, a bone marrow transplant or a solid organ transplant and is

experiencing GVHD. In some embodiments, the subject has received a hematopoietic stem cell transplant, an umbilical cord blood stem cell transplant, an inducible pluripotent stem cell-derived progenitor or differentiated cell transplant, a bone marrow transplant or a solid organ transplant and is at risk of experiencing GVHD. In some embodiments, the subject has received a hematopoietic stem cell transplant and is experiencing GVHD. In some embodiments, the subject has received a hematopoietic stem cell transplant and is at risk of experiencing GVHD. In some embodiments, the subject has received an umbilical cord blood stem cell transplant and is experiencing GVHD. In some embodiments, the subject has received an umbilical cord blood stem cell transplant and is at risk of experiencing GVHD. In some embodiments, the subject has received an inducible pluripotent stem cell-derived progenitor or differentiated cell transplant and is experiencing GVHD. In some embodiments, the subject has received an inducible pluripotent stem cell-derived progenitor or differentiated cell transplant and is at risk of experiencing GVHD. In some embodiments, the subject has received a bone marrow transplant and is experiencing GVHD. In some embodiments, the subject has received a bone marrow transplant and is at risk of experiencing GVHD. In some embodiments, the subject has received a solid organ transplant and is experiencing GVHD. In some embodiments, the subject has received a solid organ transplant and is at risk of experiencing GVHD.

[0345] In some embodiments, GVHD is suppressed or reduced by depletion of pathogenic immune cells, such as CD4 T cells. In some embodiments, GVHD is suppressed or reduced by depletion of pathogenic immune cells, such as CD8+KIR+ Tregs from the transplant. In some embodiments, the CD8+KIR+ Tregs are contacted with the binding agent in vivo. In some embodiments, the CD8+KIR+ Tregs are contacted with the binding agent ex vivo. The activated CD8+KIR+ Tregs can then be administered in an effective amount to a subject in need thereof.

[0346] In some embodiments, the binding agent is selected from any of the binding agents described herein, in each case that has reduced effector function activity or has substantially no effector function activity. In some embodiments, the reduced effector function activity is reduced or no ADCC, ADCP, or CDC effector function activity. In some embodiments, having substantially no effector function activity means having substantially no ADCC, ADCP, and CDC effector function activity. In some embodiments, a binding agent lacks an Fc domain or region and has reduced effector function or substantially no effector function. In some embodiments, a binding agent has an Fc domain or region with reduced effector function or substantially no effector function due to amino acid substitutions in the Fc domain or region. In some embodiments, a binding agent has an Fc domain or region with reduced effector function or substantially no effector function due to amino acid substitutions in the Fc domain or region, such as Fc null substitutions. In some embodiments, a binding agent lacks an Fc domain or region or has an Fc domain or region with reduced binding to one or more Fcgamma receptors or is an Fc null domain. In some embodiments, a binding agent lacks an Fc domain or region. In some embodiments, a binding agent has an Fc domain or region with reduced binding to one or more Fcgamma receptors or is an Fc null domain. In some embodiments, a binding agent has an Fc domain or region with reduced binding to one or more Fcgamma receptors due to amino acid substitutions in the Fc domain or region.

[0347] In some embodiments, the binding agent is selected from any of the binding agents described herein, in each case that has effector function activity comprising at least ADCC. In some embodiments, the effector function activity is ADCC in combination with ADCP and/or CDC effector function activity. In some embodiments, having effector function activity means having ADCC, ADCP, and CDC effector function activity. In various embodiments, such a binding agent has an Fc domain or has an Fc domain that binds to one or more Fcgamma receptors.

[0348] Without intending to be bound by any particular theory, the reduction or absence of effector function activity by a binding agent may limit the interaction of the binding agent with other cell types (*i.e.*, non CD8+KIR+ Tregs) and/or limits depletion of the CD8+KIR+ Tregs. In contrast, the presence of effector function activity by a binding agent is believed to bias the immune response towards depletion of CD8+KIR+ Tregs.

[0349] In some embodiments, the transplant is a stem cell transplant, a bone marrow transplant or a solid organ transplant. As used herein, the term "transplant" mean refers to an organ, tissue, or cell that has been transplanted from one subject to a different subject, or transplanted within the same subject (*e.g.*, to a different area within the subject). Organs such as liver, kidney, heart, or lung, or other body parts, such as bone or skeletal matrix such as bone marrow, tissue, such as skin, cornea, intestines, endocrine glands, or stem cells or various types, or hematopoietic cells including hematopoietic stem and progenitor cells, umbilical cord blood stem cells and inducible pluripotent stem cell-derived progenitor or differentiated cells, are all examples of transplants. In some embodiments, the solid organ transplant is a liver, kidney, lung, pancreas, and/or heart transplant. The term transplant includes a graft. The transplant can be an allograft (or allogeneic graft) or xenograft. The term "allograft" refers to a graft between two genetically non-identical members of a species. The term "xenograft" refers to a graft between members of different species.

[0350] In some embodiments, the transplant is a bone marrow transplant. In some embodiments, the transplant is a hematopoietic stem cell transplant. In some embodiments, the transplant is an umbilical cord blood stem cell transplant. In some embodiments, the transplant is an inducible pluripotent stem cell-derived progenitor or differentiated cell transplant.

[0351] In some embodiments, the subject has graft versus host disease or GVHD. In some embodiments, the subject is at risk of having GVHD.

[0352] The methods described herein include administering a therapeutically effective amount of a binding agent to a subject receiving a

transplant. As used herein, the phrases "therapeutically effective amount", "amount effective", "effective amount", and "effective dose" may refer to an amount of the binding agent as described herein that provides a therapeutic benefit in the management of GVHD associated with a transplant. In some embodiments, the therapeutic benefit is delay of or prevention of onset of a GVHD. In some embodiments, the therapeutic benefit is a statistically significant decrease in at least one symptom, sign, or marker of GVHD. Determination of a therapeutically effective amount is well within the capability of those skilled in the art. Generally, a therapeutically effective amount can vary with the subject's history, age, condition, and sex, as well as the severity and type of the medical condition in the subject, and administration of other pharmaceutically active agents.

[0353] It is contemplated that the methods herein reduce symptoms of, reduce, or prevent GVHD in a subject who has received a transplant. As used herein, a "subject" refers to a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal, or game animal. Primates include chimpanzees, cynomolgus monkeys, spider monkeys, and macaques, *e.g.*, Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits, and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species, *e.g.*, domestic cat, canine species, *e.g.*, dog, fox, wolf, avian species, *e.g.*, chicken, emu, ostrich, and fish, *e.g.*, trout, catfish, and salmon. In certain embodiments, the subject is a mammal, *e.g.*, a primate, *e.g.*, a human. The terms, "patient", "individual", and "subject" are used interchangeably herein.

[0354] Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but is not limited to these examples. Mammals other than humans can be advantageously used, for example, as subjects that represent animal models of, for example, various autoimmune diseases. In addition, the methods described herein can be used to treat domesticated animals, and/or pets. A

subject can be male or female. In certain embodiments, the subject is a human.

[0355] A subject can be one who will receive a transplant. Alternatively, a subject can be one who has already received a transplant. A subject can be one who will receive a transplant and is at risk of GVHD. A subject can be one who has received a transplant and is at risk of having GVHD. A subject can be one who has received a transplant and has GVHD.

[0356] As used herein, the terms "treat", "treatment", "treating", or "amelioration" when used in reference to a disease, disorder, or medical condition (*e.g.*, GVHD), refer to therapeutic treatments for a condition, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down, or stop the progression or severity of a symptom or condition of GVHD. The term "treating" includes reducing or alleviating at least one adverse effect or symptom of a condition or disease. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced. Alternatively, treatment is "effective" if the progression of a condition is reduced or halted. That is, "treatment" may include not just the improvement of symptoms or markers, but also a cessation or at least slowing of progress or worsening of symptoms that would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, reduction in one or more symptoms associated with GVHD.

[0357] As used herein, the term "administering" refers to contacting a binding agent as described herein or a nucleic acid encoding the binding agent as described herein (*e.g.*, by administration to a subject) by a method or route which results in binding of the binding agent to the CD8+KIR+ Tregs. Similarly, a pharmaceutical composition comprising a binding agent as described herein or a nucleic acid encoding the binding agent as described herein disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject.

[0358] The dosage ranges for a binding agent depend upon the potency, and encompass amounts large enough to produce the desired effect, *e.g.*, reduction in one or more symptoms of GVHD or reducing or preventing GVHD. The dosage should not be so large as to cause unacceptable adverse side effects. Generally, the dosage will vary with the age, condition, and sex of the subject and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of any complication. In some embodiments, the dosage ranges from about 0.01 mg/kg body weight to about 20 mg/kg body weight. In some embodiments, the dosage ranges from about 0.5 mg/kg body weight to about 15 mg/kg body weight. In some embodiments, the dose range is from about 0.5 mg/kg body weight to about 5 mg/kg body weight. Alternatively, the dose range can be titrated to maintain serum levels between 1 ug/mL and 1000 ug/mL.

[0359] In some embodiments, a subject receives a single dose of any of the binding agents described herein, such as for treatment of acute GVHD following a transplant. In some embodiments, a subject receives repeated doses of any of the binding agents described herein, such as for treatment of a chronic GVHD. In some embodiments, the doses are administered weekly, biweekly, every three weeks, monthly, bi-monthly, or every 6 months for several weeks, months, or years. The duration of treatment depends upon the subject's clinical progress and responsiveness to treatment.

[0360] In some embodiments, a dose can be administered intravenously. In some embodiments, an intravenous administration can be an infusion occurring over a period of from about 10 minutes to about 4 hours. In some embodiments, an intravenous administration can be an infusion occurring over a period of from about 30 minutes to about 90 minutes. In some embodiments, a dose can be administered subcutaneously.

[0361] Pharmaceutical compositions containing any of the binding agents described herein can be administered in a unit dose. The term "unit dose" when used in reference to a pharmaceutical composition refers to physically

discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material (*e.g.*, a binding agent), calculated to produce the desired therapeutic effect in association with the required physiologically acceptable diluent, *i.e.*, carrier, or vehicle.

[0362] In some embodiments, administration of any of the binding agents described herein can result in an improved treatment outcome, such as reduced systemic inflammatory cytokines, reduced pathology in tissues impacted by disease, reduced self reporting of symptoms associated with an immune response associated with adverse effects on host tissues, improved or extended transplant engraftment, alleviation of one or more symptom(s), and/or prevention, delay, or slowing of onset or progression of rejection of the transplant, or extended transplant engraftment with decreased use of broad spectrum immunosuppressive agents, such as corticosteroids.

[0363] In some embodiments, a binding agent or a pharmaceutical composition of any of the binding agents described herein, is administered with an immunosuppressive agent, such as a corticosteroid(s).

Treatment of Infectious Disease

[0364] In some aspects, the binding agents as described herein can be used in a method(s) comprising administering a binding agent or pharmaceutical composition as described herein to a subject having an infection. In some embodiments, the subject is in need of treatment for an infection. In some embodiments, provided is a method of treating an infection by administering any of the binding agents or pharmaceutical compositions described herein to a subject in need thereof in an amount effective to activate or stimulate CD8+KIR+ Tregs and thereby ameliorate a symptom of the infection. In some embodiments, the method comprises stimulating an immune response against an infectious agent by contacting CD8+KIR+ T regulatory cells (Tregs) with any of the binding agents or pharmaceutical compositions described herein in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby the immune response against an infectious

agent is stimulated. In some embodiments, the method comprises stimulating an immune response against a cell(s) infected with an infectious agent by contacting CD8+KIR+ T regulatory cells (Tregs) with any of the binding agents or pharmaceutical compositions described herein in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby infected cells are depleted. In some embodiments, the method comprises stimulating an immune response against a cell infected with an infectious agent by contacting CD8+KIR+ T regulatory cells (Tregs) with any of the binding agents or pharmaceutical compositions described herein in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby the immune response against the infected cell is stimulated.

[0365] In some embodiments of the methods of treating infection, the CD8+KIR+ Tregs are contacted with any of the binding agents described herein in vivo. In some embodiments, the CD8+KIR+ Tregs are contacted with any of the binding agents described herein ex vivo. The activated CD8+KIR+ Tregs are subsequently administered in an effective amount to a subject in need thereof. In some embodiments, the immune response comprises a reduction in immune suppressive immune cells. As used herein, immune suppressive immune cells include CD4 Tregs and tolerizing DCs. In some embodiments, the number of infected cells in the subject is decreased.

[0366] In some embodiments, the binding agent is selected from any of the binding agents described herein, in each case that has reduced effector function activity or has substantially no effector function activity. In some embodiments, the reduced effector function activity is reduced or no ADCC, ADCP, or CDC effector function activity. In some embodiments, having substantially no effector function activity means having substantially no ADCC, ADCP, and CDC effector function activity. In some embodiments, a binding agent lacks an Fc domain or region and has reduced effector function or substantially no effector function. In some embodiments, a binding agent has an Fc domain or region with reduced effector function or substantially no

effector function due to amino acid substitutions in the Fc domain or region. In some embodiments, a binding agent has an Fc domain or region with reduced effector function or substantially no effector function due to amino acid substitutions in the Fc domain or region, such as Fc null substitutions. In some embodiments, a binding agent lacks an Fc domain or region or has an Fc domain or region with reduced binding to one or more Fcgamma receptors or is an Fc null domain. In some embodiments, a binding agent lacks an Fc domain or region. In some embodiments, a binding agent has an Fc domain or region with reduced binding to one or more Fcgamma receptors or is an Fc null domain. In some embodiments, a binding agent has an Fc domain or region with reduced binding to one or more Fcgamma receptors due to amino acid substitutions in the Fc domain or region.

[0367] Without intending to be bound by any particular theory, the reduction or absence of effector function activity by a binding agent may limit the interaction of the binding agent with other cell types (*i.e.*, non-CD8+KIR+ Tregs) and/or limits depletion of the CD8+KIR+ Tregs bound by the binding agent.

[0368] In some embodiments, the subject is in need of treatment for an infectious disease or is infected with an infectious agent, such as for example, a bacterial disease, a systemic fungal disease, rickettsial disease, a parasitic disease, or a viral disease. In some embodiments, the infection is a bacterial disease, such as Diphtheria, Pertussis, Occult Bacteremia, Urinary Tract Infection, Gastroenteritis, Cellulitis, Epiglottitis, Tracheitis, Adenoid Hypertrophy, Retropharyngeal Abscess, Impetigo, Ecthyma, Pneumonia, Endocarditis, Septic Arthritis, Pneumococcal Pneumonia, Peritonitis, Bacteremia, Meningitis, Acute Purulent Meningitis, Urethritis, Cervicitis, Proctitis, Pharyngitis, Salpingitis, Epididymitis, Gonorrhea, Syphilis, Listeriosis, Anthrax, Nocardiosis, Salmonella, Typhoid Fever, Dysentery, Conjunctivitis, Sinusitis, Brucellosis Tularemia, Cholera, Bubonic Plague, Tetanus, Necrotizing Enteritis, Actinomycosis, Mixed Anaerobic Infections, Syphilis, Relapsing Fever,

Leptospirosis, Lyme Disease, Rat Bite Fever, Tuberculosis, including mycobacterium tuberculosis, Lymphadenitis, Leprosy, Chlamydia, Chlamydial Pneumonia, Trachoma, or Inclusion Conjunctivitis; a systemic fungal disease such as Histoplasmosis, Coccicidoidomycosis, Blastomycosis, Sporotrichosis, Cryptococcosis, Systemic Candidiasis, Aspergillosis, Mucormycosis, Mycetoma, or Chromomycosis; a rickettsial disease, such as Typhus, Rocky Mountain Spotted Fever Ehrlichiosis, Eastern Tick-Borne Rickettsioses, Rickettsialpox, Q Fever, or Bartonellosis; a parasitic disease such as Malaria, Babesiosis, African Sleeping Sickness, Chagas' Disease, Leishmaniasis, Dum-Dum Fever, Toxoplasmosis Meningoencephalitis, Keratitis, Dientamebiasis, Giardiasis, Cryptosporidiosis, Isosporiasis, Cyclosporiasis, Microsporidiosis, Ascariasis, Whipworm Infection, Hookworm Infection, Threadworm Infection, Ocular Larva Migrans, Trichinosis, Guinea Worm Disease, Lymphatic Filariasis, Loiasis, River Blindness, Canine Heartworm Infection, Schistosomiasis, Swimmer's Itch, Oriental Lung Fluke, Oriental Liver Fluke, Fascioliasis, Fasciolopsiasis, Opisthorchiasis, Tapeworm Infections, Hydatid Disease, and Alveolar Hydatid Disease; and Viral Disease such as Measles, Subacute sclerosing panencephalitis, Common Cold, Mumps, Rubella, Roseola, Fifth Disease, Chickenpox, Coronavirus Infection, Covid19 Disease, Respiratory syncytial virus infection, Croup, Bronchiolitis, Infectious Mononucleosis, Poliomyelitis, Herpangina, Hand-Foot-and-Mouth Disease, Bornholm Disease, Genital Herpes, Genital Warts, Aseptic Meningitis Myocarditis, Echovirus Infection, Epstein-Barr Virus Pericarditis, Gastroenteritis, Hepatitis A infection, Hepatitis B infection, Hepatitis C infection, HIV infection, human papillomavirus (HPV) infection, Reye's Syndrome, Kawasaki Syndrome, Influenza, Bronchitis, Viral "Walking" Pneumonia, Acute Febrile Respiratory Disease, Acute pharyngoconjunctival fever, Epidemic keratoconjunctivitis, Herpes Simplex Virus 1 (HSV-1) infection, Herpes Simples Virus 2 (HSV-2) infection, Shingles, Cytomegalic inclusion Disease, Rabies, Progressive Multifocal Leukoencephalopathy, Kuru, Fatal Familial Insomnia, Creutzfeldt-Jakob

Disease, Gerstmann-Sfraussler-Scheinker Disease, Tropical Spastic Paraparesis, Western Equine Encephalitis, California Encephalitis, St. Louis Encephalitis, Yellow Fever, Dengue, Lymphocytic choriomeningitis, Lassa Fever, Hemorrhagic Fever, Hantavirus Pulmonary Syndrome, Marburg Virus Infections, Ebola Virus Infections, and Smallpox.

[0369] In some embodiments, the infection is a viral infection. In some embodiments, the infection is a viral infection, such as an HIV infection, Hepatitis A infection, Hepatitis B infection, Hepatitis C infection, an Epstein Bar Virus infection, a coronavirus infection such as a SARS-COV2 infection (Covid-19), and flu virus infection (influenza). In some embodiments, an infection is cause by an infectious agent, such as a coronavirus, diphtheria, ebola, flu (influenza), HIV, human papillomavirus (HPV), Hepatitis A, Hepatitis B, Hepatitis C, measles virus, respiratory syncytial virus, rotavirus, and herpes virus.

[0370] The methods described herein include administering a therapeutically effective amount of a binding agent to a subject having an infection or having cells infected by an infectious agent. As used herein, the phrases "therapeutically effective amount", "amount effective", "effective amount", or "effective dose" may refer to an amount of any of the binding agents or pharmaceutical compositions as described herein that provide a therapeutic benefit in the treatment of, management of or prevention of relapse of an infectious disease, *e.g.*, an amount that provides a statistically significant decrease in at least one symptom, sign, or marker of an infection. Determination of a therapeutically effective amount is well within the capability of those skilled in the art. Generally, a therapeutically effective amount can vary with the subject's history, age, condition, and sex, as well as the severity and type of the medical condition in the subject, and administration of other pharmaceutically active agents.

[0371] As used herein, a "subject" refers to a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal, or game

animal. Primates include chimpanzees, cynomolgus monkeys, spider monkeys, and macaques, *e.g.*, Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits, and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species, *e.g.*, domestic cat, canine species, *e.g.*, dog, fox, wolf, avian species, *e.g.*, chicken, emu, ostrich, and fish, *e.g.*, trout, catfish, and salmon. In certain embodiments, the subject is a mammal, *e.g.*, a primate, *e.g.*, a human. The terms, "patient", "individual", and "subject" are used interchangeably herein.

[0372] Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but is not limited to these examples. Mammals other than humans can be advantageously used, for example, as subjects that represent animal models of, for example, various infections. In addition, the methods described herein can be used to treat domesticated animals and/or pets. A subject can be male or female. In certain embodiments, the subject is a human.

[0373] A subject can be one who has been previously diagnosed with or identified as suffering from an infection and in need of treatment, but need not have already undergone treatment for the infection. Alternatively, a subject can also be one who has not been previously diagnosed as having an infection, but is in need of treatment. A subject can be one who exhibits one or more risk factors for a condition or one or more complications related to an infection or a subject who does not exhibit such risk factors. A "subject in need" of treatment for an infection can be a subject having that infection or diagnosed as having that infection. In other embodiments, a subject "at risk of developing" an infection refers to a subject diagnosed as being at risk for developing the infection.

[0374] As used herein, the terms "treat", "treatment", "treating", or "amelioration" when used in reference to a disease, disorder, or medical condition (*e.g.*, an infection), refer to therapeutic treatments for a condition, wherein the object is to reverse, alleviate, ameliorate, inhibit, prevent, slow

down, or stop the progression or severity of a symptom or condition. The term "treating" includes reducing or alleviating at least one adverse effect or symptom of a condition. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced. Alternatively, treatment is "effective" if the progression of a condition is reduced or halted. That is, "treatment" includes not just the improvement of symptoms or markers, but also a cessation or at least slowing of progress or worsening of symptoms that would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, reduction in infected cells in the subject, alleviation of one or more symptom(s), diminishment of extent of the deficit, stabilized (*i.e.*, not worsening) state of the infection, or a delay or slowing of the infection or progression of the infection as compared to that expected in the absence of treatment. As used herein, the term "administering" refers to providing a binding agent as described herein or a nucleic acid encoding the binding agent as described herein into contact (*e.g.*, by administration to a subject) by a method or route which results in binding to the binding agent to the CD8+KIR+ Tregs. Similarly, a pharmaceutical composition comprising a binding agent as described herein or a nucleic acid encoding the binding agent as described herein disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject.

[0375] The dosage ranges for a binding agent depend upon the potency, and encompass amounts large enough to produce the desired effect, *e.g.*, stimulation of an immune response against infected cells, reducing the number of infected cells, or slow or preventing progression of the infection. The dosage should not be so large as to cause unacceptable adverse side effects. Generally, the dosage will vary with the age, condition, and sex of the subject and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of any complication. In some embodiments, the dosage ranges from about 0.01 mg/kg body weight to about 20 mg/kg body weight. In some embodiments, the dosage ranges from about

0.01 mg/kg body weight to about 10 mg/kg body weight. In some embodiments, the dosage ranges from about 0.1 mg/kg body weight to about 10 mg/kg body weight. In some embodiments, the dosage ranges from about 0.5 mg/kg body weight to about 10 mg/kg body weight. In some embodiments, the dose range is from about 0.5 mg/kg body weight to about 5 mg/kg body weight. Alternatively, the dose range can be titrated to maintain serum levels between 1 ug/mL and 1000 ug/mL.

[0376] In some embodiments, a subject receives a single dose of any of the binding agents or pharmaceutical compositions as described herein, such as for treatment of an acute infection. In some embodiments, a subject receives a single dose of any of the binding agents or pharmaceutical compositions as described herein, such as for prevention of an immune response to an infection that may lead to an autoimmune disease or condition. In some embodiments, a subject receives repeated doses of any of the binding agents or pharmaceutical compositions as described herein, such as for treatment of a chronic infection. In some embodiments, the doses are administered weekly, biweekly, every three weeks, monthly, bi-monthly, or every 6 months for several weeks, months, or years. The duration of treatment depends upon the subject's clinical progress and responsiveness to treatment.

[0377] In some embodiments, a dose can be administered intravenously. In some embodiments, an intravenous administration can be an infusion occurring over a period of from about 10 minutes to about 4 hours. In some embodiments, an intravenous administration can be an infusion occurring over a period of from about 30 minutes to about 90 minutes. In some embodiments, a dose can be administered subcutaneously.

[0378] In some embodiments, a total of from about 2 to about 10 doses are administered to a subject. In some embodiments, a total of 4 doses are administered. In some embodiments, a total of 5 doses are administered. In some embodiments, a total of 6 doses are administered. In some embodiments, a total of 7 doses are administered. In some embodiments, a

total of 8 doses are administered. In some embodiments, a total of 9 doses are administered. In some embodiments, a total of 10 doses are administered. In some embodiments, a total of more than 10 doses are administered.

[0379] Pharmaceutical compositions containing a binding agent can be administered in a unit dose. The term "unit dose" when used in reference to a pharmaceutical composition refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material (*e.g.*, binding agent), calculated to produce the desired therapeutic effect in association with the required physiologically acceptable diluent, *i.e.*, carrier, or vehicle.

[0380] In some embodiments, administration of any of the binding agents described herein can result in an improved treatment outcome such as amelioration of clinical symptoms, or reduction of viral load, or elimination or reduction of pathogen or reduction in infected cells.

[0381] In some embodiments, a binding agent or a pharmaceutical composition of any of the embodiments described herein is administered with an infectious disease control agent, such as an anti-bacterial, anti-fungal, or an antiviral agent. Antibacterial gents can be, for example, Lactam Antibiotics, such as Penicillin G, Penicillin V, Cloxacillin, Dicloxacillin, Methicillin, Nafcillin, Oxacillin, Ampicillin, Amoxicillin, Bacampicillin, Azlocillin, Carbenicillin, Mezlocillin, Piperacillin or Ticarcillin; Aminoglycosides such as Amikacin, Gentamicin, Kanamycin, Neomycin, Netilmicin, Streptomycin, or Tobramycin; Macrolides such as Azithromycin, Clarithromycin, Erythromycin, Lincomycin or Clindamycin; Tetracyclines such as Demeclocycline, Doxycycline, Minocycline, Oxytetracycline or Tetracycline; Quinolones such as Cinoxacin or Nalidixic Acid; Fluoroquinolones such as Ciprofloxacin, Enoxacin, Grepafloxacin, Levofloxacin, Lomefloxacin, Norfloxacin, Ofloxacin, Sparfloxacin, or Trovafloxacin; Polypeptides such as Bacitracin, Colistin or Polymyxin B; Sulfonamides such as Sulfisoxazole, Sulfamethoxazole, Sulfadiazine, Sulfamethizole, or Sulfacetamide,; and other Antibacterial Agents such as

Trimethoprim, Sulfamethazole, Chloramphenicol, Vancomycin, Metronidazole, Quinupristin, Dalfopristin, Rifampin, Spectinomycin, or Nitrofurantoin; and Antiviral Agents, such as for example, General Antiviral Agents such as Idoxuridine, Vidarabine, Trifluridine, Acyclovir, Famciclovir, Penciclovir, Valacyclovir, Ganciclovir, Foscarnet, Ribavirin, Amantadine, Rimantadine, or Cidofovir; Antisense Oligonucleotides; Immunoglobulins; Interferons; and other drugs, such as Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Nevirapine, Delavirdine, Saquinavir, Oseltamivir and Peramivir, Ritonavir, Indinavir, or Nelfinavir.

Treatment of Cancer

[0382] In some aspects, the binding agents or pharmaceutical compositions as described herein can be used in a method(s) for the treatment of cancer comprising administering a binding agent or pharmaceutical compositions as described herein to a subject in need thereof. In some embodiments, provided are methods of treating cancer, comprising administering any of the binding agents described herein or any of the pharmaceutical compositions described herein, wherein the binding agent has substantially no effector function activity, to a subject in need thereof in an amount effective to activate or stimulate CD8+KIR+ Tregs and thereby ameliorate a symptom of the cancer. In some embodiments, provided are methods of stimulating an immune response against an antigen associated with a cancer (cancer antigen; *e.g.*, an antigen expressed on a cancer cell), comprising contacting CD8+KIR+ T regulatory cells (Tregs) with any of the binding agents described herein or any of the pharmaceutical compositions described herein, wherein the binding agent has substantially no effector function activity, in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby the immune response to the cancer antigen is increased.

[0383] In some embodiments, the binding agent is selected from any of the binding agents described herein, in each case that has reduced effector

function activity or has substantially no effector function activity. In some embodiments, the reduced effector function activity is reduced or no ADCC, ADCP, or CDC effector function activity. In some embodiments, having substantially no effector function activity means having substantially no ADCC, ADCP, and CDC effector function activity. In some embodiments, a binding agent lacks an Fc domain or region and has reduced effector function or substantially no effector function. In some embodiments, a binding agent has an Fc domain or region with reduced effector function or substantially no effector function due to amino acid substitutions in the Fc domain or region. In some embodiments, a binding agent has an Fc domain or region with reduced effector function or substantially no effector function due to amino acid substitutions in the Fc domain or region, such as Fc null substitutions. In some embodiments, a binding agent lacks an Fc domain or region or has an Fc domain or region with reduced binding to one or more Fcgamma receptors or is an Fc null domain. In some embodiments, a binding agent lacks an Fc domain or region. In some embodiments, a binding agent has an Fc domain or region with reduced binding to one or more Fcgamma receptors or is an Fc null domain. In some embodiments, a binding agent has an Fc domain or region with reduced binding to one or more Fcgamma receptors due to amino acid substitutions in the Fc domain or region.

[0384] In some embodiments, provided are methods of treating cancer, comprising administering any of the binding agents described herein or any of the pharmaceutical compositions described herein, wherein the binding agent has effector function activity comprising at least ADCC, to a subject in need thereof in an amount effective to deplete CD8+KIR+ Tregs and thereby ameliorate a symptom of the cancer. In some embodiments, provided are methods of stimulating an immune response against a cancer, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with any of the binding agents described herein or any of the pharmaceutical compositions described herein, wherein the binding agent has effector function activity comprising at least

ADCC, in an amount effective to deplete CD8+KIR+ Tregs, whereby the immune response to the cancer is increased. In some embodiments, provided are methods of stimulating an immune response against an antigen associated with a cancer (cancer antigen), comprising contacting CD8+KIR+ T regulatory cells (Tregs) with any of the binding agents described herein or any of the pharmaceutical compositions described herein, wherein the binding agent has effector function activity comprising at least ADCC, in an amount effective to deplete CD8+KIR+ Tregs, whereby the immune response to the cancer antigen is increased.

[0385] In some embodiments, the binding agent is selected from any of the binding agents described herein, in each case that has effector function activity comprising at least ADCC. In some embodiments, the effector function activity is ADCC in combination with ADCP and/or CDC effector function activity. In some embodiments, having effector function activity means having ADCC, ADCP, and CDC effector function activity. In various embodiments, such a binding agent has an Fc domain or has an Fc domain that binds to one or more Fcgamma receptors.

[0386] In some embodiments, wherein the CD8+KIR+ Tregs are contacted with the binding agent in vivo. In some embodiments, the CD8+KIR+ Tregs are contacted with the binding agent ex vivo. In some embodiments, the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof.

[0387] Without intending to be bound by any particular theory, the reduction or absence of effector function activity by a binding agent may limit the interaction of the binding agent with other cell types (*i.e.*, non CD8+KIR+ Tregs) and/or limits depletion of the CD8+KIR+ Tregs. In contrast, the presence of effector function activity by a binding agent is believed to bias the immune response towards depletion of CD8+KIR+ Tregs.

[0388] In some embodiments, an increased immune response comprises a reduction in cancer cells or depletion of immune suppressive immune cells.

In this context, immune suppressive immune cells refers to, for example, tumor associated macrophages, CD4+ Tregs, and/or tolerizing dendritic cells (DCs). In some embodiments, the number of cancer cells in the subject is decreased. In some embodiments, the number of immune suppressive immune cells in the subject is decreased.

[0389] The terms "cancer" and "malignancy" refer to an uncontrolled growth of cells which interferes with the normal functioning of the bodily organs and systems. A cancer or malignancy may be primary or metastatic, *i.e.*, it has become invasive, seeding tumor growth in tissues remote from the original tumor site. A "tumor" refers to an uncontrolled growth of cells which interferes with the normal functioning of the bodily organs and systems. As used herein, the term cancer includes malignancies and tumors, unless otherwise indicated by context. A subject that has a cancer is a subject having objectively measurable cancer cells present in the subject's body. Included in this definition are benign tumors and malignant cancers, as well as potentially dormant tumors and micro-metastases. Cancers that migrate from their original location and seed other vital organs can eventually lead to the death of the subject through the functional deterioration of the affected organs. Hematologic malignancies (hematopoietic cancers), such as leukemias and lymphomas, are able to for example out-compete the normal hematopoietic compartments in a subject, thereby leading to hematopoietic failure (in the form of anemia, thrombocytopenia and neutropenia) ultimately causing death.

