

LILRB1-BASED CHIMERIC ANTIGEN RECEPTOR

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional patent applications 62/946,888, filed on December 11, 2019, and 63/085,969 filed on August 30, 2020, the contents of each of which are incorporated herein by reference in their entireties.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted in ASCII format via EFS-WEB and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 11, 2020 is named A2BI-015-01WO_SeqList.txt and is 228 KB in size.

BACKGROUND

[0003] Chimeric antigen receptor (CAR) T cell therapy, and T Cell Receptor (TCR) therapy, is proving to be an effective therapeutic approach to various diseases, particularly hematological malignancies but also other cancers. CAR NK cells may also have clinical applications. Conventional CARs provide a stimulatory signal to the engineered immune cell (*e.g.* a T cell or an NK cell). In CAR-T cells, this results in killing activity towards the target cell identified by the antigen-binding domain of the CAR. Inhibitory CARs (iCARs) have been developed as a means to control cell activity or restrict the activity of an activator CAR to specific cell types. Fedorov et al. *Sci. Transl. Med.* 5(215):215ra172 (2013). The inhibitory CAR generally has the intracellular domain of an inhibitory signaling molecule (such as PD-1 or CTLA-4) fused to an antigen-binding domain (*e.g.*, a single-chain variable fragment, scFv) through a transmembrane region and optionally a hinge region.

[0004] Numerous alternative iCAR architectures have been described in the art. However, there remains an unmet need for novel alternative inhibitory receptors and identification of particular inhibitory receptor architectures having superior performance, along with associated compositions and methods of use thereof.

SUMMARY

[0005] In one aspect, the disclosure provides chimeric antigen receptors having the hinge, transmembrane region, and/or intracellular domain of LILRB1, or functional fragments or variants

thereof. The chimeric antigen receptor may include single polypeptide, or more than one polypeptide. The receptors may include one or more, or all of the following: (a) an LILRB1 hinge domain or functional fragment or variant thereof; (b) an LILRB1 transmembrane domain or a functional variant thereof; and (c) an LILRB1 intracellular domain or a functional variant thereof, such as an LILRB1 intracellular domain and/or an intracellular domain comprising at least one immunoreceptor tyrosine-based inhibitory motif (ITIM) found in the polypeptide sequence of LILRB1. In some embodiments, the receptor comprises at least two ITIMs found in the polypeptide sequence of LILRB1. The ITIMs of LILRB1 are NLYAAV (SEQ ID NO: 8), VTYA EV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11). The receptor may include one, two, three, four, five, six, or more of these ITIMs, in any combination including multiple copies of the same ITIM.

[0006] In some embodiments of the receptors of the disclosure, the intracellular domain comprises both ITIMs NLYAAV (SEQ ID NO: 8) and VTYA EV (SEQ ID NO: 9). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 12. In some embodiments, the intracellular domain comprises both ITIMs VTYA EV (SEQ ID NO: 9) and VTYAQL (SEQ ID NO: 10). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 13. In some embodiments, the intracellular domain comprises both ITIMs VTYAQL (SEQ ID NO: 10) and SIYATL (SEQ ID NO: 11). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 14. In some embodiments, the polypeptide comprises an intracellular domain comprising at least three immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTYA EV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11). In some embodiments, the intracellular domain comprises the ITIMs NLYAAV (SEQ ID NO: 8), VTYA EV (SEQ ID NO: 9), and VTYAQL (SEQ ID NO: 10). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 15. In some embodiments, the intracellular domain comprises the ITIMs VTYA EV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 16. In some embodiments, the intracellular domain comprises the ITIMs NLYAAV (SEQ ID NO: 8), VTYA EV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ

ID NO: 17. In some embodiments, the intracellular domain comprises a sequence at least 95% identical to the LILRB1 intracellular domain (SEQ ID NO: 7). In some embodiments, the intracellular domain comprises a sequence of SEQ ID NOS: 12-17.

[0007] In some embodiments of the receptors of the disclosure, the polypeptide comprises the LILRB1 transmembrane domain or a functional variant thereof. In some embodiments, the LILRB1 transmembrane domain or a functional variant thereof comprises a sequence at least 95% identical to SEQ ID NO: 5. In some embodiments, the LILRB1 transmembrane domain comprises SEQ ID NO: 5.

[0008] In some embodiments of the receptors of the disclosure, the polypeptide comprises the LILRB1 hinge domain or functional fragment or variant thereof. In some embodiments, the LILRB1 hinge domain or functional fragment or variant thereof comprises a sequence at least 95% identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84 or SEQ ID NO: 93. In some embodiments, the LILRB1 hinge domain comprises a sequence identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84 or SEQ ID NO: 93. In some embodiments, the LILRB1 hinge domain or functional fragment or variant thereof comprises a sequence at least 95% identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83 or SEQ ID NO: 84. In some embodiments, the LILRB1 hinge domain comprises a sequence identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83 or SEQ ID NO: 84.

[0009] In some embodiments of the receptors of the disclosure, the polypeptide comprises: (a) an LILRB1 hinge domain or functional fragment or variant thereof, and (b) the LILRB1 transmembrane domain or a functional variant thereof. In some embodiments, the polypeptide comprises a sequence at least 95% identical to SEQ ID NO: 20.

[0010] In some embodiments of the receptors of the disclosure, the polypeptide comprises: (a) the LILRB1 transmembrane domain or a functional variant thereof, and (b) an LILRB1 intracellular domain and/or an intracellular domain comprising at least two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTYA EV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11). In some embodiments, the polypeptide comprises a sequence at least

95% identical to SEQ ID NO: 21. In some embodiments, the polypeptide comprises a sequence of SEQ ID NO: 21.

[0011] In some embodiments of the receptors of the disclosure, the polypeptide comprises: (a) an LILRB1 hinge domain or functional fragment or variant thereof; (b) an LILRB1 transmembrane domain or a functional variant thereof; and (c) an LILRB1 intracellular domain and/or an intracellular domain comprising at least two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0012] In some embodiments of the receptors of the disclosure, the polypeptide comprises a sequence at least 95% identical to SEQ ID NO: 2 or SEQ ID NO: 3. In some embodiments, the polypeptide comprises a sequence at least 99% identical to SEQ ID NO: 20. In some embodiments, the polypeptide comprises a sequence at least 99% identical to SEQ ID NO: 21. In some embodiments, the polypeptide comprises a sequence at least 99% identical to SEQ ID NO: 2 or SEQ ID NO: 3. In some embodiments, the polypeptide comprises a sequence identical to SEQ ID NO: 20. In some embodiments, the polypeptide comprises a sequence identical to SEQ ID NO: 21. In some embodiments, the polypeptide comprises a sequence identical to SEQ ID NO: 2 or SEQ ID NO: 3.

[0013] In some embodiments of the receptors of the disclosure, the polypeptide comprises antigen-binding domain. In some embodiments, the antigen-binding domain is an antigen-binding domain other than the LILRB1 extracellular ligand binding protein. In some embodiments, the polypeptide comprises two or more antigen-binding domains. In some embodiments, the antigen-binding domain comprises a single chain variable fragment (scFv). In some embodiments, the receptor comprises a second polypeptide. In some embodiments, the first polypeptide comprises a first chain of an antibody and the second polypeptide comprise a second chain of said antibody. In some embodiments, the receptor comprises a Fab fragment of an antibody. In some embodiments, (a) the first polypeptide comprises an antigen-binding fragment of the heavy chain of the antibody, and (b) the second polypeptide comprises an antigen-binding fragment of the light chain of the antibody. In some embodiments, (a) the first polypeptide comprises an antigen-binding fragment of the light chain of the antibody, and (b) the second polypeptide comprises an antigen-binding fragment of the heavy chain of the antibody. In some embodiments, the first polypeptide comprises a first chain of a T-cell receptor (TCR) and the second polypeptide comprises a second chain of

said TCR. In some embodiments, the receptor comprises an extracellular fragment of a T cell receptor (TCR). In some embodiments, (a) the first polypeptide comprises an antigen-binding fragment of an alpha chain of the TCR, and (b) the second polypeptide comprises an antigen-binding fragment of the beta chain of the TCR. In some embodiments, (a) the first polypeptide comprises an antigen-binding fragment of the beta chain of the TCR, and (b) the second polypeptide comprises an antigen-binding fragment of the alpha chain of the TCR. In some embodiments, the receptor comprises a single-chain TCR. In some embodiments, the scFv comprises the complementarity determined regions (CDRs) of any one of SEQ ID NOS: 22-33. In some embodiments, the scFv comprises a sequence at least 95% identical to any one of SEQ ID NOS: 35-46 or 125. In some embodiments, the scFv comprises a sequence at least 95% identical to any one of SEQ ID NOS: 35, 39, 46 or 125. In some embodiments, the scFv comprises a sequence identical to any one of SEQ ID NOS: 35-46 or 125. In some embodiments, the scFv comprises a sequence identical to any one of SEQ ID NOS: 35, 39, 46 or 125. In some embodiments, the heavy chain of the antibody comprises the heavy chain CDRs of any one of SEQ ID NOS: 25-27 or 31-33, and wherein the light chain of the antibody comprises the light chain CDRs of any one of SEQ ID NOS: 22-24 or 28-30. In some embodiments, the heavy chain of the antibody comprises a sequence at least 95% identical to the heavy chain portion of any one of SEQ ID NOS: 35-46 or 125, and wherein the light chain of the antibody comprises a sequence at least 95% identical to the light chain portion of any one of SEQ ID NOS: 35-46 or 125. In some embodiments, the heavy chain of the antibody comprises a sequence identical to the heavy chain portion of any one of SEQ ID NOS: 35-46 or 125, and wherein the light chain of the antibody comprises a sequence identical to the light chain portion of any one of SEQ ID NOS: 35-46 or 125. In some embodiments, the heavy chain of the antibody comprises a sequence identical to the heavy chain portion of any one of SEQ ID NOS: 35, 39, 46 or 125, and wherein the light chain of the antibody comprises a sequence identical to the light chain portion of any one of SEQ ID NOS: 35, 39, 46 or 125.

[0014] In some embodiments of the receptors of the disclosure, the receptor comprises an amino acid sequence at least 95% identical to any one of SEQ ID NOS: 47-71, 77-79, 89-92, 120 or 122. In some embodiments, the receptor comprises an amino acid sequence of SEQ ID NOS: 47-71, 77-79, 89-92, 120 or 122.

[0015] In some embodiments of the receptors of the disclosure, the receptor is an inhibitory receptor.

[0016] The disclosure provides a polynucleotide comprising a nucleic acid sequence encoding the receptor or polypeptide of the disclosure.

[0017] The disclosure provides a vector comprising the polynucleotide of the disclosure. In some embodiments, the vector further comprises a sequence encoding a promoter operably linked to the polynucleotide.

[0018] The disclosure provides an immune cell comprising the receptor, polynucleotide, polypeptide or receptor of the disclosure. In some embodiments, the immune cell activation is reduced when the cell is contacted with the antigen or a cell expressing the antigen on its surface. In some embodiments, immune cell activation comprises expression of a gene operatively linked to an NFAT promoter. In some embodiments, the immune cell is a T cell. In some embodiments, further comprises an activator receptor. In some embodiments, the activator receptor is a chimeric antigen receptor or a T cell receptor.

[0019] The disclosure provides methods making an immune cell, comprising introducing the polynucleotide or vector of the disclosure into the immune cell. In some embodiments, the immune cell expresses the receptor. In some embodiments, the cell is an immune cell. In some embodiments, the immune cell is a T cell. In some embodiments, immune cell activation is reduced when the cell is contacted with an antigen specific to the chimeric antigen receptor, or a cell expressing the antigen on its surface. In some embodiments, immune cell activation comprises expression of a gene operatively linked to an NFAT promoter.

[0020] The disclosure provides methods of treating a subject with a disease or a disorder, comprising administering to the subject a plurality of the immune cells of the disclosure. In some embodiments, the disease or disorder is cancer.

[0021] The disclosure provides a kit, comprising the receptor, polypeptide, polynucleotide, vector or immune cell of the disclosure.

[0022] The disclosure provides an immune cell comprising a chimeric antigen receptor comprising a polypeptide, wherein the polypeptide sequence shares at least 95% identity or at least 100% identity to SEQ ID NO: 21.

[0023] In some embodiments of the immune cells of the disclosure, the polypeptide sequence shares at least 95% identity or at least 100% identity to SEQ ID NO: 3. In some embodiments,

the polypeptide sequence shares at least 95% identity or at least 100% identity to SEQ ID NO: 2. In some embodiments, the chimeric antigen receptor comprises an antigen-binding domain comprising CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, CDR-H3 sequences according to SEQ ID NO: 22-27, respectively. In some embodiments, the chimeric antigen receptor comprises an antigen-binding domain comprising CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, CDR-H3 sequences according to SEQ ID NO: 28-33, respectively. In some embodiments, the polypeptide sequence shares at least 95% identity or at least 100% identity to SEQ ID NO: 122. In some embodiments, the polypeptide sequence shares at least 95% identity or at least 100% identity with any one of SEQ ID NOS: 35, 39, 46 or 125 in combination with SEQ ID NO: 2.

[0024] In some embodiments, the immune cell is a T cell. In some embodiments, the T cell comprises a chimeric antigen receptor or T cell receptor that specifically binds to a target expressed on tumor cells. In some embodiments, the T cell comprises a chimeric antigen receptor or T cell receptor that specifically binds to a target selected from etiolate receptor, $\alpha\beta\beta$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD19, CD20, CD22, CD30, CD33, CD37, CD44, CD44v6, CD44v7/8, CD70, CD123, CD138, CD171, CEA, DLL4, EGP-2, EGP-40, CSPG4, EGFR, EGFR family including ErbB2 (HER2), EGFRvIII, EPCAM, EphA2, EpCAM, FAP, FBP, fetal acetylcholine receptor, Fzd7, GD2, GD3, Glypican-3 (GPC3), h5T4, IL-11R, IL13R-a2, KDR, κ light chain, λ light chain, LeY, LI CAM, MAGE-A1, mesothelin, MHC presented peptides, MUC1, MUC16, NCAM, NKG2D ligands, Notch1, Notch2/3, NY-ESO-1, PRAME, PSCA, PSMA, Survivin, TAG-72, TEMs, TERT, VEGFR2, and ROR1.

[0025] The disclosure provide methods of treating and/or preventing cancer in a subject in need thereof, comprising administering to the subject the immune cells of the disclosure. In some embodiments, the method comprises treating and/or preventing cancer in a subject in need thereof, comprising administering to the subject the immune cells of the disclosure.

[0001] Illustrative CARs provided herein include, without limitation, antibody-based CARs such as single-chain variable fragment (scFv) CARs, Fab CARs, or others; and T cell receptor (TCR)-based CARs.

[0002] In other aspects, the disclosure provides polynucleotides encoding such receptors; vectors for delivery of such polynucleotides; and immune cells with such polynucleotide and receptors.

[0003] In further aspects, the disclosure provides methods of introducing polynucleotide or vectors encoding such receptors into a cell. Advantageously, immune cell activation is reduced when the cell is contacted with the antigen or a cell expressing the antigen on its surface.

[0004] Yet further aspects and embodiments of the invention are provided in the detailed description that follows.

BRIEF DESCRIPTION OF DRAWINGS

[0005] FIG. 1 shows an illustrative diagram of domain arrangement in an embodiment having a ligand binding domain (LBD), hinge, transmembrane (TM), and intracellular signaling domain (ICD).

[0006] FIGS. 2A-2B show illustrative diagrams of domain arrangements in embodiments having a ligand binding domain (LBD), hinge, transmembrane (TM), and intracellular signaling domain (ICD). When the ligand binding domain comprises two peptides, for example a heterodimeric LBD from a T Cell Receptor, each peptide may be fused with a hinge, TM and intracellular domain (FIG. 2A). Alternatively, only one peptide of the ligand binding domain may be fused to the hinge, TM and intracellular domain (FIG. 2B).

[0007] FIG. 3 shows four illustrative embodiments of immune cells having an activator scFv-based chimeric antigen receptor (CAR) [101 and 102] or activator T cell receptor [103 and 104] and an inhibitory scFv based CAR [101 and 103] or inhibitory TCR-based CAR [102 and 104].

[0008] FIG. 4 shows a chart of luminescence in an NFAT-based reporter assay (relative luminescence units, RLU) at varying concentrations of MAGE-A3 activating peptide 1 (MP1, microliters, μ M) for the indicated constructs in the presence of 50 μ M NY-ESO-1 peptide.

[0009] FIG. 5 shows a chart of luminescence in an NFAT-based reporter assay (RLU) at varying concentrations of MAGE-A3 peptide 1 (MP1, nM) for the indicated constructs in the presence of various concentrations (μ M) of NY-ESO-1 peptide.

[0010] FIG. 6 shows a chart of luminescence in an NFAT-based reporter assay (RLU) at varying concentrations of MAGE-A3 peptide 1 (MP1, nM) for the indicated constructs in the presence of various concentrations (μ M) of NY-ESO-1 peptide.

[0011] FIG. 7 shows a chart of luminescence in an NFAT-based reporter assay (RLU) at varying concentrations of MAGE-A3 peptide 1 (MP1, nM) for the indicated constructs in the presence of 50 μ M NY-ESO-1 peptide.

[0012] FIG. 8 shows a chart of luminescence in an NFAT-based reporter assay (RLU) at varying concentrations of MAGE-A3 peptide 1 (MP1, nM) for the indicated constructs in the presence of 50 μ M NY-ESO-1 peptide.

[0013] FIG. 9 shows a chart of luminescence in an NFAT-based reporter assay (RLU) at varying concentrations of MAGE-A3 peptide 1 (MP1, nM) for the indicated constructs in the presence of 5 μ M NY-ESO-1 peptide.

[0014] FIG. 10 shows a chart of luminescence in an NFAT-based reporter assay (RLU) at varying concentrations of MAGE-A3 peptide 1 (MP1, nM) for the indicated constructs in the presence of 50 μ M NY-ESO-1 peptide.

[0015] FIG. 11 shows a chart of luminescence in an NFAT-based report assay (RLU) at varying concentrations of MAGE-A3 peptide 1 (MP1, nM) for the indicated constructs in the presence of 50 μ M NY-ESO-1 peptide.

[0016] FIG. 12A is a plot showing the effect of LIR-1 hinge on the ability of an HLA-A*02 scFv inhibitory receptor to block activation of Jurkat cells by a KRAS TCR. H: hinge, T: transmembrane domain, ICD: intracellular domain, s: short. LIR-1 constructs are described in more detail in FIG. 12B. Humanized PA2.1 and humanized BB7.2 with shorter LIR-1 hinge block similarly to original, longer hinge.

[0017] FIG. 12B is a plot and a table showing EC50 shift (+/- HLA-A*02 target cells) for Jurkat cells expressing a KRAS TCR activator and the HLA-A*02 scFv LIR-1 inhibitory receptor shown in the table at bottom.

[0018] FIG. 13A is a plot showing the effect of LIR-1 hinge on the ability of an HLA-A*02 inhibitory receptor to block activation of Jurkat cells by a KRAS TCR. H: hinge, TM: transmembrane domain, ICD: intracellular domain, s: short; tr: truncated. LIR-1 constructs are described in more detail in FIG. 13B. Mouse PA2.1 with slightly longer hinges function similarly to original LIR-1 hinge in T2-Jurkat assay.

[0019] FIG. 13B is a plot and a pair of tables showing EC50 shift (+/- HLA-A*02 target cells) for Jurkat cells expressing a KRAS TCR activator and the HLA-A*02 scFv LIR-1 inhibitory receptors shown in the table at bottom, with hinge lengths shown in the table at left.

[0020] FIG. 14A is a diagram showing a schematic of T2-Jurkat experiments to evaluate blocker constructs.

[0021] FIG. 14B is a plot, table and diagram showing the effect of various NY-ESO-1 scFv LBD blocker modules (PD-1, CTLA-4, LIR-1) on EC50 of MAGE-A3 CAR activator (MP1-LBD 1-CAR), measured by MAGE peptide titration of cells loaded with a fixed (50 μ M) NY-ESO-1 blocker peptide concentration. In each of FIGS. 14B-14F, NFAT-luciferase signal of Jurkat cells transfected with either activator CAR alone or in combination with each blocker receptor after 6 hours of co-culture with activator and blocker peptide-loaded T2 cells was assayed. The baseline (Jurkat only) varies with different activator alone constructs and can be especially high with CARs; in most cases expression of the blocker receptor absent its ligand suppresses the baseline. Activator peptide concentrations range from 0 then 10^{-6} to 10^2 μ M and luminescence measurements ranged from 0 to 80000 RLU.

[0022] FIG. 14C is a plot, table and diagram showing the effect of LIR-1 blocker receptor with various scFv LBDs (ESO, MP1 LBD 1, MP1 LBD 2, HPV E6 LBD 1, HPV E6 LBD 2, HPV E7) on EC50 of MAGE-A3 CAR activator (MP1-CAR) when loaded with corresponding blocker peptide at fixed (50 μ M) peptide concentration, as in FIG. 14B. RLU = relative light units; error bars indicate $\pm$ SD (n=2). Activator peptide concentrations range from 0 then 10^{-3} to 10^2 μ M and luminescence measurements ranged from 0 to 100000 RLU.

[0023] FIG. 14D is a plot, table and diagram showing the effect of LIR-1 blocker receptor with NY-ESO-1 scFv LBD on EC50 of different MAGE-A3 CAR activators (MP1-LBD 1-CAR or MP2-CAR) when loaded with 50uM NY-ESO-1 blocker peptide. Activator peptide concentrations range from 0 then 10^{-4} to 10^2 μ M and luminescence measurements ranged from 0 to 80000 RLU.

[0024] FIG. 14E is a plot, table and diagram showing the effect of LIR-1 blocker receptor with NY-ESO-1 scFv LBD on EC50 of different TCR activators (MP1-TCR, MP2-TCR, HPV E6-TCR) when loaded with 50uM NY-ESO-1 blocker peptide. Activator peptide concentrations range from 0 then 10^{-4} to 10^2 μ M and normalized luminescence measurements ranged from 0 to 150 RLU.

[0025] FIG. 14F is a plot, table and diagram showing the effect of LIR-1 blocker receptor with NY-ESO-1 TCR LBDs on EC50 of MAGE-A3 CAR and TCR activators (MP1-LBD 1-CAR, MP1-TCR) when loaded with 50uM NY-ESO-1 blocker peptide. RLU = relative light units; error bars indicate $\pm$ SD (n=2). Activator peptide concentrations range from 0 then 10^{-7} to 10^1 μ M and normalized luminescence measurements ranged from 0 to 150 RLU.