[0390] Examples of cancers include, but are not limited to, carcinomas, lymphomas, blastomas, sarcomas, myelomas, and leukemias. More particular examples of such cancers include, but are not limited to, basal cell carcinoma, biliary tract cancer, bladder cancer, bone cancer, brain and CNS cancer, breast cancer (*e.g.*, triple negative breast cancer), cancer of the peritoneum, cervical cancer; cholangiocarcinoma, choriocarcinoma, chondrosarcoma, colon and rectum cancer (colorectal cancer), connective tissue cancer, cancer of the digestive system, endometrial cancer, esophageal cancer, eye cancer, cancer

of the head and neck, gastric cancer (including gastrointestinal cancer and stomach cancer), glioblastoma (GBM), hepatic carcinoma, hepatoma, intra-epithelial neoplasm, kidney or renal cancer (*e.g.*, clear cell kidney cancer or non-clear cell kidney cancer), larynx cancer, leukemia, liver cancer, lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), lymphoma including Hodgkin's and non-Hodgkin's lymphoma, melanoma, mesothelioma, myelomas, neuroblastoma, oral cavity cancer (*e.g.*, lip, tongue, mouth, and pharynx), ovarian cancer, pancreatic cancer, prostate cancer, retinoblastoma, rhabdomyosarcoma, cancer of the respiratory system, salivary gland carcinoma, sarcoma, skin cancer, squamous cell cancer, testicular cancer, thyroid cancer, uterine or endometrial cancer, uterine serious carcinoma, cancer of the urinary system, vulval cancer; as well as other carcinomas and sarcomas, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL), small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, mantle cell lymphoma, AIDS-related lymphoma, and Waldenstrom's Macroglobulinemia), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), Hairy cell leukemia, chronic myeloblastic leukemia, and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome; and myelomas, such as multiple myeloma.

[0391] In some embodiments, the cancer is selected from a solid tumor, including but not limited to, hepatocellular carcinoma, lung carcinoma such as small cell lung cancer and large cell lung cancer, colorectal carcinoma, esophageal carcinoma, cervical carcinoma, ovarian carcinoma, renal cell carcinoma, prostate carcinoma, and bladder carcinoma.

[0392] The methods described herein include administering a therapeutically effective amount of a binding agent or a pharmaceutical composition as described herein to a subject having a cancer or malignancy. As used herein, the phrases "therapeutically effective amount", "effective amount" "amount effective", or "effective dose" may refer to an amount of the binding agent or pharmaceutical composition as described herein that provides a therapeutic benefit in the treatment of, management of, or prevention of relapse of a cancer or malignancy, *e.g.*, an amount that provides a statistically significant decrease in at least one symptom, sign, or marker of a cancer, tumor, or malignancy. Determination of a therapeutically effective amount is well within the capability of those skilled in the art. Generally, a therapeutically effective amount can vary with the subject's history, age, condition, and sex, as well as the severity and type of the medical condition in the subject, and administration of other pharmaceutically active agents.

[0393] In some embodiments, the methods described herein reduce tumor size or tumor burden in the subject, and/or reduce metastasis in the subject. In various embodiments, tumor size in the subject is decreased by about 25-50%, about 40-70%, or about 50-90% or more. In various embodiments, the methods reduce the tumor size by 10%, 20%, 30%, or more. In various embodiments, the methods reduce tumor size by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[0394] As used herein, a "subject" refers to a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal, or game animal. Primates include chimpanzees, cynomolgus monkeys, spider monkeys, and macaques, *e.g.*, Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits, and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species, *e.g.*, domestic cat, canine species, *e.g.*, dog, fox, wolf, avian species, *e.g.*, chicken, emu, ostrich, and fish, *e.g.*, trout, catfish, and salmon. In certain embodiments, the

subject is a mammal, *e.g.*, a primate, *e.g.*, a human. The terms, "patient", "individual", and "subject" are used interchangeably herein.

[0395] Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but is not limited to these examples. Mammals other than humans can be advantageously used, for example, as subjects that represent animal models of, for example, various cancers. In addition, the methods described herein can be used to treat domesticated animals and/or pets. A subject can be male or female. In certain embodiments, the subject is a human.

[0396] A subject can be one who has been previously diagnosed with or identified as suffering from a cancer and in need of treatment, but need not have already undergone treatment for the cancer. Alternatively, a subject can also be one who has not been previously diagnosed as having a cancer in need of treatment. A subject can be one who exhibits one or more risk factors for a condition or one or more complications related to a cancer or a subject who does not exhibit risk factors. A "subject in need" of treatment for a particular cancer can be a subject having that condition or diagnosed as having that condition. In other embodiments, a subject "at risk of developing" a condition refers to a subject diagnosed as being at risk for developing the condition.

[0397] As used herein, the terms "treat", "treatment", "treating", or "amelioration" when used in reference to a disease, disorder, or medical condition (*e.g.*, a cancer), refer to therapeutic treatments for a condition, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down, or stop the progression or severity of a symptom or condition. The term "treating" includes reducing or alleviating at least one adverse effect or symptom of a condition. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced. Alternatively, treatment is "effective" if the progression of a condition is reduced or halted. That is, "treatment" includes not just the improvement of symptoms or markers, but also a cessation or at least slowing of progress or worsening of symptoms that would be expected in the absence

of treatment. Beneficial or desired clinical results include, but are not limited to, reduction in cancer cells in the subject, alleviation of one or more symptom(s), diminishment of extent of the deficit, stabilized (*i.e.*, not worsening) state of a cancer or malignancy, delay, or slowing of tumor growth and/or metastasis, and an increased lifespan as compared to that expected in the absence of treatment. As used herein, the term "administering" refers to contacting a binding agent or pharmaceutical composition as described herein or a nucleic acid encoding the binding agent as described herein (*e.g.*, by administration to a subject) by a method or route which results in binding to the binding agent to the CD8+KIR+ Tregs. Similarly, a pharmaceutical composition comprising a binding agent as described herein or a nucleic acid encoding the binding agent as described herein disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject.

[0398] The dosage ranges for a binding agent depend upon the potency, and encompass amounts large enough to produce the desired effect, *e.g.*, slowing of tumor growth or a reduction in tumor size. The dosage should not be so large as to cause unacceptable adverse side effects. Generally, the dosage will vary with the age, condition, and sex of the subject and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of any complication. In some embodiments, the dosage ranges from about 0.01 mg/kg body weight to about 20 mg/kg body weight. In some embodiments, the dosage ranges from about 0.01 mg/kg body weight to about 10 mg/kg body weight. In some embodiments, the dosage ranges from about 0.1 mg/kg body weight to about 10 mg/kg body weight. In some embodiments, the dosage ranges from about 0.5 mg/kg body weight to about 10 mg/kg body weight. In some embodiments, the dose range is from about 0.5 mg/kg body weight to about 5 mg/kg body weight. Alternatively, the dose range can be titrated to maintain serum levels between 1 ug/mL and 1000 ug/mL.

[0399] Administration of the doses recited above can be repeated. In a preferred embodiment, the doses recited above are administered weekly, biweekly, every three weeks, or monthly for several weeks or months. The duration of treatment depends upon the subject's clinical progress and responsiveness to treatment.

[0400] In some embodiments, a dose can be administered intravenously. In some embodiments, an intravenous administration can be an infusion occurring over a period of from about 10 minutes to about 4 hours. In some embodiments, an intravenous administration can be an infusion occurring over a period of from about 30 minutes to about 90 minutes. In some embodiments, a dose can be administered subcutaneously.

[0401] Pharmaceutical compositions containing a binding agent can be administered in a unit dose. The term "unit dose" when used in reference to a pharmaceutical composition refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material (*e.g.*, binding agent), calculated to produce the desired therapeutic effect in association with the required physiologically acceptable diluent, *i.e.*, carrier, or vehicle.

[0402] In some embodiments, administration of any of the binding agents described herein can result in an improved treatment outcome as an objective response selected from stable disease, a partial response, or a complete response as determined by standard medical criteria for the cancer being treated. In some embodiments, the improved treatment outcome is reduced tumor burden. In some embodiments, the improved treatment outcome is progression-free survival or disease-free survival.

[0403] In some embodiments, a binding agent or a pharmaceutical composition of any of the embodiments described herein is administered with an immunotherapy or a chemotherapy. As used herein, "immunotherapy" refers to therapeutic strategies designed to induce or augment the subject's own immune system to fight the cancer or malignancy. Examples of an

immunotherapy include, but are not limited to, antibodies such as check point inhibitors. In some embodiments, the chemotherapy is, for example, alkylating agents, such as for example, Nitrogen mustards such as cyclophosphamide, Ifosfamide, trofosfamide, or Chlorambucil; Nitrosoureas such as carmustine (BCNU) and Lomustine (CCNU); Alkylsulphonates such as busulfan and Treosulfan; Triazenes such as Dacarbazine; Platinum containing compounds such as Cisplatin and carboplatin; Plant Alkaloids such as Vinca alkaloids such as vincristine, Vinblastine, Vindesine and Vinorelbine; Taxoids such as paclitaxel and Docetaxol; DNA Topoisomerase Inhibitors; Epipodophyllins such as etoposide, Teniposide, Topotecan, 9-aminocamptothecin, camptothecin, exatecan, and crisnatol; mitomycins such as Mitomycin C; Anti-metabolites such as Anti-folates such as DHFR inhibitors such as methotrexate and Trimetrexate; IMP dehydrogenase Inhibitors such as mycophenolic acid, Tiazofurin, Ribavirin and EICAR; Ribonuclotide reductase Inhibitors such as hydroxyurea, and deferoxamine; and Pyrimidine analogs such as Uracil analogs such as 5-Fluorouracil, Floxuridine, Doxifluridine and Ratitrexed; Cytosine analogs such as cytarabine (ara C), Cytosine arabinoside, and fludarabine; Purine analogs such as mercaptopurine and Thioguanine; Hormonal therapies such as Receptor antagonists such as Anti-estrogens such as Tamoxifen, Raloxifene and megestrol; LHRH agonists such as goscrclin and Leuprolide acetate; Anti-androgens such as flutamide and bicalutamide; Retinoids/Deltoids; Vitamin D3 analogs such as EB 1089, CB 1093 and KH 1060; Photodynamic therapies such as vertoporfm (BPD-MA), Phthalocyanine, photosensitizer Pc4, Demethoxy-hypocrellin A and (2BA-2-DMHA); Cytokines such as Interferon- alpha and Interferon- gamma; Tumor necrosis factor; and others such as Isoprenylation inhibitors such as Lovastatin; Dopaminergic neurotoxins such as l-methyl-4-phenylpyridinium ion; Cell cycle inhibitors such as staurosporine; Actinomycins such as Actinomycin D, and Dactinomycin; Bleomycins such as bleomycin A2, Bleomycin B2, and Peplomycin; Anthracyclines such as daunorabicin, Doxorubicin (adriamycin), Idarubicin,

Epirabicin, Pirarabicin, Zorabicin, and Mitoxantrone; MDR inhibitors such as verapamil and Ca2+ATPase inhibitors such as thapsigargin.

Exemplary Embodiments

[0404] The present invention is further illustrated by the following embodiments which should not be construed as limiting.

1. A binding agent comprising:

a first binding domain that specifically binds to a first antigen, the first antigen selected from antigens expressed on CD8+KIR+ T regulatory cells (Tregs) other than a KIR protein; and

a second binding domain that specifically binds to an inhibitory KIR protein, wherein the binding agent binds to CD8+KIR+ Tregs.

2. The binding agent of the preceding embodiment, wherein the first antigen is selected from the group consisting of CD3, CD5, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB.

3. The binding agent of any of the preceding embodiments, wherein the first antigen is selected from the group consisting of CD3, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB.

4. The binding domain of any of the preceding embodiments, wherein the first antigen is selected from the following groups of antigens:

a. CD3, CD5, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD122, ICOS, OX-40, 2B4, 41BB, and HLA-DR;

b. LAG-3/CD223, TIM-3, PD-1, S1000A8/9, and TLT2;

c. CD3, CD5, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB;

d. CD103 (ITGAE), CD166, CD177, CXCR3, and S1000A8/9;

e. CCR7, CXCR3, and CXCR5;

f. PD-1, ICOS, and CXCR3;

g. CD3, CD5, and CD8; and

h. CD3 and CD8.

5. The binding domain of any of the preceding embodiments, wherein the first antigen is selected from the following groups of antigens:

a. CD3, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD122, ICOS, OX-40, 2B4, 41BB, and HLA-DR;

b. LAG-3/CD223, TIM-3, PD-1, S1000A8/9, and TLT2;

c. CD3, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB;

d. CD103 (ITGAE), CD166, CD177, CXCR3, and S1000A8/9;

e. CCR7, CXCR3, and CXCR5; and

f. CD3 and CD8.

6. The binding agent of any of the preceding embodiments, wherein the binding agent is a bispecific antibody, a diabody, an antibody Fc fusion, an scFv1-ScFv2, an ScFv12-Fc-scFv22, an IgG-scFv, a DVD-Ig, a triomab/quadroma, a two-in-one IgG, a scFv2-Fc, a TandAb, an scFv-HSA-scFv, an scFv-VHH, a Fab-scFv-Fc, a Fab-VHH-Fc, a dAb-IgG, an IgG-VHH, a Tandem scFv-Fc, a (scFv1)2-Fc-(VHH)2, a BiTe, a DART, a CrossMab, a scFv-Fc, a one-armed tandem scFv-Fc, a DART-Fc, an anticalin, an affibody, an avimer, a DARPin, or an adnectin.

7. The binding agent of any of the preceding embodiments, wherein either the first or second binding domain is selected from an antibody or antigen binding portion thereof, and the other binding domain is an antibody fragment.

8. The binding agent of embodiment 7, wherein the antigen binding portion is a Fab, Fab', F(ab')2, Fv, scFv, or a single domain antibody, such as a VHH, VNAR, sdAb, or nanobody.

9. The binding agent of any of the preceding embodiments, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region.

10. The binding agent of any of the preceding embodiments, wherein the second binding domain comprises a heavy chain variable region, and a light chain variable region.

11. The binding agent of any of the preceding embodiments, wherein the first binding domain specifically binds to CD3 or a subunit of CD3, optionally CD3epsilon.

12. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL), the VH and VL amino acid sequences selected from the pairs of amino acid sequences set forth in the group consisting of:

a. SEQ ID NO:1 and SEQ ID NO:2, respectively;
b. SEQ ID NO:9 and SEQ ID NO:10, respectively;
c. SEQ ID NO:17 and SEQ ID NO:18, respectively;
d. SEQ ID NO:25 and SEQ ID NO:26, respectively;
e. SEQ ID NO:33 and SEQ ID NO:34, respectively;
f. SEQ ID NO:41 and SEQ ID NO:34, respectively;
g. SEQ ID NO:45 and SEQ ID NO:34, respectively;
h. SEQ ID NO:49 and SEQ ID NO:50, respectively;
i. SEQ ID NO:57 and SEQ ID NO:58, respectively;
j. SEQ ID NO:65 and SEQ ID NO:66, respectively; and
k. SEQ ID NO:65 and SEQ ID NO:166, respectively.

13. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence

set forth in SEQ ID NO:1 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:2.

14. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:9 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:10.

15. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:17 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:18.

16. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:25 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:26.

17. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:33 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:34.

18. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:41 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:34.

19. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:45 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:34.

20. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:49 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:50.

21. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:57 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:58.

22. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:65 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:66.

23. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:65 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:166.

24. The binding agent of embodiment 11, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3, and lCDR1, lCDR2, and lCDR3, respectively, the CDRs having amino acid sequences selected from the sets of amino acid sequences set forth in the group consisting of:

a. SEQ ID NO:3 to SEQ ID NO:8, respectively;

b. SEQ ID NO:11 to SEQ ID NO:16, respectively;

c. SEQ ID NO:19 to SEQ ID NO:24, respectively;

d. SEQ ID NO:27 to SEQ ID NO:32, respectively;

e. SEQ ID NO:35 to SEQ ID NO:40, respectively;

f. SEQ ID NO:42 to SEQ ID NO:44 and SEQ ID NO:38 to SEQ ID NO:40, respectively;

g. SEQ ID NO:46 to SEQ ID NO:48 and SEQ ID NO:38 to SEQ ID NO:40, respectively;

h. SEQ ID NO:51 to SEQ ID NO:56, respectively;

i. SEQ ID NO:59 to SEQ ID NO:64, respectively;

j. SEQ ID NO:67 to SEQ ID NO:72, respectively; and

k. SEQ ID NOs:67-69 and 167-169, respectively.

25. The binding agent of any of embodiments 1 to 10, wherein the first binding domain specifically binds to CD8 or a subunit of CD8, optionally CD8alpha.

26. The binding agent of embodiment 25, wherein the first binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL), the VH and VL regions having amino acid sequences selected from the pairs of amino acid sequences set forth in the group consisting of:

a. SEQ ID NO:73 and SEQ ID NO:74, respectively; and

b. SEQ ID NO:81 and SEQ ID NO:82, respectively;

or the first binding domain comprises a VHH chain, the VHH chain having the amino acid sequence selected from the amino acid sequences set forth in the group consisting of:

c. SEQ ID NO:89;

d. SEQ ID NO:93; and

e. SEQ ID NO:97.

27. The binding agent of embodiment 25, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:73 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:74.

28. The binding agent of embodiment 25, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:81 and a light chain variable region (VL) having the amino acid sequence set forth in SEQ ID NO:82.

29. The binding agent of embodiment 25, wherein the first binding domain comprises a VHH chain having the amino acid sequence set forth in SEQ ID NO:89.

30. The binding agent of embodiment 25, wherein the first binding domain comprises a VHH chain having the amino acid sequence set forth in SEQ ID NO:93.

31. The binding agent of embodiment 25, wherein the first binding domain comprises a VHH chain having the amino acid sequence set forth in SEQ ID NO:97.

32. The binding agent of embodiment 25, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3, and lCDR1, lCDR2, and lCDR3, respectively, the CDRs having amino acid sequences selected from the sets of amino acid sequences set forth in the group consisting of:

a. SEQ ID NO:75 to SEQ ID NO:80, respectively; and

b. SEQ ID NO:83 to SEQ ID NO:88, respectively;

or the first binding domain includes a VHH chain having hCDR1, hCDR2, and hCDR3, the amino acid sequences of the VHH CDRs selected from the sets of amino acid sequences set forth in the group consisting of:

c. SEQ ID NO:90 to SEQ ID NO:92, respectively;

d. SEQ ID NO:94 to SEQ ID NO:96, respectively; and

e. SEQ ID NO:98 to SEQ ID NO:100, respectively.

33. The binding agent of any of embodiments 1 to 10, wherein the first binding domain specifically binds to ICOS.

34. The binding agent of embodiment 33, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence of SEQ ID NO:170 and a light chain variable region (VL) having the amino acid sequence of SEQ ID NO:171.

35. The binding agent of embodiment 33, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 amino acid sequences according to SEQ ID NOs:172, 173, and 174, respectively, and lCDR1, lCDR2, and lCDR3 amino acid sequences according to SEQ ID NOs: 175, 176, and 177, respectively.

36. The binding agent of any of embodiments 1 to 10, wherein the first binding domain specifically binds to PD-1.

37. The binding agent of embodiment 36, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence of SEQ ID NO:178 and a light chain variable region (VL) having the amino acid sequence of SEQ ID NO:179.

38. The binding agent of embodiment 36, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 amino acid sequences according to SEQ ID NOs:180, 181, and 182, respectively, and lCDR1, lCDR2, and lCDR3 amino acid sequences according to SEQ ID NOs: 183, 184, and 185, respectively.

39. The binding agent of any of embodiments 1 to 10, wherein the first binding domain specifically binds to CXCR3.

40. The binding agent of embodiment 39, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence of SEQ ID NO:186 and a light chain variable region (VL) having the amino acid sequence of SEQ ID NO:187.

41. The binding agent of embodiment 39, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 amino acid sequences according to SEQ ID NOs:188, 189, and 190, respectively, and lCDR1, lCDR2, and lCDR3 amino acid sequences according to SEQ ID NOs: 191, 192, and 193, respectively.

42. The binding agent of any of embodiments 1 to 10, wherein the first binding domain specifically binds to CD5.

43. The binding agent of embodiment 42, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence of SEQ ID NO:194 and a light chain variable region (VL) having the amino acid sequence of SEQ ID NO:195.

44. The binding agent of embodiment 42, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 amino acid sequences according to SEQ ID NOs:196, 197, and 198, respectively, and lCDR1, lCDR2, and lCDR3 amino acid sequences according to SEQ ID NOs: 199, 200, and 201, respectively.

45. The binding agent of any of the preceding embodiments, wherein the inhibitory KIR protein is selected from KIR3DL1, KIR3DL2, KIR2DL1, KIR2DL2, and KIR2DL3 or a combination thereof.

46. The binding agent of embodiment 45, wherein the second binding domain specifically binds to KIR2DL1/2/3 or KIR2DL1/2.

47. The binding agent of any of the preceding embodiments, wherein the second binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL), the VH and VL regions having amino acid sequences selected from the pairs of amino acid sequences set forth in the group consisting of:

a. SEQ ID NO:101 and SEQ ID NO:102, respectively;
b. SEQ ID NO:109 and SEQ ID NO:110, respectively;
c. SEQ ID NO:117 and SEQ ID NO:118, respectively;
d. SEQ ID NO:125 and SEQ ID NO:126, respectively;
e. SEQ ID NO:133 and SEQ ID NO:134, respectively;
f. SEQ ID NO:141 and SEQ ID NO:142, respectively;
g. SEQ ID NO:149 and SEQ ID NO:150, respectively; and
h. SEQ ID NO:157 and SEQ ID NO:158, respectively.

48. The binding agent of any of the preceding embodiments, wherein the second binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:101 and a light chain variable region (VL) having the amino acid sequence set forth inSEQ ID NO:102.

49. The binding agent of any of claims 1 to 47, wherein the second binding domain comprises a heavy chain variable region (VH) having the amino acid

sequence set forth in SEQ ID NO:109 and a light chain variable region (VL) having the amino acid sequence set forth inSEQ ID NO:110.

50. The binding agent of any of claims 1 to 47, wherein the second binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:117 and a light chain variable region (VL) having the amino acid sequence set forth inSEQ ID NO:118.

51. The binding agent of any of claims 1 to 47, wherein the second binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:125 and a light chain variable region (VL) having the amino acid sequence set forth inSEQ ID NO:126.

52. The binding agent of any of claims 1 to 47, wherein the second binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:133 and a light chain variable region (VL) having the amino acid sequence set forth inSEQ ID NO:134.

53. The binding agent of any of claims 1 to 47, wherein the second binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:141 and a light chain variable region (VL) having the amino acid sequence set forth inSEQ ID NO:142.

54. The binding agent of any of claims 1 to 47, wherein the second binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:149 and a light chain variable region (VL) having the amino acid sequence set forth inSEQ ID NO:150.

55. The binding agent of any of claims 1 to 47, wherein the second binding domain comprises a heavy chain variable region (VH) having the amino acid sequence set forth in SEQ ID NO:157 and a light chain variable region (VL) having the amino acid sequence set forth inSEQ ID NO:158.

56. The binding agent of any of claims 1 to 47, wherein the second binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3, and lCDR1, lCDR2, and lCDR3, respectively, the CDRs having

amino acid sequences selected from the sets of amino acid sequences set forth in the group consisting of the CDRs having amino acid sequence selected from the sets of amino acid sequences set forth in the group consisting of:

a. SEQ ID NO:103 to SEQ ID NO:108, respectively;
b. SEQ ID NO:111 to SEQ ID NO:116, respectively;
c. SEQ ID NO:119 to SEQ ID NO:124, respectively;
d. SEQ ID NO:127 to SEQ ID NO:132, respectively;
e. SEQ ID NO:135 to SEQ ID NO:140, respectively;
f. SEQ ID NO:143 to SEQ ID NO:148, respectively;
g. SEQ ID NO:151 to SEQ ID NO:156, respectively; and
h. SEQ ID NO:159 and SEQ ID NO:164, respectively.

57. The binding agent of any of the preceding embodiments, where the binding agent does not contain an Fc domain.

58. The binding agent of any of embodiments 1 to 56, further comprising an Fc domain.

59. The binding agent of embodiment 58, wherein the Fc domain is selected from an IgG1 and an IgG4 Fc domain.

60. The binding agent of embodiment 59, wherein the binding agent has substantially no effector function activity.

61. The binding agent of any of embodiments 58 to 60, wherein the Fc domain is an IgG1 Fc domain.

62. The binding agent of any of embodiments 58 to 61, wherein the Fc domain is an IgG1 Fc null.

63. The binding agent of any of the preceding embodiments, wherein the binding agent is bivalent or tetravalent.

64. The binding agent of any of the preceding embodiments, wherein the binding agent is bispecific.

65. A pharmaceutical composition comprising the binding agent of any of embodiments 1 to 64 and a pharmaceutically acceptable carrier.

66. A nucleic acid encoding the binding agent of any of embodiments 1 to 64.

67. A vector comprising the nucleic acid of embodiment 66.

68. A cell line comprising the vector of embodiment 67.

69. A method of treating an autoimmune disease, comprising administering the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65 to a subject in need thereof in an amount effective to decrease the number or activity of pathogenic immune cells in the subject and thereby ameliorate a symptom of the autoimmune disease.

70. A method of suppressing an immune response mediated by pathogenic immune cells, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65 in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby the number or activity of pathogenic immune cells is decreased.

71. A method of suppressing an immune response to an antigen, such as an autoantigen, comprising administering to a subject in need thereof the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65 in an amount effective to activate or stimulate CD8+KIR+ Tregs, whereby the number or activity of pathogenic immune cells that are responsive to the antigen or autoantigen is decreased.

72. The method of embodiment 70, wherein the CD8+KIR+ Tregs are contacted with the binding agent in vivo.

73. The method of embodiment 70, wherein the CD8+KIR+ Tregs are contacted with the binding agent ex vivo.

74. The method of embodiment 73, wherein the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof.

75. The method of any of embodiments 69 to 74, wherein the pathogenic immune cells are autoreactive CD4 T cells, autoantibody producing B cells, or self antigen presenting dendritic cells.

76. The method of any of embodiments 69 to 74, wherein the pathogenic immune cells are self antigen presenting cells.

77. The method of embodiment 71, whereby the titer of autoantibodies is decreased in the subject.

78. The method of any of embodiments 69 and 74 to 77, wherein the subject has an autoimmune disease selected from the group consisting of celiac disease, Crohn's disease, juvenile idiopathic arthritis, inflammatory bowel disease (IBD), insulin-dependent diabetes mellitus (IDDM or type 1 diabetes), lupus nephritis, myasthenia gravis, myocarditis, multiple sclerosis (MS), pemphigus/pemphigoid, rheumatoid arthritis (RA), scleroderma/systemic sclerosis, Sjögren's syndrome (SjS), systemic lupus erythematosus (SLE), and ulcerative colitis.

79. The method of embodiment 78, wherein the autoimmune disease is selected from the group consisting of celiac disease, Crohn's disease, inflammatory bowel disease (IBD), insulin-dependent diabetes mellitus (IDDM or type 1 diabetes), lupus nephritis, multiple sclerosis (MS), rheumatoid arthritis (RA), scleroderma/systemic sclerosis, Sjögren's syndrome (SjS), systemic lupus erythematosus (SLE), and ulcerative colitis.

80. The method of any of embodiments 69 to 79, wherein the binding agent specifically binds to CD8 and the inhibitory KIR protein on CD8+KIR+ Tregs.

81. The method of any of embodiments 69 to 79, wherein the binding agent specifically binds to CD3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

82. The method of any of embodiments 69 to 79, wherein the binding agent specifically binds to CD5 and the inhibitory KIR protein on CD8+KIR+ Tregs.

83. The method of any of embodiments 69 to 79, wherein the binding agent specifically binds to PD-1 and the inhibitory KIR protein on CD8+KIR+ Tregs.

84. The method of any of embodiments 69 to 79, wherein the binding agent specifically binds to ICOS and the inhibitory KIR protein on CD8+KIR+ Tregs.

85. The method of any of embodiments 69 to 79, wherein the binding agent specifically binds to CXCR3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

86. The method of any of embodiments 69 to 85 wherein the CD8+KIR+ Tregs are MHC class I restricted.

87. The method of any of embodiments 69 to 86, wherein the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

88. The method of any of embodiments 69 to 87, further comprising administering an immunosuppressive agent to the subject.

89. The method of any of embodiments 69 to 88, wherein the administration of the binding agent to the subject results in an improved treatment outcome in the subject.

90. The method of embodiment 89, wherein the improved treatment outcome is reduced frequency or severity disease flares, reduced systemic inflammatory cytokines, or reduced self reporting of symptoms associated the autoimmune disease.

91. The method of any of embodiments 69 to 90, wherein the binding agent is administered intravenously.

92. The method of any of embodiments 69 to 91, wherein the binding agent is administered subcutaneously.

93. The method of any of embodiments 69 to 92, wherein the binding agent is administered in a dose of about 0.01 mg/kg to about 20 mg/kg.

94. The method of any of embodiments 69 to 93, wherein the binding agent has substantially no effector function activity.

95. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65 for the treatment of autoimmune disease in a subject by activating or stimulating CD8+KIR+ Tregs.

96. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65 for the reduction of an immune response by pathogenic immune cells by activating or stimulating CD8+KIR+ Tregs.

97. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65 for the reduction of autoantibody titer in a subject by activating or stimulating CD8+KIR+ Tregs.

98. A method of treating cancer, comprising administering the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has substantially no effector function activity, to a subject in need thereof in an amount effective to activate or stimulate CD8+KIR+ Tregs and thereby ameliorate a symptom of the cancer.

99. A method of stimulating an immune response against an antigen associated with a cancer (cancer antigen), comprising contacting CD8+KIR+ T regulatory cells (Tregs) with the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has substantially no effector function activity, in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby the immune response to the cancer antigen is increased.

100. A method of treating cancer, comprising administering the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has effector function activity comprising at least ADCC, to a subject in need thereof in an amount effective to deplete CD8+KIR+ Tregs and thereby ameliorate a symptom of the cancer.

101. A method of stimulating an immune response against an antigen associated with a cancer (cancer antigen), comprising contacting CD8+KIR+ T regulatory cells (Tregs) with the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has effector function activity comprising at least ADCC, in an amount effective to deplete CD8+KIR+ Tregs, whereby the immune response to the cancer antigen is increased.

102. The method of embodiment 99 or 101, wherein the CD8+KIR+ Tregs are contacted with the binding agent in vivo.

103. The method of embodiment 99, wherein the CD8+KIR+ Tregs are contacted with the binding agent ex vivo.

104. The method of embodiment 103, wherein the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof.

105. The method of any of embodiments 99 and 101 to 104, wherein the increased immune response comprises a reduction in cancer cells or depletion of immune suppressive immune cells.

106. The method of any of embodiments 98 to 105, whereby the cancer cells in the subject are decreased.

107. The method of any of embodiments 98 to 106, wherein the cancer is selected from the group consisting of carcinomas, lymphomas, blastomas, sarcomas, myelomas and leukemias.

108. The method of any of embodiments 98 to 107, wherein the cancer is selected from the group consisting of solid tumors such as breast, cervical, ovary, lung, CRC (and other cancers of the bowel), skin, esophageal, adenocarcinoma, bladder, and prostate; and lymphomas.

109. The method of any of embodiments 98 to 108, wherein the binding agent specifically binds to CD8 and the inhibitory KIR protein on CD8+KIR+ Tregs.

110. The method of any of embodiments 98 to 108, wherein the binding agent specifically binds to CD3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

111. The method of any of embodiments 98 to 108, wherein the binding agent specifically binds to CD5 and the inhibitory KIR protein on CD8+KIR+ Tregs.

112. The method of any of embodiments 98 to 108, wherein the binding agent specifically binds to PD-1 and the inhibitory KIR protein on CD8+KIR+ Tregs.

113. The method of any of embodiments 98 to 108, wherein the binding agent specifically binds to ICOS and the inhibitory KIR protein on CD8+KIR+ Tregs.