[0026] FIG. 15 is a plot showing the effect of blocker peptide loading (50 μ M each of NY-ESO-1, MAGE-A3, HPV E6, and HPV E7) on activating MAGE-A3 CAR. MP2-CAR [0 μ M], EC50 = 44 nM; MP2-CAR [50 μ M HPVp2], EC50 = 495 nM. RLU = relative light units; error bars indicate $\pm$ SD (n = 2).

[0027] FIG. 16A is a series of plots showing NFAT-luciferase signal of Jurkat cells transfected with either activator MAGE-A3 CAR alone or in combination with various amounts of NY-ESO-1 scFv LBD blocker (DNA ratios of activator and blocker receptors components shown on left as A:B, activator receptor: blocker receptor after 6 h of co-culture with activator and blocker peptide-loaded T2 cells. T2 cells were loaded with titrated amounts of activator MAGE-A3 peptide and a fixed amount of blocker NY-ESO-1 peptide concentration. Activator and/or blocker peptide concentrations range from 0 then 10^{-6} to 10^2 μ M and luminescence measurements ranged from 0 to 200000 RLU.

[0028] FIG. 16B is a series of plots showing NFAT-luciferase signal of Jurkat cells transfected with either activator MAGE-A3 CAR alone or in combination with various amounts of NY-ESO-1 scFv LBD blocker. T2 cells were loaded with titrated amounts of blocker NY-ESO-1 peptide and a fixed amount of activator MAGE-A3 peptide concentration above the Emax concentration (~ 0.1 mM). Activator and/or blocker peptide concentrations range from 10^{-5} to 10^2 μ M and luminescence measurements ranged from 0 to 200000 RLU.

[0029] FIG. 16C is a series of plots and two tables showing NFAT-luciferase signal of Jurkat cells transfected with either activator MAGE-A3 CAR alone or in combination with various amounts of NY-ESO-1 scFv LBD blocker. The x-value blocker NY-ESO-1 peptide concentrations from FIG. 16B were normalized to the constant activator MAGE peptide concentrations used for each curve and plotted on the x-axis. The ratio of blocker peptide to activator peptide required for 50 % blocking (IC50) are indicated for each curve. For all DNA ratios, the B:A peptide ratio required is less than 1 indicating that, for this pair of activator CAR and blocker, similar (or fewer) blocker pMHC antigens are required on target cells to block activator pMHC antigens. Activator and/or blocker peptide concentrations range from 10^{-10} to 10^4 μ M and luminescence measurements ranged from 0 to 200000 RLU.

[0030] FIG. 16D is a table and a plot showing that blocking CD19-CAR activator is possible with pMHC blockers at blocker pMHC antigen densities similar to those required to activate pMHC CARs. NFAT-luciferase signal of Jurkat cells transfected with either activator CD19 CAR alone

or in combination with various amounts of NY-ESO-1 blocker (DNA ratios shown) after 6 h of co-culture with blocker peptide-loaded T2 cells which express endogenous levels of CD19 antigen. The IC₅₀ is estimated from the inhibition curves to range from 0.1-1.0 mM, corresponding to ~1,500 - 3,500 pMHCs/cell. RLU = relative light units; error bars indicate $\pm$ SD (n = 2).

[0031] FIG. 17A is a plot showing the effect of NY-ESO-1-LIR-1 blocker on EC₅₀ of activating MAGE-A3 CAR (MP1-CAR) when loaded with various concentrations of NY-ESO-1 blocker peptide. The EC₅₀ shifts are greater as the concentration of blocker peptide (NY-ESO-1) increases. The shift in the presence of a negative-control HPV peptide (binds HLA-A*02 but not NY-ESO-1 blocker scFv) is routinely seen and believed to be caused by competition of the control peptide for binding sites on the T2 HLA-A*02 molecules, reducing the number of activator targets. Activator concentrations range from 0 then 10⁻⁴ to 10² μ M and luminescence measurements ranged from 0 to 140000 RLU.

[0032] FIG. 17B is a plot showing the effect of modified LIR-1 blocker receptors containing no ICD or a mutated ICD with NY-ESO-1 scFv LBD on EC₅₀ of MAGE-A3 CAR activator (MP2-CAR) when loaded with 10 μ M of NY-ESO-1 blocker peptide. Activator concentrations range from 0 then 10⁻⁴ to 10² μ M and luminescence measurements ranged from 0 to 140000 RLU.

[0033] FIGS. 17C-17E are a series of plots showing the effect of various NY-ESO-1 scFv LBD blocker receptors (CTLA-4 (FIG. 17C), PD-1 (FIG. 17D) and LIR-1 (FIG. 17E)) on EC₅₀ of MAGE-A3 CAR activator (MP1-LBD 1-CAR) when blockers are stimulated or unstimulated. NFAT-luciferase signal of Jurkat cells transfected with either activator CAR alone or in combination with each blocker after 6 h of co-culture with peptide-loaded T2 cells. T2 cells were loaded with titrated amounts of activating MAGE peptide and tested with and without loading additional constant amount (50 μ M) of NY-ESO-1 blocker peptide. RLU = relative light units; error bars indicate $\pm$ SD (n = 2). Activator concentrations range from 0 then 10⁻⁶ to 10² μ M and luminescence measurements ranged from 0 to 100000 RLU.

[0034] FIG. 18A is a diagram and a pair of plots showing that Jurkat cells transfected with either HPV E7-CAR or HPV E7-CAR & A2-LIR-1 co-cultured with beads displaying various ratios of activator (HPV E7) and blocker (NY-ESO-1) antigen demonstrates blocking in cis but not trans.

[0035] FIG. 18B is a plot showing that HLA-A*02-LIR-1 blocker receptor blocks CD19-CAR activator at various activator to blocker ratios. Ratios ranged from 0 to 10 and luminescence (RLU) ranged from 0 to 70000.

[0036] FIG. 18C is a plot showing surface expression of titrated HLA-A*02 (A2) LIR-1 blocker receptor.

[0037] FIG. 18D is a plot showing that an scFv against HLA-A*02 can also serve as an activator when fused to activator CAR. T2 cells expressing endogenous HLA-A*02 serve as the target. RLU = relative light units; error bars indicate $\pm$ SD (n=2).

[0038] FIG. 19A is a plot showing the effect of LIR-1 blocker receptor with NY-ESO-1 scFv LBD on EC50 of different TCR activators (MP1-TCR, MP2-TCR, HPV E6-TCR) when loaded with NY-ESO-1 blocker peptide. For FIG. 14E each set was normalized to the Emax of the curve showing response of the activator only. Activator concentrations range from 0 then 10^{-4} to 10^2 μ M and luminescence measurements ranged from 0 to 140000 RLU.

[0039] FIG. 19B is a plot showing the effect of LIR-1 blocker receptor with NY-ESO-1 TCR LBDs on EC50 of MAGE-A3 CAR and TCR activators (MP1-LBD 1-CAR, MP1-TCR). Each set was normalized to the Emax of the curve showing response of the activator only. RLU = relative light units; error bars indicate $\pm$ SD (n = 2). Activator concentrations range from 0 then 10^{-7} to 10^1 μ M and luminescence measurements ranged from 0 to 140000 RLU.

[0040] FIG. 20A is a plot showing primary T cells (donor 1) transduced with HPV E7-TCR activator and ESO-LIR-1 blocker shifts EC50 ~25x in primary T cell killing assay (HPV E7 TCR EC50 = 0.044 nM; HPV E7 TCR + ESO-LIR-1, EC50 = 1.1 nM). The assay was performed using peptide-loaded MCF7 target cells at a 3:1 E:T. Luciferase measurement represents live target cells at 48 hours.

[0041] FIG. 20B is a plot showing that HLA-A*02-LIR-1 blocks NY-ESO-1 CAR activator at various activator: blocker DNA ratios in Jurkat cells using NY-ESO-1 peptide-loaded T2 target cells. RLU = relative light units; error bars indicate $\pm$ SD (n=2).

[0042] FIG. 21A is a series of images and plots showing that primary T cells (donor 1) transduced with CD19 CAR activator and HLA-A*02 blocker distinguish “tumor” cells from “normal” cells in in vitro cytotoxicity assay and demonstrate selective killing of “tumor” cells in mixed target cell assay at 3:1 E:T. Images shown were captured at 72 hours. Shown are Untransduced, CD19-CAR T cells, and CD19-CAR T + A2-LIR-1. Measurements were taken between 0 and 150 hours and normalized fluorescent protein intensity (GFP or RFP) ranged from 0 to 10.

[0043] FIG. 21B is a series of plots showing that primary T cells (donor 1) selectively kill tumor cells similarly at various tumor to “normal” cell ratios at 3:1 E:T ratios in Incucyte imaging

assay. RLU values were normalized against target cell mixtures grown in the absence of primary T cells. Shown are Untransduced, CD19-CAR T cells, and CD19-CAR T + A2-LIR-1. Measurements were taken between 0 and 150 hours and normalized fluorescent protein intensity (GFP or RFP) ranged from, on the top row, from left to right: 0 to 3×10^7 , 0 to 2×10^7 , 0 to 1.6×10^7 , 0 to 7×10^6 , 0 to 2×10^6 ; bottom row, from left to right: 0 to 7×10^5 , 0 to 2×10^5 , 0 to 4×10^5 , 0 to 6×10^5 , 0 to 7×10^5 .

[0044] FIG. 21C is a series of plots showing that primary T cells (donor 1) selectively kill tumor cells similarly at various tumor to “normal” cell ratios at 3:1 E:T ratios in quantitative target cell lysis and IFN γ secretion. Shown are Untransduced, CD19-CAR T cells, and CD19-CAR T + A2-LIR-1.

[0045] FIG. 22A is a pair of plots showing that Jurkat cells transfected with MSLN LBD1-CAR or MSLN LBD1-CAR & A2-LIR-1 co-cultured with K562 cells expressing either MSLN or MSLN & HLA-A*02 shows blocking of activation by a high-density antigen with A2-LIR-1 blocker only in the presence of HLA-A*02.

[0046] FIG. 22B is a pair of plots showing that killing of endogenous MSLN+ HeLa cells by MSLN LBD1-CAR T cells is blocked in the presence of HLA-A*02 with the A2-LIR-1 blocker.

[0047] FIG. 22C is a pair of plots showing killing of endogenous MSLN+ HeLa cells by MSLN LBD2-CAR T cells. The effect of A2-LIR-1 blocker on T cell killing is in part controlled by the activator LBD.

[0048] FIG. 23 is a plot showing that LIR-1 blocker receptors have little effect on killing efficacy of activator in absence of blocker antigen. In the absence of NY-ESO-1 blocker antigen, primary T cells (donor 1) transduced with HPV E7-TCR activator and ESO-LIR-1 blocker display similar killing efficacy to primary T cells transduced with only the HPV E7-TCR activator. Luciferase measurement represents live target cells. RLU = relative light units; error bars indicate $\pm$ SD (n = 2).

[0049] FIG. 24A is a pair of plots showing that A2-LIR-1 blocks Jurkat activation in A2+ Raji cells but not WT Raji cells. Histograms show Raji WT “tumor” cells and Raji A2+ “normal” cells have identical CD19 surface expression while HLA-A*02 is expressed only in Raji A2 “normal” cells.

[0050] FIG. 24B is a plot showing that A2-LIR-1 blocks Jurkat activation in A2+ Raji cells but not WT Raji cells. Jurkat cells transfected with either CD19 or CD19 + A2-LIR-1 were co-cultured

with either WT (A2-) Raji cells or A2+ Raji cells at various cell ratios. RLU = relative light units; error bars indicate $\pm$ SD (n = 2).

[0051] FIGS. 25A-25B are each a series of images showing the reversibility of blockade by LIR-1 inhibitory receptors. Primary T cells (donor 2) transduced with CD19 CAR activator and HLA-A*02 blocker demonstrate reversible blockade (FIG. 25A) and activation (FIG. 25B) after 3 rounds of antigen exposure (AB-A-AB and A-AB-A) in in vitro cytotoxicity assay at 3:1 E:T. Primary T cell cytotoxicity assay was reproduced with three HLA-A*02-negative donors. Images shown were captured at 72 hours.

[0052] FIGS. 25C-25D are each a pair of plots showing quantification of target cell lysis (FIG. 25C) and IFN γ (FIG. 25D) in response to repeated exposure to multiple rounds of normal and target cells, 3:1 E:T (T cells from donor 2). Shown conditions are Untransduced, CD19-CAR T cells, and CD19-CAR T + A2-LIR-1. Error bars indicate $\pm$ SEM (n=2). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, determined using a two-way ANOVA followed by Tukey's multiple-comparisons test. In this experiment, IFN γ response diminished over time, while cytotoxicity remained robust.

[0053] FIGS. 26A-26B are each a pair of plots showing cytotoxic T cell killing and secretion of IFN γ in co-culture with a separate donor (donor 3). Cytotoxic CD19 CAR activator and HLA-A*02 blocker transduced T cells demonstrate reversible blocking after multiple rounds of antigen exposure in cytotoxic assays and IFN γ at 9:1 E:T. We noted that this donor's T cells survival and activity tailed off over time in culture. Shown conditions are Untransduced, CD19-CAR T cells, and CD19-CAR T + A2-LIR-1. Cytotoxicity (FIG. 26A) and IFN γ (FIG. 26B) results correspond to FIGS. 25C-25D. Error bars indicate $\pm$ SEM (n = 2). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, determined using a two-way ANOVA followed by Tukey's multiple-comparisons test.

[0054] FIG. 27A is a plot showing primary T cells transduced with CD19 CAR activator and HLA-A*02 blocker demonstrates ~20-fold expansion with CD3/28 stimulation over 10 days.

[0055] FIG. 27B is a diagram showing an experiment to show that CAR-T cells expressing a LIR-1 blocker receptor selectively kill tumors in a xenograft model. HLA-A*02 NSG mice were administered either "tumor cells" (A2-negative Raji cells) or "normal cells" (A2-positive Raji cells) subcutaneously and primary T cells (human, HLA-A*02-negative donor 4) were injected into the tail vein when Raji xenografts averaged ~70 mm³.

[0056] FIGS. 27C-27E are each a pair of plots that show readouts by caliper measurement (FIG. 27C), human T cell counts in peripheral blood by flow cytometry (FIG. 27D), and survival (FIG. 27E). Error bars in C-D indicate $\pm$ SEM (n=7). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, determined using a two-way ANOVA followed by Tukey's multiple-comparisons test.

[0057] FIG. 28A is a series of plots showing flow cytometry analysis of primary T cell post-enrichment and expansion prepared for mouse tail vein injection. Tumor volume was measured at the number of days from T cell injection, starting 10 days prior to T cell injection (-10) to 40 days following T cell injection (40). Tumor volumes ranged from 0 to 2500 mm³.

[0058] FIG. 28B is a series of plots showing tumor measurement by caliper plotted for individual mice in each group.

[0059] FIG. 28C is a series of plots showing correlation of huCD3+ T cells in mouse blood to tumor growth. Graph of huCD3+ T cells compared to tumor volume 10 days and 17 days after T cell injection.

[0060] FIG. 28D is a pair of plots showing huCD4+ and huCD8+ T cell counts in peripheral blood by flow cytometry. Open circles, mice grafted with "normal" cells; closed circle, mice grafted with tumor cells. Samples with fewer than 100 cells were excluded from the analysis. Error bars indicate $\pm$ SEM (n = 7 in all groups except n = 6 in CD19+/A2- Raji group treated with CD19-CAR + A2-LIR-1 T cells).

[0061] FIG. 29A is a series of images showing histological analysis of T cell infiltration in tumors. Representative images of tumor samples collected at study termination, sectioned and stained for huCD3 are shown.

[0062] FIG. 29B is a plot showing quantification of T cell infiltration using ImageJ. T cell infiltration was significantly higher for T cells with CD19-CAR or CD19-CAR + A2-LIR-1 in CD19+/A2- tumors compared to untransduced cells. However, in CD19+/A2+ tumors, CD19-CAR + A2-LIR-1 T cells were not significantly different compared to untransduced cells. There was also a significant drop in infiltration of CD19-CAR + A2-LIR-1 T cells between CD19+/A2- and CD19+/A2+ tumors. Qualitatively, CD19-CAR + A2-LIR-1 T cells were less prevalent in CD19+/A2+ tumors compared to CD19-CAR only T cells; however, this difference was not statistically significant. Saline samples were similarly quantified to show background staining levels. Groups of data were analyzed using an ordinary one-way ANOVA, while individual pairs

between "tumor" and "normal" were analyzed using an unpaired t test. ns = not significant, * $p < 0.05$, ** $p < 0.01$.

DETAILED DESCRIPTION

[0063] The present disclosure describes receptors having one or more domains from Leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1, sometimes referred to as LIR1 or LIR-1). Numerous receptors, engineered cells, and uses thereof are contemplated herein. The inventors have found that chimeric receptors comprising an antigen-binding domain and one or more LILRB1 domains, including the LILRB1 intracellular domain, can inhibit immune cell signaling even in the presence of activatory chimeric antigen receptors (CARs) or T cell receptors (TCRs).

[0064] The term "chimeric antigen receptors" or "CARs" as used herein, may refer to artificial T-cell receptors, chimeric T-cell receptors, or chimeric immunoreceptors, for example, and encompass engineered receptors that graft an artificial specificity onto a particular immune effector cell, such as a helper T cell (CD4+), cytotoxic T cell (CD8+) or NK cell. CARs may be employed to impart the specificity of a monoclonal antibody onto a T cell, thereby allowing a large number of specific T cells to be generated, for example, for use in adoptive cell therapy. In specific embodiments, CARs direct specificity of the cell to a tumor associated antigen. In some embodiments, CARs comprise an intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an antigen-binding region. In some embodiments, CARs comprise fusions of single-chain variable fragments (scFvs) or scFabs derived from monoclonal antibodies, fused to a transmembrane domain and intracellular signaling domain(s). The fusion may also comprise a hinge. Either heavy-light (H-L) and light-heavy (L-H) scFvs may be used. The specificity of CAR designs may be derived from ligands of receptors (*e.g.*, peptides). Depending on the type of intracellular domain, a CAR can be an activatory receptor or an inhibitory receptor. In some embodiments, for example when the CAR is an activatory receptor, the CAR comprises domains for additional co-stimulatory signaling, such as CD3, FcR, CD27, CD28, CD137, DAP10, and/or OX40. In some embodiments, molecules can be co-expressed with the CAR, including co-stimulatory molecules, reporter genes for imaging (*e.g.*, for positron emission tomography), gene products that conditionally ablate the T cells upon addition of a pro-drug, homing receptors, cytokines, and cytokine receptors. As used herein, characteristics

attributed to a chimeric antigen receptor may be understood to refer to the receptor itself or to a host cell comprising the receptor.

[0065] As used herein, a “TCR”, sometimes also called a “TCR complex” or “TCR/CD3 complex” refers to a protein complex comprising a TCR alpha chain, a TCR beta chain, and one or more of the invariant CD3 chains (zeta, gamma, delta and epsilon), sometimes referred to as subunits. The TCR alpha and beta chains can be disulfide-linked to function as a heterodimer to bind to peptide-MHC complexes. Once the TCR alpha/beta heterodimer engages peptide-MHC, conformational changes in the TCR complex in the associated invariant CD3 subunits are induced, which leads to their phosphorylation and association with downstream proteins, thereby transducing a primary stimulatory signal. In an exemplary TCR complex, the TCR alpha and TCR beta polypeptides form a heterodimer, CD3 epsilon and CD3 delta form a heterodimer, CD3 epsilon and CD3 gamma form a heterodimer, and two CD3 zeta form a homodimer.

[0066] The term “stimulation” refers to a primary response induced by binding of a stimulatory domain or stimulatory molecule (*e.g.*, a TCR/CD3 complex) with its cognate ligand thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex. Stimulation can mediate altered expression of certain molecules, and/or reorganization of cytoskeletal structures, and the like.

[0067] The term “stimulatory molecule” or “stimulatory domain” refers to a molecule or portion thereof that, when natively expressed by a T-cell, provides the primary cytoplasmic signaling sequence(s) that regulate activation of the TCR complex in a stimulatory way for at least some aspect of the T-cell signaling pathway. TCR alpha and/or TCR beta chains of wild type TCR complexes do not contain stimulatory domains and require association with CD3 subunits such as CD3 zeta to initiate signaling. In one aspect, the primary stimulatory signal is initiated by, for instance, binding of a TCR/CD3 complex with an a major histocompatibility complex (MHC) bound to peptide, and which leads to mediation of a T-cell response, including, but not limited to, proliferation, activation, differentiation, and the like. One or more stimulatory domains, as described herein, can be fused to the intracellular portion of any one or more subunits of the TCR complex, including TCR alpha, TCR beta, CD3 delta, CD3 gamma and CD3 epsilon.

[0068] As used herein, a “domain capable of providing a stimulatory signal” refers to any domain that, either directly or indirectly, can provide a stimulatory signal that enhances or increases the effectiveness of signaling mediated by the TCR complex to enhance at least some

aspect of T-cell signaling. The domain capable of providing a stimulatory signal can provide this signal directly, for example with the domain capable of providing the stimulatory signal is a primary stimulatory domain or co-stimulatory domain. Alternatively, or in addition, the domain capable of providing the stimulatory signal can act indirectly. For example, the domain can be a scaffold that recruits stimulatory proteins to the TCR, or provide an enzymatic activity, such as kinase activity, that acts through downstream targets to provide a stimulatory signal.

[0069] As used herein, a “domain capable of providing an inhibitory signal” refers to any domain that, either directly or indirectly, can provide an inhibitory signal that inhibits or decreases the effectiveness signaling mediated by the TCR complex. The domain capable of providing an inhibitory signal can reduce, or block, totally or partially, at least some aspect of T-cell signaling or function. The domain capable of providing an inhibitory signal can provide this signal directly, for example with the domain capable of providing the inhibitory signal provides a primary inhibitory signal. Alternatively, or in addition, the domain capable of providing the stimulatory signal can act indirectly. For example, the domain can recruit additional inhibitory proteins to the TCR, or can provide an enzymatic activity that acts through downstream targets to provide an inhibitory signal.

[0070] Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. As another example, a range such as 95-99% identity, includes something with 95%, 96%, 97%, 98% or 99% identity, and includes subranges such as 96-99%, 96-98%, 96-97%, 97-99%, 97-98% and 98-99% identity. This applies regardless of the breadth of the range.

[0071] In general, “sequence identity” or “sequence homology” refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Typically, techniques for determining sequence identity include determining the nucleotide sequence of a polynucleotide and/or determining the amino acid

sequence encoded thereby and comparing these sequences to a second nucleotide or amino acid sequence. Two or more sequences (polynucleotide or amino acid) can be compared by determining their “percent identity.” The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100. Percent identity may also be determined, for example, by comparing sequence information using the advanced BLAST computer program, including version 2.2.9, available from the National Institutes of Health. The BLAST program is based on the alignment method of Karlin and Altschul, Proc. Natl. Acad. Sci. USA 87:2264-2268 (1990) and as discussed in Altschul, et al., J. Mol. Biol. 215:403-410 (1990); Karlin And Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5877 (1993); and Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997). Briefly, the BLAST program defines identity as the number of identical aligned symbols (generally nucleotides or amino acids), divided by the total number of symbols in the shorter of the two sequences. The program may be used to determine percent identity over the entire length of the proteins being compared. Default parameters are provided to optimize searches with short query sequences in, for example, with the blastp program. Ranges of desired degrees of sequence identity are approximately 80% to 100% and integer values therebetween. Typically, the percent identities between a disclosed sequence and a claimed sequence are at least 80%, at least 85%, at least 90%, at least 95%, or at least 98%.

[0072] As used herein, a “subsequence” refers to a length of contiguous amino acids or nucleotides that form a part of a sequence described herein. A subsequence may be identical to a part of a full length sequence when aligned to the full length sequence, or less than 100% identical to the part of the full length sequence to which it aligns (e.g., 90% identical to 50% of the full sequence, or the like).

[0073] The term “exogenous” is used herein to refer to any molecule, including nucleic acids, protein or peptides, small molecular compounds, and the like that originate from outside the organism. In contrast, the term “endogenous” refers to any molecule that originates from inside the organism (*i.e.*, naturally produced by the organism).

[0074] A polynucleotide is “operably linked” to another polynucleotide when it is placed into a functional relationship with the other polynucleotide. For example, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. A peptide is

"operably linked" to another peptide when the polynucleotides encoding them are operably linked, preferably they are in the same open reading frame.

[0075] A "promoter" is a sequence of DNA needed to turn a gene on or off. Promoters are located immediately upstream and/or overlapping the transcription start site, and are usually between about one hundred to several hundred base pairs in length.

[0076] All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment, or any form of suggestion, that they constitute valid prior art or form part of the common general knowledge in any country in the world.

[0077] In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. The term "about", when immediately preceding a number or numeral, means that the number or numeral ranges plus or minus 10%.

[0078] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

Leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1)

[0079] The present disclosure describes receptors having one or more domains from Leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1, or LIR1). Numerous receptors, engineered cells, and uses thereof are contemplated herein.

[0080] Leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1), also known as Leukocyte immunoglobulin-like receptor B1, as well as ILT2, LIR1, MIR7, PIRB, CD85J, ILT-2 LIR-1, MIR-7 and PIR-B, is a member of the leukocyte immunoglobulin-like receptor (LIR) family. The LILRB1 protein belongs to the subfamily B class of LIR receptors. These receptors contain two to four extracellular immunoglobulin domains, a transmembrane domain, and two to four cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs).

The LILRB1 receptor is expressed on immune cells, where it binds to MHC class I molecules on antigen-presenting cells and transduces a negative signal that inhibits stimulation of an immune response. LILRB1 is thought to regulate inflammatory responses, as well as cytotoxicity, and to play a role in limiting auto-reactivity. Multiple transcript variants encoding different isoforms of LILRB1 exist, all of which are contemplated as within the scope of the instant disclosure.

[0081] In some embodiments of the receptors having one or domains of LILRB1, the one or more domains of LILRB1 comprise an amino acid sequence that is at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or is identical to a sequence or subsequence of SEQ ID NO: 1. In some embodiments, the one or more domains of LILRB1 comprise an amino acid sequence that is identical to a sequence or subsequence of SEQ ID NO: 1. In some embodiments, the one or more domains of LILRB1 consist of an amino acid sequence that is at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or is identical to a sequence or subsequence of SEQ ID NO: 1. In some embodiments, the one or more domains of LILRB1 consist of an amino acid sequence that is identical to a sequence or subsequence of SEQ ID NO: 1.

[0082] In some embodiments of the receptors having one or domains of LILRB1, the one or more domains of LILRB1 are encoded by a polynucleotide sequence that is at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or is identical to a sequence or subsequence of SEQ ID NO: 34.

[0083] In some embodiments of the receptors having one or domains of LILRB1, the one or more domains of LILRB1 are encoded by a polynucleotide sequence that is identical to a sequence or subsequence of SEQ ID NO: 34.

Receptors

[0084] In various embodiments, a chimeric antigen receptor is provided, comprising a polypeptide, wherein the polypeptide comprises one or more of: an LILRB1 hinge domain or functional fragment or variant thereof; an LILRB1 transmembrane domain or a functional variant thereof; and an LILRB1 intracellular domain or an intracellular domain comprising at least one, or at least two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

Intracellular Domain

[0085] The disclosure provides chimeric antigen receptors, the chimeric antigen receptors comprising a polypeptide. In some embodiments, the polypeptide comprises an intracellular domain. In some embodiments, the intracellular domain is an LILRB1 intracellular domain or a functional variant thereof.

[0086] As used herein, “intracellular domain” refers to the cytoplasmic or intracellular domain of a protein, such as a receptor, that interacts with the interior of the cell, and carries out a cytosolic function. As used herein, “cytosolic function” refers to a function of a protein or protein complex that is carried out in the cytosol of a cell. For example, intracellular signal transduction cascades are cytosolic functions.

[0087] As used herein an “immunoreceptor tyrosine-based inhibitory motif” or “ITIM” refers to a conserved sequence of amino acids with a consensus sequence of S/I/V/LxYxxI/V/L (SEQ ID NO: 124), or the like, that is found in the cytoplasmic tails of many inhibitory receptors of the immune system. After ITIM-possessing inhibitory receptors interact with their ligand, the ITIM motif is phosphorylated, allowing the inhibitory receptor to recruit other enzymes, such as the phosphotyrosine phosphatases SHP-1 and SHP-2, or the inositol-phosphatase called SHIP.

[0088] In some embodiments, the polypeptide comprises an intracellular domain comprising at least one immunoreceptor tyrosine-based inhibitory motif (ITIM), at least two ITIMs, at least 3 ITIMs, at least 4 ITIMs, at least 5 ITIMs or at least 6 ITIMs. In some embodiments, the intracellular domain has 1, 2, 3, 4, 5, or 6 ITIMs.

[0089] In some embodiments, the polypeptide comprises an intracellular domain comprising at least one ITIM selected from the group of ITIMs consisting of NLYAAV (SEQ ID NO: 8), VTIAEV (SEQ ID NO: 9), VTIAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0090] In further particular embodiments, the polypeptide comprises an intracellular domain comprising at least two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTIAEV (SEQ ID NO: 9), VTIAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0091] In some embodiments, the intracellular domain comprises both ITIMs NLYAAV (SEQ ID NO: 8) and VTIAEV (SEQ ID NO: 9). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 12. In some embodiments, the intracellular domain comprises or consists essentially of a sequence identical to SEQ ID NO: 12.

[0092] In some embodiments, the intracellular domain comprises both ITIMs VTIAEV (SEQ ID NO: 9) and VTIAQL (SEQ ID NO: 10). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 13. In some embodiments, the intracellular domain comprises or consists essentially of a sequence identical to SEQ ID NO: 13.

[0093] In some embodiments, the intracellular domain comprises both ITIMs VTIAQL (SEQ ID NO: 10) and SIATL (SEQ ID NO: 11). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 14. In some embodiments, the intracellular domain comprises or consists essentially of a sequence identical to SEQ ID NO: 14.

[0094] In some embodiments, the intracellular domain comprises the ITIMs NLYAAV (SEQ ID NO: 8), VTIAEV (SEQ ID NO: 9), and VTIAQL (SEQ ID NO: 10). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 15. In some embodiments, the intracellular domain comprises or consists essentially of a sequence identical to SEQ ID NO: 15.

[0095] In some embodiments, the intracellular domain comprises the ITIMs VTIAEV (SEQ ID NO: 9), VTIAQL (SEQ ID NO: 10), and SIATL (SEQ ID NO: 11). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 16. In some embodiments, the intracellular domain comprises or consists essentially of a sequence identical to SEQ ID NO: 16.

[0096] In some embodiments, the intracellular domain comprises the ITIMs NLYAAV (SEQ ID NO: 8), VTIAEV (SEQ ID NO: 9), VTIAQL (SEQ ID NO: 10), and SIATL (SEQ ID NO: 11). In some embodiments, the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 17. In some embodiments, the intracellular domain comprises or consists essentially of a sequence identical to SEQ ID NO: 17.

[0097] In some embodiments, the intracellular domain comprises a sequence at least 95% identical to the LILRB1 intracellular domain (SEQ ID NO: 7). In some embodiments, the intracellular domain comprises or consists essentially of a sequence identical to the LILRB1 intracellular domain (SEQ ID NO: 7).

[0098] LILRB1 intracellular domains or functional variants thereof of the disclosure can have at least 1, at least 2, at least 4, at least 4, at least 5, at least 6, at least 7, or at least 8 ITIMs. In some embodiments, the LILRB1 intracellular domain or functional variant thereof has 2, 3, 4, 5, or 6 ITIMs.

[0099] In particular embodiments, the polypeptide comprises an intracellular domain comprising two, three, four, five, or six immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0100] In particular embodiments, the polypeptide comprises an intracellular domain comprising at least three immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0101] In particular embodiments, the polypeptide comprises an intracellular domain comprising three immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0102] In particular embodiments, the polypeptide comprises an intracellular domain comprising four immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0103] In particular embodiments, the polypeptide comprises an intracellular domain comprising five immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0104] In particular embodiments, the polypeptide comprises an intracellular domain comprising six immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0105] In particular embodiments, the polypeptide comprises an intracellular domain comprising at least seven immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0106] In some embodiments, the intracellular domain comprises a TCR alpha intracellular domain. In some embodiments, the intracellular domain comprises a TCR alpha intracellular domain and an LILRB1 intracellular domain, as described herein. In some embodiments, a TCR

alpha intracellular domain comprises Ser-Ser. In some embodiments, a TCR alpha intracellular domain is encoded by a sequence of TCCAGC.

[0107] In some embodiments, the intracellular domain comprises a TCR beta intracellular domain. In some embodiments, the intracellular domain comprises a TCR beta intracellular domain and an LILRB1 intracellular domain, as described herein. In some embodiments, the TCR beta intracellular domain comprises an amino acid sequence having at least 80% identity, at least 90% identity, or is identical to a sequence of: MAMVKRKDSR (SEQ ID NO: 94). In some embodiments, the TCR beta intracellular domain comprises, or consists essentially of MAMVKRKDSR (SEQ ID NO: 94). In some embodiments, the TCR beta intracellular domain is encoded by a sequence of ATGGCCATGGTCAAGAGAAAGGATTCCAGA (SEQ ID NO: 95).

Transmembrane Domain

[0108] The disclosure provides chimeric antigen receptors the receptors comprising a polypeptide. In some embodiments, the polypeptide comprises a transmembrane domain. In some embodiments, the transmembrane domain is a LILRB1 transmembrane domain or a functional variant thereof.

[0109] A “transmembrane domain”, as used herein, refers to a domain of a protein that spans membrane of the cell. Transmembrane domains typically consist predominantly of non-polar amino acids, and may traverse the lipid bilayer once or several times. Transmembrane domains usually comprise alpha helices, a configuration which maximizes internal hydrogen bonding.

[0110] Transmembrane domains isolated or derived from any source are envisaged as within the scope of the fusion proteins of the disclosure.

In particular embodiments, the polypeptide comprises an LILRB1 transmembrane domain or a functional variant thereof.

[0111] In some embodiments, the LILRB1 transmembrane domain or a functional variant thereof comprises a sequence at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical or at least 99% to SEQ ID NO: 5. In some embodiments, the LILRB1 transmembrane domain or a functional variant thereof comprises a sequence at least 95% identical to SEQ ID NO: 5. In some embodiments, the LILRB1 transmembrane domain comprises a sequence identical to SEQ ID NO: 5. In embodiments, the LILRB1 transmembrane domain consists essentially of a sequence identical to SEQ ID NO: 5.

[0112] In some embodiments of the chimeric antigen receptors of the disclosure, the transmembrane domain is not a LILRB1 transmembrane domain. In some embodiments, the transmembrane domain is one that is associated with one of the other domains of the fusion protein, or isolated or derived from the same protein as one of the other domains of the fusion protein.

[0113] The transmembrane domain may be derived either from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Exemplary transmembrane domains may include at least the transmembrane region(s) of *e.g.*, the alpha, beta or zeta chain of the TCR, CD3 delta, CD3 epsilon or CD3 gamma, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154.

[0114] In some embodiments, the transmembrane comprises a TCR alpha transmembrane domain. In some embodiments, the TCR alpha transmembrane domain comprises an amino acid sequence having at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity or is identical to a sequence of: VIGFRILLKLVAGFNLLMTLRLW (SEQ ID NO: 96). In some embodiments, the TCR alpha transmembrane domain comprises, or consists essentially of, VIGFRILLKLVAGFNLLMTLRLW (SEQ ID NO: 96). In some embodiments, the TCR alpha transmembrane domain is encoded by a sequence of: GTGATTGGGTTCCGAATCCTCCTGAAAGTGGCCGGGTTAATCTGCTCATGACG CTGCGGCTGTGG (SEQ ID NO: 97).

[0115] In some embodiments, the transmembrane comprises a TCR beta transmembrane domain. In some embodiments, the TCR beta transmembrane domain comprises an amino acid sequence having at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity or is identical to a sequence of: TILYEILLGKATLYAVLVSAVL (SEQ ID NO: 98). In some embodiments, the TCR beta transmembrane domain comprises, or consists essentially of, TILYEILLGKATLYAVLVSAVL (SEQ ID NO: 98). In some embodiments, the TCR beta transmembrane domain is encoded by a sequence of: ACCATCCTCTATGAGATCTTGCTAGGGAAGGCCACCTTGATGCCGTGCTGGTCAGT GCCCTCGTGCTG (SEQ ID NO: 99).

[0116] In some embodiments, the TCR alpha and/or TCR beta transmembrane domain comprises one or more mutations that attenuate or abolish interaction of the TCR with the TCR CD3 subunit. In some embodiments, the TCR alpha transmembrane domain comprises a R253L mutation. In some embodiments, the TCR beta transmembrane domain comprises a K288L mutation.

[0117] In some embodiments the transmembrane domain comprise a CD28 transmembrane domain. In some embodiments, the CD28 transmembrane domain comprises an amino acid sequence having at least 80% identity, at least 90% identity, at least 95% identity, at least 99% identity or is identical to a sequence of FWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO: 100). In some embodiments, the CD28 transmembrane domain comprises or consists essentially of FWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO: 100). In some embodiments, the CD28 transmembrane domain is encoded by a nucleotide sequence having at least 80% identity, at least 90% identity, at least 95% identity, at least 99% identity or is identical to a sequence of

TTCTGGGTGCTGGTCGTTGTGGGCGCGTGCTGGCCTGCTACAGCCTGCTGGTGACAGTGGCCTTCATCATCTTTTGGTG (SEQ ID NO: 101).

[0118] In some embodiments, the transmembrane domain can be attached to the extracellular region chimeric antigen receptor, *e.g.*, the antigen-binding domain or ligand binding domain, via a hinge, *e.g.*, a hinge from a human protein. For example, in some embodiments, the hinge can be a human immunoglobulin (Ig) hinge, *e.g.*, an IgG4 hinge, a CD8a hinge or an LILRB1 hinge.

Hinge Domain

[0119] The disclosure provides chimeric antigen receptors, the receptors comprising a polypeptide. In some embodiments, the polypeptide comprises a hinge domain. In some embodiments, the hinge domain is a LILRB1 hinge domain or a functional variant thereof.

[0120] The LILRB1 protein has four immunoglobulin (Ig) like domains termed D1, D2, D3 and D4. In some embodiments, the LILRB1 hinge domain comprises an LILRB1 D3D4 domain or a functional variant thereof. In some embodiments, the LILRB1 D3D4 domain comprises a sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or identical to SEQ ID NO: 18. In some embodiments, the LILRB1 D3D4 domain comprises or consists essentially of SEQ ID NO: 18.

[0121] In some embodiments, the polypeptide comprises the LILRB1 hinge domain or functional fragment or variant thereof. In embodiments, the LILRB1 hinge domain or functional

fragment or variant thereof comprises a sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical or identical to SEQ ID NO: 4, SEQ ID NO: 18, or SEQ ID NO: 19. In embodiments, the LILRB1 hinge domain or functional fragment or variant thereof comprises a sequence at least 95% identical to SEQ ID NO: 4, SEQ ID NO: 18, or SEQ ID NO: 19.

[0122] In some embodiments, the LILRB1 hinge domain comprises a sequence identical to SEQ ID NO: 4, SEQ ID NO: 18, or SEQ ID NO: 19.

[0123] In some embodiments, the LILRB1 hinge domain consists essentially of a sequence identical to SEQ ID NO: 4, SEQ ID NO: 18, or SEQ ID NO: 19.

[0124] In some embodiments the chimeric antigen receptors of the disclosure, the polypeptide comprises a hinge that is not isolated or derived from LILRB1.

[0125] In some embodiments, the hinge is isolated or derived from CD8 α or CD28. In some embodiments, the CD8 α hinge comprises an amino acid sequence having at least 80% identity, at least 90% identity, at least 95% identity, at least 99% identity or is identical to a sequence of TTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 102). In some embodiments, the CD8 α hinge comprises TTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 102). In some embodiments, the CD8 α hinge consists essentially of TTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 102). In some embodiments, the CD8 α hinge is encoded by a nucleotide sequence having at least 80% identity, at least 90% identity, at least 95% identity, at least 99% identity or is identical to a sequence of

accacgacgccagcgccgcgaccaccaacaccggcgcccaccatcgcgctgcagccccctgtccc
tgcgcccagaggcgtgccggccagcgggcgggggcgcgagtgcacacgagggggctggacttcgc
ctgtgat (SEQ ID NO: 103).

[0126] In some embodiments, the CD28 hinge comprises an amino acid sequence having at least 80% identity, at least 90% identity, at least 95% identity, at least 99% identity or is identical to a sequence of CTIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKP (SEQ ID NO: 104). In some embodiments, the CD28 hinge comprises or consists essentially of CTIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKP (SEQ ID NO: 104). In some embodiments, the CD28 hinge is encoded by a nucleotide sequence having at least 80% identity, at least 90% identity, at least 95% identity, at least 99% identity or is identical to a sequence of

tgtaccattgaagttatgtatcctcctccttacctagacaatgagaagagcaatggaaccattatccatg
tgaaagggaaacacctttgtccaagtcccctatttcccggaccttctaagccc (SEQ ID NO: 105).

Combinations of LILRB1 Domains

[0127] In some embodiments, the chimeric antigen receptors of the disclosure comprise a polypeptide comprising more than one LILRB1 domain or functional equivalent thereof. For example, in some embodiments, the polypeptide comprises an LILRB1 transmembrane domain and intracellular domain, or an LILRB1 hinge domain, transmembrane domain and intracellular domain.

[0128] In particular embodiments, the polypeptide comprises an LILRB1 hinge domain or functional fragment or variant thereof, and the LILRB1 transmembrane domain or a functional variant thereof. In some embodiments, the polypeptide comprises a sequence at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical or identical to SEQ ID NO: 20. In some embodiments, the polypeptide comprises a sequence at least 95% identical to SEQ ID NO: 20. In some embodiments, the polypeptide comprises a sequence identical to SEQ ID NO: 20.

[0129] In further embodiments, the polypeptide comprises: the LILRB1 transmembrane domain or a functional variant thereof, and an LILRB1 intracellular domain and/or an intracellular domain comprising at least one immunoreceptor tyrosine-based inhibitory motif (ITIM), wherein the ITIM is selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11). In some embodiments, the polypeptide comprises the LILRB1 transmembrane domain or a functional variant thereof, and an LILRB1 intracellular domain and/or an intracellular domain comprising at least two ITIM, wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0130] In some embodiments, the polypeptide comprises a LILRB1 transmembrane domain and intracellular domain. In some embodiments, the polypeptide comprises a sequence at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical or identical to SEQ ID NO: 21. In some embodiments, the polypeptide comprises a sequence at least 95% identical to SEQ ID NO: 21. In some embodiments, the polypeptide comprises a sequence identical to SEQ ID NO: 21.

[0131] In preferred embodiments, the polypeptide comprises: an LILRB1 hinge domain or functional fragment or variant thereof; an LILRB1 transmembrane domain or a functional variant thereof; and an LILRB1 intracellular domain and/or an intracellular domain comprising at least two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from LYAAV (SEQ ID NO: 8), VTYAE (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

[0132] In some embodiments, the polypeptide comprises a sequence at least 95% identical to SEQ ID NO: 2 or SEQ ID NO: 3, or at least 99% identical to SEQ ID NO: 2 or SEQ ID NO: 3, or identical to SEQ ID NO: 2 or SEQ ID NO: 3.

[0133] In some embodiments, the polypeptide comprises a sequence at least 99% identical to SEQ ID NO: 20, or at least 99% identical to SEQ ID NO: 20, or identical to SEQ ID NO: 20.

[0134] In some embodiments, the polypeptide comprises a sequence at least 99% identical to SEQ ID NO: 21, or at least 99% identical to SEQ ID NO: 21, or identical to SEQ ID NO: 21.

Extracellular Domains

[0135] The disclosure provides chimeric antigen receptors comprising a polypeptide. In some embodiments, the polypeptide comprises a ligand binding domain, such as an antigen-binding domain. Suitable antigen-binding domains include, but are not limited to antigen-binding domains from antibodies, antibody fragments, scFv, antigen-binding domains derived from T cell receptors, and the like. All forms of antigen-binding domains known in the art are envisaged as within the scope of the disclosure.

[0136] An “extracellular domain”, as used herein, refers to the extracellular portion of a protein. For example, the TCR alpha and beta chains each comprise an extracellular domain, which comprise a constant and a variable region involved in peptide-MHC recognition. The “extracellular domain” can also comprise a fusion domain, for example of fusions between additional domains capable of binding to and targeting a specific antigen and the endogenous extracellular domain of the TCR subunit.

[0137] The term “antibody,” as used herein, refers to a protein, or polypeptide sequences derived from an immunoglobulin molecule, which specifically binds to an antigen. Antibodies can be intact immunoglobulins of polyclonal or monoclonal origin, or fragments thereof and can be derived from natural or from recombinant sources.

[0138] The terms “antibody fragment” or “antibody binding domain” refer to at least one portion of an antibody, or recombinant variants thereof, that contains the antigen-binding domain, *i.e.*, an antigenic determining variable region of an intact antibody, that is sufficient to confer recognition and specific binding of the antibody fragment to a target, such as an antigen and its defined epitope. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments, single-chain (sc)Fv (“scFv”) antibody fragments, linear antibodies, single domain antibodies (abbreviated “sdAb”) (either VL or VH), camelid VHH domains, and multi-specific antibodies formed from antibody fragments.