114. The method of any of embodiments 98 to 108, wherein the binding agent specifically binds to CXCR3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

115. The method of any of embodiments 98 to 114 wherein the CD8+KIR+ Tregs are MHC class I restricted.

116. The method of any of embodiments 98 to 115, wherein the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

117. The method of any of embodiments 98 to 116, further comprising administering a chemotherapeutic agent or an immunotherapy, such as a check point inhibitor, to the subject.

118. The method of any of embodiments 98 to 117, wherein the administration of the binding agent to the subject results in an improved treatment outcome in the subject.

119. The method of embodiment 118, wherein the improved treatment outcome is a partial response or complete response.

120. The method of embodiment 118, wherein the improved treatment outcome is remission.

121. The method of any of embodiments 98 to 120, wherein the binding agent is administered intravenously.

122. The method of any of embodiments 98 to 120, wherein the binding agent is administered subcutaneously.

123. The method of any of embodiments 98 to 122, wherein the binding agent is administered in a dose of about 0.01 mg/kg to about 20 mg/kg.

124. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has substantially no effector function activity, for the treatment of cancer in a subject by activating or stimulating CD8+KIR+ Tregs.

125. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has substantially no effector function activity, for the reduction of immune suppression by immune suppressive immune cells by activating or stimulating CD8+KIR+ Tregs.

126. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has substantially no effector function activity, for the reduction of tumor burden in a subject by activating or stimulating CD8+KIR+ Tregs.

127. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has effector function activity comprising at least ADCC, for the treatment of cancer in a subject by depleting CD8+KIR+ Tregs.

128. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has effector function activity comprising at least ADCC, for the depletion of CD8+KIR+ Tregs.

129. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has effector function activity comprising at least ADCC, for the reduction of tumor burden in a subject.

130. A method of treating an infection, comprising administering the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65 to a subject in need thereof in an amount effective to activate or stimulate CD8+KIR+ Tregs and thereby ameliorate a symptom of the infection.

131. A method of stimulating an immune response against infected cells caused by an infection, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65 in an amount effective to

activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby the immune response against the infected cells.

132. The method of embodiment 131, wherein the CD8+KIR+ Tregs are contacted with the binding agent in vivo.

133. The method of embodiment 131, wherein the CD8+KIR+ Tregs are contacted with the binding agent ex vivo.

134. The method of embodiment 133, wherein the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof.

135. The method of any of embodiments 131 to 134, wherein the immune response comprises a reduction in infected cells or reduction in immune suppressive immune cells selected from CD4+ T regulatory cells and tolerizing DCs.

136. The method of embodiment 135, whereby the number of infected cells in the subject is decreased.

137. The method of any of embodiments 130 to 136, wherein the infection is selected from a bacterial disease, a systemic fungal disease, rickettsial disease, a parasitic disease, and a viral disease.

138. The method of embodiment 137, wherein the infection is selected from the group consisting of an HIV infection, hepatitis C virus (HCV) infection, human papillomavirus (HPV) infection, Epstein Bar Virus (EBV) infection, coronavirus infection such as a SARS-COV2 infection (Covid-19), cytomegalovirus (CMV) infection, and flu virus infection.

139. The method of any of embodiments 130 to 138, wherein the binding agent specifically binds to CD8 and the inhibitory KIR protein on CD8+KIR+ Tregs.

140. The method of any of embodiments 130 to 138, wherein the binding agent specifically binds to CD3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

141. The method of any of embodiments 130 to 138, wherein the binding agent specifically binds to CD5 and the inhibitory KIR protein on CD8+KIR+ Tregs.

142. The method of any of embodiments 130 to 138, wherein the binding agent specifically binds to PD-1 and the inhibitory KIR protein on CD8+KIR+ Tregs.

143. The method of any of embodiments 130 to 138, wherein the binding agent specifically binds to ICOS and the inhibitory KIR protein on CD8+KIR+ Tregs.

144. The method of any of embodiments 130 to 138, wherein the binding agent specifically binds to CXCR3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

145. The method of any of embodiments 130 to 144 wherein the CD8+KIR+ Tregs are MHC class I restricted.

146. The method of any of embodiments 130 to 145, wherein the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

147. The method of any of embodiments 130 to 146, further comprising administering an anti-microbial or an anti-viral agent to the subject.

148. The method of any of embodiments 130 to 147, wherein the administration of the binding agent to the subject results in an improved treatment outcome in the subject.

149. The method of embodiment 148, wherein the improved treatment outcome is a reduction in infection or infected cells.

150. The method of any of embodiments 130 to 149, wherein the binding agent is administered intravenously.

151. The method of any of embodiments 130 to 149, wherein the binding agent is administered subcutaneously.

152. The method of any of embodiments 130 to 151, wherein the binding agent is administered in a dose of about 0.01 mg/kg to about 20 mg/kg.

153. The method of any of embodiments 130 to 149, wherein he binding agent has substantially no effector function activity.

154. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65 for the treatment of an infection in a subject by activating or stimulating CD8+KIR+ Tregs.

155. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65 for the stimulation of an immune response by activating or stimulating CD8+KIR+ Tregs and thereby suppressing immune suppressive immune cells.

156. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65 for the reduction of infection or infected cells in a subject by activating or stimulating CD8+KIR+ Tregs.

157. A method of reducing or preventing onset of graft versus host disease (GVHD) following a transplant, comprising administering the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has substantially no effector function activity, to a subject in need thereof in an amount effective to activate or stimulate CD8+KIR+ Tregs and thereby reduce or ameliorate at least one symptom of GVHD.

158. A method of treating a subject who has received a transplant, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has substantially no effector function activity, in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby GVHD is reduced or suppressed.

159. A method of treating a subject who has received a transplant, comprising administering the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has effector function activity comprising at least ADCC, to a subject in need

thereof in an amount effective to deplete CD8+KIR+ Tregs and thereby ameliorate a symptom of GVHD.

160. A method of suppressing GVHD against a transplant, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has effector function activity comprising at least ADCC, in an amount effective to deplete CD8+KIR+ Tregs, whereby GVHD or a symptom thereof is decreased.

161. The method of embodiment 158 or 160, wherein the CD8+KIR+ Tregs are contacted with the binding agent in vivo.

162. The method of embodiment 158, wherein the CD8+KIR+ Tregs are contacted with the binding agent ex vivo.

163. The method of embodiment 162, wherein the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof.

164. The method of any of embodiments 157 and 160 to 163, wherein the decreased GVHD comprises a reduction in CD4+ T cells active in GVHD.

165. The method of any of embodiments 157 to 164, wherein the transplant is selected from the group consisting of an organ transplant, a hematopoietic stem cell transplant, an umbilical cord blood stem cell transplant, an inducible pluripotent stem cell-derived progenitor or differentiated cell transplant, and a bone marrow transplant.

166. The method of any of embodiment 165, wherein the transplant is a hematopoietic stem cell transplant, an umbilical cord blood stem cell transplant, an inducible pluripotent stem cell-derived progenitor or differentiated cell transplant, or a bone marrow transplant.

167. The method of any of embodiments 157 to 166, wherein the transplant is allogeneic.

168. The method of any of embodiments 157 to 167, wherein the binding agent specifically binds to CD8 and the inhibitory KIR protein on CD8+KIR+ Tregs.

169. The method of any of embodiments 157 to 167, wherein the binding agent specifically binds to CD3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

170. The method of any of embodiments 157 to 167, wherein the binding agent specifically binds to CD5 and the inhibitory KIR protein on CD8+KIR+ Tregs.

171. The method of any of embodiments 157 to 167, wherein the binding agent specifically binds to PD-1 and the inhibitory KIR protein on CD8+KIR+ Tregs.

172. The method of any of embodiments 157 to 167, wherein the binding agent specifically binds to ICOS and the inhibitory KIR protein on CD8+KIR+ Tregs.

173. The method of any of embodiments 157 to 167, wherein the binding agent specifically binds to CXCR3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

174. The method of any of embodiments 157 to 173 wherein the CD8+KIR+ Tregs are MHC class I restricted.

175. The method of any of embodiments 157 to 174, wherein the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

176. The method of any of embodiments 157 to 175, further comprising administering an immunosuppressive agent to the subject.

177. The method of any of embodiments 157 to 176, wherein the administration of the binding agent to the subject results in an improved treatment outcome in the subject.

178. The method of embodiment 177, wherein the improved treatment outcome is a reduction in a symptom associated with GVHD, reduced systemic inflammatory cytokines, reduced pathology in tissues impacted by GVHD, reduced self reporting of symptoms associated with an immune response associated with adverse effects on host tissues, improved or extended transplant engraftment, alleviation of one or more symptom(s), and/or

prevention, delay, or slowing of onset or progression of rejection of the transplant, or extended transplant engraftment with decreased use of broad spectrum immunosuppressive agents, such as corticosteroids.

179. The method of any of embodiments 157 to 178, wherein the binding agent is administered intravenously.

180. The method of any of embodiments 157 to 178, wherein the binding agent is administered subcutaneously.

181. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has substantially no effector function activity, for the treatment of GVHD associated with transplant in a subject.

182. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has substantially no effector function activity, for the treatment of GVHD associated with transplant in a subject by activating or stimulating CD8+KIR+ Tregs.

183. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has substantially no effector function activity, for the reduction of GVHD associated with a transplant by activating or stimulating CD8+KIR+ Tregs.

184. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has substantially no effector function activity, for the reduction of GVHD to a transplant.

185. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has effector function activity comprising at least ADCC, for the treatment of GVHD associated with a transplant in a subject by depleting CD8+KIR+ Tregs.

186. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has

effector function activity comprising at least ADCC, for the depletion of CD8+KIR+ Tregs.

187. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, wherein the binding agent has effector function activity comprising at least ADCC, for the depletion of CD8+KIR+ Tregs in a subject who has received a transplant to reduce GVHD.

188. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, in any of the methods of embodiments 69 to 94, 98 to 123, 130 to 153, and 157 to 180.

189. Use of the binding agent of any of embodiments 1 to 64 or the pharmaceutical composition of embodiment 65, in the manufacture of a medicament for use in any of the methods of embodiments 69 to 94, 98 to 123, 130 to 153, and 157 to 180.

[0405] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. These and other changes can be made to the disclosure in light of the detailed description.

[0406] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may

also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

[0407] All patents and other publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

EXAMPLES

EXAMPLE 1: Testing of mono and bispecific molecules on CD8+KIR+ Treg Cell Activation and Cytotoxicity Against Pathogenic Immune Cells in an Autoimmune Disorder

[0408] To test a panel of monospecific and bispecific molecules (including inhibitory KIR blockers) for the functional recovery of CD8 KIR+ Treg cell activation and cytotoxicity-mediated elimination of pathogenic immune cells, such as autoreactive CD4 T cells, primary CD8+ KIR+ T cells will be incubated with escalating concentrations of a CD3 agonist antibody, which will mimic peptide/MHC binding of CD8 KIR+ T cell receptors in the presence of increasing concentrations of inhibitory KIR blocking molecules. It can be expected that blockade of the inhibitory KIR signal will reduce the activation threshold of TCR engagement required for CD8 KIR+ Treg cell activation resulting in a specific and increased activation status of CD8 KIR+ T cells. The increased activation of CD8+KIR+ Treg cells will be confirmed by the increase of secretion of certain cytokines (*e.g.*, IFNgamma, IL-10, TNFalpha, IL-35 or subunits thereof, etc), the increase of expression markers associated with

activation (*e.g.*, CD69, CD25, CD62L, CD44, CD45) and an increase of proliferation.

[0409] The panel of mono- and bi-specific molecules will be examined based on their potency by inducing CD8 KIR+ Treg cell mediated functional consequences, and will be subsequently tested using Celiac patient peripheral blood mononuclear cell-derived CD8 KIR+ Treg cells in response to gluten restimulated CD4 T cells. Increased activation and enhanced activity toward pathogenic immune cells by CD8KIR+ Treg cells will be confirmed by an increase of secretion of certain cytokines (*e.g.*, IFNgamma, IL-10, TNFalpha, and/or IL-35 or subunits thereof, etc.), an increase of expression markers (*e.g.*, CD69, CD25, CD62L, CD44, and/or CD45), a decrease in inhibitory molecules (LAG-3, TIM-3, and/or PD-1), an increase of proliferation, increased inhibition of autoreactive CD4+ T cells and other pathogenic immune cells, such as autoantibody producing B cells, self-antigen presenting dendritic cells and self-APC.

EXAMPLE 2: Testing of mono and bispecific molecules on CD8+KIR+ Treg Cell Activation and Cytotoxicity In An Infection Model

[0410] A panel of monospecific and bispecific molecules, (including inhibitory KIR blockers) will be tested for the functional recovery of CD8 KIR+ Treg cell activation and cytotoxicity mediated direct and indirect elimination of pathogen infected cells. Human CMV specific T cells (Cellero) will be cultured with increasing doses of a virally derived dominant epitope (pp65), which binds the MHC class I molecule HLA-A2. Peptides will be loaded onto an HLA-A2 expressing lymphoma cell line T2. For testing of specific inhibitory KIR molecule functions, either the MHC I deficient lymphoma T1 cell line or the K562 cell line will be transfected with relevant cognate MHC class I molecules (*e.g.*, HLA-C2 when blocking KIR2DL1). To confirm that KIR blockade specifically and effectively reestablishes the elimination of pathogen infected cells, CD8 Treg cell activation, cytotoxicity, cytokine production, and proliferation will be examined. The degree of target cell elimination and

apoptosis using Annexin V staining and proliferation will also be determined. As controls we will use, for example, irrelevant influenza hemaglutinin peptides (negative control), CD3 antibody bead activation (positive control), or the MHC deficient lymphoma T1 cell line or parent K562 cells (negative controls). To detect activation with greater sensitivity we will transfect, for example, Jurkat cells with SHP1/2 or NFAT reporter to show enhanced activation upon inhibitory KIR blockade and/or agonist binding to prioritize molecules for use.

EXAMPLE 3: Testing of mono and bispecific molecules on CD8+KIR+ Treg Cell Activation and Cytotoxicity In A Cancer Model

[0411] CD8 KIR+ Treg cells will be tested against a panel of tumor cell lines with high antigenic burden in the presence of a panel of KIR bispecific molecules to determine if KIR blockade improves CD8 KIR Treg cell activation and target cell killing. KIR bispecific molecules will be tested alone or in combination with other KIR bispecific molecules and in combination with other immune checkpoint inhibitors. As an example, studies will test anti-CD3 agonist antibody dose escalation in conjunction with KIR molecule dose escalation in coculture with tumor cell lines to include, for example: A549 (NSCLC), H1229 (NSCLC), A375 (Melanoma), SK-Mel 3 (Melanoma), Caki-1 (RCC), and/or 786- O (RCC). Dependence of haplotype on responses will be determined using for example primary NY-ESO-1 specific T cells (Cellero) vs NY-ESO1 peptide pulsed T2 (HLA-A2 restricted cell line) and/or HLA-A2 K562. To assess if a subset of HLA expression by tumor cell targets is required for optimal responses (*e.g.*, HLA-B binding KIR3DL1 or KIR2DL1/2/3 binding HLA-C), the relevant HLA molecules will be overexpressed in either K562 or T1 cells and pulsed with relevant dominant epitopes (https://antibodies.cancer.gov/detail/MajorHistocompatibilityComplexClassICPeptide1).

EXAMPLE 4: Testing of mono- and bispecific molecules on CD8+KIR+ Treg Cell Activation and Cytotoxicity In A GVHD Transplantation Model

[0412] Following hematopoietic stem cell transplant and other transplant procedures, a serious and life threatening complication can occur in which donor-derived cells recognize allogeneic host tissues as foreign and become activated, destroying healthy cells of the recipient, which is known as graft vs. host disease (GVHD). Alloreactive GVHD results in transplant associated morbidity in up to 50% of transplant recipients, and accounts for approximately 20% of deaths following transplant. KIR blockade on CD8+KIR+ Tregs may reduce the severity of graft vs host disease in the event that transplanted cells destroy healthy tissues and recognize it as foreign. To test the impact of KIR blockade on GVHD severity, a well-characterized GVHD model will be used, in which human immune cells are injected into NOD/SCID/gamma chain (NSG) deficient mice, and subsequent multi-organ acute pathology observed as a result of human cell activation and destruction of mouse tissues. KIR blocking mono- and bispecific molecules will be injected every 72 hours for the duration of the 30-45 day study, and endpoint analysis will include serum pro-inflammatory cytokines, activation marker expression of human T cells, disease scoring (including survival and body weight), and histopathological analysis of gut tissues for inflammation and epithelial cell killing. This study will support the utility of KIR blockade as a method to reduce severity of GVHD while preserving transplant engraftment, as well as determine the effect of KIR blockade on systemic diseases that may impact multiple organs and tissues.

EXAMPLE 5: Ly49 Blockade Increases the Activity of CD8+Ly49+ T Regulatory Cells

[0413] The effects of Ly49 blockade on CD8+Ly49+ Tregs were confirmed in vitro. Briefly, cells were isolated from the spleens and lymph nodes of C57BL/6 mice 10 days after EAE induction using a standard MOG peptide protocol at day 10 (*see* Saligrama et al., Nature 572:481-487 (2019)). CD4+ T cells, CD8+ CD28- regulatory T cells, and CD8+CD28+ were isolated

using magnetic separation and stimulated with CD3/CD28 in the presence of no blocking antibody (control) or $F(ab')_2$ fragments of blocking antibody LY49 C/I (clone 5e6; lacking the Fc portion of the antibody) (anti-Ly49) and cultured 1:1 with CD4 T cells.

[0414] Referring to Figures 4A to 4D, in the presence of Ly49 blockade there was a statistically significant increase in CD8+ Treg activation (Figure 4A), production of immunosuppressive cytokines (Figure 4B), cytolytic activity (Granzyme B) (Figure 4C), and an increase in CD4 T cell production of anti-inflammatory IL-10 cytokine (Figure 4D). Similar results were observed with cells stimulated with CD3/CD28 in the presence of full length Ly49 C/I blocking antibody (clone 5E6) (data not shown). These results confirm that CD8+Ly49+ T cells exhibit an increase in activation by Ly49/KIR blockade.

[0415] Supernatants from the cells (above) were collected at 48 hours after the initiation of the co-culture and analyzed for a variety of analytes (cytokines) using a Bioplex assay.

[0416] The results of this analysis indicated that Ly49 blockade suppresses the following proinflammatory cytokines in samples from mice treated with MOG and suppressor peptide as compared to mice treated with MOG peptide alone: RANTES, IL-6, IL-18, GM-CSF, TNFalpha, and IFNgamma (data not shown). In addition, IL-2 and IL-15 levels were decreased in samples from mice treated with MOG and suppressor peptide as compared to mice treated with MOG peptide (data not shown). Levels of the anti-inflammatory cytokines IL-22 and MCP-3 were decreased in samples from mice treated with MOG and suppressor peptide as compared to mice treated with MOG peptide (data not shown).

[0417] The effects of Ly49 blockade were assessed in vivo in a murine EAE model. Briefly, EAE was induced in C57BL/6 mice using a standard MOG injection protocol (*see* Saligrama et al., Nature 572:481-487 (2019)). Mice were administered MOG alone or in combination with a "surrogate peptide" cocktail ("SP") or F(ab')2 fragments of blocking antibody LY49 C/I (clone 5E6; lacking

the Fc portion of the antibody) (anti-Ly49 or "Ly49 blockade") (Figure 5). Ly49 blockade delayed onset of disease and diminished severity of disease (Figure 6), suggesting that CD8+ Treg mobilization at the time of autoimmune trigger contributed to control of disease.

EXAMPLE 6: CD8 KIR+ T Cells Have Greater Cytolytic Potential than CD8 T Cells Negative for KIR Expression in Celiac Patients

[0418] Peripheral blood mononuclear cells (PBMCs) were obtained from Celiac patients and from healthy donors. The PBMCs were enriched for CD8+ T cells, and then stained for several surface markers, including CD8 and a mix of pan-inhibitory KIR reactive peptides, and sorted to obtain CD8+KIR+ T cells and CD8+KIR- cells. After sorting, the PBMCs were stimulated with gluten peptides. Six days after stimulation, the CD8 Treg cells were evaluated for intracellular Granzyme, perforin, and IFNgamma levels.

[0419] PBMCs from Celiac patients had a greater percentage of CD8+KIR+ Treg cells (Figure 7A). The KIR+ CD8+ T cells had a greater percentage of cells with perforin, and intracellular IFNgamma and Granzyme B as compared to CD8+KIR- T cells (Figures 7 B and 7C). These results indicate that Celiac patients harbor CD8+KIR+ T cells with greater cytolytic potential than CD8+ T cells negative for KIR.

EXAMPLE 7: Celiac Patients Have More KIR+ CD8+ T cells and More ICOS Expression on KIR+ CD8+ T Cells Than From Healthy Controls

[0420] PBMCs from Celiac patients (six) or healthy donors were analyzed by flow cytometry and gated on CD8+ T cells. PBMCs from Celiac patients had more CD3+/PanKIR+ T cells than PBMCs from healthy donors (Figure 8A). PBMCs from Celiac patients had more CD3+/PanKIR+/ICOS+ cells than PBMCs from healthy donors (Figure 8B). These results indicate that Celiac patients have more ICOS expression on KIR+ CD8+ T cells.

EXAMPLE 8: Gluten Restimulation Increases Granzyme B Levels and Degranulation of CD8+KIR+ Tregs and Loss of CD4+ T Cells

[0421] To determine the effects of the gluten restimulation on the CD8+KIR+ T cells, PMBCs from Celiac patients were stimulated with gluten peptides for 12 days to enrich for both CD4 reactive cells and CD8+ Treg cells in the presence of IL-7 and 15. CD8 Tregs and CD4 T cells were then selected and combined 1:1 with autologous APCs pulsed with no peptide, flu peptides, or gluten peptides. 48 hours later, the cells were analyzed by flow cytometry

[0422] Restimulation with the gluten peptides increased degranulation, as measured by CD107 (Figure 9A, left), and Granzyme B levels (Figure 9A, right), as compared to restimulation with control flu peptides or unstimulated cells. (598 refers to PBMCs from patient 598.) Restimulation with gluten peptides also caused a reduction of the percentage of viable CD4+ cells, while restimulation with flu peptide did not (Figure 9B). These results indicate that the antigenic response by CD8+KIR+ Tregs is specific, consistent with the source of the Tregs from Celiac patients, and are rapid and sustained. These results show that CD8+ Treg cells are up-regulated and CD4+ T cell activation is down-regulated, and that pathogenic CD4+ T cells are eliminated.

EXAMPLE 9: KIR Blockade Increases Granzyme B Content and Degranulation of CD8+ T Cells

[0423] CD8+CD16+ T cells were selected from 3 patients diagnosed with Celiac disease and cultured 1:1 with CD4+ T cells and 1ug/ml anti-CD3 agonist antibody (clone OKT3) in the presence or absence of 100ug/ml KIR2DL1/2/3 and KIR3DL1 antagonist antibodies (50ug each). 48 hours later, CD8+ Treg cells were analyzed using flow cytometry. KIR blockade ("KIR block") increased intracellular Granzyme B levels (Figure 10A) and degranulation (CD107) (Figure 10B).

[0424] The effects of KIR blockade were assessed *in vitro* in another experiment using PBMCs from celiac patients. Briefly, CD8+ Treg were enriched from Celiac patient PBMCs and cultured with autologous CD4 T cells

and antigen presenting cells pulsed with a gliadin peptide cocktail, and analyzed using flow cytometry. Upon restimulation, cells administered anti-inhibitory KIR antibodies (anti-KIR2DL1/2/3, anti-KIR3DL1 or a cocktail of anti-KIR2DL1/2/3 and KIR3DL1) exhibited an induction of cytolytic activity of CD8+ T cells, a decrease in CD4+ T cell activation, and an increase in CD4+ T cell death (Figure 11). These results were consistently observed across several patient samples upon mono- and bi-specific KIR blockade.

EXAMPLE 10: KIR Blockade Decreases CD4+ T Cell Activation

[0425] CD8+ CD16+ T cells were selected from 3 patients diagnosed with Celiac disease and cultured 1:1 with CD4+ T cells and 1ug/ml anti-CD3 agonist antibody (clone OKT3) in the presence or absence of 100ug/ml KIR2DL1/2/3 and KIR3DL1 antagonist antibodies (50ug each). 48 hours later, CD8+ Treg cells were analyzed using flow cytometry. KIR blockade reduced CD4+ T cell activation and proliferation (CD69) in samples from all three patients (Figure 12).

EXAMPLE 11: Association Between Select KIR Proteins and HLA Ligand Expression in CD8+ Treg Cells in Celiac Disease

[0426] Celiac patient PBMCs were stained with antibodies directed toward KIR2DL1/2/3 and KIR3DL1. After gating on CD8 T cells, the percentage positivity of the cells for the KIR ligands and HLA haplotype was determined. (HLA and KIR typing were performed in collaboration with Scisco Genetics.)

[0427] CD8 T cells from patients expressed KIRs as follows: 3 of 3 expressed KIR2DL and 2 of 3 expressed KIR3DL in peripheral blood. HLA ligands for the select KIRs are overrepresented in the Celiac patient samples. 9 of 10 patients had at least one copy of HLA-C 07:01:01 and 10 of 10 patients had at least one copy of HLA-B 08:01:01.

EXAMPLE 12: Characterization of Bispecific Molecules That Co-Bind to CD8 and KIR2DL

[0428] A CrossMab was prepared using a Fab that binds to KIR2L1/2/3 (prepared from IPH2102 IgG1r mAb (parental antibody VH and VL sequences,

SEQ ID NOs:101 and 102, respectively)) and an scFv that binds to CD8alpha (prepared from Mb1b IgG1r mAb (parental antibody VH and VL sequences, SEQ ID NOs:81 and 82)). The Fab and scFv were attached to an IgG1 hinge-CH2-CH3 in which the CH3 domain was engineered to contain the "knobs-into-holes" mutations to enforce correct association of the two heterodimeric heavy chains. The "knob" heavy chain included mutations S354C and T366W. The "hole" heavy chain included mutations Y349C, T366S, L368A, and Y407V.

[0429] The KIR2L1/2/3 - CD8alpha CrossMAb was tested for co-binding to KIR2DL1 or KIR2DL3 and CD8alpha by biolayer interferometry using an Octet instrument. For the co-binding studies, the CrossMAb was captured to AHC (anti-human Fc) biosensors using 2-fold dilutions ranging from 0.3125 ug/ml to 20 ug/ml. The analytes (KIR2DL1, KIR2DL3, and CD8alpha) were kept constant at 100 nM. Analyte co-binding following capture was analyzed in two ways: first the association of KIR2DL1 or KIR2DL3 followed directly by the association of CD8alpha, or the association of CD8alpha followed by the direct association of KIRDL1 or KIR2DL3. KIR2DL1, KIR2DL3, and CD8alpha were tagged with a hexahistidine peptide. The CrossMab was able to co-bind targets KIR2DL1 or KIR2DL3 and CD8alpha.

[0430] CrossMAb affinity for KIR2DL1, KIR2DL3, and CD8alpha ligands was measured and compared to the anti-CD8alpha and anti-KIR2DL1/L2/L3 parental antibodies using the Octet instrument. For kinetic analysis, the CrossMAb was captured to AHC (anti-human Fc) biosensors using a load concentration of 1.25 ug/ml. Each analyte (KIR2DL1, KIR2DL3, and CD8alpha) concentration ranged from 6.25 nM to 200 nM. Analyte binding following capture was analyzed first for the association of KIR2DL1, KIR2DL3, or CD8alpha followed by the dissociation of each analyte independently. This ensured that ka (on rate), kd (off rate), and KD values could be obtained and directly compared to the parental antibodies. The kinetic analysis revealed that the CrossMab retained affinity for targets KIR2DL1, KIR2DL3, and CD8alpha.

[0431] The affinities of the parental antibodies anti-KIR2DL1/L2/L3 IPH2102 IgG1r mAb and anti-CD8alpha Mb1b IgG1r mAb were also analyzed. For kinetic analysis, the parental antibodies were separately captured to AHC (anti-human Fc) biosensors using a load concentration of 1.25 ug/ml. For IPH2102 IgG1r mAb, the KIR2DL1, or KIR2DL3 analytes ranged from 6.25 nM to 200 nM. Analyte binding following capture was analyzed first for association of KIR2DL1 or KIR2DL3 followed by the dissociation of each analyte independently. Likewise, for the Mb1b IgG1r mAb, the CD8alpha analyte ranged from 6.25 nM to 200 nM. Analyte binding following capture was analyzed first for association followed by the dissociation of CD8alpha.

Table 1. Comparison of affinities between the parental antibodies and the CrossMAb

Protein	**Target**	**Affinity KD (nM)**
IPH2102 parental antibody	KIR2DL1/L2/L3	KIR2DL1: 1.38nM KIR2DL3: 0.78nM
Mb1b parental antibody	CD8alpha	CD8alpha: 2.6nm
IPH2102/Mb1b CrossMAb	KIR2DL1/L2/L3 and CD8alpha	KIR2DL1: 1.52nM KIR2DL3: 0.88nM CD8alpha: 2.07nM

EXAMPLE 13: Analyses of PBMC Samples From Patients Diagnosed with Other Autoimmune Diseases

[0432] PBMCs from patients having Lupus, Ulcerative colitis, Crohn's Disease, Multiple Sclerosis, and Type 1 Diabetes were analyzed using flow cytometry and bioplex assays. CD8+KIR+ Treg cells were identified in these patient samples using a cocktail of antibodies directed toward a subset of inhibitory KIR surface receptors, KIR2DL1/2/3 and KIR3DL1 (Figure 13). The Tregs were found to express CXCR3, CD39, and other cells surface markers, consistent with the CD8+ Tregs from Celiac disease patients (data not shown). The CD8+ Tregs were found to produce soluble analytes associated with CD8+ Treg cell function, including IFNgamma and IL-22 (data not shown).

EXAMPLE 14: Phenotypic and Functional Characterization of CD8+KIR+ Treg Cells

[0433] PBMCs from individuals with celiac disease and the HLA DQ2.5 haplotype were analyzed using flow cytometry and bioplex assays to detect soluble analytes in supernatant. CD8+KIR+ Treg cells were identified in these patient samples, and were found to express the surface markers CD39, KLRG1, NKG2D, NKG2C, KLRB, CXCR3, and CD122 (Figure 14).

[0434] When cultured under optimized conditions with autologous CD4 T cells and antigen presenting cells pulsed with gliadin peptides, the CD8+KIR+ Tregs produced soluble analytes associated with CD8+ Treg cell function, including the cytolytic markers granzyme B, perforin, and CD107a, the intracellular anti-inflammatory cytokines IL-10, IFNγ, and TNFα, and secreted cytokines (*see, e.g.*, Figure 15). Stimulation of isolated CD8+KIR+ Treg cells with increasing doses of anti-CD3 antibodies produced a number of cytokines and chemokines in a dose-dependent manner, including RANTES and TNFβ that titrated with the strength of TCR signal delivered (Figure 16). When co-cultured with CD4+ T cells restimulated with gliadin, CD8+KIR+ Treg cells controlled CD4+ T cell activation and decreased proinflammatory cytokines produced by CD4+ cells (Figures 17-19). CD4+ T cell markers of activation

show that addition of CD8+KIR+ Treg cells during gliadin restimulation specifically decreases gliadin-reactive CD4+ T cell activation (Figure 18). Anti-inflammatory cytokines produced by CD4 T cells also increased (Figure 19) and certain inflammatory cytokines and chemokines detected in the co-culture supernatant were down-regulated. These data indicate that CD8+KIR+ Tregs isolated from PBMCs derived from celiac patients are able to survive, expand, and function when cultured under optimized conditions, and thus functional defects in celiac patients appear to be reversible.

[0435] Additionally, enriched recall of the CD8+ Treg cell response and prevalence was observed upon repeated antigenic exposure, suggesting a potentially long lasting and disease-modifying effect (Figure 20). Although patient-to-patient variability was observed, consistent effects on the functional response of CD8+ Treg cells over repeated antigenic exposure were observed across multiple data readouts. These findings suggest a possible induced memory function of CD8+ Treg cells.

EXAMPLE 15: Association Between Select KIR Proteins and HLA Ligand Expression in CD8+ Treg Cells in Celiac Disease

[0436] Celiac patient PBMCs were stained with antibodies directed toward KIR2DL1/2/3 and KIR3DL1 and analyzed using flow cytometry. Narrow expression of particular inhibitory KIR proteins on CD8+ Treg cells was observed, with patients selectively expressing KIR2DL1/2/3, KIR3DL1, or both KIR2DL1/2/3 and KIR3DL1 (Figure 21), an observation that extended to patients with other AI indications, including having Systemic Lupus Erythematosus, Ulcerative colitis, Crohn's Disease, Multiple Sclerosis, and Type 1 Diabetes (Figure 13). This expression pattern was associated with nearly ubiquitous genomic expression of inhibitory KIR proteins, and overrepresented haplotype expression of their respective HLA ligands across celiac patients relative to a healthy control population (Table 2).