[0139] The term “scFv” refers to a fusion protein comprising at least one antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked via a short flexible polypeptide linker, and capable of being expressed as a single polypeptide chain, and wherein the scFv retains the specificity of the intact antibody from which it is derived.

[0140] “Heavy chain variable region” or “VH” (or, in the case of single domain antibodies, *e.g.*, nanobodies, “VHH”) with regard to an antibody refers to the fragment of the heavy chain that contains three CDRs interposed between flanking stretches known as framework regions, these framework regions are generally more highly conserved than the CDRs and form a scaffold to support the CDRs.

[0141] Unless specified, as used herein a scFv may have the VL and VH variable regions in either order, *e.g.*, with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

[0142] The term “antibody light chain,” refers to the smaller of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations. Kappa (“K”) and lambda (“λ”) light chains refer to the two major antibody light chain isotypes.

[0143] The term “recombinant antibody” refers to an antibody that is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage or yeast expression system. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the

DNA or amino acid sequence has been obtained using recombinant DNA or amino acid sequence technology which is available and well known in the art.

[0144] In some embodiments, for example those embodiments wherein the receptor comprises a first and a second polypeptide, the antigen-binding domain is isolated or derived from a T cell receptor (TCR) extracellular domain or an antibody.

[0145] In preferred embodiments, the polypeptide comprises antigen-binding domain, *e.g.*, an antigen-binding domain other than the LILRB1 antigen-binding protein. An illustrative embodiments of receptor having a single antigen-binding domain is depicted in **FIG. 1**. The disclosure contemplated chimeric antigen receptors have two, three, four or more antigen-binding domains. The antigen-binding domains may be provided on the same or a different chain of the chimeric antigen receptor. In embodiments, the chimeric antigen receptor is a DARIC as described, for example in Leung et al. *JCI Insight*. 2019 Jun 6; 4(11):e124430, WO2015017214A1; and WO2017156484A1.

[0146] In some embodiments, the receptor is an inhibitory chimeric antigen receptor (iCAR). Various methods and composition suitable for use with the embodiments disclosure herein include those provided in US2018/0044399A1; WO2018148454A1; and WO2017087723A1, each of which is incorporated herein for all purposes.

[0147] In some embodiments, the antigen-binding domain comprises a single chain variable fragment (scFv).

[0148] In some embodiments, the receptor comprises a second polypeptide. The disclosure provides receptors having two polypeptides each having a part of a ligand-binding domain (*e.g.* cognates of a heterodimeric LDB, such as a TCR α/β - or Fab-based LBD) and each having an intracellular domain, as depicted in **FIG. 2A**. The disclosure further provides receptors having two polypeptides, each having a part of a ligand-binding domain (*e.g.* cognates of a heterodimeric LDB, such as a TCR α/β - or Fab-based LBD) and one part of the ligand binding domain is fused to a hinge or transmembrane domain, while the other part of the ligand binding domain has no intracellular domain, as depicted in **FIG. 2B**. Further variations include receptors where each polypeptide has a hinge domain, and where each polypeptide has a hinge and transmembrane domain. In some embodiments, the hinge domain is absent. In other embodiments, the hinge domain is a membrane proximal extracellular region (MPER), such as the LILRB1 D3D4 domain.

In any of the embodiments disclosed herein, the domains may be fused adjacent to one another with linkers between them.

[0149] In some embodiments, the first polypeptide comprises a first chain of an antibody and the second polypeptide comprise a second chain of said antibody.

[0150] In some embodiments, the receptor comprises a Fab fragment of an antibody. In embodiments, an antigen-binding fragment of the heavy chain of the antibody, and the second polypeptide comprises an antigen-binding fragment of the light chain of the antibody. In embodiments, the first polypeptide comprises an antigen-binding fragment of the light chain of the antibody, and the second polypeptide comprises an antigen-binding fragment of the heavy chain of the antibody.

[0151] In some embodiments, the first polypeptide comprises a first chain of a T-cell receptor (TCR) and the second polypeptide comprises a second chain of said TCR. In embodiments, the receptor comprises an extracellular fragment of a T cell receptor (TCR). In embodiments, the first polypeptide comprises an antigen-binding fragment of the alpha chain of the TCR, and the second polypeptide comprises an antigen-binding fragment of the beta chain of the TCR. In some embodiments, the first polypeptide comprises an antigen-binding fragment of the beta chain of the TCR, and the second polypeptide comprises an antigen-binding fragment of the alpha chain of the TCR.

[0152] In some embodiments, the receptor comprises a single-chain TCR, such as, without limitation, those disclosed in WO2017091905A1.

Illustrative Antigen-Binding Domains

[0153] Various single variable domains known in the art or disclosed herein are suitable for use in embodiments. Such scFv's include, for example and without limitation the following mouse and humanized scFv antibodies that bind HLA-A*02 in a peptide-independent way (complementarity determining regions underlined):

C-001765

MMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSG
 VPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHPVPRTSGGGTELEIKGGGSGGGG
 SGGGSGGGQVQLQQSGPELVKPGASVRISCKASGYTFTSYHIHWVKQRPGQGLEWIGWI
 YPGNVNTEYNEKFKGKATLTADKSSSTAYMHLSSLTSEDSAVYFCAREEITYAMDYWG
 QGTSVTVSSYG (SEQ ID NO: 35); or

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFS
SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPRTSGGGTKLEIKGGGGSGG
GGSGGGSGGGQVQLQQSGPELVKPGASVRISCKASGYTFTSYHHWVKQRPQGGLWEI
GWIYPGNVNTEYNEKFKGKATLTADKSSSTAYMHLSSLTSEDSAVYFCAREEITYAMD
YWGQGTSTVTVSS (SEQ ID NO: 125, the corresponding polynucleotide sequence is provided as
SEQ ID NO: 127)

C-002159

QLVQSGAEVKKPGSSVKVSCKASGYTFTSYHHWVRQAPGQGLEWMGWIYPGNVNTE
YNEKFKGKATITADKSTSTAYMELSSLRSEDTAVYYCAREEITYAMDYWGQGTSTVTVS
SGGGGSGGGGSGGGGSGGEIVLTQSPGTLSPGERATLSCRSSQSIVHSNGNTYLEWYQ
QKPGQAPRLLIYKVSNRFSGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCFQGSHVPRTF
GGGKVEIK (SEQ ID NO: 36)

C-002160

QLVQSGAEVKKPGSSVKVSCKASGYTFTSYHHWVRQAPGQGLEWMGWIYPGNVNTE
YNEKFKGKATITADKSTSTAYMELSSLRSEDTAVYYCAREEITYAMDYWGQGTSTVTVS
SGGGGSGGGGSGGGGSGGDIVMTQTPLSLPVTGPGEPAISCRSSQSIVHSNGNTYLEWYL
QKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT
FGGKVEIK (SEQ ID NO: 37)

C-002161

QLVESGGGLVKPGGSLRLSCAASGYTFTSYHHWVRQAPGKGLEWVGWIYPGNVNTEY
NEKFKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCAREEITYAMDYWGQGTSTVTVSS
GGGGSGGGGSGGGGSGGDIQMTQSPSSLSASVGDRTTITCRSSQSIVHSNGNTYLEWYQ
QKPGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCFQGSHVPRTF
GGGKVEIK (SEQ ID NO: 38)

C-002162

QLVQSGAEVKKPGSSVKVSCKASGYTFTSYHHWVRQAPGQGLEWIGWIYPGNVNTEY
NEKFKGKATITADESTNTAYMELSSLRSEDTAVYYCAREEITYAMDYWGQGTSLVTVSS
GGGGSGGGGSGGGGSGGDIQMTQSPSTLSASVGDRTTITCRSSQSIVHSNGNTYLEWYQ
QKPGKAPKLLIYKVSNRFSGVPARFSGSGSGTEFTLTISLQPDFATYYCFQGSHVPRTF
GQGTKVEVK (SEQ ID NO: 39)

C-002163

QLVQSGAEVKKPGSSVKVSCKASGYTFTSYHMHWVRQAPGQGLEWIGYIYPGNVNTE
YNEKFKGKATLTADKSTNTAYMELSSLRSEDTAVYFCAREEITYAMDYWGQGTSLVTVS

SGGGGSGGGGSGGGGSGGDVQMTQSPSTLSASVGDRVITITCSSSQSIVHSNGNTYMEW
YQKPKGKAPKLLIYKVSNRFSGVPDRFSGSGSGTEFTLTISLQPD~~DF~~ATYYCHQGSHVP
RTFGQGTKVEVK (SEQ ID NO: 40)

C-002164

QVQLQQSGPELVKPGASVKMSCKASGYTFTSYHIQWVKQRPQGGLEWIGWIYPGDGST
QYNEKFKGKTTLTADKSSSTAYMLLSSLTSEDSAIYFCAREGTYYAMDYWGQGTSTV
SSGGGSGGGGSGGGGSGGDVLMQTPLSLPVSLGDQVSISCRSSQSIVHSNGNTYLEW
YLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVP
RTFGGGTKLEIK (SEQ ID NO: 41)

C-002165

QLQLQESGPGLVKPSETLSLTCTVSGYTFTSYHIQWIRQPPGKGLEWIGWIYPGDGSTOY
NEKFKGRATISVDTSKNQFSLNLDSVSAADTAIYYCAREGTYYAMDYWGKGSTVTVSS
GGGGSGGGGSGGGGSGGDIQMTQSPSSLSASVGDRVITITCRSSQSIVHSNGNTYLEWYQ
QKPKGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTFTISLQPEDATYYCFQGSHVPRTF
GPGTKVDIK (SEQ ID NO: 42)

C-002166

EVQLVQSGAELKKPGSSVKVSCKASGYTFTSYHIQWVKQAPGQGLEWIGWIYPGDGST
QYNEKFKGKATLTVDKSTNTAYMELSSLRSEDTAVYYCAREGTYYAMDYWGQGT~~LV~~T
VSSGGGSGGGGSGGGGSGGDIQMTQSPSTLSASVGDRVITITCRSSQSIVHSNGNTYLE
WYQKPKGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISLQPD~~DF~~ATYYCFQGSHV
PRTFGGTKVEVK (SEQ ID NO: 43)

C-002167

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYHIQWVRQAPGQGLEWMGWIYPGDGS
TQYNEKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCAREGTYYAMDYWGQGT~~TV~~
TVSSGGGSGGGGSGGGGSGGEIVLTQSPGTLSPGERATLSCRSSQSIVHSNGNTYLE
WYQKPGQAPRLLIYKVSNRFSGIPPDRFSGSGSGTDFTLTISRLEPEDFAVYYCFQGSHV
PRTFGGGTKVEIK (SEQ ID NO: 44)

C-002168

QVTLKQSGAEVKKPGSSVKVSCTASGYTFTSYHVS~~WVR~~QAPGQGLEWLGRIYPGDGST
QYNEKFKGKVTITADKSMDTSFMELTSLTSEDTAVYYCAREGTYYAMD~~L~~WGQGT~~LV~~T
VSSGGGSGGGGSGGGGSGGEIVLTQSPGTLSPGERATLSCRSSQSIVHSNGNTYLAW
YQKPGQAPRL~~LIS~~KVSNRFSGVPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGSHVP
RTFGGGTKVEIK (SEQ ID NO: 45)

C-002169

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHMHWVRQAPGQRLEWMGWIYPGDG
 STQYNEKFKGKVTITRDTSASTAYMELSSLRSEDVAVYYCAREGTYYAMDYWGQGLV
 TVSSGGGGSGGGSGGGSGGGDIVMTQTPLSLPVTTPGEPASISCRSSQSIVHSNGNTYLD
 WYLQKPGQSPQLLIYKVSNNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQGS
 HVPRTFGGGTKVEIK (SEQ ID NO: 46)

CDR-L1	CDR-L2	CDR-L3	CDR-H1	CDR-H2	CDR-H3
RSSQSIVH SNGNTYLE (SEQ ID NO: 22)	KVSNRFSG VPDR (SEQ ID NO: 23)	FQGSHPVR T (SEQ ID NO: 24)	ASGYTFTS YHIH (SEQ ID NO: 25)	WIYPGNVN TEYNEKFK GK (SEQ ID NO: 26)	EEITYAMD Y (SEQ ID NO: 27)
RSSQSIVH SNGNTYLD (SEQ ID NO: 28)	KVSNRFSG VPDR (SEQ ID NO: 29)	MQGSHPVR T (SEQ ID NO: 30)	SGYTFTSY HMH (SEQ ID NO: 31)	WIYPGDGS TQYNEKFK G (SEQ ID NO: 32)	EGTYIAM DY (SEQ ID NO: 33)

[0154] In some embodiments, the scFv comprises the complementarity determined regions (CDRs) of any one of SEQ ID NOS: 22-33. In some embodiments, the scFv comprises a sequence at least 95% identical to any one of SEQ ID NOS: 22-33. In some embodiments, the scFv comprises a sequence identical to any one of SEQ ID NOS: 22-33. In some embodiments, the heavy chain of the antibody comprises the heavy chain CDRs of any one of SEQ ID NOS: 25-27 or 31-33, and the light chain of the antibody comprises the light chain CDRs of any one of SEQ ID NOS: 22-24 or 28-30. In some embodiments, the heavy chain of the antibody comprises a sequence at least 95% identical to the heavy chain portion of any one of SEQ ID NOS: 35-46 or 125, and wherein the light chain of the antibody comprises a sequence at least 95% identical to the light chain portion of any one of SEQ ID NOS: 35-46 or 125. In some embodiments, the heavy chain comprises all of SEQ ID NOS: 25-27, and the light chain comprises all of SEQ ID NOS: 22-24. In some embodiments, the heavy chain comprises all of SEQ ID NOS: 31-33, and the light chain comprises all of SEQ ID NOS: 28-30.

[0155] In some embodiments, the heavy chain of the antibody comprises a sequence identical to the heavy chain portion of any one of SEQ ID NOS: 35-46 or 125, and wherein the light chain

of the antibody comprises a sequence identical to the light chain portion of any one of SEQ ID NOS: 35-46 or 125.

[0156] In some embodiments, the ScFv comprises a sequence at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical or identical to any one of SEQ ID NOS: 35-46 or 125.

B- and T-lymphocyte attenuator (BTLA) Domains

[0157] In some embodiments, the polypeptide comprises a B- and T-lymphocyte attenuator (BTLA) hinge domain, transmembrane domain, intracellular domain or a functional variant, derivative or combination thereof.

[0158] In some embodiments, the polypeptide comprises a BTLA intracellular domain. In some embodiments, the BTLA intracellular domain comprises a sequence of RRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYDNDPDLCFRMQEGSEVYSNPCLEENKPGIVYASLNHVSIGPNSRLARNVKEAPTEYASICVRS (SEQ ID NO: 87). In some embodiments, the BTLA intracellular domain comprises SEQ ID NO: 87, or a sequence with at least 95% identity thereto. In some embodiments, the BTLA intracellular domain consists essentially SEQ ID NO: 87.

[0159] In some embodiments, the BTLA transmembrane domain and intracellular domain comprises a sequence at least 95% identical to a sequence of LLPLGGLPLLITTCFCLFCCLRRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYDNDPDLCFRMQEGSEVYSNPCLEENKPGIVYASLNHVSIGPNSRLARNVKEAPTEYASICVRS (SEQ ID NO: 88). In some embodiments, the BTLA transmembrane domain and intracellular domain comprises or consists essentially of a sequence of SEQ ID NO: 88.

[0160] *Signal Peptides*

[0161] In some embodiments, the polypeptide comprises a signal peptide. For example, the polypeptide comprises a VK1 signal peptide. In some embodiments, the signal peptide is an N-terminal signal peptide. In some embodiments, the signal peptide comprises a sequence at least 95% identical to a sequence of MDMRVPAQLLGLLLLWLRGARC (SEQ ID NO: 128). In some embodiments, the signal peptide comprises a sequence of MDMRVPAQLLGLLLLWLRGARC (SEQ ID NO: 128). In some embodiments, the signal peptide is encoded by a sequence at least 95% identical to a sequence of

ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTACTCTGGCTCCGAGGT
GCCAGATGT (SEQ ID NO: 129), or a sequence identical thereto.

Antigens

[0162] The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen for the LILRB1-based receptors described herein. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an “antigen” as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. Moreover, a skilled artisan will understand that an antigen need not be encoded by a “gene” at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample, or might be macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a fluid with other biological components.

[0163] In some embodiments, the antigen-binding domain specifically binds to a target selected from etiolate receptor, $\alpha\text{v}\beta\beta$ integrin, TNF receptor superfamily member 17 (BCMA), CD276 molecule (B7-H3), natural killer cell cytotoxicity receptor 3 ligand 1 (B7-H6), carbonic anhydrase 9 (CAIX), CD19 molecule (CD19), membrane spanning 4-domains A1 (CD20), CD22 molecule (CD22), TNF receptor superfamily member 8 (CD30), CD33 molecule (CD33), CD37 molecule (CD37), CD44 molecule (CD44), CD44v6, CD44v7/8, CD70 molecule (CD70), interleukin 3 receptor subunit alpha (CD123), syndecan 1 (CD138), L1 cell adhesion molecule (CD171), CEA cell adhesion molecule (CEA), delta like canonical Notch ligand 4 (DLL4), epithelial cell adhesion molecule (EGP-2), epithelial cell adhesion molecule (EGP-40), chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor receptor (EGFR), EGFR family including ErbB2 (HER2), EGFRvIII, epithelial cell adhesion molecule (EPCAM), EPH receptor A2 (EphA2), EpCAM, fibroblast activation protein alpha (FAP), folate receptor alpha (FBP), fetal acetylcholine receptor, frizzled class receptor 7 (Fzd7), diganglioside GD2 (GD2), ganglioside GD3 (GD3), Glypican-3 (GPC3), trophoblast glycoprotein (h5T4), interleukin 11 receptor subunit alpha (IL-11R), interleukin 13 receptor subunit alpha 2 (IL13R-a2), kinase insert domain receptor (KDR), κ light chain, λ light chain, LeY, L1 cell adhesion molecule (L1 CAM),

MAGE-A1, mesothelin, MHC presented peptides, mucin 1, cell surface associated (MUC1), mucin 16, cell surface associated (MUC16), neural cell adhesion molecule 1 (NCAM), killer cell lectin like receptor K1 (NKG2D) ligands, Notch1, Notch2/3, NY-ESO-1, PRAME nuclear receptor transcriptional regulator (PRAME), prostate stem cell antigen (PSCA), folate hydrolase 1 (PSMA), Survivin, TAG-72, TEMs, telomerase reverse transcriptase (TERT), kinase insert domain receptor (VEGFR2), and receptor tyrosine kinase like orphan receptor 1 (ROR1).

[0164] In some embodiments, the antigen-binding domain specifically binds to a target selected from CD33, CD38, a human leukocyte antigen (HLA), an organ specific antigen, a blood-brain barrier specific antigen, an Epithelial-mesenchymal transition (EMT) antigen, E-cadherin, cytokeratin, Opioid-binding protein/cell adhesion molecule (OPCML), HYLA2, Deleted in Colorectal Carcinoma (DCC), Scaffold/Matrix attachment region-binding protein 1 (SMAR1), cell surface carbohydrate and mucin type O-glycan.

[0165] In some embodiments, the extracellular domain of the LILRB1-based receptors described herein comprises an antigen-binding domain specific to an antigen that is lost through loss of heterozygosity in cells of a subject.

[0166] As used herein, “loss of heterozygosity (LOH)” refers to a genetic change that occurs at high frequency in cancers, whereby one of the two alleles is deleted, leaving a single mono-allelic (hemizygous) locus.

[0167] In some embodiments, the LILRB1-based receptor comprises an antigen-binding domain specific to a minor histocompatibility antigen (MiHA). MiHAs are peptides derived from proteins that contain nonsynonymous differences between alleles and are displayed by common HLA alleles. The non-synonymous differences can arise from SNPs, deletions, frameshift mutations or insertions in the coding sequence of the gene encoding the MiHA. Exemplary MiHAs can be about 9-12 amino acids in length and can bind to MHC class I and MHC class II proteins. Binding of the TCR to the MHC complex displaying the MiHA can activate T cells. The genetic and immunological properties of MiHAs will be known to the person of ordinary skill in the art, and specific MiHAs described in PCT/US2020/045228, the contents of which are incorporated by reference.

[0168] In some embodiments, the LILRB1-based receptor comprises an antigen-binding domain specific to an antigen that is lost in cancer cells of a subject through loss of Y chromosome.

[0169] In some embodiments, the LILRB1-based receptor comprises an antigen-binding domain specific to an HLA class I allele. The major histocompatibility complex (MHC) class I is a protein complex that displays antigens to cells of the immune system, triggering immune response. The Human Leukocyte Antigens (HLAs) corresponding to MHC class I are HLA-A, HLA-B and HLA-C. HLA-E is known in the art as a non-classical MHC class I molecule. In some embodiments, the antigen for the LILRB1-based receptor comprises an HLA class I allele. In some embodiments, allele of HLA class I is lost in a target cell, such as a cancer cell, through loss of heterozygosity (LOH).

[0170] HLA-A is a group of human leukocyte antigens (HLA) of the major histocompatibility complex (MHC) that are encoded by the HLA-A locus. HLA-A is one of three major types of human MHC class I cell surface receptors. The receptor is a heterodimer comprising a heavy α chain and smaller β chain. The α chain is encoded by a variant of HLA-A, while the β chain (β 2-microglobulin) is invariant. There are several thousand HLA-A variants, all of which fall within the scope of the instant disclosure.

[0171] In some embodiments, the LILRB1-based receptor comprises an antigen-binding domain specific to an HLA-B allele. The HLA-B gene has many possible variations (alleles). Hundreds of versions (alleles) of the HLA-B gene are known, each of which is given a particular number (such as HLA-B27).

[0172] In some embodiments, the LILRB1-based receptor comprises an antigen-binding domain specific to an HLA-C allele. HLA-C belongs to the HLA class I heavy chain paralogues. This class I molecule is a heterodimer consisting of a heavy chain and a light chain (β 2 microglobulin).

[0173] In some embodiments, the HLA class I allele has broad or ubiquitous RNA expression.

[0174] In some embodiments, the HLA class I allele has a known, or generally high minor allele frequency.

[0175] In some embodiments, the HLA class I allele does not require a peptide-MHC antigen, for example when the HLA class I allele is recognized by a pan-HLA ligand binding domain.

[0176] In some embodiments, the LILRB1-based receptor comprises an antigen-binding domain specific to an HLA-A allele. In some embodiments the HLA-A allele comprises HLA-A*02. Various single variable domains known in the art or disclosed herein that bind to and recognize HLA-A*02 are suitable for use in embodiments, and are described herein.