Table 2. Receptor-Ligand Pairings are Over-Represented in Celiac Patients

Receptor/Ligand Pair		**Celiac Patients**	**Prevalence***
Inhibitory KIR	KIR3DL1	14 out of 15	
Ligand Haplotype	HLA-B 08:01:01	42 out of 53	US: ~25% *(<< in other countries)*
Inhibitory KIR	KIR2DL1	15 out of 15	
Ligand Haplotype	HLA-C 07:01:01	43 out of 53	US: ~30%

* http://www.allelefrequencies.net/hla.asp

EXAMPLE 16: T Cell Expression and Interaction Patterns in PBMC and Gut Tissue from Celiac Patients Pre- and Post-Challenge with Gluten

[0437] Formalin fixed, paraffin embedded celiac patient duodenal biopsies were sectioned and stained using a proprietary platform and custom antibody cocktail that detects eight cell markers simultaneously in fixed tissues (developed in collaboration with Ultivue, Inc.). The antibody cocktail included antibodies directed toward CD3, CD4, CD8, CD28, PD-1, Ki-67, Granzyme B, and KIR2DL1/2/3.

[0438] CD8+ KIR+ Treg cells are increased in celiac disease (Figure 22). Expression of inhibitory KIR was confirmed in celiac patient peripheral blood and tissue and is a marker of CD8+ Treg cells (Figure 23). Only a subset of CD8+ T cells co-localize with KIR expression, so KIR and CD8 co-expression is indicative of CD8+ Treg cells in gut tissue.

[0439] Duodenal tissue samples from a celiac patient on a gluten-free diet, and prior to challenge with gluten, were analyzed for T cell markers. Granzyme-positive CD8+ T cells were observed interacting directly with CD4+ T cells (*see* Figure 24, in which granzyme B is shown as white, CD8+ T cells

are shown as green, CD4+ T cells are shown as ochre, and the interaction between CD8+ T cells and CD4+ T cells is shown as yellow).

[0440] Upon challenge with gluten, CD8+ Treg cells increased in peripheral blood (Figure 25) and tissue in celiac patients (Figure 26). Fourteen days after gluten challenge, CD4+ T cell number, costimulatory molecule expression, and proliferation in tissue decreased, while inhibitory KIR2DL expressing T cells increased (relative to matched patient tissues pre-gluten challenge) (Figure 27).

EXAMPLE 17: Bi-specific KIR Binders

[0441] Bi-specific KIR binders, such as bi-specific antibodies or fragments thereof that have binding domains targeting KIR and another molecule, can be used to engineer signal strength to reach a functional threshold of activation and to achieve function without toxicity (Figure 28).

[0442] Briefly, CD8 Treg were enriched from Celiac patient PBMCs and cultured with autologous CD4 T cells and antigen presenting cells pulsed with a gliadin peptide cocktail and analyzed using flow cytometry (Figures 29A, 29B, and 29C), bioplex assay detecting 34 analytes (ProCarta Plex; Figure 30), and longitudinal imaging using incucyte, followed by flow cytometry (Figure 31). Gluten restimulation of celiac patient-derived PBMCs in the presence of a bi- and mono-specific KIR blockade indicated that the bi-specific blockade (anti-KIR2DL1/2/3 and anti-CD8) resulted in greater effects on CD8+ Treg cell activity than blocking KIR alone (Figures 29A, 29B, and 29C). These effects were also dose-dependent (Figures 29B and 29C). Dose-dependent reductions were also observed for an extensive panel of proinflammatory cytokines (Figure 30) and chemokines. Bi-specific KIR-CD8 blockade was also associated with greater and dose-dependent effects on CD4+ T cell survival (Figures 31 and 32). Bi-specifics binding KIR3DL1 and CD8 were similarly effective (data not shown). Preferential binding of the bi-specific blocker (anti-KIR2DL1/2/3 and anti-CD8) to CD8+ T cells was demonstrated, despite 10-fold fewer CD8+ T cells expressing KIR compared to NK cells (Figures 33 and 34). Similar

preferential binding was observed for bi-specifics targeting KIR3DL1 rather than KIR2DL1/2/3 (data not shown).

[0443] While specific embodiments have been illustrated and described, it will be readily appreciated that the various embodiments described above can be combined to provide further embodiments, and that various changes can be made therein without departing from the spirit and scope of the invention.

[0444] All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications, and non-patent publications referred to in this specification or listed in the Application Data Sheet, including U.S. Provisional Patent Application No. 63/145,394 filed February 3, 2021, U.S. Provisional Patent Application No. 63/148,016 filed February 10, 2021, U.S. Provisional Patent Application No. 63/161,325 filed March 15, 2021, U.S. Provisional Patent Application No. 63/209,949 filed June 11, 2021, and U.S. Provisional Patent Application No. 63/298,028 filed January 10, 2022, are incorporated herein by reference, in their entirety, unless explicitly stated otherwise. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications, and publications to provide yet further embodiments.

[0445] These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

What is claimed is:

1. A binding agent comprising:

a first binding domain that specifically binds to a first antigen, the first antigen selected from antigens expressed on CD8+KIR+ T regulatory cells (Tregs) other than a KIR protein; and

a second binding domain that specifically binds to an inhibitory KIR protein, wherein the binding agent binds to CD8+KIR+ Tregs.

2. The binding agent of claim 1, wherein the first antigen is selected from the group consisting of CD3, CD5, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB.

3. The binding domain of any one of the preceding claims, wherein the first antigen is selected from the following groups of antigens:

a. CD3, CD5, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD122, ICOS, OX-40, 2B4, 41BB, and HLA-DR;

b. LAG-3/CD223, TIM-3, PD-1, S1000A8/9, and TLT2;

c. CD3, CD5, CD8, CD27, CD38, CD39, CD40L, CD45RA, CD45RB, CD45RO, CD73, CD103 (ITGAE), CD122, CD166, CD177, CCR7, CXCR3, CXCR5, HLA-DR, ICOS, LAG-3/CD223, OX-40, PD-1, S1000A8/9, TIM-3, TLT-2, 2B4, and 41BB;

d. CD103 (ITGAE), CD166, CD177, CXCR3, and S1000A8/9;

e. CCR7, CXCR3, and CXCR5;

f. PD-1, ICOS, and CXCR3;

g. CD3, CD5, and CD8; and

h. CD3 and CD8.

4. The binding agent of any one of the preceding claims, wherein the binding agent is a bispecific antibody, a diabody, an antibody Fc fusion, an scFv1-ScFv2, an $ScFv1_2$-Fc-$scFv2_2$, an IgG-scFv, a DVD-Ig, a triomab/quadroma, a two-in-one IgG, a $scFv_2$-Fc, a TandAb, an scFv-HSA-scFv, an scFv-VHH, a Fab-scFv-Fc, a Fab-VHH-Fc, a dAb-IgG, an IgG-VHH, a Tandem scFv-Fc, a $(scFv1)_2$-Fc-$(VHH)_2$, a BiTe, a DART, a crossmab, a scFv-Fc, a one-armed tandem scFv-Fc, a DART-Fc, an anticalin, an affibody, an avimer, a DARPin, or an adnectin.

5. The binding agent of any one of the preceding claims, wherein either the first or second binding domain is selected from an antibody or antigen binding portion thereof, and the other binding domain is an antibody fragment.

6. The binding agent of claim 5, wherein the antigen binding portion is a Fab, Fab', F(ab')2, Fv, scFv, or a single domain antibody.

7. The binding agent of any one of the preceding claims, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region.

8. The binding agent of any one of the preceding claims, wherein the second binding domain comprises a heavy chain variable region, and a light chain variable region.

9. The binding agent of any one of the preceding claims, wherein the first binding domain specifically binds to CD3 or a subunit of CD3, optionally CD3epsilon.

10. The binding agent of claim 9, wherein the first binding domain comprises a heavy chain variable region (VH) and a light chain variable region

(VL), the VH and VL amino acid sequences selected from the pairs of amino acids sequences set forth in the group consisting of:

a. SEQ ID NO:1 and SEQ ID NO:2, respectively;
b. SEQ ID NO:9 and SEQ ID NO:10, respectively;
c. SEQ ID NO:17 and SEQ ID NO:18, respectively;
d. SEQ ID NO:25 and SEQ ID NO:26, respectively;
e. SEQ ID NO:33 and SEQ ID NO:34, respectively;
f. SEQ ID NO:41 and SEQ ID NO:34, respectively;
g. SEQ ID NO:45 and SEQ ID NO:34, respectively;
h. SEQ ID NO:49 and SEQ ID NO:50, respectively;
i. SEQ ID NO:57 and SEQ ID NO:58, respectively;
j. SEQ ID NO:65 and SEQ ID NO:66, respectively; and
k. SEQ ID NO:65 and SEQ ID NO:166, respectively.

11. The binding agent of claim 9, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3, and lCDR1, lCDR2, and lCDR3, respectively, the CDRs having amino acid sequences selected from the sets of amino acid sequences set forth in the group consisting of:

a. SEQ ID NO:3 to SEQ ID NO:8, respectively;
b. SEQ ID NO:11 to SEQ ID NO:16, respectively;
c. SEQ ID NO:19 to SEQ ID NO:24, respectively;
d. SEQ ID NO:27 to SEQ ID NO:32, respectively;
e. SEQ ID NO:35 to SEQ ID NO:40, respectively;
f. SEQ ID NO:42 to SEQ ID NO:44 and SEQ ID NO:38 to SEQ ID NO:40, respectively;
g. SEQ ID NO:46 to SEQ ID NO:48 and SEQ ID NO:38 to SEQ ID NO:40, respectively;
h. SEQ ID NO:51 to SEQ ID NO:56, respectively;

i. SEQ ID NO:59 to SEQ ID NO:64, respectively;

j. SEQ ID NO:67 to SEQ ID NO:72, respectively; and

k. SEQ ID NOs:67-69 and 167-169, respectively.

12. The binding agent of any one of claims 1 to 8, wherein the first binding domain specifically binds to CD8 or a subunit of CD8, optionally CD8alpha.

13. The binding agent of claim 12, wherein the first binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL), the VH and VL regions having amino acid sequences selected from the pairs of amino acid sequences set forth in the group consisting of:

a. SEQ ID NO:73 and SEQ ID NO:74, respectively; and

b. SEQ ID NO:81 and SEQ ID NO:82, respectively;

or the first binding domain comprises a VHH chain, the VHH chain having the amino acid sequence selected from the amino acid sequences set forth in the group consisting of:

c. SEQ ID NO:89;

d. SEQ ID NO:93; and

e. SEQ ID NO:97.

14. The binding agent of claim 12, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3, and lCDR1, lCDR2, and lCDR3, respectively, the CDRs having amino acid sequences selected from the sets of amino acid sequences set forth in the group consisting of:,

a. SEQ ID NO:75 to SEQ ID NO:80, respectively; or

b. SEQ ID NO:83 to SEQ ID NO:88, respectively;

or the first binding domain includes a VHH chain having hCDR1, hCDR2 and hCDR3, the VHH CDRs having the amino acid sequences selected from the sets of amino acid sequences set forth in the group consisting of:

c. SEQ ID NO:90 to SEQ ID NO:92, respectively;

d. SEQ ID NO:94 to SEQ ID NO:96, respectively; and

e. SEQ ID NO:98 to SEQ ID NO:100, respectively.

15. The binding agent of any one of claims 1 to 8, wherein the first binding domain specifically binds to ICOS.

16. The binding agent of claim 15, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence of SEQ ID NO:170 and a light chain variable region (VL) having the amino acid sequence of SEQ ID NO:171.

17. The binding agent of claim 15, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 amino acid sequences according to SEQ ID NOs:172, 173, and 174, respectively, and lCDR1, lCDR2, and lCDR3 amino acid sequences according to SEQ ID NOs: 175, 176, and 177, respectively.

18. The binding agent of any one of claims 1 to 8, wherein the first binding domain specifically binds to PD-1.

19. The binding agent of claim 18, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence of SEQ ID NO:178 and a light chain variable region (VL) having the amino acid sequence of SEQ ID NO:179.

20. The binding agent of claim 18, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 amino acid sequences according to SEQ ID NOs:180, 181, and 182, respectively, and lCDR1, lCDR2, and lCDR3 amino acid sequences according to SEQ ID NOs: 183, 184, and 185, respectively.

21. The binding agent of any one of claims 1 to 8, wherein the first binding domain specifically binds to CXCR3.

22. The binding agent of claim 21, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence of SEQ ID NO:186 and a light chain variable region (VL) having the amino acid sequence of SEQ ID NO:187.

23. The binding agent of claim 21, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 amino acid sequences according to SEQ ID NOs:188, 189, and 190, respectively, and lCDR1, lCDR2, and lCDR3 amino acid sequences according to SEQ ID NOs: 191, 192, and 193, respectively.

24. The binding agent of any one of claims 1 to 8, wherein the first binding domain specifically binds to CD5.

25. The binding agent of claim 24, wherein the first binding domain comprises a heavy chain variable region (VH) having the amino acid sequence of SEQ ID NO:194 and a light chain variable region (VL) having the amino acid sequence of SEQ ID NO:195.

26. The binding agent of claim 24, wherein the first binding domain comprises a heavy chain variable region and a light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3 amino acid sequences according to SEQ ID NOs:196, 197, and 198, respectively, and lCDR1, lCDR2, and lCDR3 amino acid sequences according to SEQ ID NOs: 199, 200, and 201, respectively.

27. The binding agent of any one of the preceding claims, wherein the inhibitory KIR protein is selected from KIR3DL1, KIR3DL2, KIR2DL1, KIR2DL2, and KIR2DL3 or a combination thereof.

28. The binding agent of claim 27, wherein the second binding domain specifically binds to KIR2DL1/2/3 or KIR2DL1/2.

29. The binding agent of any one of the preceding claims, wherein the second binding domain comprises a heavy chain variable region (VH) and a light chain variable region (VL), the VH and VL having amino acid sequences selected from the pairs of amino acid sequences set forth in the group consisting of:

a. SEQ ID NO:101 and SEQ ID NO:102, respectively;
b. SEQ ID NO:109 and SEQ ID NO:110, respectively;
c. SEQ ID NO:117 and SEQ ID NO:118, respectively;
d. SEQ ID NO:125 and SEQ ID NO:126, respectively;
e. SEQ ID NO:133 and SEQ ID NO:134, respectively;
f. SEQ ID NO:141 and SEQ ID NO:142, respectively;
g. SEQ ID NO:149 and SEQ ID NO:150, respectively; and
h. SEQ ID NO:157 and SEQ ID NO:158, respectively.

30. The binding agent of any one of the preceding claims, wherein the second binding domain comprises a heavy chain variable region (VH) and a

light chain variable region, the heavy and light chain variable regions comprising hCDR1, hCDR1, and hCDR3, and lCDR1, lCDR2, and lCDR3, respectively, the CDRs having the amino acid sequence selected from the sets of amino acid sequences set forth in the group consisting of:

a. SEQ ID NO:103 to SEQ ID NO:108, respectively;
b. SEQ ID NO:111 to SEQ ID NO:116, respectively;
c. SEQ ID NO:119 to SEQ ID NO:124, respectively;
d. SEQ ID NO:127 to SEQ ID NO:132, respectively;
e. SEQ ID NO:135 to SEQ ID NO:140, respectively;
f. SEQ ID NO:143 to SEQ ID NO:148, respectively;
g. SEQ ID NO:151 to SEQ ID NO:156, respectively; and
h. SEQ ID NO:159 and SEQ ID NO:164, respectively.

31. The binding agent of any one of the preceding claims, where the binding agent does not contain an Fc domain.

32. The binding agent of any one of claims 1 to 30, further comprising an Fc domain.

33. The binding agent of claim 32, wherein the Fc domain is selected from an IgG1 and an IgG4 Fc domain.

34. The binding agent of claim 33, wherein the binding agent has substantially no effector function activity.

35. The binding agent of any one of claims 32 to 34, wherein the Fc domain is an IgG1 Fc domain.

36. The binding agent of any one of claims 32 to 35, wherein the Fc domain is an IgG1 Fc null.

37. The binding agent of any one of the preceding claims, wherein the binding agent is bivalent or tetravalent.

38. The binding agent of any one of the preceding claims, wherein the binding agent is bispecific.

39. A pharmaceutical composition comprising the binding agent of any one of claims 1 to 38 and a pharmaceutically acceptable carrier.

40. A nucleic acid encoding the binding agent of any one of claims 1 to 38.

41. A vector comprising the nucleic acid of claim 40.

42. A cell line comprising the vector of claim 41.

43. A method of treating an autoimmune disease, comprising administering the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39 to a subject in need thereof in an amount effective to decrease the number or activity of pathogenic immune cells in the subject and thereby ameliorate a symptom of the autoimmune disease.

44. A method of suppressing an immune response mediated by pathogenic immune cells, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39 in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby the number or activity of pathogenic immune cells is decreased.

45. A method of suppressing an immune response to an antigen, such as an autoantigen, comprising administering to a subject in need thereof the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39 in an amount effective to activate or stimulate CD8+KIR+ Tregs, whereby the number or activity of pathogenic immune cells that are responsive to the antigen or autoantigen is decreased.

46. The method of claim 44, wherein the CD8+KIR+ Tregs are contacted with the binding agent in vivo.

47. The method of claim 44, wherein the CD8+KIR+ Tregs are contacted with the binding agent ex vivo.

48. The method of claim 47, wherein the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof.

49. The method of any one of claims 43 to 48, wherein the pathogenic immune cells are autoreactive CD4+ T cells, autoantibody producing B cells, or self antigen presenting dendritic cells.

50. The method of any one of claims 43 to 48, wherein the pathogenic immune cells are self antigen presenting cells.

51. The method of claim 45, whereby the titer of autoantibodies is decreased in the subject.

52. The method of any one of claims 43 to 51, wherein the subject has an autoimmune disease selected from the group consisting of autoimmune disease is selected from the group consisting of celiac disease, Crohn's disease, juvenile idiopathic arthritis, inflammatory bowel disease (IBD), insulin-

dependent diabetes mellitus (IDDM or type 1 diabetes), lupus nephritis, myasthenia gravis, myocarditis, multiple sclerosis (MS), pemphigus/pemphigoid, rheumatoid arthritis (RA), scleroderma/systemic sclerosis, Sjögren's syndrome (SjS), systemic lupus erythematosus (SLE), and ulcerative colitis.

53. The method of claim 52, wherein the autoimmune disease is selected from the group consisting of celiac disease, Crohn's disease, inflammatory bowel disease (IBD), insulin-dependent diabetes mellitus (IDDM or type 1 diabetes), lupus nephritis, multiple sclerosis (MS), rheumatoid arthritis (RA), scleroderma/systemic sclerosis, Sjögren's syndrome (SjS), systemic lupus erythematosus (SLE), and ulcerative colitis.

54. The method of any one of claims 43 to 53, wherein the binding agent specifically binds to CD8 and the inhibitory KIR protein on CD8+KIR+ Tregs.

55. The method of any one of claims 43 to 53, wherein the binding agent specifically binds to CD3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

56. The method of any one of claims 43 to 53, wherein the binding agent specifically binds to CD5 and the inhibitory KIR protein on CD8+KIR+ Tregs.

57. The method of any one of claims 43 to 53, wherein the binding agent specifically binds to PD-1 and the inhibitory KIR protein on CD8+KIR+ Tregs.

58. The method of any one of claims 43 to 53, wherein the binding agent specifically binds to ICOS and the inhibitory KIR protein on CD8+KIR+ Tregs.

59. The method of any one of claims 43 to 53, wherein the binding agent specifically binds to CXCR3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

60. The method of any one of claims 43 to 59, wherein the CD8+KIR+ Tregs are MHC class I restricted.

61. The method of any one of claims 43 to 60, wherein the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

62. The method of any one of claims 43 to 61, further comprising administering an immunosuppressive agent to the subject.

63. The method of any one of claims 43 to 62, wherein the administration of the binding agent to the subject results in an improved treatment outcome in the subject.

64. The method of claim 63, wherein the improved treatment outcome is reduced frequency or severity disease flares, reduced systemic inflammatory cytokines, or reduced self reporting of symptoms associated the autoimmune disease.

65. The method of any one of claims 43 to 64, wherein the binding agent is administered intravenously.

66. The method of any one of claims 43 to 65, wherein the binding agent is administered subcutaneously.

67. The method of any one of claims 43 to 66, wherein the binding agent is administered in a dose of about 0.01 mg/kg to about 20 mg/kg.

68. The method of any one of claims 43 to 67, wherein the binding agent has substantially no effector function activity.

69. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39 for the treatment of autoimmune disease in a subject by activating or stimulating CD8+KIR+ Tregs.

70. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39 for the reduction of an immune response by pathogenic immune cells by activating or stimulating CD8+KIR+ Tregs.

71. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39 for the reduction of autoantibody titer in a subject by activating or stimulating CD8+KIR+ Tregs.

72. A method of treating cancer, comprising administering the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has substantially no effector function activity, to a subject in need thereof in an amount effective to activate or stimulate CD8+KIR+ Tregs and thereby ameliorate a symptom of the cancer.

73. A method of stimulating an immune response against an antigen associated with a cancer (cancer antigen), comprising contacting CD8+KIR+ T

regulatory cells (Tregs) with the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has substantially no effector function activity, in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby the immune response to the cancer antigen is increased.

74. A method of treating cancer, comprising administering the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has effector function activity comprising at least ADCC, to a subject in need thereof in an amount effective to deplete CD8+KIR+ Tregs and thereby ameliorate a symptom of the cancer.

75. A method of stimulating an immune response against an antigen associated with a cancer (cancer antigen), comprising contacting CD8+KIR+ T regulatory cells (Tregs) with the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has effector function activity comprising at least ADCC, in an amount effective to deplete CD8+KIR+ Tregs, whereby the immune response to the cancer antigen is increased.

76. The method of claim 73 or claim 75, wherein the CD8+KIR+ Tregs are contacted with the binding agent in vivo.

77. The method of claim 73, wherein the CD8+KIR+ Tregs are contacted with the binding agent ex vivo.

78. The method of claim 77, wherein the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof.

79. The method of any one of claims 73 and 75 to 78, wherein the increased immune response comprises a reduction in cancer cells or depletion of immune suppressive immune cells.

80. The method of any one of claims 72 to 79, whereby the cancer cells in the subject are decreased.

81. The method of any one of claims 72 to 80, wherein the cancer is selected from the group consisting of carcinomas, lymphomas, blastomas, sarcomas, myelomas, and leukemias.

82. The method of any one of claims 72 to 81, wherein the cancer is selected from the group consisting of solid tumors such as breast, cervical, ovary, lung, CRC (and other cancers of the bowel), skin, esophageal, adenocarcinoma, bladder, and prostate; and lymphomas.

83. The method of any one of claims 72 to 82, wherein the binding agent specifically binds to CD8 and the inhibitory KIR protein on CD8+KIR+ Tregs.

84. The method of any one of claims 72 to 82, wherein the binding agent specifically binds to CD3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

85. The method of any one of claims 72 to 82, wherein the binding agent specifically binds to CD5 and the inhibitory KIR protein on CD8+KIR+ Tregs.

86. The method of any one of claims 72 to 82, wherein the binding agent specifically binds to PD-1 and the inhibitory KIR protein on CD8+KIR+ Tregs.

87. The method of any one of claims 72 to 82, wherein the binding agent specifically binds to ICOS and the inhibitory KIR protein on CD8+KIR+ Tregs.

88. The method of any one of claims 72 to 82, wherein the binding agent specifically binds to CXCR3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

89. The method of any one of claims 72 to 88 wherein the CD8+KIR+ Tregs are MHC class I restricted.

90. The method of any one of claims 72 to 89, wherein the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

91. The method of any one of claims 72 to 90, further comprising administering a chemotherapeutic agent to the subject.

92. The method of any one of claims 72 to 91, wherein the administration of the binding agent to the subject results in an improved treatment outcome in the subject.

93. The method of claim 92, wherein the improved treatment outcome is a partial response or complete response.

94. The method of claim 92, wherein the improved treatment outcome is remission.

95. The method of any one of claims 72 to 94, wherein the binding agent is administered intravenously.

96. The method of any one of claims 72 to 94, wherein the binding agent is administered subcutaneously.

97. The method of any one of claims 72 to 96, wherein the binding agent is administered in a dose of about 0.01 mg/kg to about 20 mg/kg.

98. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has substantially no effector function activity, for the treatment of cancer in a subject by activating or stimulating CD8+KIR+ Tregs.

99. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has substantially no effector function activity, for the reduction of immune suppression by immune suppressive immune cells by activating or stimulating CD8+KIR+ Tregs.

100. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has substantially no effector function activity, for the reduction of tumor burden in a subject by activating or stimulating CD8+KIR+ Tregs.

101. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has effector function activity comprising at least ADCC, for the treatment of cancer in a subject by depleting CD8+KIR+ Tregs.

102. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has effector function activity comprising at least ADCC, for the depletion of CD8+KIR+ Tregs.

103. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has effector function activity comprising at least ADCC, for the reduction of tumor burden in a subject.

104. A method of treating an infection, comprising administering the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39 to a subject in need thereof in an amount effective to activate or stimulate CD8+KIR+ Tregs and thereby ameliorate a symptom of the infection.

105. A method of stimulating an immune response against infected cells caused by an infection, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39 in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby the immune response against the infected cells.

106. The method of claim 105, wherein the CD8+KIR+ Tregs are contacted with the binding agent in vivo.

107. The method of claim 105, wherein the CD8+KIR+ Tregs are contacted with the binding agent ex vivo.

108. The method of claim 107, wherein the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof.

109. The method of any one of claims 104 to 108, wherein the immune response comprises a reduction in infected cells or reduction in immune suppressive immune cells selected from CD4+ T regulatory cells and tolerizing DCs.

110. The method of claim 109, whereby the number of infected cells in the subject is decreased.

111. The method of any one of claims 104 to 110, wherein the infection is selected from a bacterial disease, a systemic fungal disease, a rickettsial disease, a parasitic disease, and a viral disease.

112. The method of claim 111, wherein the infection is selected from the group consisting of an HIV infection, hepatitis C virus (HCV) infection, human papillomavirus (HPV) infection, Epstein Bar Virus (EBV) infection, coronavirus infection such as a SARS-COV2 infection (Covid-19), cytomegalovirus (CMV) infection, and flu virus infection.

113. The method of any one of claims 104 to 112, wherein the binding agent specifically binds to CD8 and the inhibitory KIR protein on CD8+KIR+ Tregs.

114. The method of any one of claims 104 to 112, wherein the binding agent specifically binds to CD3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

115. The method of any one of claims 104 to 112, wherein the binding agent specifically binds to CD5 and the inhibitory KIR protein on CD8+KIR+ Tregs.

116. The method of any one of claims 104 to 112, wherein the binding agent specifically binds to PD-1 and the inhibitory KIR protein on CD8+KIR+ Tregs.

117. The method of any one of claims 104 to 112, wherein the binding agent specifically binds to ICOS and the inhibitory KIR protein on CD8+KIR+ Tregs.

118. The method of any one of claims 104 to 112, wherein the binding agent specifically binds to CXCR3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

119. The method of any one of claims 104 to 118, wherein the CD8+KIR+ Tregs are MHC class I restricted.

120. The method of any one of claims 104 to 119, wherein the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

121. The method of any one of claims 104 to 120, further comprising administering an anti-microbial or an anti-viral agent to the subject.

122. The method of any one of claims 104 to 121, wherein the administration of the binding agent to the subject results in an improved treatment outcome in the subject.

123. The method of claim 122, wherein the improved treatment outcome is a reduction in infection or infected cells.

124. The method of any one of claims 104 to 123, wherein the binding agent is administered intravenously.

125. The method of any one of claims 104 to 123, wherein the binding agent is administered subcutaneously.

126. The method of any one of claims 104 to 125, wherein the binding agent is administered in a dose of about 0.01 mg/kg to about 20 mg/kg.

127. The method of any one of claims 104 to 126, wherein the binding agent has substantially no effector function activity.

128. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39 for the treatment of an infection in a subject by activating or stimulating CD8+KIR+ Tregs.

129. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39 for the stimulation of an immune response by activating or stimulating CD8+KIR+ Tregs and thereby suppressing immune suppressive immune cells.

130. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39 for the reduction of infection or infected cells in a subject by activating or stimulating CD8+KIR+ Tregs.

131. A method of reducing or preventing onset of graft versus host disease (GVHD) following a transplant, comprising administering the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has substantially no effector function activity, to a subject in need thereof in an amount effective to activate or stimulate CD8+KIR+ Tregs and thereby reduce or ameliorate at least one symptom of GVHD.

132. A method of treating a subject who has received a transplant, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has substantially no effector function activity, in an amount effective to activate or stimulate CD8+KIR+ Tregs (activated Tregs), whereby GVHD is reduced or suppressed.

133. A method of treating a subject who has received a transplant, comprising administering the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has effector function activity comprising at least ADCC, to a subject in need thereof in an amount effective to deplete CD8+KIR+ Tregs and thereby ameliorate a symptom of GVHD.

134. A method of suppressing GVHD against a transplant, comprising contacting CD8+KIR+ T regulatory cells (Tregs) with the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has effector function activity comprising at least ADCC, in an amount effective to deplete CD8+KIR+ Tregs, whereby GVHD or a symptom thereof is decreased.

135. The method of claim 132 or claim 134, wherein the CD8+KIR+ Tregs are contacted with the binding agent in vivo.

136. The method of claim 132, wherein the CD8+KIR+ Tregs are contacted with the binding agent ex vivo.

137. The method of claim 136, wherein the activated CD8+KIR+ Tregs are administered in an effective amount to a subject in need thereof.

138. The method of any one of claims 132 and 134 to 137, wherein the reduced or decreased GVHD comprises a reduction in CD4+ T cells active in GVHD.

139. The method of any one of claims 131 to 138, wherein the transplant is selected from the group consisting of an organ transplant, a hematopoietic stem cell transplant, an umbilical cord blood stem cell transplant, an inducible pluripotent stem cell-derived progenitor or differentiated cell transplant, and a bone marrow transplant.

140. The method of claim 139, wherein the transplant is a hematopoietic stem cell transplant, an umbilical cord blood stem cell transplant, an inducible pluripotent stem cell-derived progenitor or differentiated cell transplant, or a bone marrow transplant.

141. The method of any one of claims 131 to 140, wherein the transplant is allogeneic.

142. The method of any one of claims 131 to 141, wherein the binding agent specifically binds to CD8 and the inhibitory KIR protein on CD8+KIR+ Tregs.

143. The method of any one of claims 131 to 141, wherein the binding agent specifically binds to CD3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

144. The method of any one of claims 131 to 141, wherein the binding agent specifically binds to CD5 and the inhibitory KIR protein on CD8+KIR+ Tregs.

145. The method of any one of claims 131 to 141, wherein the binding agent specifically binds to PD-1 and the inhibitory KIR protein on CD8+KIR+ Tregs.

146. The method of any one of claims 131 to 141, wherein the binding agent specifically binds to ICOS and the inhibitory KIR protein on CD8+KIR+ Tregs.

147. The method of any one of claims 131 to 141, wherein the binding agent specifically binds to CXCR3 and the inhibitory KIR protein on CD8+KIR+ Tregs.

148. The method of any one of claims 131 to 147, wherein the CD8+KIR+ Tregs are MHC class I restricted.

149. The method of any one of claims 131 to 148, wherein the CD8+KIR+ Tregs are not MHC HLA E (Qa-1b) restricted.

150. The method of any one of claims 131 to 149, further comprising administering an immunosuppressive agent to the subject.

151. The method of any one of claims 131 to 150, wherein the administration of the binding agent to the subject results in an improved treatment outcome in the subject.

152. The method of claim 151, wherein the improved treatment outcome is a reduction in a symptom associated with GVHD, reduced systemic inflammatory cytokines, reduced pathology in tissues impacted by GVHD, reduced self reporting of symptoms associated with an immune response associated with adverse effects on host tissues, improved or extended

transplant engraftment, alleviation of one or more symptom(s), and/or prevention, delay, or slowing of onset or progression of rejection of the transplant, or extended transplant engraftment with decreased use of broad spectrum immunosuppressive agents, such as corticosteroids.