[0177] In some embodiments, the antigen-binding domain specifically binds to an HLA-A*02 antigen. In some embodiments, the antigen-binding domain specifically binds to an HLA-A*02 antigen in a peptide-independent manner.

Polynucleotides and Vectors

[0178] In other aspects, the disclosure provides polynucleotides comprising a nucleic acid sequence encoding receptors of the disclosure. In some embodiments, the polynucleotides encode one or more of an LILRB1 hinge domain, an LILRB1 transmembrane domain and an LILRB1 intracellular domain or a functional derivative or fragment thereof.

[0179] In some embodiments, the polynucleotide comprises a nucleic acid sequence that encodes a polypeptide that is at least 95% identical to any one of SEQ ID NOS: 1-7 or 12-21. In some embodiments, the polynucleotide comprises a nucleic acid sequence that encodes a polypeptide that is at least 95% identical to any one of SEQ ID NOS: 47-71, 77-79, 89-92, 120 or 122. In some embodiments, the polynucleotide comprises a nucleic acid sequence that encodes a polypeptide that is at least 95% identical to the heavy chain portion or the light chain portion of any one of SEQ ID NOS: 35-46 or 125. In some embodiments, the polynucleotide comprises a nucleic acid sequence that encodes a polypeptide that is at least 95% identical to the heavy chain portion or the light chain portion of any one of SEQ ID NOS: 35, 39, 46 or 125. In some embodiments, the polynucleotide comprises a nucleic acid sequence that encodes a polypeptide that is identical to the heavy chain portion or the light chain portion of any one of SEQ ID NOS: 35, 39, 46 or 125. In another aspect, the disclosure provides vectors comprising the polynucleotides encoding receptors of the disclosure.

[0180] In some embodiments, the polynucleotide comprises a sequence at least 95% identical to SEQ ID NO: 121 or 123. In some embodiments, the polynucleotide comprises SEQ ID NO: 121 or 123.

[0181] In some embodiments, the polynucleotide comprises a sequence of a LILRB1 hinge, transmembrane and intracellular domain. In some embodiments, the polynucleotide comprises a sequence at least 95% identical to SEQ ID NO: 126. In some embodiments, the polynucleotide comprises a sequence of SEQ ID NO: 126.

[0182] Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its

propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity.

[0183] The expression of natural or synthetic nucleic acids encoding receptors is typically achieved by operably linking a nucleic acid encoding receptor or portions thereof to a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration in eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

[0184] The polynucleotides encoding the receptors can be cloned into a number of types of vectors. For example, the polynucleotides can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[0185] Further, the expression vector may be provided to cells, such as immune cells, in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2001, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (*e.g.*, WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[0186] A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivirus vectors are used.

[0187] In some embodiments, the vector comprises a promoter. Vectors can also include additional regulatory elements. Additional regulatory elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 basepairs (bp) upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

[0188] One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor-1 α (EF-1 α). However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionein promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

[0189] In order to assess the expression of receptor the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and

reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

[0190] Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

[0191] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (2001, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York). One method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection.

[0192] Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[0193] Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g., an artificial membrane vesicle).

[0194] Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, “molecular biological” assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; “biochemical” assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

Engineered cells

[0195] In another aspect, the disclosure provides immune cells comprising a nucleic acid sequence or vector encoding receptors of the disclosure and/or expressing receptors of the disclosure.

[0196] In embodiments, immune cell activation is reduced when the cell is contacted with the antigen of the LILRB1 based receptors of the disclosure, or a cell expressing the antigen on its surface. In embodiments, immune cell activation comprises expression of a gene operatively linked to an NFAT promoter. Immune cell activation and/or inhibition of activation can be measured by various other methods known in the art. In some embodiments, the immune cell comprises an additional exogenous receptor, for example an activator receptor such as a chimeric antigen receptor (CAR) or TCR.

[0197] In embodiments, the immune cell is a T cell.

[0198] As used herein, the term “immune cell” refers to a cell involved in the innate or adaptive (acquired) immune systems. Exemplary innate immune cells include phagocytic cells such as neutrophils, monocytes and macrophages, Natural Killer (NK) cells, polymorphonuclear leukocytes such as neutrophils eosinophils and basophils and mononuclear cells such as monocytes, macrophages and mast cells. Immune cells with roles in acquired immunity include lymphocytes such as T-cells and B-cells.

[0199] As used herein, a “T-cell” refers to a type of lymphocyte that originates from a bone marrow precursor that develops in the thymus gland. There are several distinct types of T-cells which develop upon migration to the thymus, which include, helper CD4⁺ T-cells, cytotoxic CD8⁺ T cells, memory T cells, regulatory CD4⁺ T-cells and stem memory T-cells. Different types of T-cells can be distinguished by the ordinarily skilled artisan based on their expression of markers. Methods of distinguishing between T-cell types will be readily apparent to the ordinarily skilled artisan.

Method of making engineered cells

[0200] In another aspect, the disclosure provides methods comprising introducing a polynucleotide of the disclosure into cells, optionally using vectors of the disclosure. In

embodiments, the resulting cell expresses LILRB1 based receptor encoded by the polynucleotide. In embodiments, the cell is an immune cell. In embodiments, the immune cell is a T cell.

[0201] Methods of transforming populations of immune cells, such as T cells, with the vectors of the instant disclosure will be readily apparent to the person of ordinary skill in the art. For example, CD3+ T cells can be isolated from PBMCs using a CD3+ T cell negative isolation kit (Miltenyi), according to manufacturer's instructions. T cells can be cultured at a density of 1×10^6 cells/mL in X-Vivo 15 media supplemented with 5% human A/B serum and 1% Pen/strep in the presence of CD3/28 Dynabeads (1:1 cell to bead ratio) and 300 Units/mL of IL-2 (Miltenyi). After 2 days, T cells can be transduced with viral vectors, such as lentiviral vectors using methods known in the art. In some embodiments, the viral vector is transduced at a multiplicity of infection (MOI) of 5. Cells can then be cultured in IL-2 or other cytokines such as combinations of IL-7/15/21 for an additional 5 days prior to enrichment. Methods of isolating and culturing other populations of immune cells, such as B cells, or other populations of T cells, will be readily apparent to the person of ordinary skill in the art. Although this method outlines a potential approach it should be noted that these methodologies are rapidly evolving. For example excellent viral transduction of peripheral blood mononuclear cells can be achieved after 5 days of growth to generate a >99% CD3+ highly transduced cell population.

[0202] Methods of activating and culturing populations of T cells comprising the receptors, polynucleotides or vectors of the disclosure will be readily apparent to the person of ordinary skill in the art.

[0203] Whether prior to or after genetic modification, T cells can be activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041, 10040846; and U.S. Pat. Appl. Pub. No. 2006/0121005.

[0204] In some embodiments, T cells of the instant disclosure are expanded and activated in vitro. Generally, the T cells of the instant disclosure are expanded in vitro by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated as described herein, such as by contact with an anti-CD3 antibody. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the

accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either CD4⁺ T cells or CD8⁺ T cells, an anti-CD3 antibody and an anti-CD28 antibody can be used. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besançon, France) can be used as can other methods commonly known in the art (Berg et al., Transplant Proc. 30(8):3975-3977, 1998; Haanen et al., J. Exp. Med. 190(9):1319-1328, 1999; Garland et al., J. Immunol Meth. 227(1-2):53-63, 1999).

[0205] In some embodiments, the primary stimulatory signal and the co-stimulatory signal for the T cell may be provided by different protocols. For example, the agents providing each signal may be in solution or coupled to a surface. When coupled to a surface, the agents may be coupled to the same surface (i.e., in “cis” formation) or to separate surfaces (i.e., in “trans” formation). Alternatively, one agent may be coupled to a surface and the other agent in solution. In some embodiments, the agent providing the co-stimulatory signal is bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain embodiments, both agents can be in solution. In another embodiment, the agents may be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example, U.S. Patent Application Publication Nos. 20040101519 and 20060034810 for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T cells in the present invention.

[0206] In some embodiments, the two agents are immobilized on beads, either on the same bead, i.e., “cis,” or to separate beads, i.e., “trans.” By way of example, the agent providing the primary activation signal is an anti-CD3 antibody or an antigen-binding fragment thereof and the agent providing the co-stimulatory signal is an anti-CD28 antibody or antigen-binding fragment thereof; and both agents are co-immobilized to the same bead in equivalent molecular amounts. In one embodiment, a 1:1 ratio of each antibody bound to the beads for CD4⁺ T cell expansion and T cell growth is used. In some embodiments, the ratio of CD3:CD28 antibody bound to the beads ranges from 100:1 to 1:100 and all integer values there between. In one aspect of the present invention, more anti-CD28 antibody is bound to the particles than anti-CD3 antibody, i.e., the ratio of CD3:CD28 is less than one. In certain embodiments of the invention, the ratio of anti CD28 antibody to anti CD3 antibody bound to the beads is greater than 2:1.

[0207] Ratios of particles to cells from 1:500 to 500:1 and any integer values in between may be used to stimulate T cells or other target cells. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells may depend on particle size relative to the target cell. For example, small sized beads could only bind a few cells, while larger beads could bind many. In certain embodiments the ratio of cells to particles ranges from 1:100 to 100:1 and any integer values in-between and in further embodiments the ratio comprises 1:9 to 9:1 and any integer values in between, can also be used to stimulate T cells. In some embodiments, a ratio of 1:1 cells to beads is used. One of skill in the art will appreciate that a variety of other ratios may be suitable for use in the present invention. In particular, ratios will vary depending on particle size and on cell size and type.

[0208] In further embodiments of the present invention, the cells, such as T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative embodiment, prior to culture, the agent-coated beads and cells are not separated but are cultured together. In a further embodiment, the beads and cells are first concentrated by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

[0209] By way of example, cell surface proteins may be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached to contact the T cells. In one embodiment the cells (for example, CD4⁺ T cells) and beads (for example, DYNABEADS CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer. Again, those of ordinary skill in the art can readily appreciate any cell concentration may be used. In certain embodiments, it may be desirable to significantly decrease the volume in which particles and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and particles. For example, in one embodiment, a concentration of about 2 billion cells/ml is used. In another embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. In some embodiments, cells that are cultured at a density of 1×10^6 cells/mL are used.

[0210] In some embodiments, the mixture may be cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. In another embodiment, the beads and T

cells are cultured together for 2-3 days. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN- γ , IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGF β , and TNF- α or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α -MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. In some embodiments, the media comprises X-VIVO-15 media supplemented with 5% human A/B serum, 1% penicillin/streptomycin (pen/strep) and 300 Units/ml of IL-2 (Miltenyi).

[0211] The T cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (*e.g.*, 37° C.) and atmosphere (*e.g.*, air plus 5% CO₂).

[0212] In some embodiments, the T cells comprising receptors of the disclosure are autologous. Prior to expansion and genetic modification, a source of T cells is obtained from a subject. Immune cells such as T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present invention, any number of T cell lines available in the art, may be used. In certain embodiments of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation.

[0213] In some embodiments, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In some embodiments, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In alternative embodiments, the wash solution lacks calcium and may lack magnesium or may lack many if not

all divalent cations. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated “flow-through” centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca²⁺-free, Mg²⁺-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[0214] In some embodiments, immune cells such as T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. Specific subpopulations of immune cells, such as T cells, B cells, or CD4⁺ T cells can be further isolated by positive or negative selection techniques. For example, in one embodiment, T cells are isolated by incubation with anti-CD4 -conjugated beads, for a time period sufficient for positive selection of the desired T cells.

[0215] Enrichment of an immune cell population, such as a T cell population, by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immune-adherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4⁺ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD 14, CD20, CD 11b, CD 16, HLA-DR, and CD8.

[0216] For isolation of a desired population of immune cells by positive or negative selection, the concentration of cells and surface (e.g., particles such as beads) can be varied. In certain embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and beads.

[0217] In some embodiments, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either 2-10° C or at room temperature.

[0218] T cells for stimulation, or PBMCs from which immune cells such as T cells are isolated, can also be frozen after a washing step. Wishing not to be bound by theory, the freeze and

subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80°C . at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20°C . or in liquid nitrogen.

Assaying Signaling

[0219] In some embodiments, immune cell activation is reduced when the cell is contacted with the antigen corresponding to the LILRB1 based receptor of the disclosure, or a cell expressing the antigen on its surface. In some embodiments, immune cell activation comprises expression of a gene operatively linked to an NFAT promoter. Nuclear factor of activated T-cells (NFAT) is a family of transcription factors shown to be important in immune response. The NFAT transcription factor family consists of five members NFATc1, NFATc2, NFATc3, NFATc4, and NFAT5. NFAT plays a role in regulating inflammation.

[0220] As used herein, an NFAT promoter is a promoter that is regulated (i.e., activated or repressed) when NFAT is expressed in a cell. NFAT target promoters are described in Badran, B. M. et al.(2002) J. Biological Chemistry Vol. 277: 47136-47148, and contain NFAT consensus sequences such as GGAAA.

[0221] Methods of assessing the effects of receptor activation on gene expression are known in the art, and include the use of reporter genes, whose expression can be quantified. Reporter genes are used for identifying potentially transfected or transduced cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells.

Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription. In exemplary embodiments, an NFAT promoter operably linked to a reporter gene is used to evaluate the expression of the receptors of the disclosure on NFAT signaling.

Pharmaceutical Compositions

[0222] The disclosure provides pharmaceutical compositions comprising immune cells comprising the LILRB1-based receptors of the disclosure a pharmaceutically acceptable diluent, carrier or excipient.

[0223] Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; and preservatives.

Methods of Treating Disease

[0224] Provided herein are methods of treating a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a composition comprising a plurality of immune cells comprising the LILRB1-based receptors described herein. In some embodiments, the immune cells further comprise an activator receptor, such as an activator CAR or TCR.

[0225] Additional methods of treating subjects, and activator receptors combination combined with inhibitory receptors, are described in PCT/US2020/045228, the contents of which are incorporated by reference herein in their entirety.

[0226] In some embodiments, the subject in need thereof has cancer. In some embodiments, the methods of treating the subject comprise administering to the subject a plurality of immune cells comprising the LILRB1-receptors of the disclosure. In some embodiments, the plurality of immune cells further comprises an activator receptor, such as a CAR or a TCR. In some

embodiments, the CAR or TCR comprises an antigen-binding domain specific to a cancer antigen. Activator receptors specific for cancer antigens can comprise antigen-binding domains isolated or derived from any antibody or antigen-binding domain known in the art, including, but not limited to, urelumab, utomilumab, oleclumab, naptumomab, ascrinvacumab, tacatuzumab, nesvacumab, vanucizumab, belimumab, tabalumab, tibulizumab, belantamab, igovomab, oregovomab, sofituzumab, mogamulizumab, talacotuzumab, tavolimab, vonlerolizumab, ipilimumab, duvortuxizumab, blinatumomab, coltuximab, denintuzumab, inebilizumab, loncastuximab, taplitumomab, ibritumomab, obinutuzumab, ocaratuzumab, ocrelizumab, ofatumumab, rituximab, tositumomab, veltuzumab, samalizumab, bectumomab, epratuzumab, inotuzumab, moxetumomab, pinatuzumab, gomiliximab, lumiliximab, camidanlumab, basiliximab, inolimomab, daclizumab, varlilumab, enoblituzumab, omburtamab, brentuximab, iratumumab, gemtuzumab, lintuzumab, vadastuximab, lilotomab, otlertuzumab, tetulomab, daratumumab, isatuximab, bivatumab, abituzumab, intetumumab, lorvotuzumab, itolizumab, cusatumumab, vorsetuzumab, milatumab, polatumab, iladatuzumab, galixima, altumomab, arcitumomab, labetuzumab, cibisatamab, zolbetuximab, lacnotuzumab, cabiralizumab, emactuzumab, gimsilumab, lenzilumab, otilimab, mavrilimumab, tremelimumab, ulocuplumab, tepoditamab, rovalpituzumab, demcizumab, drozitumab, parsatumab, cetuximab, depatuxizumab, futuximab, imgatuzumab, laprituximab, matuzumab, necitumumab, nimotuzumab, panitumumab, zalutumumab, modotuximab, amivantamab, tomuzotuximab, losatuxizumab, adecatumumab, citatumab, edrecolomab, oportuzumab, solitomab, tucotuzumab, catumaxomab, ifabotuzumab, duligotuzumab, elgantumab, lumretuzumab, patritumab, seribantumab, zenocutuzumab, aprutumab, bemarituzumab, vantictumab, dinutuximab, ecomeximab, mitumomab, codrituzumab, glembatumumab, zatuximab, ertumaxomab, margetuximab, timigutuzumab, gancotamab, pertuzumab, trastuzumab, ficlatuzumab, rilotumumab, telisotuzumab, emibetuzumab, cixutumumab, dalotuzumab, figitumumab, ganitumab, robatumumab, teprotumumab, flotetuzumab, bermekimab, cergutuzumab, volociximab, etaracizumab, relatlimab, carlumab, amatuximab, clivatuzumab, gatipotuzumab, pentumomab, cantuzumab, pankomab, racotumomab, brontictuzumab, tarextumab, vesencumab, camrelizumab, cetrelimab, nivolumab, pembrolizumab, pidilizumab, cemiplimab, spartalizumab, atezolizumab, avelumab, durvalumab, cirmtuzumab, tenatumomab, fresolimumab, brolocizumab, bevacizumab, ranibizumab, varisacumab, faricimab, icrucumab, alacizumab, and ramucirumab.

[0227] In some embodiments, the LILRB1-based receptor of the disclosure comprises an antigen-binding domain specific to an antigen that is lost in the cancer cells through loss of heterozygosity. In some embodiments, the antigen is a minor histocompatibility antigen (MiHA). In some embodiments, the antigen is an HLA class I allele. In some embodiments, the HLA class I allele comprises HLA-A, HLA-B or HLA-C. In some embodiments, the HLA class I allele comprises HLA-E. In some embodiments, the HLA class I allele is an HLA-A*02 allele. In some embodiments, the antigen is not expressed in the target cell due to loss of Y chromosome. In some embodiments, the antigen specific to the LILRB1-based receptor is an HLA-A*02 antigen.

[0228] In some embodiments, the subject in need thereof has cancer. Cancer is a disease in which abnormal cells divide without control and spread to nearby tissue. In some embodiments, the cancer comprises a liquid tumor or a solid tumor. Exemplary liquid tumors include leukemias and lymphomas. Further cancers that are liquid tumors can be those that occur, for example, in blood, bone marrow, and lymph nodes, and can include, for example, leukemia, myeloid leukemia, lymphocytic leukemia, lymphoma, Hodgkin's lymphoma, melanoma, and multiple myeloma. Leukemias include, for example, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), and hairy cell leukemia. Exemplary solid tumors include sarcomas and carcinomas. Cancers can arise in virtually an organ in the body, including blood, bone marrow, lung, breast, colon, bone, central nervous system, pancreas, prostate and ovary. Further cancers that are solid tumors include, for example, prostate cancer, testicular cancer, breast cancer, brain cancer, pancreatic cancer, colon cancer, thyroid cancer, stomach cancer, lung cancer, ovarian cancer, Kaposi's sarcoma, skin cancer, squamous cell skin cancer, renal cancer, head and neck cancers, throat cancer, squamous carcinomas that form on the moist mucosal linings of the nose, mouth, throat, bladder cancer, osteosarcoma, cervical cancer, endometrial cancer, esophageal cancer, liver cancer, and kidney cancer. In some embodiments, the condition treated by the methods described herein is metastasis of melanoma cells, prostate cancer cells, testicular cancer cells, breast cancer cells, brain cancer cells, pancreatic cancer cells, colon cancer cells, thyroid cancer cells, stomach cancer cells, lung cancer cells, ovarian cancer cells, Kaposi's sarcoma cells, skin cancer cells, renal cancer cells, head or neck cancer cells, throat cancer cells, squamous carcinoma cells, bladder cancer cells, osteosarcoma cells, cervical cancer cells, endometrial cancer cells, esophageal cancer cells, liver cancer cells, or kidney cancer cells.

[0229] Any cancer wherein a plurality of the cancer cells express the first, activator ligand and do not express the second, inhibitor ligand is envisaged as within the scope of the instant disclosure. For example, CEA positive cancers that can be treated using the methods described herein include colorectal cancer, pancreatic cancer, esophageal cancer, gastric cancer, lung adenocarcinoma, head and neck cancer, diffuse large B cell cancer or acute myeloid leukemia cancer.

[0230] Treating cancer can result in a reduction in size of a tumor. A reduction in size of a tumor may also be referred to as “tumor regression”. Preferably, after treatment, tumor size is reduced by 5% or greater relative to its size prior to treatment; more preferably, tumor size is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75% or greater. Size of a tumor may be measured by any reproducible means of measurement. The size of a tumor may be measured as a diameter of the tumor.

[0231] Treating cancer can result in a reduction in tumor volume. Preferably, after treatment, tumor volume is reduced by 5% or greater relative to its size prior to treatment; more preferably, tumor volume is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75% or greater. Tumor volume may be measured by any reproducible means of measurement.

[0232] Treating cancer results in a decrease in number of tumors. Preferably, after treatment, tumor number is reduced by 5% or greater relative to number prior to treatment; more preferably, tumor number is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75%. Number of tumors may be measured by any reproducible means of measurement. The number of tumors may be measured by counting tumors visible to the naked eye or at a specified magnification. Preferably, the specified magnification is 2x, 3x, 4x, 5x, 10x, or 50x.

[0233] Treating cancer can result in a decrease in number of metastatic lesions in other tissues or organs distant from the primary tumor site. Preferably, after treatment, the number of metastatic lesions is reduced by 5% or greater relative to number prior to treatment; more preferably, the

number of metastatic lesions is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75%. The number of metastatic lesions may be measured by any reproducible means of measurement. The number of metastatic lesions may be measured by counting metastatic lesions visible to the naked eye or at a specified magnification. Preferably, the specified magnification is 2x, 3x, 4x, 5x, 10x, or 50x.

[0234] Treating cancer can result in an increase in average survival time of a population of treated subjects in comparison to a population receiving carrier alone. Preferably, the average survival time is increased by more than 30 days; more preferably, by more than 60 days; more preferably, by more than 90 days; and most preferably, by more than 120 days. An increase in average survival time of a population may be measured by any reproducible means. An increase in average survival time of a population may be measured, for example, by calculating for a population the average length of survival following initiation of treatment with an active compound. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival following completion of a first round of treatment with an active compound.

[0235] Treating cancer can result in an increase in average survival time of a population of treated subjects in comparison to a population of untreated subjects. Preferably, the average survival time is increased by more than 30 days; more preferably, by more than 60 days; more preferably, by more than 90 days; and most preferably, by more than 120 days. An increase in average survival time of a population may be measured by any reproducible means. An increase in average survival time of a population may be measured, for example, by calculating for a population the average length of survival following initiation of treatment with an active compound. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival following completion of a first round of treatment with an active compound.