153. The method of any one of claims 131 to 152, wherein the binding agent is administered intravenously.

154. The method of any one of claims 131 to 152, wherein the binding agent is administered subcutaneously.

155. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has substantially no effector function activity, for the treatment of GVHD associated with transplant in a subject.

156. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has substantially no effector function activity, for the treatment of GVHD associated with transplant in a subject by activating or stimulating CD8+KIR+ Tregs.

157. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has substantially no effector function activity, for the reduction of GVHD associated with a transplant by activating or stimulating CD8+KIR+ Tregs.

158. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has substantially no effector function activity, for the reduction of GVHD to a transplant.

159. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has effector function activity comprising at least ADCC, for the treatment of GVHD associated with a transplant in a subject by depleting CD8+KIR+ Tregs.

160. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has effector function activity comprising at least ADCC, for the depletion of CD8+KIR+ Tregs.

161. Use of the binding agent of any one of claims 1 to 38 or the pharmaceutical composition of claim 39, wherein the binding agent has effector function activity comprising at least ADCC, for the depletion of CD8+KIR+ Tregs in a subject who has received a transplant to reduce GVHD.

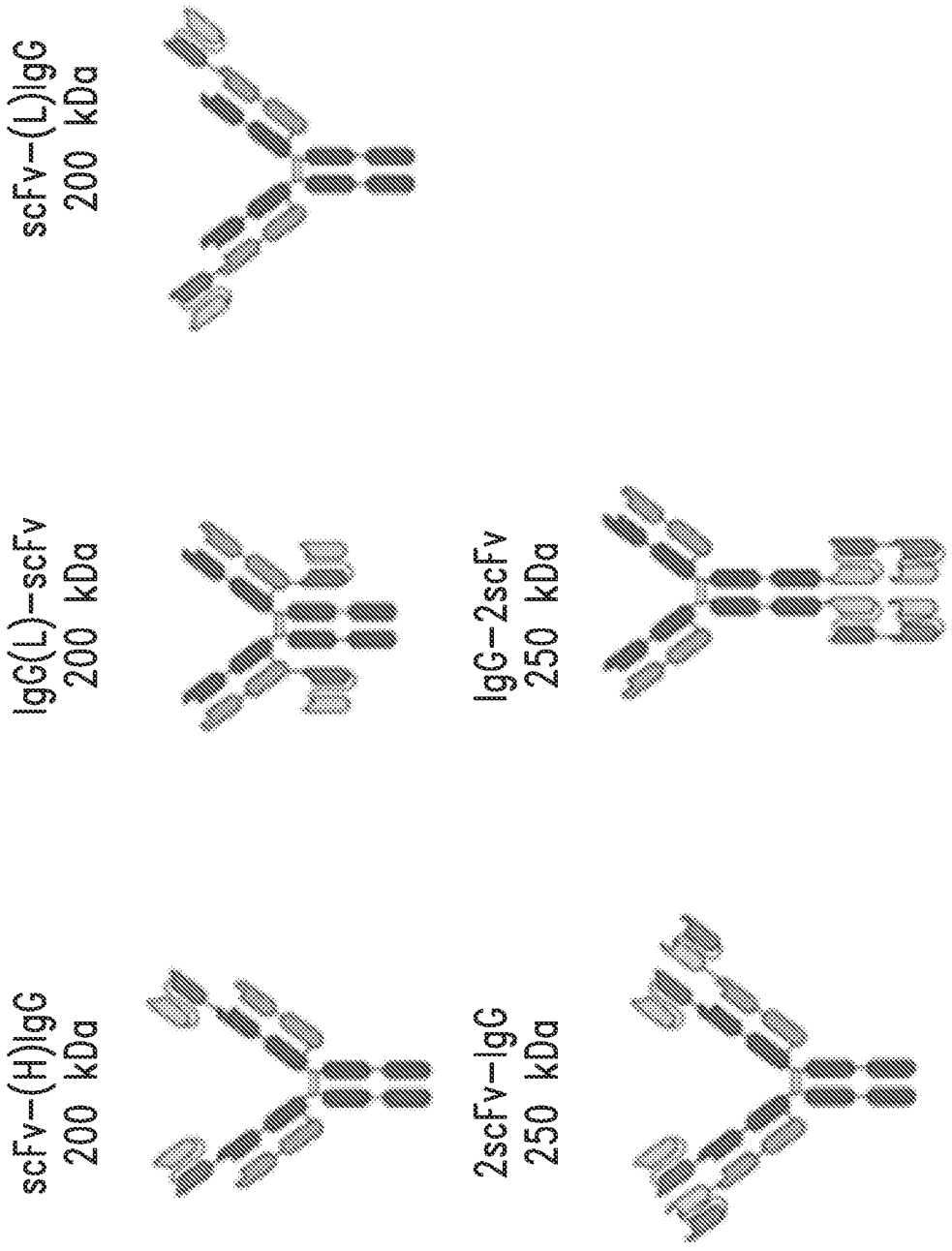

IgG(H)–scFv
200 kDa
scFv–(H)IgG
200 kDa
2scFv–IgG
250 kDa
IgG(L)–scFv
200 kDa
IgG–2scFv
250 kDa
scFv–(L)IgG
200 kDa

FIG. 1

Triomab/quadroma
DVD-Ig
CrossMAb
Two-in-one IgG
scFv2-Fc
TandAb
scFv-HSA-scFv
BiTE
DART
Nanobody

FIG. 2

scFv-VHH
Fab-scFv-Fc
Fab-VHH-Fc
scFv-Fc
DART-Fc
dAb-IgG
(DVD-Ig with VHH in place of scFv)
IgG-VHH
Tandem scFv-Fc
(scFv1)2-Fc-(VHH)2
One-armed tandem scFv-Fc

FIG. 3

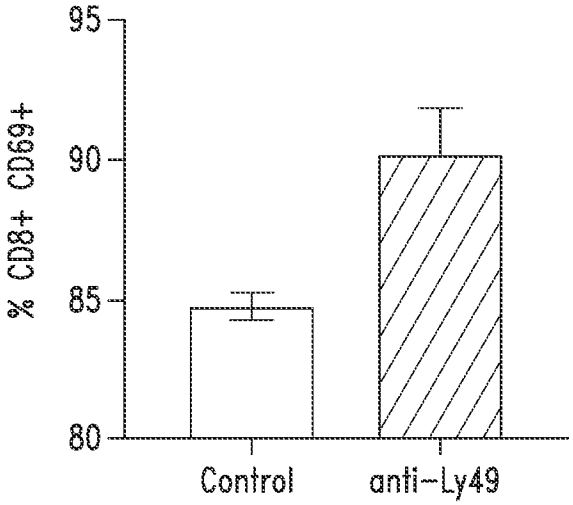


FIG. 4A

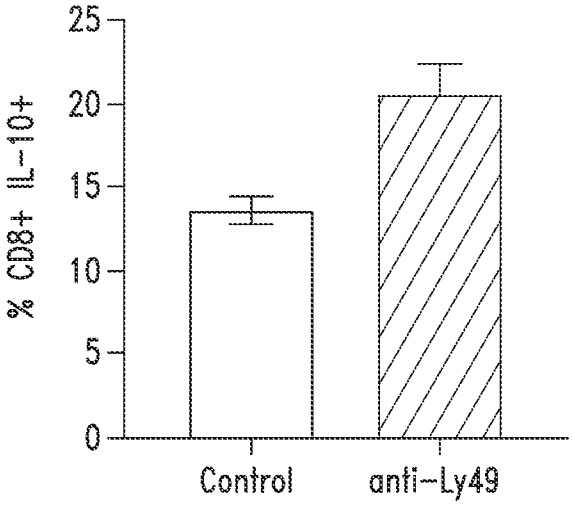


FIG. 4B

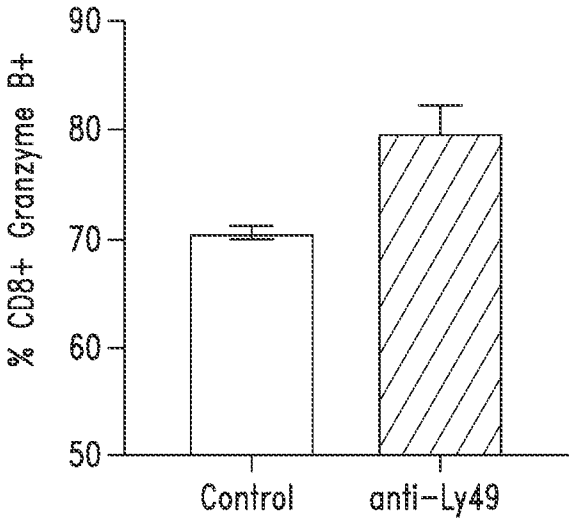


FIG. 4C

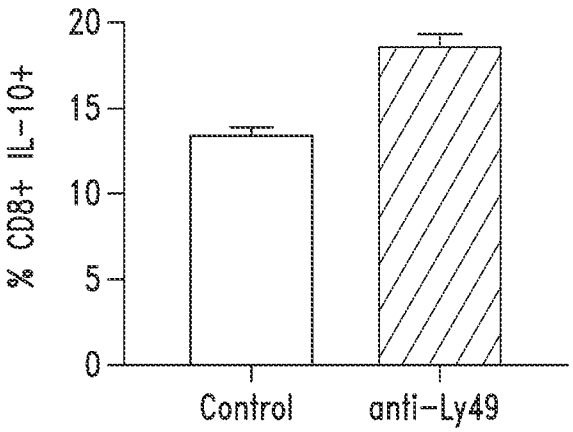


FIG. 4D

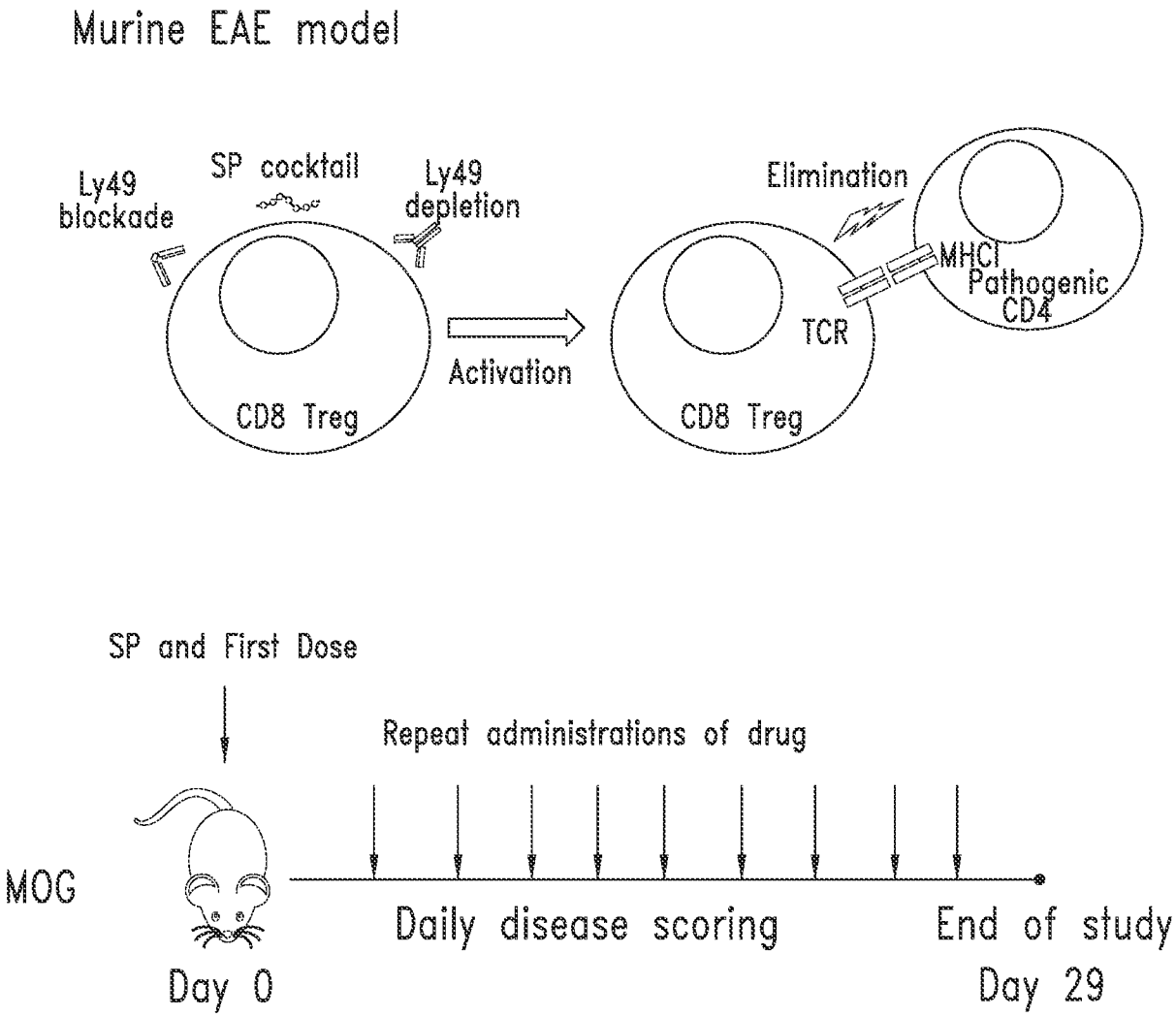

Murine EAE model
Ly49 blockade
SP cocktail
Ly49 depletion
CD8 Treg
Activation
Elimination
MHCI
Pathogenic CD4
TCR
CD8 Treg
SP and First Dose
Repeat administrations of drug
MOG
Daily disease scoring
End of study
Day 0
Day 29

FIG. 5

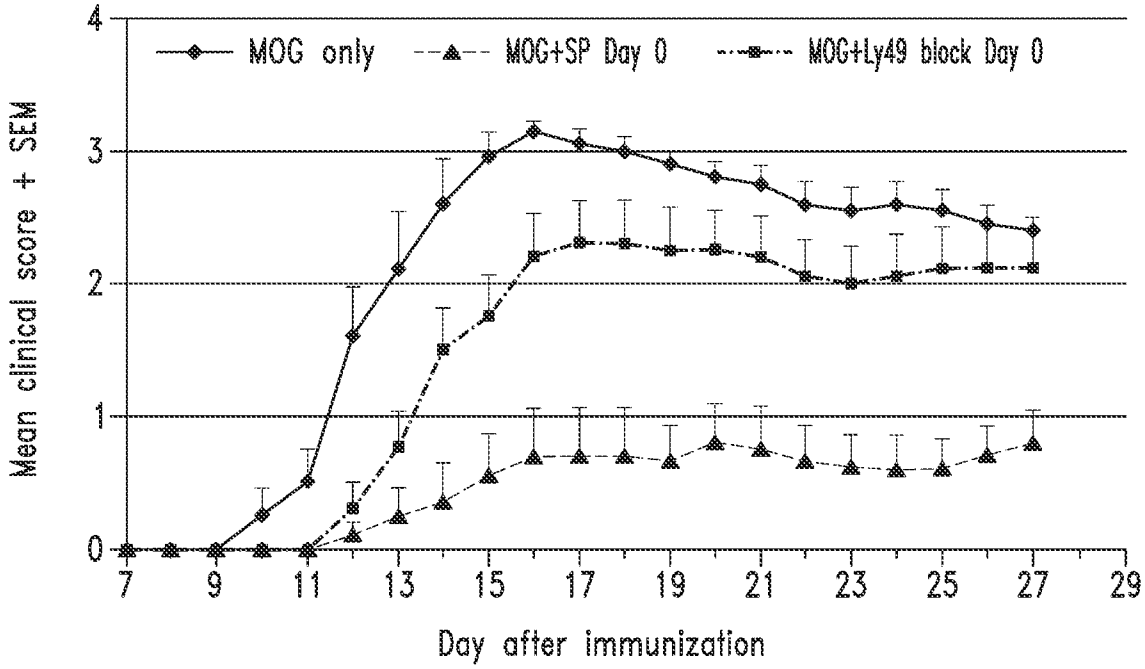

MOG only
MOG+SP Day 0
MOG+Ly49 block Day 0
Mean clinical score + SEM
Day after immunization
0
1
2
3
4
7
9
11
13
15
17
19
21
23
25
27
29

FIG. 6

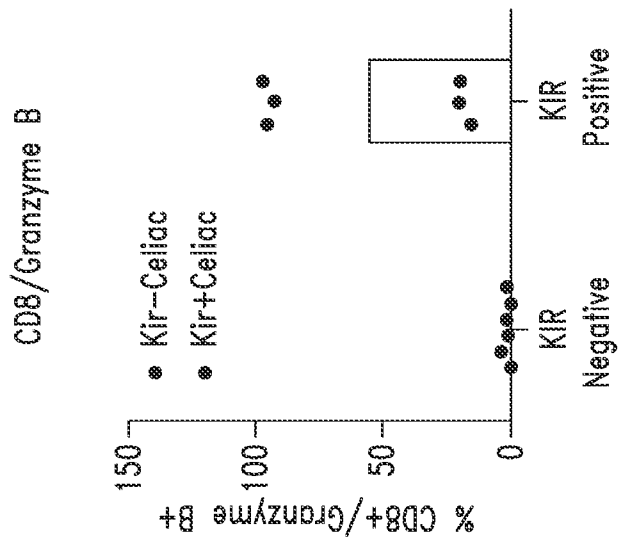


FIG. 7C

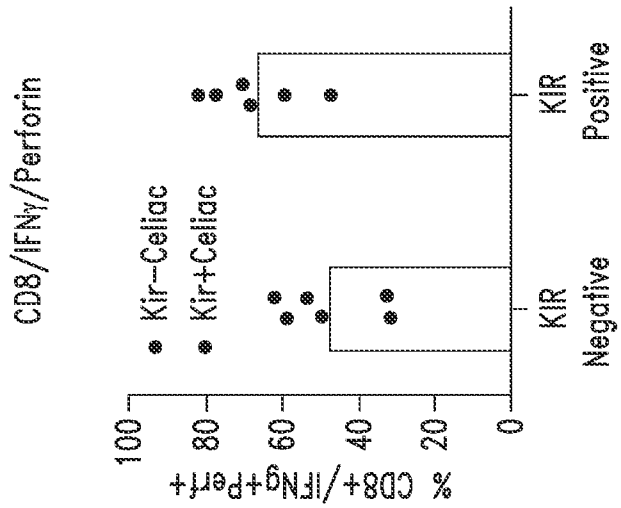


FIG. 7B

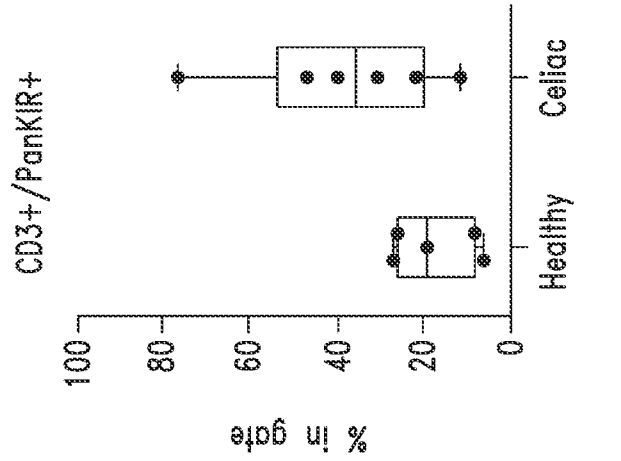


FIG. 7A

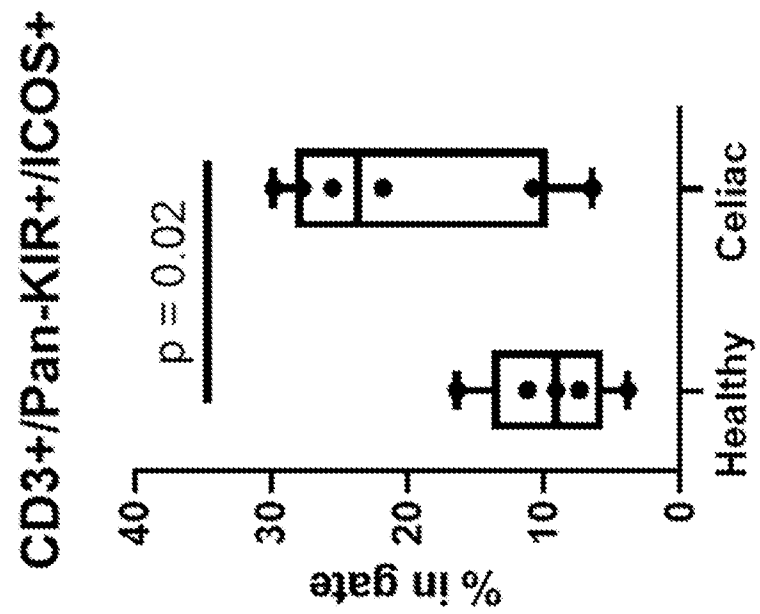

CD3+/Pan-KIR+/ICOS+
p = 0.02
% in gate
40
30
20
10
0
Healthy
Celiac

FIG. 8B

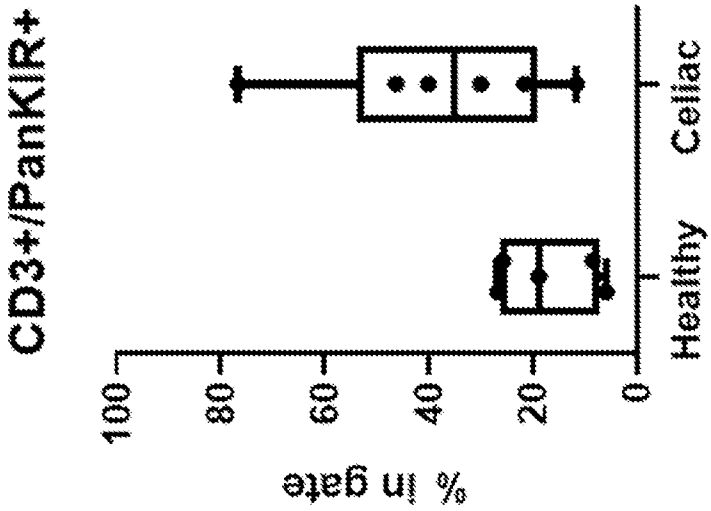

CD3+/PanKIR+
% in gate
100
80
60
40
20
0
Healthy
Celiac

FIG. 8A

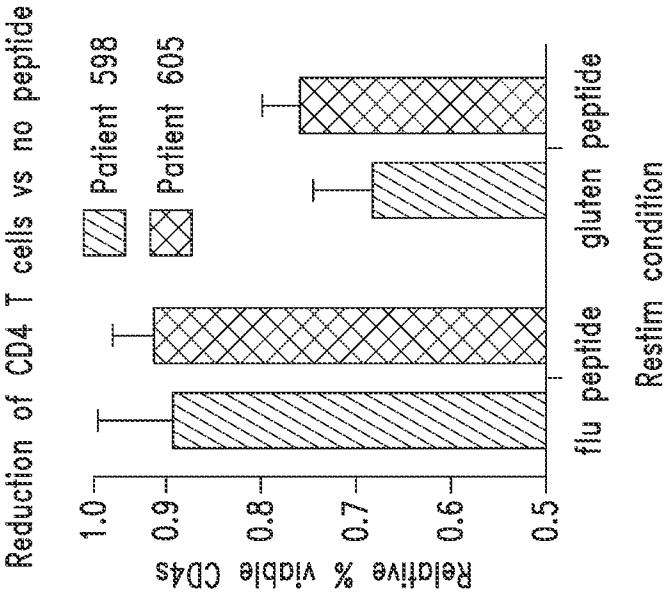

Reduction of CD4 T cells vs no peptide
Patient 598
Patient 605
Relative % viable CD4s
1.0
0.9
0.8
0.7
0.6
0.5
flu peptide
gluten peptide
Restim condition

FIG. 9B

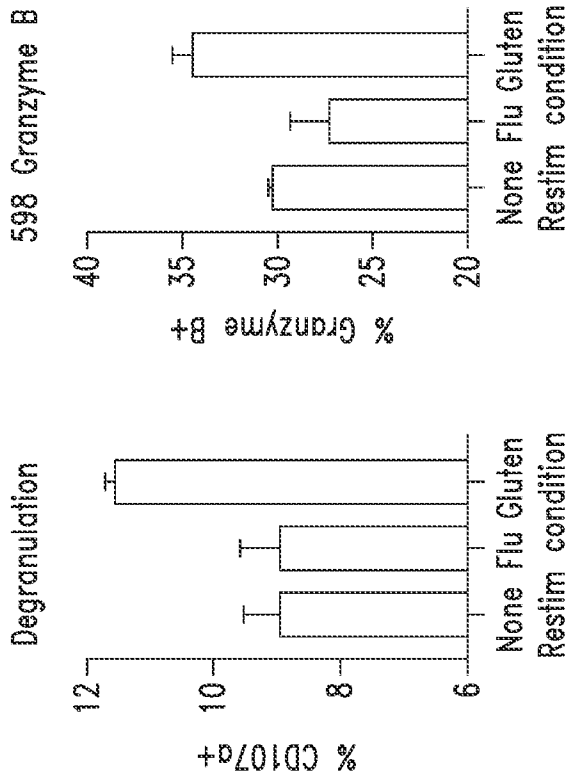

Degranulation
% CD107a+
12
10
8
6
None Flu Gluten
Restim condition
598 Granzyme B
% Granzyme B+
40
35
30
25
20
None Flu Gluten
Restim condition

FIG. 9A

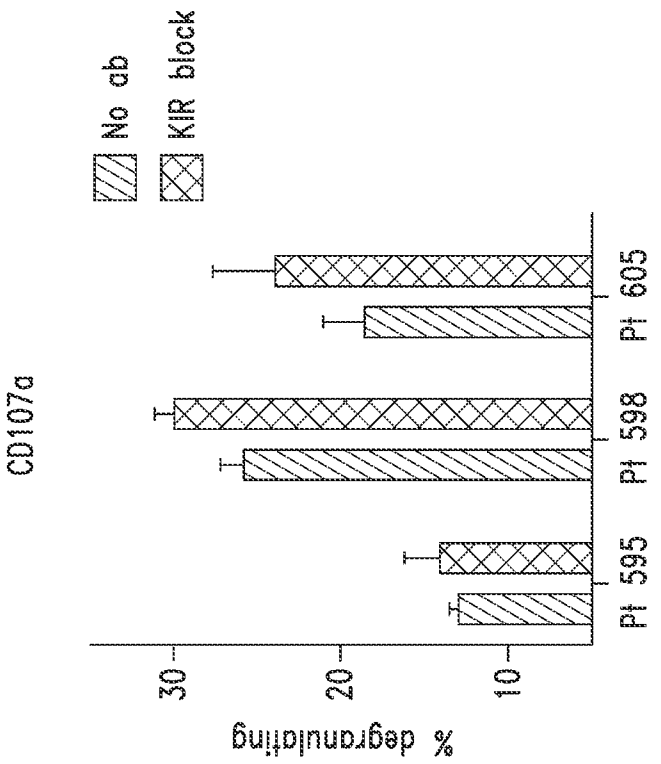


FIG. 10B

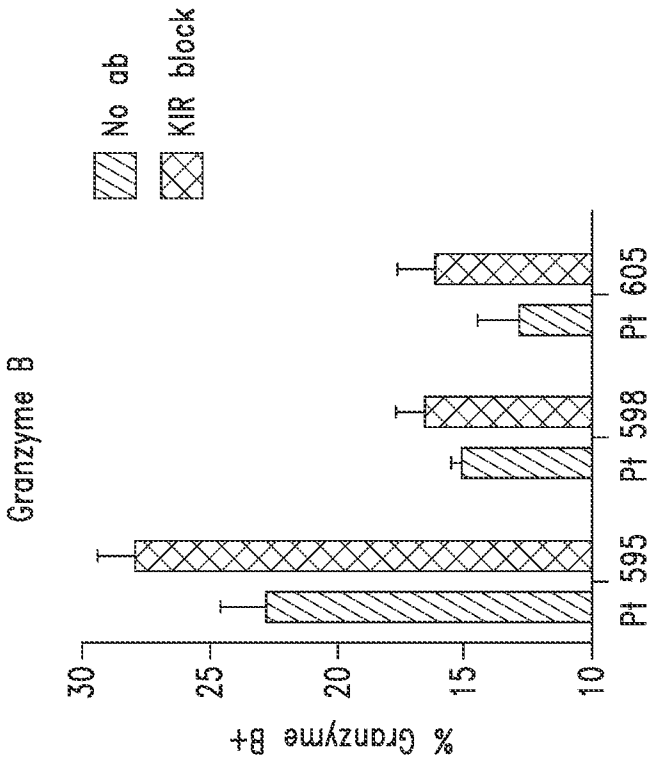


FIG. 10A

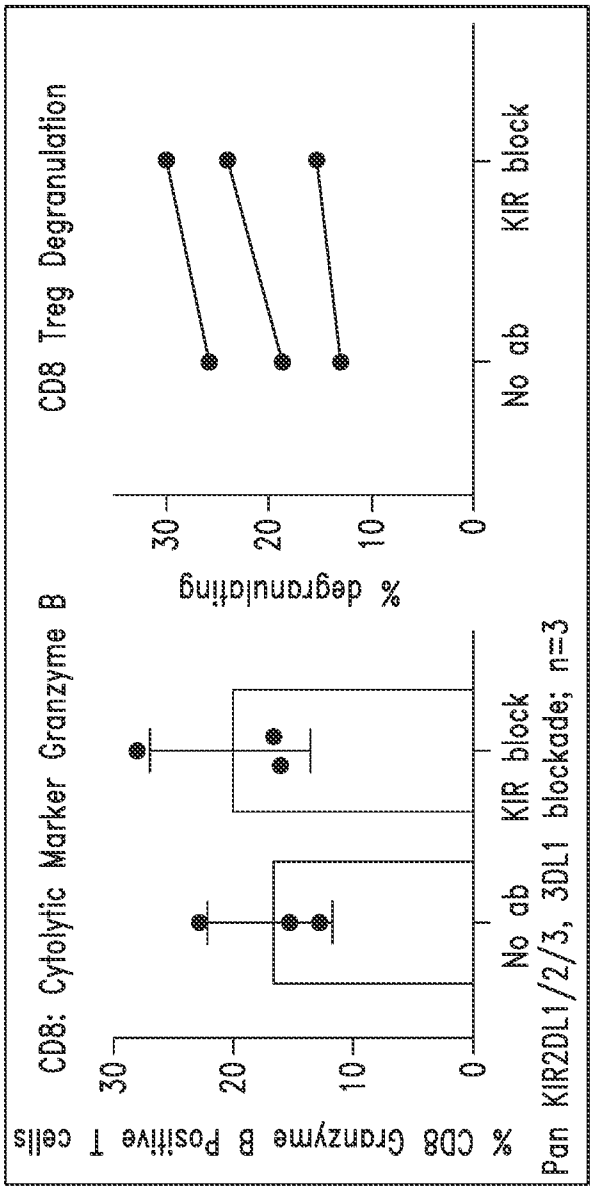

% CD8 Granzyme B Positive T cells
Pan KIR2DL1/2/3, 3DL1 blockade; n=3
No ab
KIR block
CD8: Cytolytic Marker Granzyme B
% degranulating
CD8 Treg Degranulation
No ab
KIR block
% of Caspase+ CD4 CD25+
KIR3DL1 blockade; n=1
No block
anti-KIR3DL1
Dead CD4 T Cells

FIG. 11

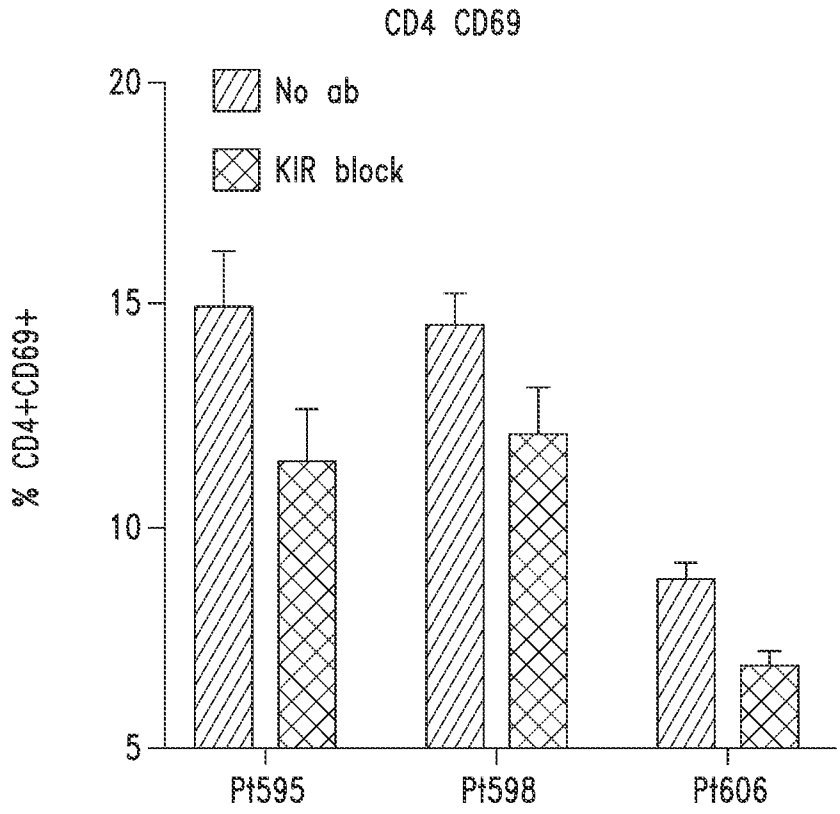


FIG. 12

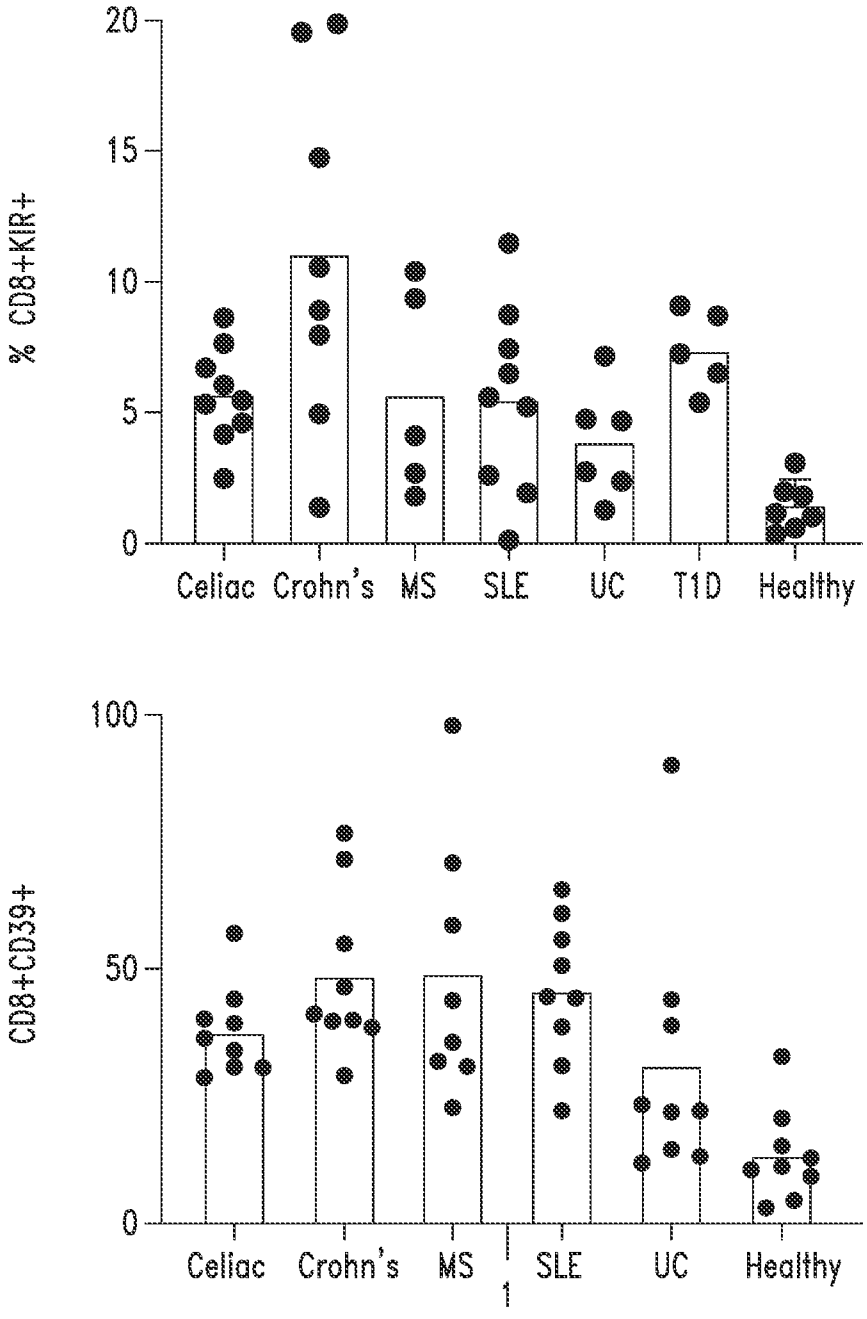

% CD8+KIR+
20
15
10
5
0
Celiac
Crohn's
MS
SLE
UC
T1D
Healthy
CD8+CD39+
100
50
0
Celiac
Crohn's
MS
SLE
UC
Healthy

FIG. 13

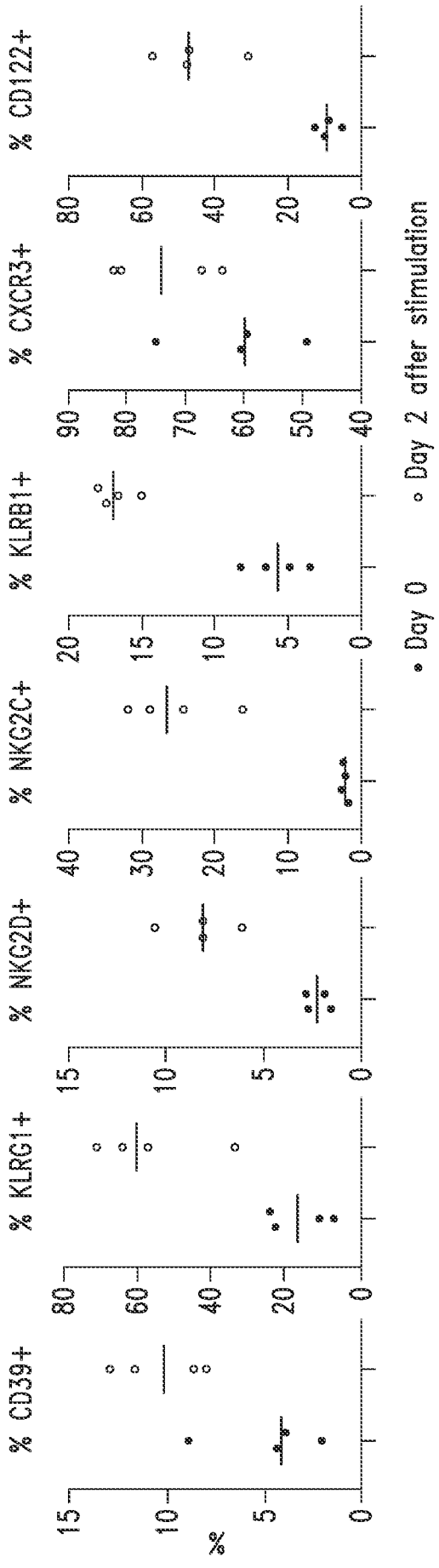

% CD39+
% KLRG1+
% NKG2D+
% NKG2C+
% KLRB1+
% CXCR3+
% CD122+
%
Day 0
Day 2 after stimulation

FIG. 14

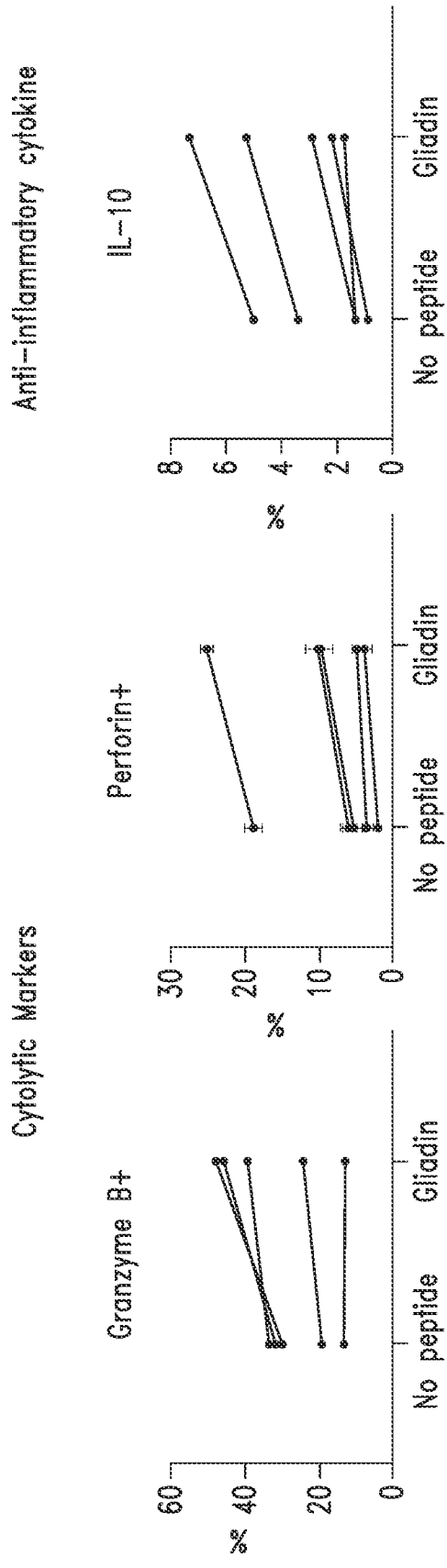

Cytolytic Markers
Granzyme B+
Perforin+
Anti-inflammatory cytokine
IL-10
%
60
40
20
0
30
20
10
0
8
6
4
2
0
No peptide
Gliadin

FIG. 15

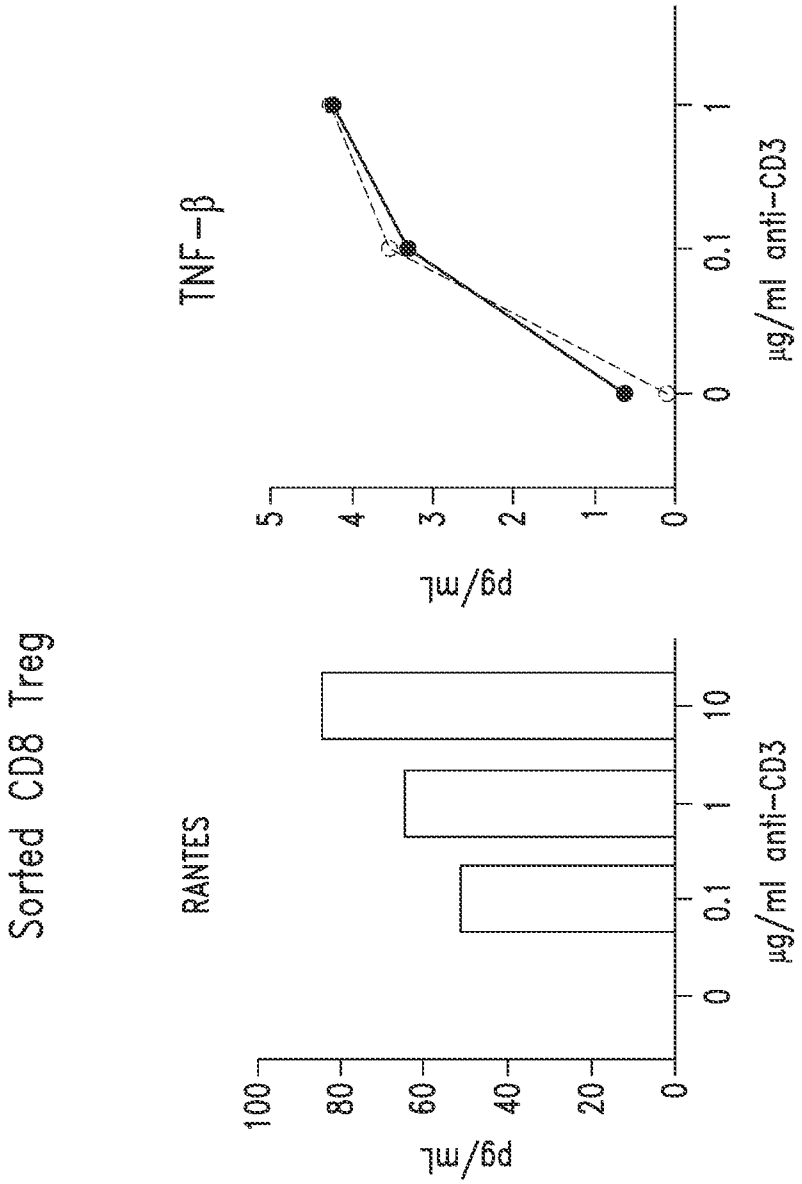

Sorted CD8 Treg
RANTES
pg/mL
100
80
60
40
20
0
0
0.1
1
10
µg/ml anti-CD3
TNF-β
pg/mL
5
4
3
2
1
0
0
0.1
1
µg/ml anti-CD3

FIG. 16

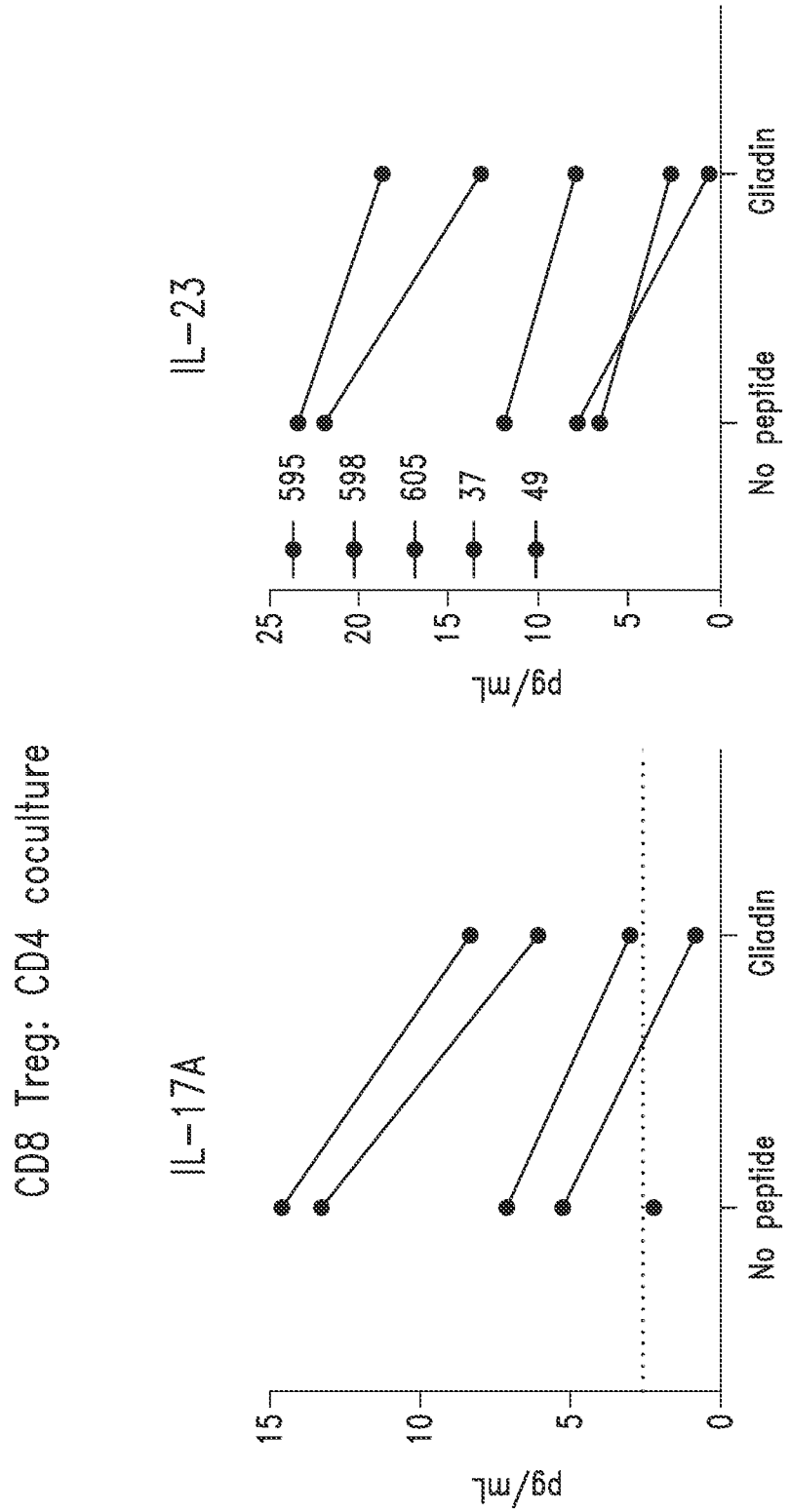

CD8 Treg: CD4 coculture
IL-17A
IL-23
pg/mL
15
10
5
0
25
20
15
10
5
0
595
598
605
37
49
No peptide
Gliadin

FIG. 17

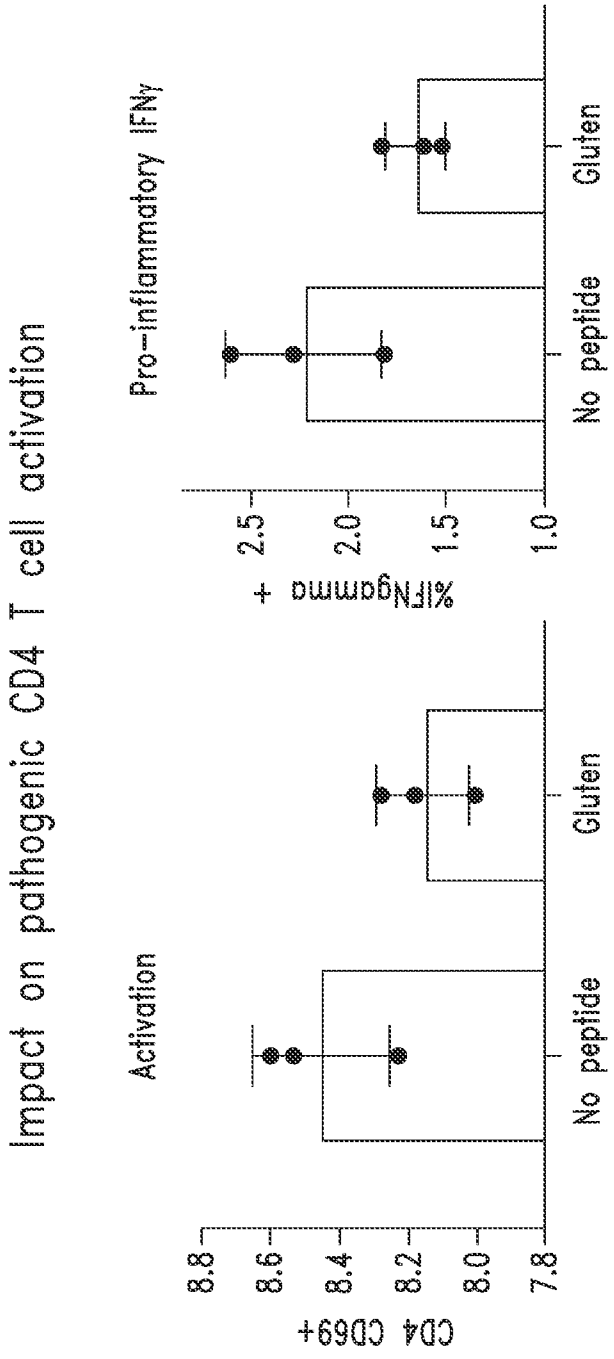

Impact on pathogenic CD4 T cell activation
Activation
Pro-inflammatory IFNγ
CD4 CD69+
%IFNgamma +
8.8
8.6
8.4
8.2
8.0
7.8
2.5
2.0
1.5
1.0
No peptide
Gluten
No peptide
Gluten
Representative patient, n=5

FIG. 18

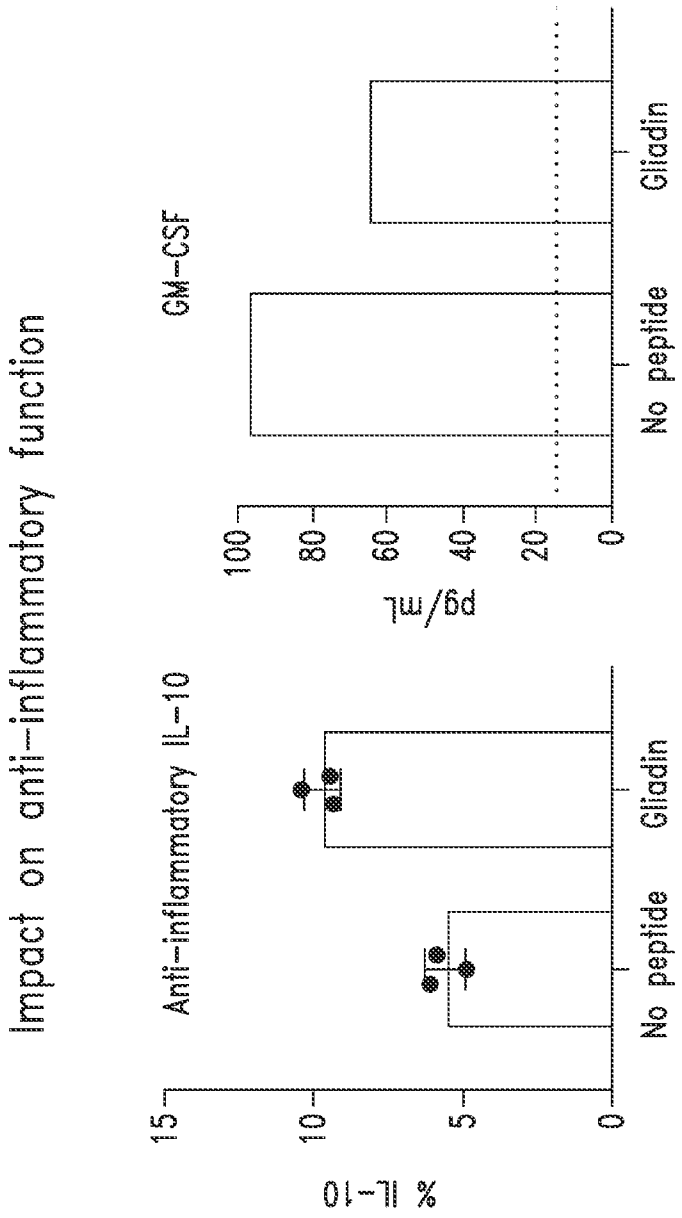

Impact on anti-inflammatory function
Anti-inflammatory IL-10
% IL-10
15
10
5
0
No peptide
Gliadin
GM-CSF
pg/mL
100
80
60
40
20
0
No peptide
Gliadin

FIG. 19

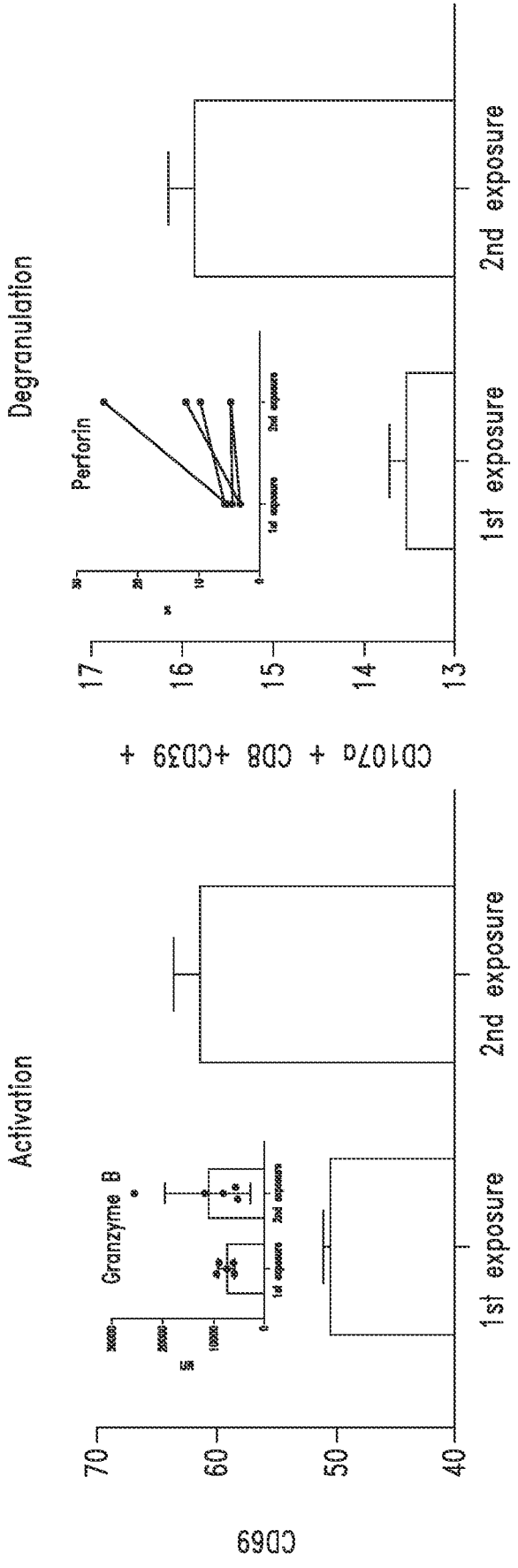

Activation
CD69
70
60
50
40
Granzyme B
1st exposure
2nd exposure
Degranulation
CD107a + CD8 +CD39 +
17
16
15
14
13
Perforin
1st exposure
2nd exposure