[0236] Treating cancer can result in increase in average survival time of a population of treated subjects in comparison to a population receiving monotherapy with a drug that is not a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, analog or derivative thereof. Preferably, the average survival time is increased by more than 30 days; more

preferably, by more than 60 days; more preferably, by more than 90 days; and most preferably, by more than 120 days. An increase in average survival time of a population may be measured by any reproducible means. An increase in average survival time of a population may be measured, for example, by calculating for a population the average length of survival following initiation of treatment with an active compound. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival following completion of a first round of treatment with an active compound.

[0237] Treating cancer can result in a decrease in the mortality rate of a population of treated subjects in comparison to a population receiving carrier alone. Treating cancer can result in a decrease in the mortality rate of a population of treated subjects in comparison to an untreated population. Treating cancer can result in a decrease in the mortality rate of a population of treated subjects in comparison to a population receiving monotherapy with a drug that is not a compound of the present invention, or 1a pharmaceutically acceptable salt, prodrug, metabolite, analog or derivative thereof. Preferably, the mortality rate is decreased by more than 2%; more preferably, by more than 5%; more preferably, by more than 10%; and most preferably, by more than 25%. A decrease in the mortality rate of a population of treated subjects may be measured by any reproducible means. A decrease in the mortality rate of a population may be measured, for example, by calculating for a population the average number of disease-related deaths per unit time following initiation of treatment with an active compound. A decrease in the mortality rate of a population may also be measured, for example, by calculating for a population the average number of disease-related deaths per unit time following completion of a first round of treatment with an active compound.

[0238] Treating cancer can result in a decrease in tumor growth rate. Preferably, after treatment, tumor growth rate is reduced by at least 5% relative to number prior to treatment; more preferably, tumor growth rate is reduced by at least 10%; more preferably, reduced by at least 20%; more preferably, reduced by at least 30%; more preferably, reduced by at least 40%; more preferably, reduced by at least 50%; even more preferably, reduced by at least 50%; and most preferably, reduced by at least 75%. Tumor growth rate may be measured by any reproducible means of measurement. Tumor growth rate can be measured according to a change in tumor diameter per unit time.

[0239] Treating cancer can result in a decrease in tumor regrowth. Preferably, after treatment, tumor regrowth is less than 5%; more preferably, tumor regrowth is less than 10%; more preferably, less than 20%; more preferably, less than 30%; more preferably, less than 40%; more preferably, less than 50%; even more preferably, less than 50%; and most preferably, less than 75%. Tumor regrowth may be measured by any reproducible means of measurement. Tumor regrowth is measured, for example, by measuring an increase in the diameter of a tumor after a prior tumor shrinkage that followed treatment. A decrease in tumor regrowth is indicated by failure of tumors to reoccur after treatment has stopped.

[0240] Treating or preventing a cell proliferative disorder can result in a reduction in the rate of cellular proliferation. Preferably, after treatment, the rate of cellular proliferation is reduced by at least 5%; more preferably, by at least 10%; more preferably, by at least 20%; more preferably, by at least 30%; more preferably, by at least 40%; more preferably, by at least 50%; even more preferably, by at least 50%; and most preferably, by at least 75%. The rate of cellular proliferation may be measured by any reproducible means of measurement. The rate of cellular proliferation is measured, for example, by measuring the number of dividing cells in a tissue sample per unit time.

[0241] Treating or preventing a cell proliferative disorder can result in a reduction in the proportion of proliferating cells. Preferably, after treatment, the proportion of proliferating cells is reduced by at least 5%; more preferably, by at least 10%; more preferably, by at least 20%; more preferably, by at least 30%; more preferably, by at least 40%; more preferably, by at least 50%; even more preferably, by at least 50%; and most preferably, by at least 75%. The proportion of proliferating cells may be measured by any reproducible means of measurement. Preferably, the proportion of proliferating cells is measured, for example, by quantifying the number of dividing cells relative to the number of nondividing cells in a tissue sample. The proportion of proliferating cells can be equivalent to the mitotic index.

[0242] Treating or preventing a cell proliferative disorder can result in a decrease in size of an area or zone of cellular proliferation. Preferably, after treatment, size of an area or zone of cellular proliferation is reduced by at least 5% relative to its size prior to treatment; more preferably, reduced by at least 10%; more preferably, reduced by at least 20%; more preferably, reduced by at least 30%; more preferably, reduced by at least 40%; more preferably, reduced by at least 50%; even more preferably, reduced by at least 50%; and most preferably, reduced by at least 75%. Size of an area or zone of cellular proliferation may be measured by any reproducible means of

measurement. The size of an area or zone of cellular proliferation may be measured as a diameter or width of an area or zone of cellular proliferation.

[0243] Treating or preventing a cell proliferative disorder can result in a decrease in the number or proportion of cells having an abnormal appearance or morphology. Preferably, after treatment, the number of cells having an abnormal morphology is reduced by at least 5% relative to its size prior to treatment; more preferably, reduced by at least 10%; more preferably, reduced by at least 20%; more preferably, reduced by at least 30%; more preferably, reduced by at least 40%; more preferably, reduced by at least 50%; even more preferably, reduced by at least 50%; and most preferably, reduced by at least 75%. An abnormal cellular appearance or morphology may be measured by any reproducible means of measurement. An abnormal cellular morphology can be measured by microscopy, e.g., using an inverted tissue culture microscope. An abnormal cellular morphology can take the form of nuclear pleiomorphism.

Kits and Articles of Manufacture

[001] The disclosure provides kits and articles of manufacture comprising the polynucleotides and vectors encoding the receptors described herein. In some embodiments, the kit comprises articles such as vials, syringes and instructions for use.

[002] In some embodiments, the kit comprises a polynucleotide or vector comprising a sequence encoding one or more chimeric antigen receptors of the disclosure. For example, the polynucleotide or vector comprises a sequence one or more LILRB1 domains as described herein.

[003] In some embodiments, the kit comprises a plurality of immune cells comprising a chimeric antigen receptor as described herein. In some embodiments, the plurality of immune cells comprises a plurality of T cells.

Polypeptide Sequences for Elements of Illustrative Chimeric Antigen Receptors

Name	Sequence
LILRB1	MTPILTVLICLGLSLGPRTHVQAGHLPKPTLWAEPGSVITQ GSPVTLRCQGGQETQEYRLYREKKTALWITRIPQELVKKG QFPIPSITWEHAGRYRCYYGSDTAGRSESSDPLELVVTGA YIKPTLSAQSPVVNSGGNVILQCDSQVAFDGFSLCKEGED EHPQCLNSQPHARGSSRAIFSVGPVSPSRRWWYRCYAYDS NSPYEWSLPSDLLELLVLGVSKKPSLSVQPGPIVAPEETLT

	LQCGSDAGYNRFVLYKDGGERDFLQLAGAQPQAGLSQANF TLGPVSRSYGGQYRCYGAHNLSSEWSAPSDPLDILIAQGF YDRVSLSVQPGPTVASGENVTLLCQSQGWMQTFLLTKEG AADDPWRLRSTYQSQKYQAEFPMGPVTSAHAGTYRCY <u>YG</u> <u>SQSSKPYLLTHPSDPLEL</u> VVSGPSGGPSSPTTGPTSTSGPE DQPLTPTGSDPQSGGLGRHLGVVIGILVAVILLLLLLLLLLFLI LRHRRQGKHWSTSTQRKADFQHPAGAVGPEPTDRGLQWR SSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQA <u>VTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQ</u> <u>MDTEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGPSP</u> <u>AVPSIYATLAIHPSQEGPSPAVPSIYATLAIH</u> SEQ ID NO: 1
LILRB1 hinge- transmembrane- intracellular domain (SEQ ID NO: 126 is the polynucleotide sequence)	<u>YGSQSSKPYLLTHPSDPLEL</u> VVSGPSGGPSSPTTGPTSTSG PEDQPLTPTGSDPQSGGLGRHLGVVIGILVAVILLLLLLLLLL <u>FLILRHRRQGKHWSTSTQRKADFQHPAGAVGPEPTDRGLQ</u> <u>WRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDEDP</u> <u>QAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDR</u> <u>QMDTEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGPS</u> <u>PAVPSIYATLAIH</u> SEQ ID NO: 2
LILRB1 hinge- transmembrane- intracellular domain (w/o YGSQSSKPYLLTHPSD PLEL, SEQ ID NO: 18)	VVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGGLGRH LGVVIGILVAVILLLLLLLLLL <u>FLILRHRRQGKHWSTSTQRK</u> <u>ADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAV</u> <u>KHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMA</u> <u>SPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYA</u> <u>QLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH</u> SEQ ID NO: 3
LILRB1 hinge domain	<u>YGSQSSKPYLLTHPSDPLEL</u> VVSGPSGGPSSPTTGPTSTSG PEDQPLTPTGSDPQSGGLGRHLG SEQ ID NO: 4
LILRB1 transmembrane domain	<u>VVIGILVAVILLLLLLLLLLFLIL</u> SEQ ID NO: 5
LILRB1 intracellular domain	RHRRQGKHWSTSTQRKADFQHPAGAVGPEPTDRGLQWRS SPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAV TYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQM DTEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGPSPA VPSIYATLAIH SEQ ID NO: 7
ITIM1	NLYAAV SEQ ID NO: 8
ITIM2	VTYAEV SEQ ID NO: 9
ITIM3	VTYAQL

	SEQ ID NO: 10
ITIM4	SIYATL SEQ ID NO: 11
ITIM1-2	<u>NLYAAVKHTQPEDGVEMDTRSPHDEDPQA</u> <u>VTYAEV</u> SEQ ID NO: 12
ITIM2-3	<u>VTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQ</u> <u>MDTEAAASEAPQD</u> <u>VTYAQL</u> SEQ ID NO: 13
ITIM3-4	<u>VTYAQLHSLTLRREATEPPPSQEGPSPAVP</u> <u>SIYATL</u> SEQ ID NO: 14
ITIM1-3	<u>NLYAAVKHTQPEDGVEMDTRSPHDEDPQA</u> <u>VTYAEVKHS</u> <u>RPRREMASPPSPLSGEFLDTKDRQAEEDRQ</u> <u>MDTEAAASEA</u> <u>PQD</u> <u>VTYAQL</u> SEQ ID NO: 15
ITIM2-4	<u>VTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQ</u> <u>MDTEAAASEAPQD</u> <u>VTYAQLHSLTLRREATEPPPSQEGPSP</u> <u>AVP</u> <u>SIYATL</u> SEQ ID NO: 16
ITIM1-4	<u>NLYAAVKHTQPEDGVEMDTRSPHDEDPQA</u> <u>VTYAEVKHS</u> <u>RPRREMASPPSPLSGEFLDTKDRQAEEDRQ</u> <u>MDTEAAASEA</u> <u>PQD</u> <u>VTYAQLHSLTLRREATEPPPSQEGPSPAVP</u> <u>SIYATL</u> SEQ ID NO: 17
D3D4 domain	<u>YGSQSSKPYLLTHPSDPLEL</u> SEQ ID NO: 18
Short hinge	VVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRH LG SEQ ID NO: 19
Hinge (iTIM hinge)	YGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTTGPTSTSGP EDQPLTPTGSDPQSGLGRHLGV (SEQ ID NO: 80)
Short hinge 2	VVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRH LGV (SEQ ID NO: 81)
Long hinge 1	AGSGGSGGSGGSPVPSTPPTPSPSTPPTPSPSGGSGNSSGSG GSPVPSTPPTPSPSTPPTPSPSASV (SEQ ID NO: 82)
Long hinge 2	AGSGGSGGSGGSPVPSTPPTNSSSTPPTPSPSPVPSTPPTNSS STPPTPSPSPVPSTPPTNSSSTPPTPSPSASV (SEQ ID NO: 83)
2X short hinge	VVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRH VVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRH LGV (SEQ ID NO: 84)
Hinge (truncated)	TTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGV (SEQ ID NO: 93)
Hinge-transmembrane	<u>YGSQSSKPYLLTHPSDPLEL</u> VVSGPSGGPSSPTTGPTSTSG PEDQPLTPTGSDPQSGLGRHLGVVIGILVAVILLILLILL FLIL

	SEQ ID NO: 20
Transmembrane- intracellular domain.	VVIGILVAVILLLLLLLLLLFLILRHRRQGKHWTSTQRKA DFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVK HTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMAS PPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYA QLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH SEQ ID NO: 21

Polypeptide Sequences for Illustrative Chimeric Antigen Receptors

Name	Sequence
C563 (SEQ ID NO: 47)	QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQ PPGKGLEWIGYIYYSGSTYYNPSLKSRTVMSVDTSKNQFS LKLSSVTAVDTAVYYCARIPFGDWWYFDLWGRGTLVTVS SGGGGSGGGGSGGGGSGGDIQMTQSPSSLSASVGDRVITIT CRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFS GSGSGTDFTLTISLQPEDFATYYCQQSYSFVLTFGGGTKV EIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDFWVLVVVGGVLACYLLVTVAFIIFWVRSKRSL LHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF SRSADAPAYKQGQNLYNELNLGRREEYDVLDRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDGLYQGLSTATKDTYDALHMQALPPR
C1759 (SEQ ID NO: 48)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYMDVWVGKGTVT VSSFSGFRALPCVWSNSSDPLLVSVTGNPSSSWPSPTEPSS KSGICRHLHVLIGTSVVIFLFIILLFLLYRWCSNKKNAAV MDQEPAGDRTVNRQDSDEQDPQEVTYAQLDHCVFQIRKI SRPSQRPKTPLTDTSVYTELPNAEPRSKVVSCPRAPQSGLE GVF
C1760 (SEQ ID NO: 49)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYMDVWVGKGTVT VSSFSGFRALPHAWSDPDPLPVSVTGNRNHLHVLIGTSVV IIPFAILLFLLHRCANKKNAVVMQEPAGNRTVNREDS DEQDPQEVTYAQLNHCVFQIRKITRPSQRPKTPPTDTSV
C1761 (SEQ ID NO: 50)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDF

	ATYYCQQSYSTPLTFGGGTKVEIKGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDN SKNTL YLQMNSLRAEDTAVYYCARYSYYYYYYMDVWGKGT T VT VSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTTGPTST SGPEDQPLTPTGSDPQSGLGRHLGVVIGILVAVILL L L L L L L L L L LFLILRHRROGKHWTSTQRKADFQHPAGAVGPEPTDRGL QWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDE DPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAE E DRQMDTEAAASEAPQDV TYAQLHSLTLRREATEPPPSQEG PSPAVPSIYATLAIH
C1762 (SEQ ID NO: 51)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFLT TISSLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDN SKNTL YLQMNSLRAEDTAVYYCARYSYYYYYYMDVWGKGT T VT VSSTERRAEVPTAHPSPSPRPAGQFQTLVVG VVG L L GSL VLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSV DYGELDFQWREKTPEPPVPCVPEQTEYATIVFPSGMGTSSP ARRGSA DGPRSAQPLRPEDGHCSWPL
C2057 (SEQ ID NO: 52)	METLLGLLILWLQLQWVSSKQEV TQIPAALSVPEGENLVL NCSFTDSAIYNLQWFRQDPGKGLT S L L L I Q S S Q R E Q T S G R L NASLDKSSGRSTLYIAASQPGDSATYLC AVRPLYGGSYIPT FGRGTS LIVHPYIQNPDP AVYQLRDSKSSDKSVCLFTDFDS QTNVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSNKS DFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLN FQNL SVVIGILVAVILL L L L L L L L L L L FLILRHRROGKHWTSTQR KADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAV KHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMA SPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDV TYA QLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH
C2058 (SEQ ID NO: 53)	MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMT LQCAQDMNHEYMSWYRQDPGMGLRLIHYSVGAGITDQG EVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNT GELFFGEGSRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKA TLVCLATGFYPDHVELSWWVNGKEVHSGVCTDPQPLKEQ PALNDSRYCLSSRLRVSA TFWQNPRNHFR CQVQFYGLSEN DEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSV VIGILVAVILL L L L L L L L L L L FLILRHRROGKHWTSTQRKADFQ HPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKHTQP EDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPL SGEFLDTKDRQAEEDRQMDTEAAASEAPQDV TYAQLHSL TLRREATEPPPSQEGPSPAVPSIYATLAIH
C2070 (SEQ ID NO: 54)	MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMT LQCAQDMNHEYMSWYRQDPGMGLRLIHYSVGAGITDQG

	<p> EVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNT GELFFGEGSRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKA TLVCLATGFYPDHVLSWWVNGKEVHSGVCTDPQPLKEQ PALNDSRYCLSSRLRVSAFTWQNPRNHFRCQVQFYGLSEN DEWTQDRAKPVTOIVSAEAWGRADCGFTSESYQQGVLSA TILYEILLGKATLYAVLVSALVLMAMVKRKDSRGGGGGS GGGGSGGGGSRAARGTIGARRTGQPLKEDPSAVPVFSVD YGELDFQWREKTPEPPVPCVPEQTEYATIVFSPGMGTSSPA RRGSADGPRSAQPLRPEDGHCSWPL </p>
C2071 (SEQ ID NO: 55)	<p> MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMT LQCAQDMNHEYMSWYRQDPGMGLRLIHYSVGAGITDQG EVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNT GELFFGEGSRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKA TLVCLATGFYPDHVLSWWVNGKEVHSGVCTDPQPLKEQ PALNDSRYCLSSRLRVSAFTWQNPRNHFRCQVQFYGLSEN DEWTQDRAKPVTOIVSAEAWGRADCGFTSESYQQGVLSA TILYEILLGKATLYAVLVSALVLMAMVKRKDSRGGGGGS GGGGSGGGGSNKKNAAVMDQEPAGDRTVNQRDSDEQDP QEVTYAQLDHCVFQIRKISRPSQRPKTPLTDTSVYTELPNA EPRSKVVSCPRAPQSGLEGVF </p>
C2072 (SEQ ID NO: 56)	<p> MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMT LQCAQDMNHEYMSWYRQDPGMGLRLIHYSVGAGITDQG EVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNT GELFFGEGSRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKA TLVCLATGFYPDHVLSWWVNGKEVHSGVCTDPQPLKEQ PALNDSRYCLSSRLRVSAFTWQNPRNHFRCQVQFYGLSEN DEWTQDRAKPVTOIVSAEAWGRADCGFTSESYQQGVLSA TILYEILLGKATLYAVLVSALVLMAMVKRKDSRGGGGGS GGGGSGGGGSRHRRQKGHWSTQRKADFQHPAGAVGPE PTDRGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDT RSPHDEDPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTK DRQAEEDRQMDTEAAASEAPQDVITYAQLHSLTLRREATE PPPSQEGPSPAVPSIYATLAIH </p>
C2106 (SEQ ID NO: 57)	<p> DIQMTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFLTITSLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYMDVWGKTTVT VSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHDREKKPR QHSGDHENLMNVPSDKEMFSRSVTSLATDAPASSEQNGA LTNGDILSEDSTLTCMQHYEEVQTSASDLLDSQDSTGPKP CHQSRELPRIPPESAVDTMLTARSVDGDQGLGMEGPYEV KDSSSQENMVEDCLYETVKEIKEVAAAHALEKGHSGKAK STSASKELPGPQTEGKAFAEYASVDRNKKCRQSVNVESE </p>

	LGNSCDPEEEAPPPVPVKLLDENENLQEKEGGEAEESATD TTSETNKRFFSSLSYKSREEDPTLTEEEISAMYSSVNKPGQL VNKSGQSLTVPESTYTSIQGDPQRSPSSCNDLYATVKDFEK TPNSTLPPAGRPSEEPEDYEAIQTLNREEEKATLGTNGHH GLVPKENDYESISDLQQGRDITRL
C2107 (SEQ ID NO: 58)	DIQMTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYMDVWGKGTVT VSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDLWGSAAVAIFFVITFLIFLCSSCDREKKPRQHS GDHENLMNVPSDKEMFSRSVTSLATDAPASSEQNGALTN GDILSEDSTLTCMQHYEEVQTSASDLLDSQDSTGKPKCHQ SRELPRIPPESAVDTMLTARSVDGDQGLGMEGPYEVKDS SSQENMVEDCLYETVKEIKEVAAAAHLEKGHSGKAKSTS ASKELPGPQTEGKAFAEYASVDRNKKCRQSVNVESILGN SCDPEEEAPPPVPVKLLDENENLQEKEGGEAEESATDTTSE TNKRFFSSLSYKSREEDPTLTEEEISAMYSSVNKPGQLVNKS GQSLTVPESTYTSIQGDPQRSPSSCNDLYATVKDFEKTPNS TLPPAGRPSEEPEDYEAIQTLNREEEKATLGTNGHHGLVP KENDYESISDLQQGRDITRL
C2153 (SEQ ID NO: 59)	DIQMTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYMDVWGKGTVT VSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDFWVLVVVGGVLACYSLVTVAFIIFWVLRHRR QGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAA DAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYA EVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEA AASEAPQDVTYAQLHSLTLRREATEPPPSQEGPSPAVPSIY ATLAIH
C2156 (SEQ ID NO: 60)	METLLGLLILWLQLQWVSSKQEVQIPAAALSVPEGENLVL NCSFTDSAIYNLQWFRQDPGKGLTSLLLIQSSQREQTSGRL NASLDKSSGRSTLYIAASQPGDSATYLC AVRPLYGGSYIPT FGRGTS LIVHPYIQNPDP AVYQLRDSKSSDKSVCLFTDFDS QTNVSQSKSDVYITDKCVLDMRSMDFKSNSAVAWSNKS DFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLN FQNL SVIGFLILLLLVAGFNLLMTLLLWSSLILRHRRQGKH WTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEE NLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHS

	RPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEA PQDVITYAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH
C2157 (SEQ ID NO: 61)	MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMT LQCAQDMNHEYMSWYRQDPGMGLRLIHYSVGAGITDQG EVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNT GELFFGEGSRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKA TLVCLATGFYPDHVELSWVNGKEVHSGVCTDPQPLKEQ PALNDSRYCLSSRLRVSAFTWQNPRNHFRQCQVQFYGLSEN DEWTQDRAKPVTOIVSAEAWGRADCGFTSESYQQGVLSA TILYLILLGLATLYAVLVSALVLMILRHRRQGKHWTSTQ RKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAA VKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREM ASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVITY AQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH
C2158 (SEQ ID NO: 62)	QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWI PPGKGLEWIGYIYYSGSTYYNPSLKSRTMSVDTSKNQFS LKLSSVTAVDTAVYYCARIPFGDWWYFDLWGRGTLVTVS SGGGGSGGGGSGGGGSGGDIQMTQSPSSLSASVGDRV TITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFS GSGSGTDFTLTISLQPEDFATYYCQQSYSFVLTFGGGTKV EIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDFWVLVVGGLVACYSLLVTVAFIIFWVLRHRRQ GKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAAD AQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAE VKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAA ASEAPQDVITYAQLHSLTLRREATEPPPSQEGPSPAVPSIYA TLAIH
C2179 (SEQ ID NO: 63)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYMDVWGKGT VTVTVSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTT GPTSTSGPEDQPLTPTGSDPQSGLGRHLGVVIGILVAVIL LLLLLLLLLFLILRHRRQRPRREMASPPSPLSGEFLDTKDRQ AEEDRQMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEGP SPAVPSIYATLAIH
C2180 (SEQ ID NO: 64)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYMDVWGKGT VTVTVSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTT GPTSTSGPEDQPLTPTGSDPQSGLGRHLGVVIGILVAVIL LLLLLLLLL