FIG. 20

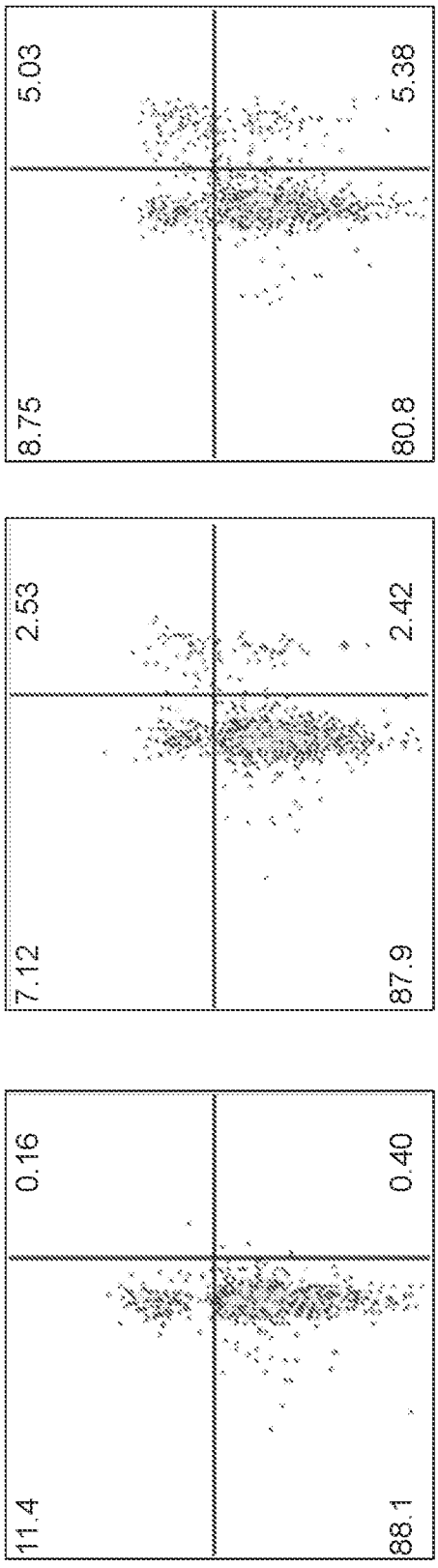

5.03
5.38
8.75
80.8
2.53
2.42
7.12
87.9
0.16
0.40
11.4
88.1
KIR3DL1
KIR2DL1/2/3

FIG. 21

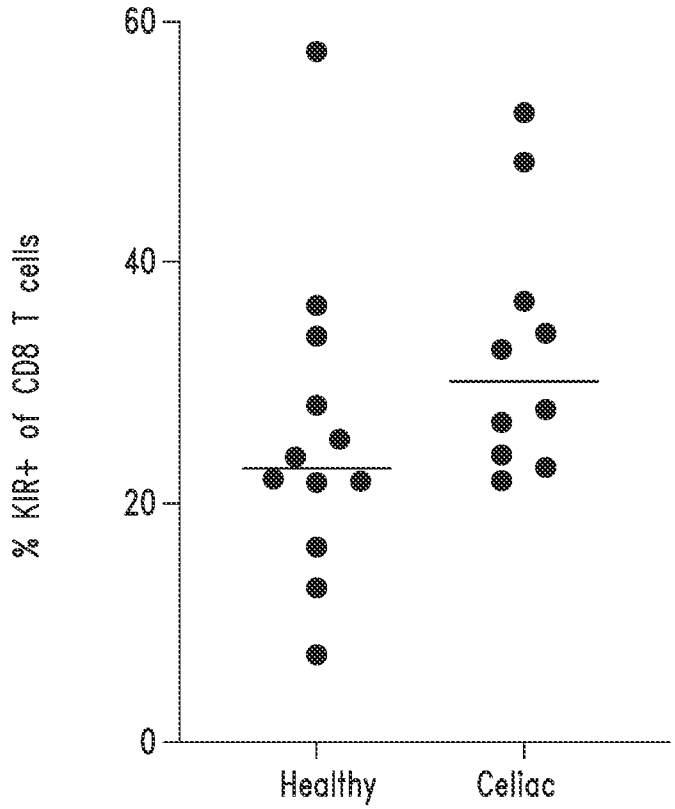


FIG. 22

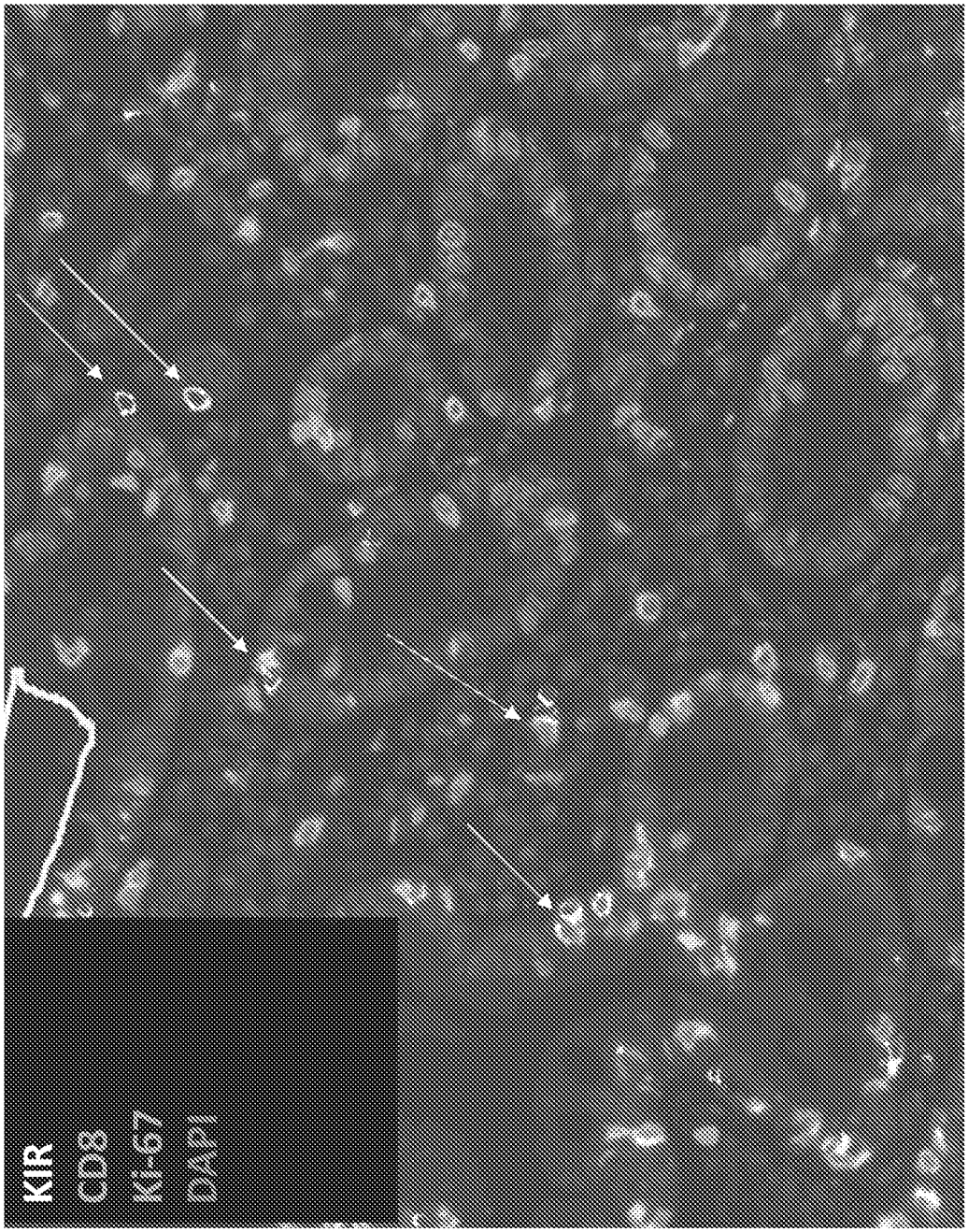


FIG. 23

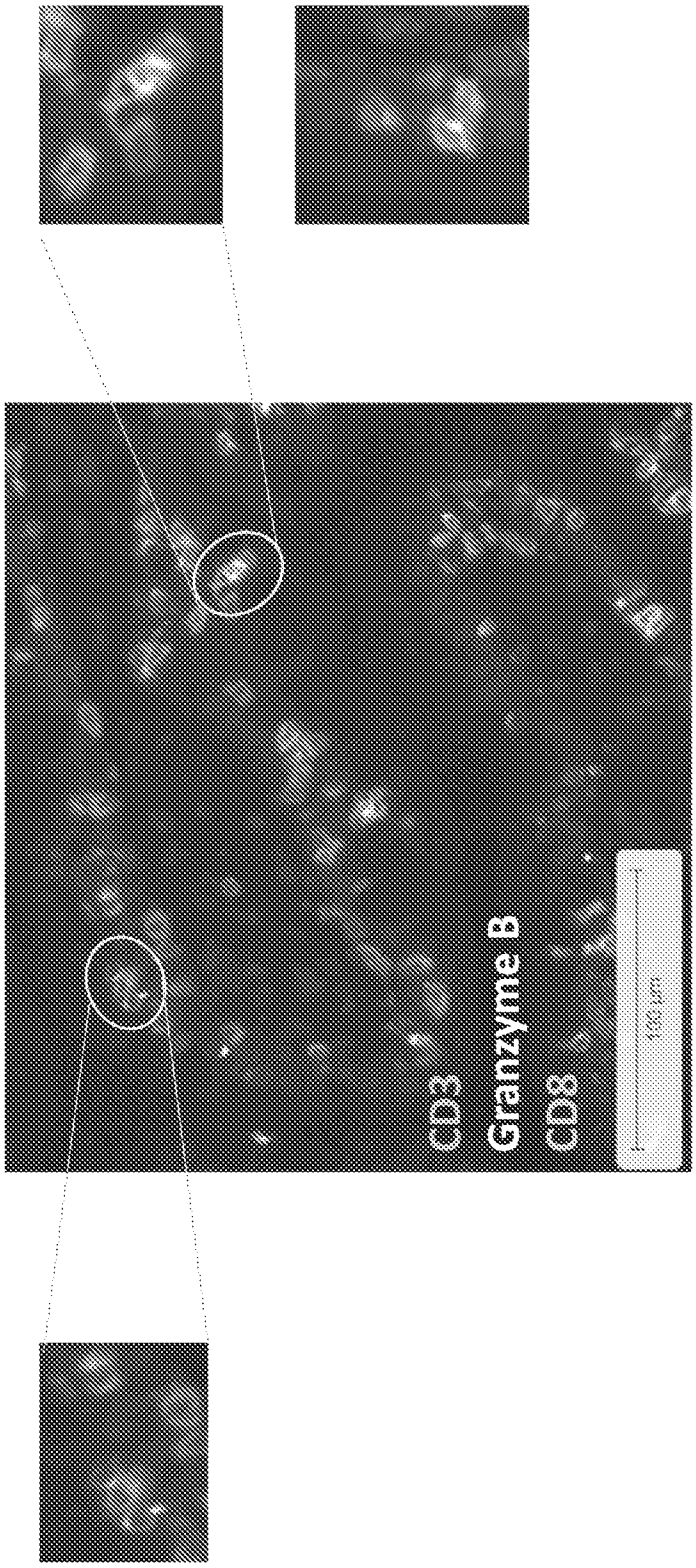


FIG. 24

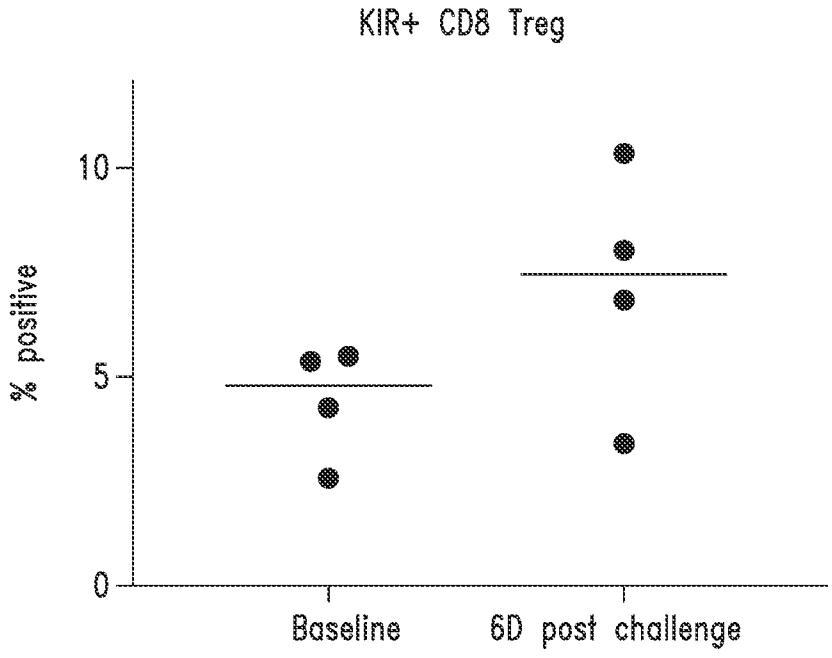


FIG. 25

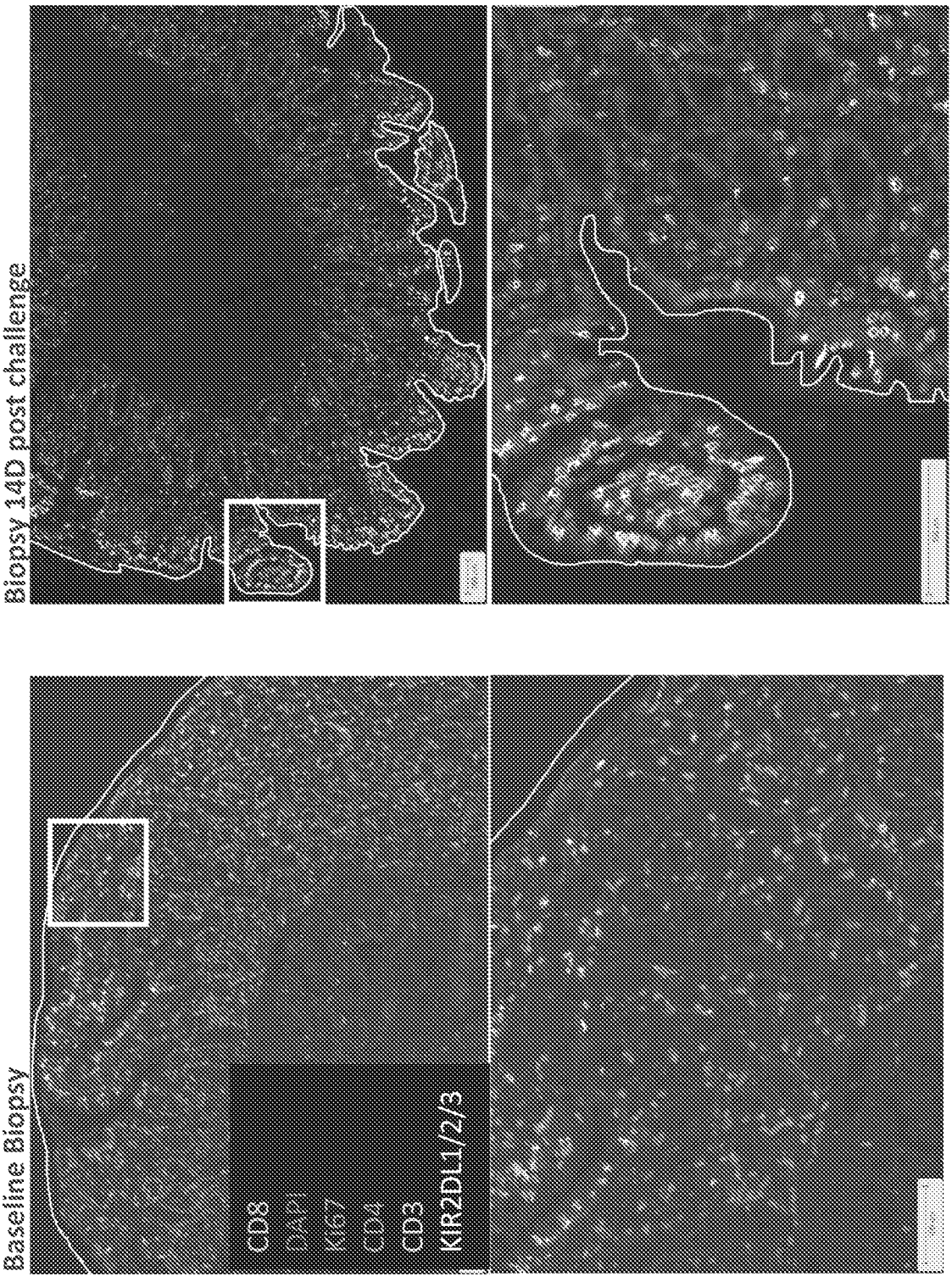

Biopsy 14D post challenge
Baseline Biopsy
CD8
DAPI
Ki67
CD4
CD3
KIR2DL1/2/3

FIG. 26

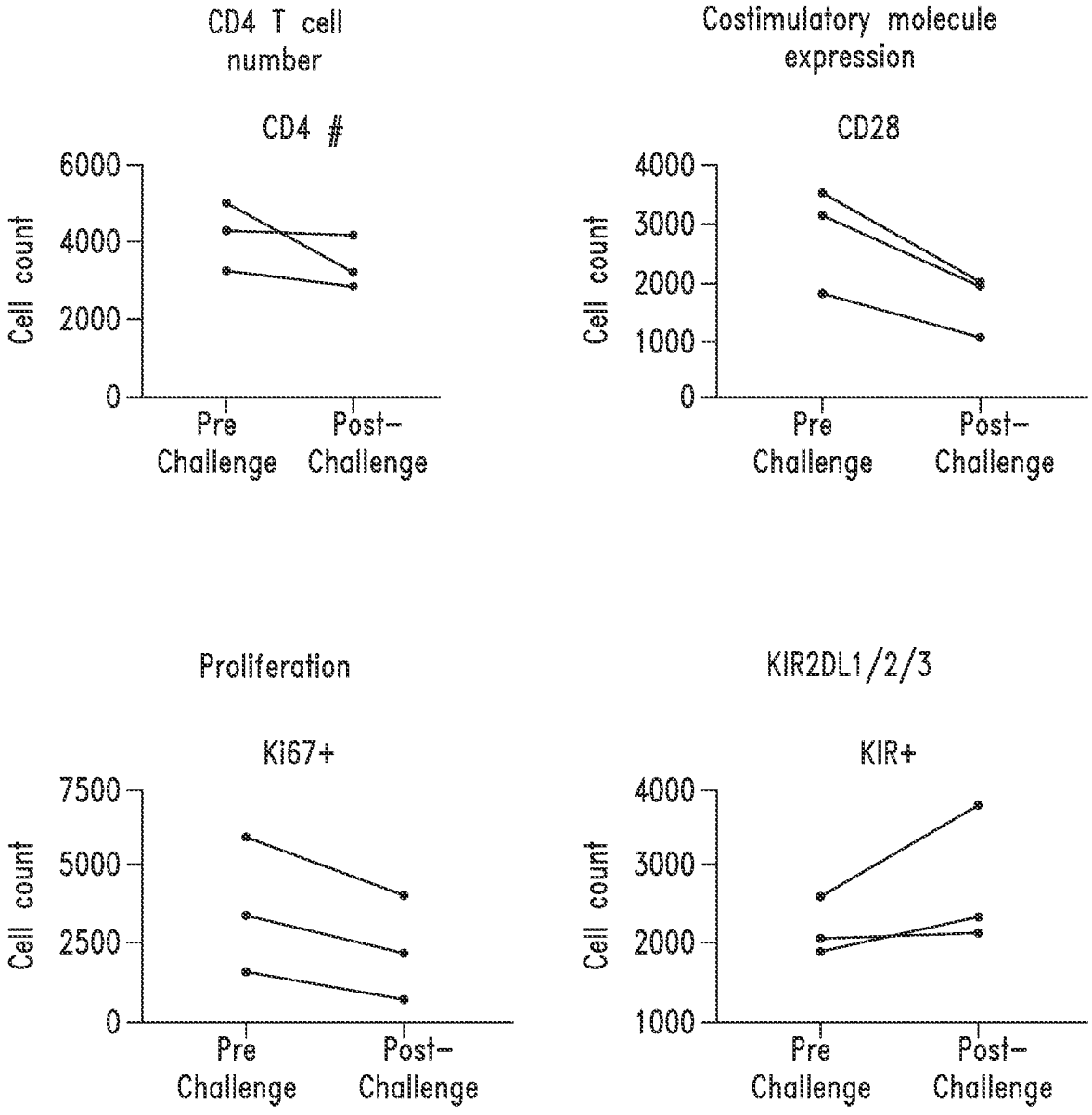

CD4 T cell number
CD4 #
Cell count
6000
4000
2000
0
Pre Challenge
Post-Challenge
Costimulatory molecule expression
CD28
Cell count
4000
3000
2000
1000
0
Pre Challenge
Post-Challenge
Proliferation
Ki67+
Cell count
7500
5000
2500
0
Pre Challenge
Post-Challenge
KIR2DL1/2/3
KIR+
Cell count
4000
3000
2000
1000
Pre Challenge
Post-Challenge

FIG. 27

KIR dependent modulation of CD8 T regulatory cells
Single Checkpoint Blockade + non-signaling tether
KIR + CD8
KIR + CXCR3
Dual Checkpoint Blockade
KIR + PD-1
KIR + CD5
Checkpoint Blockade + Costimulation
KIR + ICOS
Checkpoint Blockade + Activation
KIR + CD3
Strength of CD8 T Regulatory Cell Modulation Signal
Anti-CD8α
Anti-KIR
Anti-PD-1
Anti-KIR
Anti-ICOS
Anti-KIR
Anti-CD3
Anti-KIR

FIG. 28

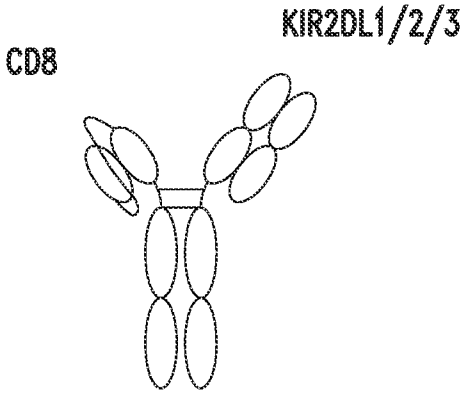


FIG. 29A

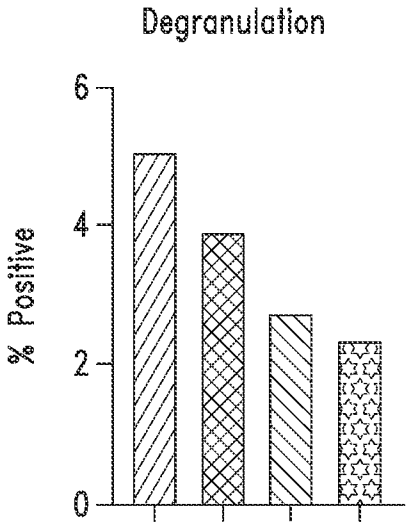


FIG. 29B

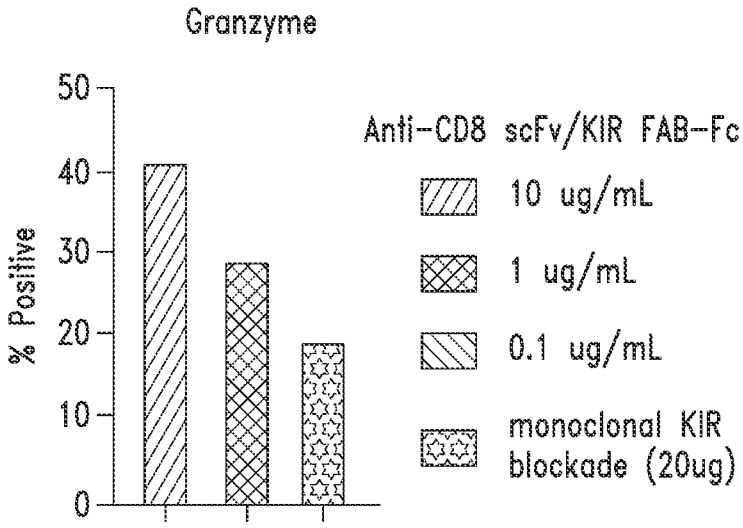


FIG. 29C

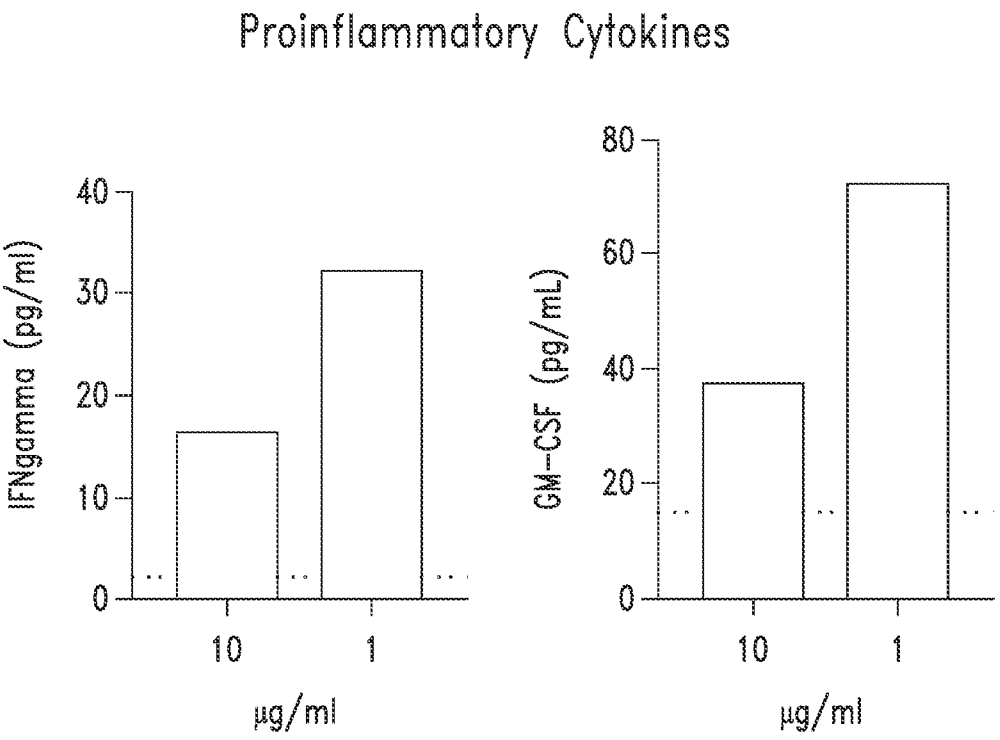

Proinflammatory Cytokines
IFNgamma (pg/ml)
40
30
20
10
0
10
1
µg/ml
GM-CSF (pg/mL)
80
60
40
20
0
10
1
µg/ml

FIG. 30

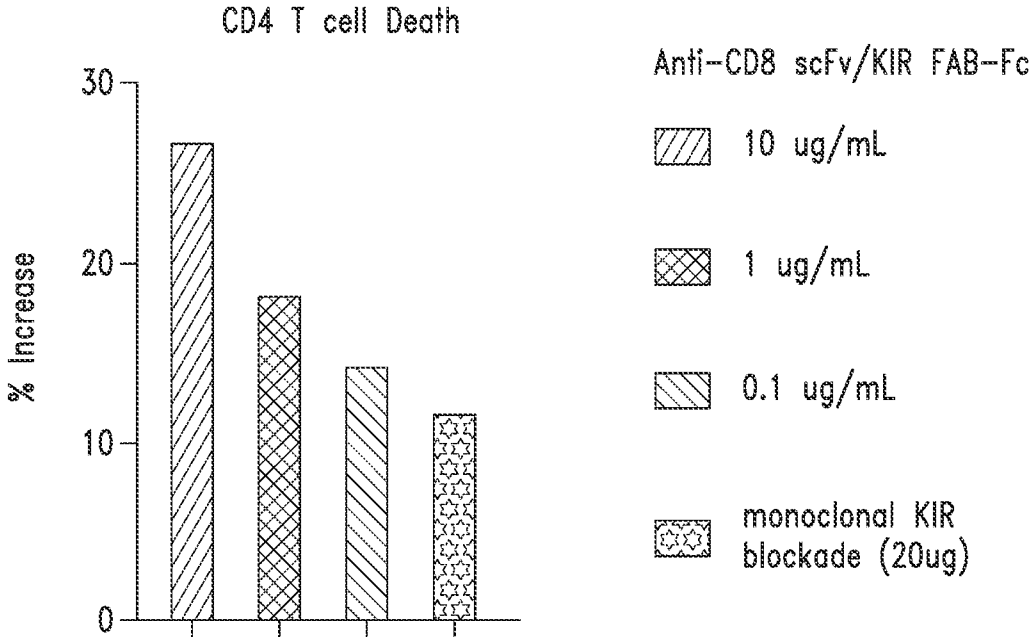

CD4 T cell Death
% Increase
30
20
10
0
Anti-CD8 scFv/KIR FAB-Fc
10 ug/mL
1 ug/mL
0.1 ug/mL
monoclonal KIR blockade (20ug)

FIG. 31

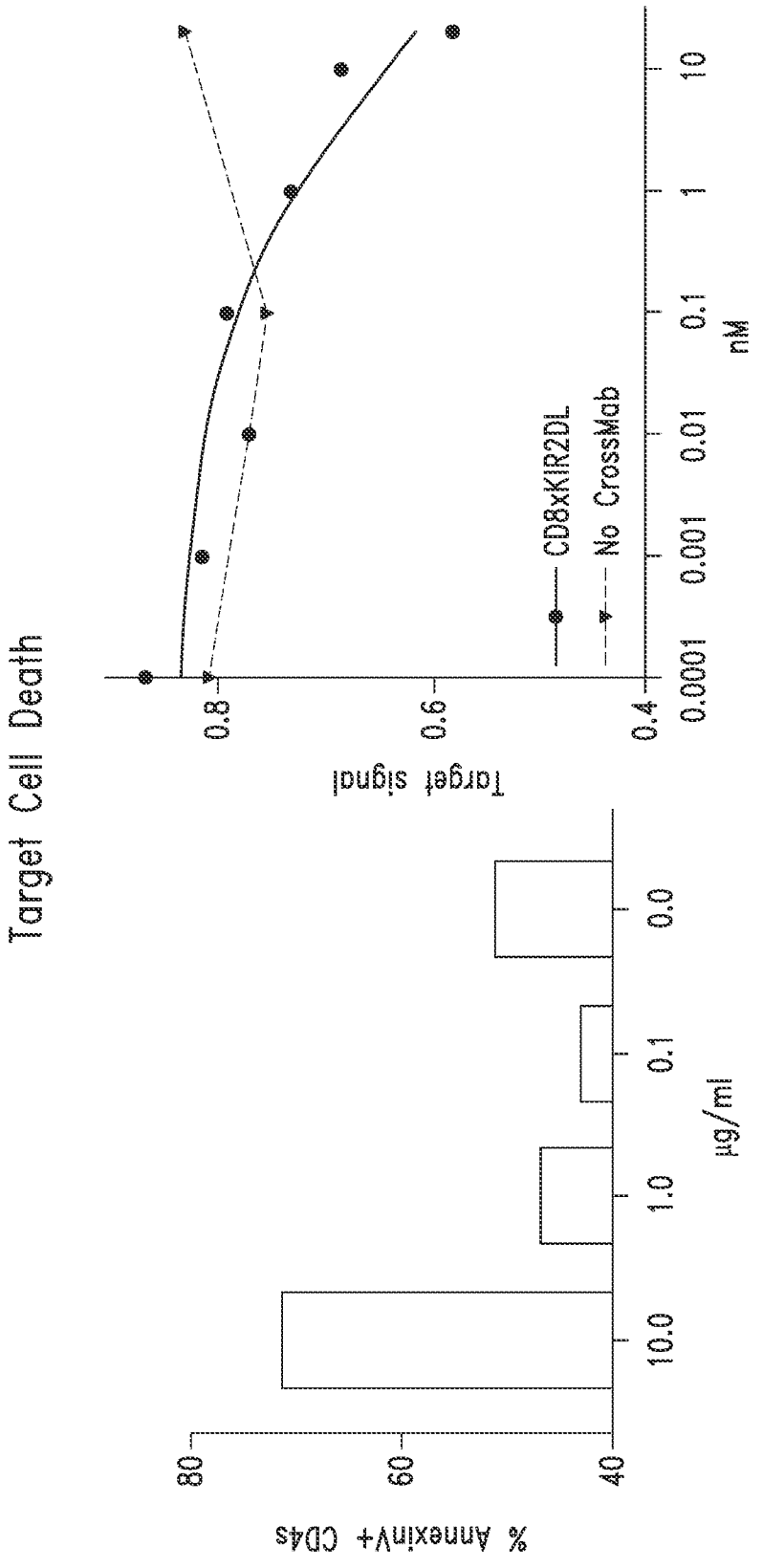

Target Cell Death
Target signal
0.8
0.6
0.4
CD8xKIR2DL
No CrossMab
0.0001
0.001
0.01
0.1
1
10
nM
% AnnexinV+ CD4s
80
60
40
10.0
1.0
0.1
0.0
µg/ml

FIG. 32

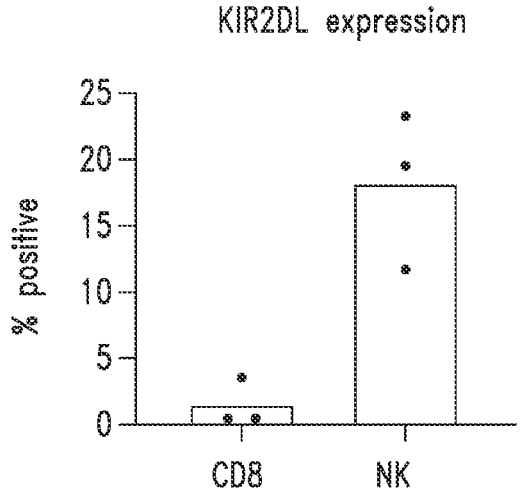

KIR2DL expression
% positive
25
20
15
10
5
0
CD8
NK

FIG. 33

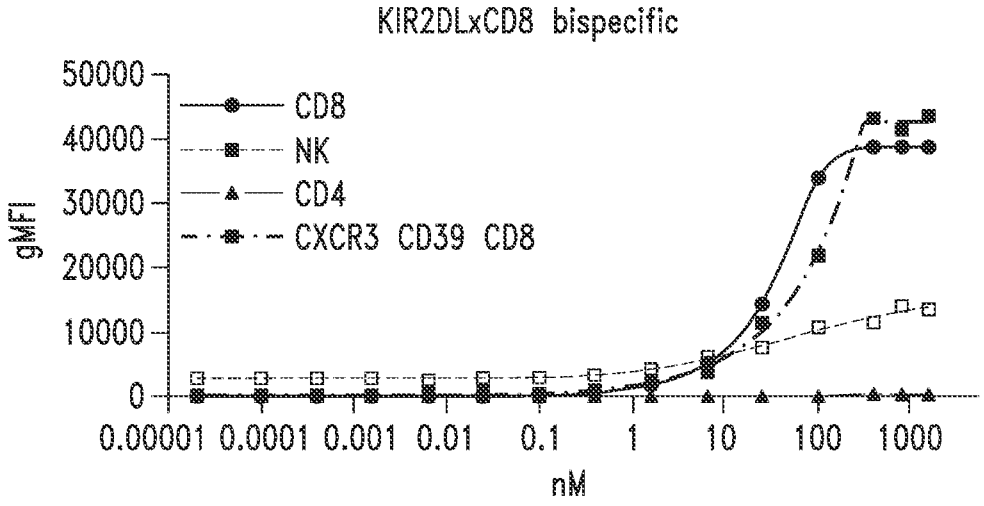

KIR2DLxCD8 bispecific
gMFI
50000
40000
30000
20000
10000
0
CD8
NK
CD4
CXCR3 CD39 CD8
0.00001 0.0001 0.001 0.01 0.1 1 10 100 1000
nM

FIG. 34

SEQUENCE LISTING

<110> Mozart Therapeutics, Inc.