	LFLILRHRQKGKHWSTQRKADFQHPAGAVGPEPTDRGL QWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDE DPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEG PSPAVPSIYATLAIHRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEG PSPAVPSIYATLAIH
C2181 (SEQ ID NO: 65)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYYMDVWGKGTITVT VSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTTGPTST SGPEDQPLTPTGSDPQSGGLGRHLGVVIGILVAVILLILLILL LFLILRHRQKGRPRREMASPPSPLSGEFLDTKDRQAEEDRQ MDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEGPSP AVPSIYATLAIHRPRREMASPPSPLSGEFLDTKDRQAEEDR QMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEGPS PAVPSIYATLAIH
C2182 (SEQ ID NO: 66)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYYMDVWGKGTITVT VSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTTGPTST SGPEDQPLTPTGSDPQSGGLGRHLGVVIGILVAVILLILLILL LFLILRHRQKGKHWSTQRKADFQHPAGAVGPEPTDRGL QWRSSPAADAQEENLFAAVKHTQPEDGVEMDTRSPHDED PQAVTFAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEED RQMDTEAAASEAPQDVTF AQLHSLTLRREATEPPPSQEGP SPAVPSIFATLAIH
C2183 (SEQ ID NO: 67)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYYMDVWGKGTITVT VSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTTGPTST SGPEDQPLTPTGSDPQSGGLGRHLGVVIGILVAVILLILLILL LFLILRHRQKGKHWSTQRKADFQHPAGAVGPEPTDRGL QWRSSPAADAQEENLFAAVKHTQPEDGVEMDTRSPHDED PQAVTFAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEED RQMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEGP SPAVPSIYATLAIH

C2184 (SEQ ID NO: 68)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYYMDVWGKGTITVT VSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTTGPTST SGPEDQPLTPTGSDPQSGGLGRHLGVVIGILVAVILLLLLLLL LFLILRHRQGHWTSTQRKADFQHPAGAVGPEPTDRGL QWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDE DPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAE DRQMDTEAAASEAPQDVTFACLHSLTLREATEPPPSQEG PSPAVPSIFATLAIH
C2218 (SEQ ID NO: 69)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYYMDVWGKGTITVT VSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTTGPTST SGPEDQPLTPTGSDPQSGGLGRHLGVVIGILVAVILLLLLLLL LFLILRRHQGKQNELSDTAGREINLVDAHLKSEQTEASTR QNSQVLLSETGIYDNDPDLCFRMQEGSEVYSNPCLEENKP GIVYASLNHNSVIGPNSRLARNVKEAPTEYASICVRS
C2219 (SEQ ID NO: 70)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYYMDVWGKGTITVT VSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTTGPTST SGPEDQPLTPTGSDPQSGGLGRHLGVLLPLGGLPLLITTCFCL FCCLRRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQ NSQVLLSETGIYDNDPDLCFRMQEGSEVYSNPCLEENKPGI VYASLNHNSVIGPNSRLARNVKEAPTEYASICVRS
C2220 (SEQ ID NO: 71)	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYMSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYYMDVWGKGTITVT VSSTDVKSASERPSKDEMASRPWLLYRLLPLGGLPLLITTC FCLFCCLRRHQGKQNELSDTAGREINLVDAHLKSEQTEAS TRQNSQVLLSETGIYDNDPDLCFRMQEGSEVYSNPCLEEN KPGIVYASLNHNSVIGPNSRLARNVKEAPTEYASICVRS

C2302 (SEQ ID NO: 77)	<p> DIQMTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSTPLTFGGGTKVEIKGGGGSGGGGSGGGGS GGEVQLVESGGGLVQPGGSLRLSCAASGFTVYDYSWVR QAPGKGLEWVSVIYSGGSTYYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARYSYYYYYYMDVWGKGTVT VSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTTGPTST SGPEDQPLTPTGSDPQSGLGRHLGVVIGILVAVILLLLLLLL LFLILRHRQRPRREMASPPSPLSGEFLDTKDRQAEEDRQ MDTEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGPSP AVPSIYATLAIHRPRREMASPPSPLSGEFLDTKDRQAEEDR QMDTEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGPS PAVPSIYATLAIHRPRREMASPPSPLSGEFLDTKDRQAEED RQMDTEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGP SPAVPSIYATLAIH </p>
CT138 (SEQ ID NO: 78)	<p> MGPVTCSVLVLLLMLRRSNGDGDSVTQTEGLVTLTEGLP VMLNCTYQTIYSNPFLFWYVQHLNESPRLLLSFTDNKRT EHQGFHATLHKSSSFHLQKSSAQLSDSALYYCAFDNTY KVIFGKGTHLHVLNPNQNPAPVYQLKDPRSQDSTLCLFTD FDSQINVPKTMESGTFITDKTVLDMKAMDSKSNGAIAWSN QTSFTCQDIFKETNTTYPSSDVPCDATLTEKSFETDMNLN QNLSVMGLRILLKLVAGFNLLMTLRLWSSRAKRSGSGAT NFSLLKQAGDVEENPGPMRVLISAVVLCSLGTGLVDMK VTQMPRYLIKRMGENVLLECGQDMSHETMYWYRQDPGL GLQLIYISYDVDSNSEGDIPKGYRVSRRKKREHFSLILDSAK TNQTSVYFCASSNTNEVFFGKGTRLTVLEDLRNVTPPKV SLFEPKAEIANKQKATLVCLARGFFPDHVELSWWVNGK EVHSGVSTDPQAYKESNYSYCLSSRLRVSAFWHNPRNH RCQVQFHGLSEEDKWPEGSPKPVTONISAEAWGRADCGIT SASYQQGVLSATILYEILLGKATLYAVLVSTLVVMAMVK RKNS </p>
CT139 (SEQ ID NO: 79)	<p> MVLVTILLLSAFFSLRGNSAQSVDPDAHVTLSEGASLELR CSYSYAAPYLFWYVQYPGQSLQFLKYITGDTVVKGTK GFEAEFRKSNSSFNKKSPAHWSDSAKYFCALEGPDTGNY KYVFGAGTRLKVIAHIQNPEPAVYQLKDPRSQDSTLCLFT DFDSQINVPKTMESGTFITDKTVLDMKAMDSKSNGAIAWS NQTSFTCQDIFKETNATYPSSDVPCDATLTEKSFETDMNLN FQNLSVMGLRILLKLVAGFNLLMTLRLWSSRAKRSGSGAT NFSLLKQAGDVEENPGPMGIQTLCCVIFYVLIANHTDAGV TQTPRHEVAEKGTIILKCEPVSGHNDLFWYRQTKIQGLE LLSYFRSKSLMEDGGAFKDRFKAEMLNSSFSTLKIQTPEPR DSAVYLCASSFGTASAETLYFGSGTRLTVLEDLRNVTPPK VSLFEPKAEIANKQKATLVCLARGFFPDHVELSWWVNG KEVHSGVSTDPQAYKESNYSYCLSSRLRVSAFWHNPRN HFRQVQFHGLSEEDKWPEGSPKPVTONISAEAWGRADC </p>

	GITSASYHQGVLSATILYEILLGKATLYAVLVSGLVLMAMVKKKNS
C1765 with signal peptide (SEQ ID NO: 120), LIR1 hinge, TM and ICD are underlined Polynucleotide sequence – SEQ ID NO: 121	MDMRVPAQLLGLLLWLRGARCDVLMQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHPVRTSGGGTKLEIKGGGGSGGGGSGGGGSGGQVQLQSGPELVKPGASVRISCKASGYTFTSYHHHWVKQRPGQGLEWIGWIYPGNVNTEYNEKFKGKATLTADKSSSTAYMHLSSLTSEDSAVYFCAREEITYAMDYWGQGTSTVTVSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGGLGRHLGVVIGILVAVILLLLLLLLLLLFLILRHRROGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPSLSGEFLDTKDRQAEEDROMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH
C1765 without signal peptide (SEQ ID NO: 122), LIR1 hinge, TM and ICD are underlined Polynucleotide sequence – SEQ ID NO: 123	DVLMQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHPVRTSGGGTKLEIKGGGGSGGGGSGGGGSGGQVQLQSGPELVKPGASVRISCKASGYTFTSYHHHWVKQRPGQGLEWIGWIYPGNVNTEYNEKFKGKATLTADKSSSTAYMHLSSLTSEDSAVYFCAREEITYAMDYWGQGTSTVTVSSYGSQSSKPYLLTHPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGGLGRHLGVVIGILVAVILLLLLLLFLILRHRROGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPSLSGEFLDTKDRQAEEDROMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH

[0244] The present description sets forth numerous exemplary configurations, methods, parameters, and the like. It should be recognized, however, that such description is not intended as a limitation on the scope of the present disclosure, but is instead provided as a description of exemplary embodiments.

EXAMPLES

Example 1: LILRB1-based Inhibitory scFv-CAR compared to PD-1, KIR3DL2, KIR3DL3

[0245] The NY-ESO-1-responsive inhibitory construct was created by fusing the NY-ESO-1 ligand binding scFv domain (C-266) to domains of receptors including hinge, transmembrane region, and/or intracellular domain of Leukocyte immunoglobulin-like receptor subfamily B member 1, LILRB1 (LILRB1); Killer cell immunoglobulin-like receptor 3DL2, KIR3DL2; Killer

cell immunoglobulin-like receptor 3DL3, KIR3DL3; and/or B- and T-lymphocyte attenuator, BTLA. Gene segments were combined using Golden Gate cloning and inserted downstream of an eF1 α promoter contained in a lentiviral expression plasmid (pLenti1).

[0246] As reporter cells, Jurkat cells encoding an NFAT Luciferase reporter were maintained in RPMI media supplemented with 10% FBS, 1% Pen/Strep and 0.4mg/mL G418/Geneticin. T2 cells (ATCC CLR-1992) were maintained in IMDM media + 20% FBS and 1% Pen/Strep. For each construct to be evaluated, Jurkat cells were transfected via 100 μ L format Neon electroporation system (Thermo Fisher) according to manufacturer's protocol using the following settings: 3 pulses, 1500V, 10 msec.

[0247] Co-transfection was performed with 3 μ g of activating CAR construct (C-563) or TCR construct (CT-139) and 3 μ g of either inactivating CAR construct or empty vector (pLenti0) per 1 million cells and recovered in RPMI media supplemented with 20% heat-inactivated FBS and 0.1% Pen/Strep.

[0248] Peptides, MAGE-A3 (FLWGPRALV) (SEQ ID NO: 106) and modified NY-ESO-1 (SLLMWITQV) (SEQ ID NO: 107), were synthesized by Genscript. Activating peptide, MAGE-A3, was serially diluted 5-fold starting at 50 μ M. Inactivating peptide, NY-ESO-1, was diluted to 50 μ M, 5 μ M, 0.5 μ M, or 0.05 μ M and these constant amounts were added to the MAGE-A3 serial dilutions and subsequently loaded onto 10,000 T2 cells in 15 μ L of RPMI supplemented with 1% BSA and 0.1 % Pen/Strep and incubated in Corning® 384-well Low Flange White Flat Bottom Polystyrene TC-treated Microplates. The following day, 10,000 Jurkat cells were resuspended in 15 μ L of RPMI supplemented with 10% heat-inactivated FBS and 0.1% Pen/Strep, added to the peptide-loaded T2 cells and co-cultured for 6 hours. ONE-Step Luciferase Assay System (BPS Bioscience) was used to evaluate Jurkat luminescence. Assays were performed in technical duplicates.

LILRB1 compared to PD-1, KIR3DL2, KIR3DL3

[0249] **FIG. 4** shows testing of the constructs provided in **Table 1**. The data show scFv-LILRB1 inhibits CAR activation *in trans*. Because the construct with an LILRB1 domain demonstrates inhibition of signaling at higher concentrations of MAGE-A3 activator peptide, the data demonstrate that a CAR with hinge, transmembrane domain, and intracellular domain from LILRB1 is superior to CARs generated with the same domains from PD-1, KIR3DL2, or KIR3DL3.

Table 1

	LBD	Hinge	TM	ICD
C563	MAGE-A3 pep1 scFv	CD8[117-161]	CD28[135-161]	CD28[136-202] 41BB[197-238] CD3z[31-127]
C1761	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1
C1759	NY-ESO-1 scFv	KIR3DL2	KIR3DL2	KIR3DL2
C1760	NY-ESO-1 scFv	KIR3DL3	KIR3DL3	KIR3DL3
C1762	NY-ESO-1 scFv	PD1	PD1	PD1
CT139	MAGE-A3 pep1 TCR (both alpha and beta chains)			

Inhibition by LILRB1-based CAR requires antigen recognition by its ligand binding domain

[0250] **FIG. 5** shows testing of selected constructs from **Table 1**. The data show that LILRB1 inhibits signaling *in trans* in a dose-dependent fashion. At increasing concentrations of the inhibitory peptide NY-ESO-1 the response to activating peptide MAGE-A3 shifts downward. This shows that the inhibitory effect of the LILRB1-based CAR is dependent on engagement of the antigen to which the ligand binding domain is specific.

LILRB1 CAR inhibits signaling through the T-cell receptor (TCR)

[0251] **FIG. 6** shows testing of selected constructs from **Table 1**, in particular a TCR specific for activating peptide MAGE-A3 rather than the CARs used in previous experiments. The LILRB1-based inhibitory CAR inhibits TCR-mediated signaling in a dose-dependent fashion.

LILRB1 CAR inhibition is preserved when two of the four native ITIMs are present

[0252] **FIG. 7** show testing of LILRB1 intracellular domains having inactivating mutations in the ITIM motifs of LILRB1. Mutation of tyrosine to phenylalanine (Y→F) was used to inactivate the ITIMs indicated by a starred number in **Table 2**.

Table 2

	LBD	Hinge	TM	ICD
C563	MAGE-A3 pep1 scFv	CD8[117-161]	CD28[135-161]	CD28[136-202] 41BB[197-238] CD3z[31-127]
C1761	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1 (ITIMs: 1,2,3,4)

C2182	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1 (ITIMs: 1*,2*,3*,4*)
C2183	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1 (ITIMs: 1*,2*,3,4)
C2184	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1 (ITIMs: 1,2,3*,4*)

[0253] Inactivation of all four ITIMs resulted in a non-functional inhibitory CAR (C2182). Inactivation of only two of the four ITIMs preserved the inhibitor function of the CAR at the concentrations tested (C1760 and C1762). Inactivation of all four ITIM (C1759) demonstrates that the ITIMs are necessary for inhibitory function. When all four ITIMs are mutated, the molecule loses inhibitory function. When only two of the four ITIMs are mutated, inhibitory activity is retained.

[0254] **FIG. 8** shows testing of LILRB1 ITIMs in combinations different from those of the native LILRB1 intracellular domain. As shown in **Table 3**, CARs having the third and fourth ITIMs of LILRB1 in four or six total copies achieve inhibitory activity comparable to the native LILRB1 intracellular domain. Inhibitory activity is also observed when only one copy each of the third and fourth ITIMs are used (C2179) or when two copies of each ITIM is used (C2180) or when multiple copies are used (C2302 or C2180).

Table 3

	LBD	Hinge	TM	ICD
C563	MAGE-A3 pep1 scFv	CD8[117-161]	CD28[135-161]	CD28[136-202] 41BB[197-238] CD3z[31-127]
C1761	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1 (ITIMs: 1,2,3,4)
C2302	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1 (ITIMs: 3,4,3,4,3,4)
C2181	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1 (ITIMs: 3,4,3,4)
C2180	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1 (ITIMs: 1,2,3,4,3,4)
C2179	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1 (ITIMs:3,4)

Example 2: LILRB1 hinge and transmembrane domains enhance the inhibitory activity of BTLA-based inhibitory CARs

BTLA-based CAR and LILRB1/BTLA-based CARs inhibit signaling via NFAT

[0255] B- and T-lymphocyte attenuator (BTLA), also known as CD272 (cluster of differentiation 272) interacts with B7 homology B7H4. Unlike CTLA-4 and PD-1, it is also a ligand for tumour necrosis factor (receptor) superfamily, member 14 (TNFRSF14). Inhibitory signaling through BTLA occurs in response to binding of B7H4 or TNFRSF14.

[0256] The full length BTLA protein was cloned into a construct having an extracellular scFv domain (C2220), a construct replacing the extracellular domain of BTLA with that of LILRB1 (C2219), and a construct replacing the extracellular and transmembrane domains of BTLA with those of LILRB1 (C2218) were generated and tested, as shown in **Table 4** and **FIG. 9**. The LILRB1-BTLA fusion exhibits inhibitory signaling comparable to the LILRB1-based CAR.

Table 4

	LBD	Hinge	TM	ICD
C563	MAGE-A3 pep1 scFv	CD8[117-161]	CD28[135-161]	CD28[136-202] 41BB[197-238] CD3z[31-127]
C1761	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1
C2218	NY-ESO-1 scFv	LILRB1	LILRB1	BTLA
C2219	NY-ESO-1 scFv	LILRB1	BTLA	BTLA
C2220	NY-ESO-1 scFv	BTLA	BTLA	BTLA

Example 3: LILRB1 hinge and transmembrane compared to CD8 or CD28

LILRB1-based CAR with LILRB1 hinge and transmembrane is superior to CD8 hinge and CD28 transmembrane region

[0257] The LILRB1-based CAR was compared to a CAR having the LILRB1 intracellular domain but CD8 hinge and CD28 transmembrane region, as shown in **Table 5** and **FIG. 10**.

Table 5

	LBD	Hinge	TM	ICD
C563	MAGE-A3 pep1 scFv	CD8[117-161]	CD28[135-161]	CD28[136-202] 41BB[197-238] CD3z[31-127]
C1761	NY-ESO-1 scFv	LILRB1	LILRB1	LILRB1
C2153	NY-ESO-1 scFv	CD8	C28	LILRB1

[0258] The LILRB1 hinge and transmembrane region generate results surprisingly superior to a construct having the CD8 hinge and CD28 transmembrane regions. E_{min} is decreased. Overall dynamic range is increased. Inhibitory potency is increased.

Table 6: Summary of results shown in Examples 1-3.

Inhibitor Construct	Activator Construct	μ M Inhibitor peptide	E_{min} [RLU]	EC_{50} [nM]	E_{max} [RLU]
-	C563	50	30,000	10	100,000
-	CT139	50	0	20	100,000
C1761	C563	50	1,000	>10,000	NA
C1761	CT139	50	400	>1,000	NA
C1759	C563	50	20,000	200	100,000
C1760	C563	50	5,000	60	100,000
C1762	C563	50	5,000	600	90,000
C2184	C563	50	1,000	>1,000	NA
C2183	C563	50	4,000	>1,000	NA
C2182	C563	50	30,000	30	90,000
C2302	C563	50	0	>10,000	NA
C2181	C563	50	0	>10,000	NA
C2180	C563	50	0	>10,000	NA
C2179	C563	50	0	>10,000	NA
C2218	C563	50	1,000	>1,000	NA
C2219	C563	50	2,000	>1,000	NA
C2220	C563	50	3,000	>1,000	NA
C2153	C563	50	20,000	2,000	60,000

C2107	CT139	5	0	100	100,000
C2106	CT139	5	0	40	100,000

Example 4: TCR-based inhibitory chimeric antigen receptors

Construct design and cloning

[0259] The NY-ESO-1-responsive inhibitory constructs were created using the high affinity anti-HLA-A*02:01/NY-ESO-1 1G4 α 95:LY T cell receptor (TCR) variant. The charged residues in the TM of TCR α (R253 and K258) and TCR β (K288) were mutated to leucine. Then, the LILRB1 ITIM (residues 484-650) was appended to the mutated TCR α or TCR β . Anti-HLA-A*02:01/MAGE-A3 single chain variable fragment (scFv) was generated in-house. The anti-HLA-A*02:01/MAGE-A3 chimeric antigen receptor (CAR) used in this study contains the anti-HLA-A*02:01/MAGE-A3 scFv, CD8 hinge, CD28 TM, and CD28, 41BB, and CD3 ζ intracellular domains (ICDs). All fragments, including 5' and 3' BsmBI sites, were amplified using Q5 polymerase (New England Biolabs) and digested with DpnI (Thermo Scientific) at 37°C for 60 min. The generated PCR fragments were purified using the Nucleospin gel and PCR cleanup kit (Macherey-Nagel). The plasmids were Golden Gate assembled in a reaction containing BsmBI (Thermo Scientific), T4 DNA ligase (Thermo Scientific), 10 mM ATP, and 1x FastDigest buffer (Thermo Scientific).

Jurkat NFAT activation assay

[0260] Jurkat T lymphocytes that contain firefly luciferase gene under the control of the nuclear factor of activator T cells (NFAT) transcription factor (BPS Bioscience) were co-transfected with plasmids encoding TCR and/or scFv-fusion constructs using the Neon transfection system (Thermo Fisher). The electroporated cells were incubated in RPMI media supplemented with 20% fetal bovine serum (FBS) heat-inactivated at 56°C for 60 min (HIA-FBS) and 0.1% penicillin-streptomycin (P/S) (Gibco). TAP deficient T2 lymphoblasts (ATCC RL-1992) were loaded with varying amounts of a modified NY-ESO-1 peptide (SLLMWITQV) (SEQ ID NO: 107) alone, MAGE-A3 peptide (FLWGPRALV) (SEQ ID NO: 106) alone, or varying amounts of MAGE-A3 peptide in addition to 50 μ M NY-ESO-1 peptide in RPMI supplemented with 1% BSA and 0.1% P/S (Gibco). Peptides used in the assay were synthesized to >95% purity assessed by mass spectrometry (Genscript). 18 hours post-transfection, Jurkats were resuspended at 0.8×10^6 cells per mL in RPMI supplemented with 10% HIA-FBS and 1% P/S. In a 384 well plate, 12000

Jurkats were co-cultured with 12000 peptide-loaded T2s per well at 37°C, 5% CO₂ for 6 hours. NFAT-mediated luciferase production was measured by adding 15 uL of ONE-Step luciferase assay reagent (BPS Bioscience). After 20 minutes, luminescence was detected using a plate reader (Tecan).