<120> BINDING AGENTS AND METHODS OF USING THE SAME

<130> 670151.403WO

<150> US 63/145394
<151> 2021-02-03

<150> US 63/148016
<151> 2021-02-10

<150> US 63/161325
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<150> US 63/209949
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<151> 2022-01-10

<160> 201

<170> PatentIn version 3.5

<210> 1
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Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe

50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

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Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45

Asp Thr Ser Lys Val Ala Ser Gly Val Pro Tyr Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr

85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

<210> 3
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Phe Thr Arg Tyr Thr Met His
1 5

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Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys
1 5 10 15

Asp

<210> 5
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Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr

1 5 10

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Arg Ala Ser Ser Ser Val Ser Tyr Met Asn
1 5 10

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Asp Thr Ser Lys Val Ala Ser
1 5

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Gln Gln Trp Ser Ser Asn Pro Leu Thr
1 5

<210> 9
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<220>
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<400> 9

```
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1               5                   10                  15


Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30


Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45


Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
    50                  55                  60


Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80


Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110


Thr Thr Leu Thr Val Ser Ser
        115
```

<210> 10
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<400> 10

```
Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1               5                   10                  15
```

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20   25   30

Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
        35   40   45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser
    50   55   60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu
65   70   75   80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85   90   95

Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn
            100   105

<210> 11
<211> 7
<212> PRT
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<400> 11

Phe Thr Arg Tyr Thr Met His
1   5

<210> 12
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<213> Artificial Sequence

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<400> 12

Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys

1 5 10 15

Asp

<210> 13
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH muromonab HCDR3

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Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr
1 5 10

<210> 14
<211> 10
<212> PRT
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<220>
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Ser Ala Ser Ser Ser Val Ser Tyr Met Asn
1 5 10

<210> 15
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Asp Thr Ser Lys Leu Ala Ser
1 5

<210> 16

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Trp Ser Ser Asn Pro Phe Thr
1 5

<210> 17
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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Asp Ser Tyr Val Ser Trp Phe
100 105 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120   125

<210> 18
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<213> Artificial Sequence

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<400> 18

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1   5   10   15

Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
            20   25   30

Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Lys Ser Pro Arg Gly
        35   40   45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
    50   55   60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Ser Gly Ala
65   70   75   80

Gln Pro Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
                85   90   95

His Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100   105

<210> 19
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<220>
<223> CD3epsilon VH XmAb13676 HCDR1

<400> 19

Thr Tyr Ala Met Asn
1   5

<210> 20
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH XmAb13676 HCDR2

<400> 20

Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser
1   5   10   15

Val Lys Gly

<210> 21
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH XmAb13676 HCDR3

<400> 21

His Gly Asn Phe Gly Asp Ser Tyr Val Ser Trp Phe Ala Tyr
1   5   10

<210> 22
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon LCDR1

<400> 22

Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn

1 5 10

<210> 23
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon LCDR2

<400> 23

Gly Thr Asn Lys Arg Ala Pro
1 5

<210> 24
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon LCDR3

<400> 24

Ala Leu Trp Tyr Ser Asn His Trp Val
1 5

<210> 25
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH gOKT3-5

<400> 25

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile

```
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Pro Val Thr Val Ser Ser
        115


<210>  26
<211>  107
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  CD3epsilon VL

<400>  26

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
```

65 70 75 80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
100 105

<210> 27
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH gOKT3-5 HCDR1

<400> 27

Arg Tyr Thr Met His
1 5

<210> 28
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH gOKT3-5 HCDR2

<400> 28

Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val Lys
1 5 10 15

Asp

<210> 29
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH gOKT3-5 HCDR3

<400> 29

Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr
1 5 10

<210> 30
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR1

<400> 30

Ser Ala Ser Ser Ser Val Ser Tyr Met Asn
1 5 10

<210> 31
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR2

<400> 31

Asp Thr Ser Lys Leu Ala Ser
1 5

<210> 32
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR3

<400> 32

Gln Gln Trp Ser Ser Asn Pro Phe Thr
1 5

<210> 33

<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH JA185

<400> 33

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

<210> 34
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL

<400> 34

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30


Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45


Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60


Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80


Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95


Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105


<210>  35
<211>  12
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  CD3epsilon VH JA185 HCDR1

<400>  35

Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met
1               5                   10


<210>  36
<211>  14
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  CD3epsilon VH JA185 HCDR2
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<400> 36

Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys
1 5 10

<210> 37
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH JA185 HCDR3

<400> 37

Tyr Tyr Asp Asp His Tyr Cys Leu
1 5

<210> 38
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR1

<400> 38

Ser Ala Ser Ser Ser Val Ser Tyr Met Asn
1 5 10

<210> 39
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR2

<400> 39

Asp Thr Ser Lys Leu Ala Ser
1 5

<210> 40

<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR3

<400> 40

Gln Gln Trp Ser Ser Asn Pro Phe
1 5

<210> 41
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH JA198

<400> 41

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met Val Lys Asp
50 55 60

Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe Leu Gln
65 70 75 80

Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys Ala Arg
85 90 95

Tyr Tyr Gln Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

Leu Thr Val Ser Ser
        115

<210> 42
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH JA198 HCDR1

<400> 42

Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met
1   5   10

<210> 43
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH JA198 HCDR2

<400> 43

Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met
1   5   10

<210> 44
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH JA198 HCDR3

<400> 44

Tyr Tyr Gln Asp His Tyr Cys Leu
1   5

<210> 45
<211> 119
<212> PRT

<213> Artificial Sequence

<220>
<223> CD3epsilon VH JA207

<400> 45

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1   5   10   15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20   25   30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35   40   45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50   55   60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Asn Thr Ala Phe
65   70   75   80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85   90   95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100   105   110

Thr Thr Leu Thr Val Ser Ser
        115

<210> 46
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH JA207 HCDR1

<400> 46

Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met

1 5 10

<210> 47
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH JA207 HCDR2

<400> 47

Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys
1 5 10

<210> 48
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH JA207 HCDR3

<400> 48

Tyr Tyr Asp Asp His Tyr Cys Leu
1 5

<210> 49
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH 28F11

<400> 49

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Lys Phe Ser Gly Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

35 40 45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gln Met Gly Tyr Trp His Phe Asp Leu Trp Gly Arg Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 50
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL

<400> 50

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro

65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
85 90 95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 51
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH 28F11 HCDR1

<400> 51

Gly Tyr Gly Met His
1 5

<210> 52
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH 28F11 HCDR2

<400> 52

Val Ile Trp Tyr Asp Gly Ser Lys Lys Tyr Tyr Val Asp Ser Val Lys
1 5 10 15

Gly

<210> 53
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH 28F11 HCDR3

<400> 53

Gln Met Gly Tyr Trp His Phe Asp Leu
1 5

<210> 54
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR1

<400> 54

Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 55
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR2

<400> 55

Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 56
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR3

<400> 56

Gln Gln Arg Ser Asn Trp Pro Pro Leu Thr
1 5 10

<210> 57

<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH 27H5

<400> 57

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Ile Ile Trp Tyr Asp Gly Ser Lys Lys Asn Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Thr Gly Tyr Asn Trp Phe Asp Pro Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 58
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL

<400> 58

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Glu Ile Val Leu Thr Gln Ser Pro Arg Thr Leu Ser Leu Ser Pro Gly
1               5                  10                  15


Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
            20                  25                  30


Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35                  40                  45


Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60


Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Asp
65                  70                  75                  80


Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
                85                  90                  95


Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
            100                 105


<210>  59
<211>  5
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  CD3epsilon VH 27H5 HCDR1

<400>  59

Ser Tyr Gly Met His
1               5


<210>  60
<211>  17
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  CD3epsilon VH 27H5 HCDR2
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<400> 60

Ile Ile Trp Tyr Asp Gly Ser Lys Lys Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 61
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH 27H5 HCDR3

<400> 61

Gly Thr Gly Tyr Asn Trp Phe Asp Pro
1 5

<210> 62
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR1

<400> 62

Arg Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala
1 5 10

<210> 63
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR2

<400> 63

Gly Ala Ser Ser Arg Ala Thr

1 5

<210> 64
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL LCDR3

<400> 64

Gln Gln Tyr Gly Ser Ser Pro Ile Thr
1 5

<210> 65
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH 15C3

<400> 65

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Ala Ile Trp Tyr Asn Gly Arg Lys Gln Asp Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Arg Gly Thr Gly Tyr Asn Trp Phe Asp Pro Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 66
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL2 15C3

<400> 66

Ala Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Ile
85 90 95

Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
100 105

<210> 67
<211> 5
<212> PRT

<213> Artificial Sequence

<220>
<223> CD3epsilon VH 15C3 HCDR1

<400> 67

Ser Tyr Gly Met His
1 5

<210> 68
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH 15C3 HCDR2

<400> 68

Ala Ile Trp Tyr Asn Gly Arg Lys Gln Asp Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 69
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VH 15C3 HCDR3

<400> 69

Gly Thr Gly Tyr Asn Trp Phe Asp Pro
1 5

<210> 70
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL2 15C3 LCDR1

<400> 70

Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

<210> 71
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL2 15C3 LCDR2

<400> 71

Asp Ala Ser Ser Leu Glu Ser
1 5

<210> 72
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL2 15C3 LCDR3

<400> 72

Gln Gln Phe Asn Ser Tyr Pro Ile Thr
1 5

<210> 73
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VH Mb1a

<400> 73

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr

```
            20                  25                  30

Tyr Ile His Phe Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Arg Ile Asp Pro Ala Asn Asp Asn Thr Leu Tyr Ala Ser Lys Phe
    50                  55                  60

Gln Gly Lys Ala Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Gly Arg Gly Tyr Gly Tyr Tyr Val Phe Asp His Trp Gly Gln Gly Thr
            100                 105                 110

Leu Val Thr Val Ser Ser
        115


<210>  74
<211>  107
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  CD8alpha VL

<400>  74

Asp Val Gln Ile Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Thr Ser Arg Ser Ile Ser Gln Tyr
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
```

50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln His Asn Glu Asn Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 75
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VH Mb1a HCDR1

<400> 75

Gly Phe Asn Ile Lys Asp Thr
1 5

<210> 76
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VH Mb1a HCDR2

<400> 76

Arg Ile Asp Pro Ala Asn Asp Asn Thr
1 5

<210> 77
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VH Mb1a HCDR3

<400> 77

Gly Tyr Tyr Val Phe Asp His
1 5

<210> 78
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VL LCDR1

<400> 78

Arg Thr Ser Arg Ser Ile Ser Gln Tyr Leu Ala
1 5 10

<210> 79
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VL LCDR2

<400> 79

Ser Gly Ser Thr Leu Gln Ser
1 5

<210> 80
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VL LCDR3

<400> 80

Gln Gln His Asn Glu Asn Pro Leu Thr
1 5

<210> 81

<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VH Mb1b

<400> 81

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30

Tyr Ile His Phe Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Asp Pro Ala Asn Asp Asn Thr Leu Tyr Ala Ser Lys Phe
50 55 60

Gln Gly Lys Ala Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Gly Arg Gly Tyr Gly Tyr Tyr Val Phe Asp His Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 82
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VL

<400> 82

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Asp Val Gln Ile Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15


Asp Arg Val Thr Ile Thr Cys Arg Thr Ser Arg Ser Ile Ser Gln Tyr
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln His Asn Glu Asn Pro Leu
                85                  90                  95


Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 83
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VH Mb1b HCDR1

<400> 83

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Gly Phe Asn Ile Lys Asp Thr
1               5
```

<210> 84
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VH Mb1b HCDR2

<400> 84

Arg Ile Asp Pro Ala Asn Asp Asn Thr
1 5

<210> 85
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VH Mb1b HCDR3

<400> 85

Gly Tyr Tyr Val Phe Asp His
1 5

<210> 86
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VL LCDR1

<400> 86

Arg Thr Ser Arg Ser Ile Ser Gln Tyr Leu Ala
1 5 10

<210> 87
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VL LCDR2

<400> 87

Ser Gly Ser Thr Leu Gln Ser
1 5

<210> 88

<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha VL LCDR3

<400> 88

Gln Gln His Asn Glu Asn Pro Leu Thr
1 5

<210> 89
<211> 145
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R2HCD26 VHH

<400> 89

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Cys Ile Arg Val Ser Asp Gly Ser Thr Tyr Tyr Ala Asp Pro Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ser Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Ala Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Gly Ser Leu Tyr Thr Cys Val Gln Ser Ile Val Val Val Pro
100 105 110

Ala Arg Pro Tyr Tyr Asp Met Asp Tyr Trp Gly Lys Gly Thr Gln Val
        115   120   125

Thr Val Ser Ser Ala Ala Ala Tyr Pro Tyr Asp Val Pro Asp Tyr Gly
    130   135   140

Ser
145

<210> 90
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R2HCD26 VHH HCDR1

<400> 90

Gly Phe Thr Phe Asp Asp Tyr Ala Ile Gly
1   5   10

<210> 91
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R2HCD26 VHH HCDR2

<400> 91

Cys Ile Arg Val Ser Asp Gly Ser Thr Tyr Tyr Ala Asp Pro Val Lys
1   5   10   15

Gly

<210> 92
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R2HCD26 VHH HCDR3

<400> 92

Ala Gly Ser Leu Tyr Thr Cys Val Gln Ser Ile Val Val Val Pro Ala
1   5   10   15

Arg Pro Tyr Tyr Asp Met Asp Tyr
            20

<210> 93
<211> 132
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R3HCD27 VHH

<400> 93

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
            20   25   30

Ala Met Ser Trp Val Arg Gln Val Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Ser Thr Ile Asn Trp Asn Gly Gly Ser Ala Glu Tyr Ala Glu Pro Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Lys Leu Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Lys Asp Ala Asp Leu Val Trp Tyr Asn Leu Ser Thr Gly Gln Gly
            100   105   110

Thr Gln Val Thr Val Ser Ser Ala Ala Ala Tyr Pro Tyr Asp Val Pro
        115   120   125

Asp Tyr Gly Ser
    130

<210> 94
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R3HCD27 VHH HCDR1

<400> 94

Gly Phe Thr Phe Asp Asp Tyr Ala Met Ser
1   5   10

<210> 95
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R3HCD27 VHH HCDR2

<400> 95

Thr Ile Asn Trp Asn Gly Gly Ser Ala Glu Tyr Ala Glu Pro Val Lys
1   5   10   15

Gly

<210> 96
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R3HCD27 VHH HCDR3

<400> 96

Lys Asp Ala Asp Leu Val Trp Tyr Asn Leu Ser
1 5 10

<210> 97
<211> 132
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R3HCD29 VHH

<400> 97

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Val Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Thr Ile Asn Trp Asn Gly Gly Ser Ala Glu Tyr Ala Glu Pro Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Leu Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asp Ala Asp Leu Val Trp Tyr Asn Leu Arg Thr Gly Gln Gly
100 105 110

Thr Gln Val Thr Val Ser Ser Ala Ala Ala Tyr Pro Tyr Asp Val Pro
115 120 125

Asp Tyr Gly Ser
130

<210> 98
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R3HCD29 VHH HCDR1

<400> 98

Gly Phe Thr Phe Asp Asp Tyr Ala Met Ser
1   5   10

<210> 99
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R3HCD29 VHH HCDR2

<400> 99

Thr Ile Asn Trp Asn Gly Gly Ser Ala Glu Tyr Ala Glu Pro Val Lys
1   5   10   15

Gly

<210> 100
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> CD8alpha R3HCD29 VHH HCDR3

<400> 100

Lys Asp Ala Asp Leu Val Trp Tyr Asn Leu Arg
1   5   10

<210> 101
<211> 123
<212> PRT

<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH IPH2102

<400> 101

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Phe Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Phe Ile Pro Ile Phe Gly Ala Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ile Pro Ser Gly Ser Tyr Tyr Tyr Asp Tyr Asp Met Asp Val
100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 102
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL

<400> 102

Glu Ile Val Leu Thr Gln Ser Pro Val Thr Leu Ser Leu Ser Pro Gly

1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Met Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 103
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH IPH2102 HCDR1

<400> 103

Phe Tyr Ala Ile Ser
1 5

<210> 104
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH IPH2102 HCDR2

<400> 104

Gly Phe Ile Pro Ile Phe Gly Ala Ala Asn Tyr Ala Gln Lys Phe
1 5 10 15

<210> 105
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH IPH2102 HCDR3

<400> 105

Ile Pro Ser Gly Ser Tyr Tyr Tyr Asp Tyr Asp Met Asp Val
1 5 10

<210> 106
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR1

<400> 106

Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 107
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR2

<400> 107

Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 108
<211> 10
<212> PRT

<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR3

<400> 108

Gln Gln Arg Ser Asn Trp Met Tyr Thr Phe
1 5 10

<210> 109
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH IPH2102-1

<400> 109

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Phe Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Phe Ile Pro Ile Phe Gly Ala Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ile Pro Ser Gly Ser Tyr Tyr Tyr Asp Tyr Asp Met Asp Val
100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser

115 120

<210> 110
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL

<400> 110

Glu Ile Val Leu Ile Gln Ser Pro Val Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Met Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 111
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH IPH2102-1 HCDR1

<400> 111

Phe Tyr Ala Ile Ser
1 5

<210> 112
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH IPH2102-1 HCDR2

<400> 112

Gly Phe Ile Pro Ile Phe Gly Ala Ala Asn Tyr Ala Gln Lys Phe
1 5 10 15

<210> 113
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH IPH2102-1 HCDR3

<400> 113

Ile Pro Ser Gly Ser Tyr Tyr Tyr Asp Tyr Asp Met Asp Val
1 5 10

<210> 114
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR1

<400> 114

Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu
1 5 10

<210> 115
<211> 7
<212> PRT

<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR2

<400> 115

Asp Ala Ser Asn Arg Ala Thr
1   5

<210> 116
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR3

<400> 116

Gln Gln Arg Ser Asn Trp Met Tyr Thr Phe
1   5   10

<210> 117
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH DF200

<400> 117

Gln Val Gln Leu Glu Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
1   5   10   15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Phe Thr Pro Tyr
            20   25   30

Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
        35   40   45

Gly Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Ala Ala Phe Ile
    50   55   60

```
Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65                  70                  75                  80


Lys Met Asn Ser Leu Gln Val Asn Asp Thr Ala Ile Tyr Tyr Cys Ala
                85                  90                  95


Arg Asn Pro Arg Pro Gly Asn Tyr Pro Tyr Gly Met Asp Tyr Trp Gly
            100                 105                 110


Gln Gly Thr Ser Val Thr Val Ser Ser
        115                 120
```

<210> 118
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL

<400> 118

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Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser Met Ser Val Gly
1               5                   10                  15


Glu Arg Val Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Val Thr Tyr
            20                  25                  30


Val Ser Trp Tyr Gln Gln Lys Pro Glu Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Gly Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60


Ser Gly Ser Ala Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ala
65                  70                  75                  80


Glu Asp Leu Ala Asp Tyr His Cys Gly Gln Gly Tyr Ser Tyr Pro Tyr
                85                  90                  95
```

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 119
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH DF200 HCDR1

<400> 119

Gly Phe Ser Phe Thr Pro Tyr Gly Val His
1 5 10

<210> 120
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH DF200 HCDR2

<400> 120

Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Ala Ala Phe Ile Ser
1 5 10 15

<210> 121
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH DF200 HCDR3

<400> 121

Asn Pro Arg Pro Gly Asn Tyr Pro Tyr Gly Met Asp Tyr
1 5 10

<210> 122
<211> 11
<212> PRT

<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR1

<400> 122

Lys Ala Ser Glu Asn Val Val Thr Tyr Val Ser
1 5 10

<210> 123
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR2

<400> 123

Gly Ala Ser Asn Arg Tyr Thr
1 5

<210> 124
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR3

<400> 124

Gly Gln Gly Tyr Ser Tyr Pro Tyr Thr
1 5

<210> 125
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH PAN2D

<400> 125

Glu Val Gln Leu Gln Gln Ser Gly Thr Val Leu Ala Arg Pro Gly Ala

1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Trp Met His Trp Met Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Thr Ile Tyr Pro Gly Asn Ser Asp Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Lys Leu Thr Ala Val Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Thr Asn Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ser Arg Pro Thr Thr Ala Thr Arg Ser Ser Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> 126
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL

<400> 126

Gln Ile Val Leu Thr Gln Ser Pro Ala Ser Met Ser Ala Ser Leu Gly
1 5 10 15

Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser Ser
20 25 30

Tyr Leu Tyr Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Trp

```
        35                  40                  45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
    50                  55                  60

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu
65                  70                  75                  80

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
                85                  90                  95

Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105


<210>  127
<211>  5
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  KIR2DL1/2/3 VH PAN2D HCDR1

<400>  127

Ser Tyr Trp Met His
1               5


<210>  128
<211>  16
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  KIR2DL1/2/3 VH PAN2D HCDR2

<400>  128

Thr Ile Tyr Pro Gly Asn Ser Asp Thr Asn Tyr Asn Gln Lys Phe Lys
1               5                   10                  15


<210>  129
<211>  12
<212>  PRT
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<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VH PAN2D HCDR3

<400> 129

Pro Thr Thr Ala Thr Arg Ser Ser Ala Met Asp Tyr
1 5 10

<210> 130
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR1

<400> 130

Thr Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu Tyr
1 5 10

<210> 131
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR2

<400> 131

Ser Thr Ser Asn Leu Ala Ser
1 5

<210> 132
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR2DL1/2/3 VL LCDR3

<400> 132

His Gln Tyr His Arg Ser Pro Pro Thr

1 5

<210> 133
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-42

<400> 133

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Asp Leu Phe Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

<210> 134
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VL

<400> 134

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Gln Ser Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1               5                  10                  15


Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
            20                  25                  30


Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
        35                  40                  45


Met Ile Tyr Asp Val Ser Asn Arg Pro Ser Gly Val Ser Asn Arg Phe
    50                  55                  60


Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65                  70                  75                  80


Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Ser Ser
                85                  90                  95


Asn Thr Leu Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu
            100                 105                 110
```

<210> 135
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-42 HCDR1

<400> 135

```
Gly Tyr Thr Phe Thr Gly Tyr Tyr Met
1               5
```

<210> 136
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-42 HCDR2

<400> 136

Ile Asn Pro Asn Ser Gly Gly Thr Asn
1 5

<210> 137
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-42 HCDR3

<400> 137

Ala Asp Leu Phe Tyr Trp
1 5

<210> 138
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VL LCDR1

<400> 138

Ser Ser Asp Val Gly Gly Tyr Asn Tyr
1 5

<210> 139
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VL LCDR2

<400> 139

Asp Val Ser
1

<210> 140

<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VL LCDR3

<400> 140

Ser Ser Tyr Thr Ser Ser Asn Thr Leu Val Phe
1 5 10

<210> 141
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-61

<400> 141

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Trp Asn Ser Gly Ser Ile Gly Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Ser Leu Arg Tyr Phe Glu Trp Pro Ile Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 142
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VL

<400> 142

Asn Phe Met Leu Thr Gln Pro Leu Ser Val Ser Glu Ser Pro Gly Lys
1   5   10   15

Thr Val Thr Ile Ser Cys Thr Ala Asn Gly Gly Ser Leu Ala Ser Lys
            20   25   30

Tyr Val Gln Trp Phe Gln Gln Arg Pro Gly Ser Ser Pro Thr Thr Val
        35   40   45

Ile Tyr Asp Asp Asn Leu Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60

Gly Ser Ile Asp Thr Ser Ser Asn Ser Ala Ala Leu Thr Ile Ser Gly
65   70   75   80

Leu Lys Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Asn
                85   90   95

Ser Ser Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100   105   110

<210> 143
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-61 HCDR1

<400> 143

Gly Phe Thr Phe Asp Asp Tyr Ala
1 5

<210> 144
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-61 HCDR2

<400> 144

Ser Trp Asn Ser Gly Ser Ile
1 5

<210> 145
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-61 HCDR3

<400> 145

Ala Ser Ser Leu Arg Tyr Phe Glu Trp Pro Ile Asp Tyr Trp
1 5 10

<210> 146
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VL LCDR1

<400> 146

Gly Gly Ser Leu Ala Ser Lys Tyr
1 5

<210> 147

<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VL LCDR2

<400> 147

Asp Asp Asn Leu
1

<210> 148
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VL LCDR3

<400> 148

Gln Ser Tyr Asp Asn Ser Ser Val Val Phe
1 5 10

<210> 149
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-74

<400> 149

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Met Asn Pro Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe

```
    50                  55                  60

Gln Gly Arg Val Thr Met Thr Arg Asn Thr Ser Ile 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

Ala Arg Tyr Ser Phe His Leu Asp Gly Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ser
        115


<210>  150
<211>  110
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  KIR3DL1 VL

<400>  150

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1               5                   10                  15

Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Val Gly Asn Asn
            20                  25                  30

Tyr Val Ser Trp Tyr Gln Gln Val Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45

Ile Tyr Asp Asn Asn Arg Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65                  70                  75                  80

Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Asn Ser Leu
```

85 90 95

Arg Val Glu Leu Phe Gly Gly Gly Thr Lys Val Thr Val Leu
100 105 110

<210> 151
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-74 HCDR1

<400> 151

Gly Gly Thr Phe Ser Ser Tyr Ala
1 5

<210> 152
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-74 HCDR2

<400> 152

Met Asn Pro Asn Ser Gly Asn Thr
1 5

<210> 153
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VH ET160-74 HCDR3

<400> 153

Ala Arg Tyr Ser Phe His Leu Asp
1 5

<210> 154

<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VL LCDR1

<400> 154

Ser Ser Asn Val Gly Asn Asn Tyr
1 5

<210> 155
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VL LCDR2

<400> 155

Asp Asn Asn
1

<210> 156
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL1 VL LCDR3

<400> 156

Tyr Cys Gly Thr Trp Asp Asn Ser Leu Arg Val Glu Leu Phe
1 5 10

<210> 157
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL2 VH AZ158

<400> 157

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Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1               5                   10                  15


Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Phe
            20                  25                  30


Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45


Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met
    50                  55                  60


Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65                  70                  75                  80


Lys Met Asn Ser Leu Gln Asn Asp Asp Thr Ala Met Tyr Tyr Cys Ala
                85                  90                  95


Arg Gly Asn Ser Asn His Tyr Val Ser Ser Phe Tyr Tyr Phe Asp Tyr
            100                 105                 110


Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
        115                 120


<210>  158
<211>  106
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  KIR3DL2 VL

<400>  158

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Leu Gly
1               5                   10                  15


Gly Lys Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Lys Tyr
            20                  25                  30
```

Ile Ala Trp Tyr Gln His Lys Pro Gly Lys Gly Pro Arg Leu Leu Ile
35 40 45

His Tyr Thr Ser Thr Leu Gln Pro Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Arg Asp Tyr Ser Phe Ser Ile Ser Asn Leu Glu Pro
65 70 75 80

Glu Asp Ile Thr Thr Tyr Tyr Cys Leu Gln Tyr Asp Asn Leu Trp Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 159
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL2 VH AZ158 HCDR1

<400> 159

Gly Phe Ser Leu Thr Ser Phe Gly Val His
1 5 10

<210> 160
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL2 VH AZ158 HCDR2

<400> 160

Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met Ser
1 5 10 15

<210> 161

<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL2 VH AZ158 HCDR3

<400> 161

Gly Asn Ser Asn His Tyr Val Ser Ser Phe Tyr Tyr Phe Asp Tyr
1 5 10 15

<210> 162
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL2 VL LCDR1

<400> 162

Lys Ala Ser Gln Asp Ile Asn Lys Tyr Ile Ala
1 5 10

<210> 163
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL2 VL LCDR2

<400> 163

Tyr Thr Ser Thr Leu Gln Pro
1 5

<210> 164
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> KIR3DL2 VL LCDR3

<400> 164

Leu Gln Tyr Asp Asn Leu Trp Thr
1 5

<210> 165
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<220>
<221> VARIANT
<222> (6)..(25)
<223> amino acids 6-25, 11-25, 16-25, or 21-25 may be present or absent

<400> 165

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25

<210> 166
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL1 15C3

<400> 166

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

```
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80


Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Trp
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 167
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL1 15C3 LCDR1

<400> 167

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Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1               5                   10
```

<210> 168
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL1 15C3 LCDR2

<400> 168

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Asp Ala Ser Asn Arg Ala Thr
1               5
```

<210> 169
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3epsilon VL1 15C3 LCDR3

<400> 169

Gln Gln Arg Ser Asn Trp Pro Trp Thr
1   5

<210> 170
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> ICOS VH 422 H2L5

<400> 170

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20   25   30

Ala Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45

Gly Leu Ile Ser Ile Tyr Ser Asp His Thr Asn Tyr Asn Gln Lys Phe
    50   55   60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr 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

Gly Arg Asn Asn Tyr Gly Asn Tyr Gly Trp Tyr Phe Asp Val Trp Gly
            100   105   110

Gln Gly Thr Thr Val Thr Val Ser Ser
        115   120

<210> 171
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> ICOS VL

<400> 171

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1   5   10   15

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20   25   30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
        35   40   45

Asp Thr Ser Lys Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser
    50   55   60

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

Asp Phe Ala Val Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Tyr Thr
                85   90   95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100   105

<210> 172
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> ICOS VH 422 H2L5 HCDR1

<400> 172

Asp Tyr Ala Met His

1 5

<210> 173
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> ICOS VH 422 H2L5 HCDR2

<400> 173

Leu Ile Ser Ile Tyr Ser Asp His Thr Asn Tyr Asn Gln Lys Phe Gln
1 5 10 15

Gly

<210> 174
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> ICOS VH 422 H2L5 HCDR3

<400> 174

Asn Asn Tyr Gly Asn Tyr Gly Trp Tyr Phe Asp Val
1 5 10

<210> 175
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> ICOS VL LCDR1

<400> 175

Ser Ala Ser Ser Ser Val Ser Tyr Met His
1 5 10

<210> 176

<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> ICOS VL LCDR2

<400> 176

Asp Thr Ser Lys Leu Ala Ser
1 5

<210> 177
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> ICOS VL LCDR3

<400> 177

Phe Gln Gly Ser Gly Tyr Pro Tyr Thr
1 5

<210> 178
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> PD-1 VH Pembrolizumab

<400> 178

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe

```
    50                  55                  60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser
        115                 120


<210>  179
<211>  111
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  PD-1 VL

<400>  179

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser
            20                  25                  30

Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
        35                  40                  45

Arg Leu Leu Ile Tyr Leu Ala Ser Tyr Leu Glu Ser Gly Val Pro Ala
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65                  70                  75                  80

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Ser Arg
```

85 90 95

Asp Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 180
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> PD-1 VH Pembrolizumab HCDR1

<400> 180

Asn Tyr Tyr Met Tyr
1 5

<210> 181
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> PD-1 VH Pembrolizumab HCDR2

<400> 181

Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe Lys
1 5 10 15

Asn

<210> 182
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> PD-1 VH Pembrolizumab HCDR3

<400> 182

Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr

1 5 10

<210> 183
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> PD-1 VL LCDR1

<400> 183

Arg Ala Ser Lys Gly Val Ser Thr Ser Gly Tyr Ser Tyr Leu His
1 5 10 15

<210> 184
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> PD-1 VL LCDR2

<400> 184

Leu Ala Ser Tyr Leu Glu Ser
1 5

<210> 185
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> PD-1 VL LCDR3

<400> 185

Gln His Ser Arg Asp Leu Pro Leu Thr
1 5

<210> 186
<211> 124
<212> PRT
<213> Artificial Sequence

<220>
<223> CXCR3 VH 4Hu7

<400> 186

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Lys Lys Pro Gly Gly
1   5   10   15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20   25   30

Ala Met Ser Trp Val Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Ala Thr Ile Ser Asn Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Phe
    50   55   60

Gln Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Ser Thr Leu Ser
65   70   75   80

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
                85   90   95

Ser Arg Pro Ser Glu Arg Ser His Tyr Tyr Ala Thr Ser Gln Phe Ala
            100   105   110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
        115   120

<210> 187
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> CXCR3 VL

<400> 187

Asp Ile Gln Leu Thr Gln Ser Pro Gly Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20   25   30

His Trp Tyr Gln Gln Lys Pro Gly Thr Ser Pro Lys Arg Trp Ile Tyr
        35   40   45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50   55   60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Leu Gln Pro Glu
65   70   75   80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Ser Pro Leu Thr
                85   90   95

Phe Gly Ala Gly Thr Lys Val Glu Leu Lys
            100   105

<210> 188
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CXCR3 VH 4Hu7 HCDR1

<400> 188

Gly Phe Thr Phe Ser Asn Tyr Ala
1   5

<210> 189
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CXCR3 VH 4Hu7 HCDR2

<400> 189

Ile Ser Asn Gly Gly Ser Tyr Thr

1 5

<210> 190
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> CXCR3 VH 4Hu7 HCDR3

<400> 190

Ser Arg Pro Ser Glu Arg Ser His Tyr Tyr Ala Thr Ser Gln Phe Ala
1 5 10 15

Tyr

<210> 191
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> CXCR3 VL LCDR1

<400> 191

Ser Ser Val Ser Tyr
1 5

<210> 192
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> CXCR3 VL LCDR2

<400> 192

Asp Thr Ser
1

<210> 193

<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CXCR3 VL LCDR3

<400> 193

Gln Gln Trp Ser Ser Ser Pro Leu Thr
1   5

<210> 194
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> CD5 VH 5D7 H1L1

<400> 194

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1   5   10   15

Ser Leu Arg Leu Ser Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
            20   25   30

Gly Met Gly Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
        35   40   45

Trp Val Ala His Ile Trp Trp Asp Asp Asp Val Tyr Tyr Asn Pro Ser
    50   55   60

Leu Lys Ser Arg Leu Thr Ile Thr Lys Asp Ala Ser Thr Asp Thr Val
65   70   75   80

Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr
                85   90   95

Cys Val Arg Arg Arg Ala Thr Gly Thr Gly Phe Asp Tyr Trp Gly Gln
            100   105   110

Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 195
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> CD5 VL

<400> 195

Asn Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Val Gly Thr Ala
            20   25   30

Val Ala Trp Tyr Gln Gln Lys Pro Asp Gln Ser Pro Lys Leu Leu Ile
        35   40   45

Tyr Trp Thr Ser Thr Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Ile Ala Thr Tyr Phe Cys His Gln Tyr Asn Ser Tyr Asn Thr
                85   90   95

Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
            100   105

<210> 196
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CD5 VH 5D7 H1L1 HCDR1

<400> 196

Phe Ser Leu Ser Thr Ser Gly Met Gly
1 5

<210> 197
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> CD5 VH 5D7 H1L1 HCDR2

<400> 197

Trp Trp Asp Asp Asp
1 5

<210> 198
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> CD5 VH 5D7 H1L1 HCDR3

<400> 198

Arg Arg Ala Thr Gly Thr Gly Phe Asp Tyr
1 5 10

<210> 199
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> CD5 VL LCDR1

<400> 199

Gln Asp Val Gly Thr Ala
1 5

<210> 200

<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> CD5 VL LCDR2

<400> 200

Trp Thr Ser Thr Arg His Thr
1 5

<210> 201
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> CD5 VL LCDR3

<400> 201

Tyr Asn Ser Tyr Asn Thr
1 5