TCR-based inhibitory CARs using the LILRB1 intracellular domain

[0261] As shown in **Table 7** and **FIG. 11**, co-expression of TCR-based CARs having either only the extracellular domains of the TCR or also having the transmembrane regions of the TCRs with mutations to polar residues, yield functional inhibitory CARs.

Table 7

	LBD	Hinge	TM	ICD
C563	MAGE-A3 pep1 scFv	CD8[117-161]	CD28[135-161]	CD28[136-202] 41BB[197-238] CD3z[31-127]
C2057	TCR 1G4:α95LY α		LILRB1	LILRB1
C2058	TCR 1G4:α95LY α		LILRB1	LILRB1
C2156	TCR 1G4:α95LY α		TCR 1G4:α95LY α (R253L/K258L)	LILRB1
C2157	TCR 1G4:α95LY α		TCR 1G4:α95LY β (K288L)	LILRB1

[0262] TCR-base CARs inhibit the anti-HLA-A*02:01 MAGE-A3 CAR in *trans*. Jurkat-NFAT luciferase reporter cells were transfected with (1) anti-HLA-A*02:01/MAGE-A3 CAR alone (C563), (2) co-transfected with MAGE-A3 CAR (C563) and TCRα(R253L/K258L)-LILRB1 fusion and TCRβ(K288L)-LILRB1 ICD fusion TCR-inhibitory fusion construct (C2156 + C2157) or (3) co-transfected with MAGE-A3 CAR and TCRαECD/TCRβECD-LILT1(TM+ICD) fusion (C2057 + C2058) TCR-inhibitory fusion construct. The effects of the two inhibitory variants on NFAT activation was measured by co-culturing transfected Jurkat cells with T2 cells loaded with 50 μM NY-ESO-1 peptide in combination with varying amounts of MAGE-A3 peptide. Data are summarized in **Table 8**.

Table 8: *Trans* effect of TCR-inhibitory fusion constructs on MAGE-A3 CAR

Inhibitor Construct	Activator Construct	μ M Inhibitor peptide	E_{min} [RLU]	EC_{50} [nM]	E_{max} [RLU]
-	C563	50	19949	24.52	134443
C2057 + C2058	C563	50	2072	>10,000	NA
C2156 + C2157	C563	50	9198	7108	NA

[0263] Truncated TCR alpha or TCR beta extracellular domains (ECD)- with LILRB1 TM-ICD fusions inhibit CAR activation. TCR alpha or TCR beta extracellular domains (ECD)- with TCR TM- LILRB1 ICD fusions also acted as inhibitor CARs when the TCR transmembrane mutations abolish TCR-CD3 subunit interactions. The experiment demonstrates that the TCR α chain and β chains can be used to generate inhibitory chimeric antigen receptors by interfering with recruitment of stimulatory factors by CD3 subunits.

Example 5: Methods for Examples 6-14

[0264] Cell Culture

[0265] Jurkat cells encoding an NFAT luciferase reporter were obtained from BPS Bioscience. All other cell lines used in this study were obtained from ATCC. In culture, Jurkat cells were maintained in RPMI media supplemented with 10% FBS, 1% Pen/Strep and 0.4mg/mL G418/Geneticin. T2, MCF7, Raji, K562 and HeLa cells were maintained as suggested by ATCC. “Normal” Raji cells were made by transducing Raji cells with HLA-A*02 lentivirus (custom lentivirus, Alstem) at a MOI of 5. HLA-A*02-positive Raji cells were sorted using a FACSMelody Cell Sorter (BD).

[0266] Plasmid Construction

[0267] The NY-ESO-1-responsive inhibitory construct was created by fusing the NY-ESO-1 scFv LBD to domains of receptors including hinge, transmembrane region, and/or intracellular domain of leukocyte immunoglobulin-like receptor subfamily B member 1, LILRB1 (LIR-1), programmed cell death protein 1, PDCD1 (PD-1), or cytotoxic T-lymphocyte protein 4, CTLA4 (CTLA-4). All activating CAR constructs contained an scFv fused to the CD8 α hinge, CD28 TM,

and CD28, 4-1BB and CD3zeta ICDs. The CD19-activating CAR scFv was derived from the FMC63 mouse hybridoma. MSLN-activating CAR scFvs were derived from human M5 (LBD1) as described and humanized SS1 (LBD2). Gene segments were combined using Golden Gate cloning and inserted downstream of a human EF1 α promoter contained in a lentiviral expression plasmid.

[0268] Jurkat Cell Transfection

[0269] Jurkat cells were transiently transfected via 100uL format Neon electroporation system (Thermo Fisher Scientific) according to manufacturer's protocol using the following settings: 3 pulses, 1500V, 10 msec. Cotransfection was performed with 1-3ug of activator CAR or TCR construct and 1-3ug of either scFv or TCR alpha/TCR beta LIR-1 blocker constructs or empty vector per 1e6 cells and recovered in RPMI media supplemented with 20% heat-inactivated FBS and 0.1% Pen/Strep. To confirm blocker surface expression, Jurkat cells were stained 18-24 hours post-transfection with 10ug/mL streptavidin-PE-HLA-A*02-pMHC tetramer for 60 minutes at 4°C in PBS with 1% BSA and characterized by flow cytometry (BD FACSCanto II).

[0270] Jurkat-NFAT-luciferase activation studies

[0271] Peptides, MAGE-A3 (MP1; FLWGPRALV; SEQ ID NO: 106) , MAGE-A3 (MP2; MPKVAELVHFL; SEQ ID NO: 108), HPV E6 (TIHDIILECV; SEQ ID NO: 109), HPV E7 (YMLDLQPET; SEQ ID NO: 110) and modified NY-ESO-1 ESO (ESO; SLLMWITQV; SEQ ID NO: 107), were synthesized by Genscript. Activating peptide was serially diluted starting at 50uM. Blocker peptide, NY-ESO-1, was diluted to 50uM (unless otherwise indicated) which was added to the activating peptide serial dilutions and subsequently loaded onto 1e4 T2 cells in 15uL of RPMI supplemented with 1% BSA and 0.1 % Pen/Strep and incubated in Corning® 384-well Low Flange White Flat Bottom Polystyrene TC-treated Microplates. The following day, 1e4 Jurkat cells were resuspended in 15uL of RPMI supplemented with 10% heat-inactivated FBS and 0.1% Pen/Strep, added to the peptide-loaded T2 cells and co-cultured for 6 hours. ONE-Step Luciferase Assay System (BPS Bioscience) was used to evaluate Jurkat luminescence. For assays involving high density targets, Jurkat cells were similarly transfected and cocultured with tumor cells expressing target antigens at various Jurkat:tumor cell ratios. Assays were performed in technical duplicates.

[0272] Primary T cell transduction, expansion, and enrichment

[0273] Leukopaks were purchased from AllCells®. Collection protocols and donor informed consent were approved by an Institutional Review Board (IRB), with strict oversight. HIPAA compliance and approved protocols were also followed. Frozen PBMCs were thawed in 37°C water bath and cultured at 1e6 cells/mL in LymphoONE (Takara) with 1% human serum and activated using 1:100 of T cell TransAct (Miltenyi) supplemented with IL-15 (10ng/mL) and IL-21 (10ng/mL). After 24 hours, lentivirus was added to PBMCs at MOI = 5. Activator and blocker receptors were simultaneously co-transduced at a MOI = 5 for each lentivirus. PBMCs were cultured for 2-3 additional days to allow cells to expand under TransAct stimulation. Post expansion, activator and blocker transduced primary T cells were enriched for blocker-positive T cells by positive selection using anti-PE microbeads (Miltenyi) according to manufacturer's instructions. Briefly, primary T cells were incubated with 10ug/mL streptavidin-PE-HLA-A*02-pMHC tetramer for 60 minutes at 4°C in MACS buffer (0.5% BSA + 2mM EDTA in PBS). Cells were washed 3 times in MACS buffer and passed through the LS column (Miltenyi) to separate blocker-positive cells (a mix of blocker-only and activator + blocker cells) from untransduced and activator-only cells.

[0274] Primary T cell in vitro cytotoxicity studies

[0275] For cytotoxicity studies with pMHC targets, enriched primary T cells were incubated with 2e3 MCF7 cells expressing renilla luciferase (Biosettia) loaded with a titration of target peptide as described above at an effector:target ratio of 3:1 for 48 hours. Live luciferase-expressing MCF7 cells were quantified using a Renilla Luciferase Reporter Assay System (Promega). For cytotoxicity studies with non-pMHC targets, enriched primary T cells were incubated with 2e3 WT Raji cells ("tumor" cells) or HLA-A*02 transduced Raji cells ("normal" cells) at an effector:target ratio of 3:1 for up to 6 days. WT "tumor" Raji cells stably expressing GFP and renilla luciferase (Biosettia) or HLA-A*02 transduced "normal" Raji cells stably expressing RFP and firefly luciferase (Biosettia) were imaged together with unlabeled primary T cells using an IncuCyte live cell imager. Fluorescence intensity of live Raji cells over time was quantified using IncuCyte imaging software. For reversibility studies, enriched primary T cells were similarly cocultured with "normal" or "tumor" Raji cells for 3 days and imaged. After 3 days, T cells were separated from remaining Raji cells using CD19 negative selection and reseeded with fresh "normal" or "tumor" Raji cells as described. In separate wells, live luciferase-expressing Raji cells were quantified using a Dual-Luciferase Reporter Assay System (Promega) at 72 hours. For

studies in which IFN γ secretion was assessed, supernatants collected after 48 hours of co-culture were tested for IFN γ using a BD Human IFN γ flex kit following manufacturer's exact instructions.

[0276] Mouse Xenograft Study

[0277] Frozen PBMCs were thawed in 37°C water bath and rested overnight in serum-free TexMACS Medium (Miltenyi) prior to activation. PBMCs were activated in 1.5e6 cells/mL using T cell TransAct (Miltenyi) and TexMACS Medium supplemented with IL-15 (20ng/mL) and IL-21 (20ng/mL). After 24 hours, lentivirus was added to PBMCs at a MOI of 5. PBMCs were cultured for 8-9 additional days to allow cells to expand under TransAct stimulation. Post expansion, T cells were enriched on A2-LIR-1 using anti-PE microbeads (Miltenyi) against streptavidin-PE-HLA-A*02-pMHC for 2-5 additional days prior to in vivo injection. Enriched T cells were also validated by flow cytometry (BD FACSCanto II) for expression of CD19 scFv activator and HLA-A*02 LIR-1 blocker by sequential staining with CD19-Fc (1:100; R&D Systems) and goat anti-human IgG-FITC (1:200; Invitrogen) for activator and 10ug/mL streptavidin-APC-HLA-A*02-pMHC for blocker.

[0278] In vivo experiments were conducted by Explora BioLabs under Institutional Animal Care and Use Committee (IACUC)-approved protocols. 5-6 week old female NOD.Cg-Prkdcscid Il2rgtm1Wjl Tg(HLA-A/H2-D/B2M)1Dvs/SzJ (NSG-HLA-A2/HHD) mice were purchased from The Jackson Labs. Animals were acclimated to the housing environment for at least 3 days prior to the initiation of the study. Animals were injected with 2e6 WT Raji cells or HLA-A*02 transduced Raji cells in 100 uL volume subcutaneously in the right flank. When tumors reached an average of 70 mm³ ($V = L \times W \times W/2$), animals were randomized into 5 groups (n=7) and 2e6 or 1e7 T cells were administered via the tail vein. Post T cell injection, tumor measurements were performed 3 times per week and blood was collected 10 days and 17 days after for flow analysis. One animal from the WT Raji group receiving 1e7 CD19-CAR + A2-LIR-1 T cells was excluded from the study due to a failed tail vein injection, followed by flow cytometry confirmation of the absence of human T cells in the blood. At each time point, human T cells in the blood were quantified by flow cytometry (BD FACSCanto II) post RBC lysis. Cells were stained with anti-mouse CD45-FITC (clone 30-F11), anti-human CD3-PE (clone SK7), anti-human CD4-APC (clone OKT4), and anti-human CD8-PerCP-Cy5.5 (clone RPA-T8). All antibodies were obtained from Biolegend and used at a 1:100 dilution. DAPI (Invitrogen) was used to exclude dead cells

from analysis. For histopathological analysis, tumor samples were fixed, sectioned and stained for huCD3 (clone EP449E). Image quantification was done using ImageJ software.

[0279] Statistical Analysis

[0280] Statistical analyses were performed using GraphPad Prism software. All peptide and cell titration studies are shown as mean $\pm$ standard deviation (SD), while in vitro and in vivo studies using primary T cells are shown as mean $\pm$ standard error of the mean (SEM), unless otherwise noted. Peptide and cell titration curves were fit using a four-parameter non-linear regression analysis. EC50 values were calculated directly from the curves. All other groups of data were analyzed using an ordinary two-way ANOVA followed by a Tukey's multiple-comparisons test, unless otherwise noted.

Example 6: Assaying the Effect of the LIR-1 Hinge on Blocking Activity

[0281] The effects of different LIR-1 hinges on the ability of HLA-A*02 scFv LIR-1 inhibitory receptors to block killing by Jurkat cells expressing a KRAS TCR activator was assayed using the Jurkat NFat Luciferase assays described *supra*. A humanized PA2.1 scFv LIR-1 receptor and humanized BB7.2 scFv LIR-1 with a shorter LIR-1 hinge were assayed in Jurkat cells as previously described, and the results are shown in FIGS. 12A-12B. Jurkat cells were transfected with a KRAS TCR activator receptor and/or HLA-A*02 scFv LIR-1 inhibitory receptor (humanized PA2.1 or humanized BB7.2) with a variety of LIR-1 derived hinges, and co-cultured with T2 target cells that were either HLA:A11 positive, or HA:A11 and HLA:A02 positive. Inhibitory receptors with both the shorter and longer hinge behaved similarly (FIG. 12A-12B). An inhibitory receptor with a mouse PA2.1 scFv and slightly longer hinges was also assayed functioned similarly to shorter LIR-1 hinges in the T2-Jurkat assay (FIG. 13A-13B). Hinge sequences are shown in black in FIG. 12B and FIG. 13B, with the gray SS in the sequences in FIG. 13B representing a linker between the antigen-binding domain and the hinge, and the gray VIGIL the start of the LIR-1 transmembrane domain. Hinge, transmembrane domain and intracellular domain of the inhibitory receptors were all derived from LIR-1. FIGS. 12A-12B and 13A-13B show that LIR-1 hinge length can be varied without negatively effecting the LIR-1 inhibitory receptor. Shorter hinges can provide an advantage when packaging nucleic acid sequences encoding LIR-1 inhibitory receptors in lentiviral vectors for delivery.

[0282] Table 9. Sequences of constructs

Name	Amino Acid Sequence	Nucleotide Sequence
PA2.1.1 4 (VL:VH) scFv LIR1 sHTICD	TEFTLTISLQPD DFATYYCFQGS HVPRTFGQGT KVEVKG GGGSGGGSGG GGGQVQLVQSGA EVKKPGSSVKVSC KASGYTFTSYHIH WVRQAPGQGLEWI GWYIPGNVNTEYN EKFKGKATITADE STNTAYMELSSLR SEDNAVYYCAREE ITYAMDYWGQGT LVTVSSVVS GSPSGPSSPTT GPTSTSGPEDQ PLTPTGSDP QSGLRHRLG VIVIGILVAVIL LLLLLLLLFL LILRHRQK HWTSTQRKAD FQH PAGAVGPEPTDRG LQWRSSPAADAQE ENLYAAVKHTQPE DGVEMDTRSPHDE DPQAVTYAEVKHS RPRREMASPPSPL SGEFLDTKDRQAE EDRQMDTEAAASE APQDVITYAQLHSL TLRREATPPPSQ EGPSPAVPSIYAT LAIH (SEQ ID NO: 92)	GACATTCAAATGACCCAGAGCCCATCCACCCTGAGCGCATCT GTAGGTGACCGGGTCACCATCACTTGTAGATCCAGTCAGAGT ATTGTACACAGTAATGGGAACACCTATTTGGAATGGTATCAG CAGAAACCAGGTAAAGCCCCAAAATTGCTCATCTACAAAGTC TCTAACAGATTTAGTGGTGTACCAGCCAGGTTTCAGCGGTTCC GGAAGTGGTACTGAATTCACCTCAGCATCTCCTCTCTCCAG CCAGATGATTTTCGCCACTTATTACTGTTTCAAGGTTTCACAT GTGCCGCGCACATTTCGGTCAGGGTACTAAAGTAGAAGTCAAAA GGCGGAGGTGGAAGCGGAGGGGAGGATCTGGCGGCGGAGGA AGCGGAGGCCAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTG AAGAAGCCTGGGTCCTCAGTGAAGGTTTCTGCAAGGCTTCT GGATACACCTTCACTAGCTATCATATACATTGGGTGCGCCAG GCCCCGACAAAGGGCTTGAGTGGATCGGATGGATCTACCT GGCAATGTTAAACACAGAATATAATGAGAAGTTCAAGGGCAAA GCCACCATACCGCGGACGAATCCACGAACACAGCCTACATG GAGCTGAGCAGCCTGAGATCTGAAGACACGGCTGTGTATTAC TGTGCGAGGGAGGAAATTACCTACGCTATGGACTACTGGGCG CAGGGAACCTGGTCACCGTGTCTCAGTGGTCTCAGGACCG TCTGGGGCCCCAGCTCCCCGACAACAGGCCCCACCTCCACA TCTGGCCCTGAGGACAGCCCCCTACCCCCACCGGGTCGGAT CCCCAGAGTGGTCTGGGAAGGCACCTGGGGGTGTGATCGGC ATCTTGGTGGCCGTCTCCTACTGCTCCTCCTCCTCCTCCTC CTCTTCTCATCCTCCGACATCGACGTCAGGGCAAAACACTGG ACATCGACCCAGAGAAAGGCTGATTTCCAACATCCTGCAGGG GCTGTGGGGCCAGAGCCACAGACAGAGGCTGCAGTGGAGG TCCAGCCAGCTGCCGATGCCAGGAAGAAAACCTCTATGCT GCCGTGAAGCACACACAGCCTGAGGATGGGGTGGAGATGGAC ACTCGGAGCCACACGATGAAGACCCCCAGGCAGTGACGTAT GCCGAGGTGAAACACTCCAGACCTAGGAGAGAAATGGCCTCT CCTCCTTCCCCACTGTCTGGGGAATTCTTGGACACAAAGGAC AGACAGGCGGAAGAGGACAGGCAGATGGACACTGAGGCTGCT GCATCTGAAGCCCCCAGGATGTGACCTACGCCAGCTGCAC AGCTTGACCTCAGACGGGAGGCAACTGAGCCTCCTCCATCC CAGGAAGGGCCCTCTCCAGCTGTGCCAGCATCTACGCCACT CTGGCCATCCACTAG (SEQ ID NO: 114)
PA2.1.14 (VL:VH)) scFv LIR1 HTICD	DIQMTQSPSTLSA SVGDRVTITCRSS QSIVHSNGNTYLE WYQQKPKGKAPKLL IYKVSNRFS GVPA RFSGSGSGTEFTL TISLQPDDEFATY YCFQGS HVPRTFG QGT KVEVKG GGGSGGGSGGQ VQLVQSGAEVKKP GSSVKVSC KASGYTFTSYHIH WVRQAPGQGLEWIGWYIP	GACATTCAAATGACCCAGAGCCCATCCACCCTGAGCGCATCT GTAGGTGACCGGGTCACCATCACTTGTAGATCCAGTCAGAGT ATTGTACACAGTAATGGGAACACCTATTTGGAATGGTATCAG CAGAAACCAGGTAAAGCCCCAAAATTGCTCATCTACAAAGTC TCTAACAGATTTAGTGGTGTACCAGCCAGGTTTCAGCGGTTCC GGAAGTGGTACTGAATTCACCTCAGCATCTCCTCTCTCCAG CCAGATGATTTTCGCCACTTATTACTGTTTCAAGGTTTCACAT GTGCCGCGCACATTTCGGTCAGGGTACTAAAGTAGAAGTCAAAA GGCGGAGGTGGAAGCGGAGGGGAGGATCTGGCGGCGGAGGA AGCGGAGGCCAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTG AAGAAGCCTGGGTCCTCAGTGAAGGTTTCTGCAAGGCTTCT GGATACACCTTCACTAGCTATCATATACATTGGGTGCGCCAG GCCCCGACAAAGGGCTTGAGTGGATCGGATGGATCTACCT GGCAATGTTAAACACAGAATATAATGAGAAGTTCAAGGGCAAA

	<p>GNVNTHEYNEKFKG KATITADESTNTA YMESSLRSEDTA VYYCAREEITYAM DYWGQGLTVTVSS YGSQSSKPYLLTH PSDPLELVVSGPS GGPSSPTTGPTST SGPEDQPLTPTGS DPQSGLGRHLGVV IGILVAVILLLLL LLLLFLILRHRRQ GKHWTSTQRKADF QHPAGAVGPEPTD RGLQWRSSPAADA QEENLYAAVKHTQ PEDGVEMDTRSPH DEDPQAVTYAEVK HSRPRREMASPPS PLSGEFLDTKDRQ AEEDRQMDTEAAA SEAPQDVITYAQLH SLTLRREATEPPP SQEGPSPAVPSIY ATLAIH (SEQ ID NO: 91)</p>	<p>GCCACCATTACCGCGGACGAATCCACGAACACAGCCTACATG GAGCTGAGCAGCCTGAGATCTGAAGACACGGCTGTGTATTAC TGTGCGAGGGAGGAAATTACCTACGCTATGGACTACTGGGGC CAGGGAACCCCTGGTCACCGTGTCTCATACGGCTCACAGAGC TCCAAACCCCTACCTGCTGACTCACCCCACTGACCCCTGGAG CTCGTGGTCTCAGGACCGTCTGGGGGCCCCAGCTCCCCGACA ACAGGCCCCACCTCCACATCTGGCCCTGAGGACCAGCCCCCTC ACCCCAACCGGTTCGGATCCCCAGAGTGGTCTGGGAAGGCAC CTGGGGGTGTGATCGGCATCTTGGTGGCCGTCACTCTACTG CTCCTCCTCCTCCTCCTCCTCTTCTCCTCATCCTCCGACATCGA CGTCAGGGCAAACACTGGACATCGACCCAGAGAAAGGCTGAT TTCCAACATCCTGCAGGGGCTGTGGGGCCAGAGCCCACAGAC AGAGGCCTGCAGTGGAGGTCCAGCCCAGCTGCCGATGCCAG GAAGAAAACCTCTATGCTGCCGTGAAGCACACACAGCCTGAG GATGGGGTGGAGATGGACACTCGGAGCCACACGATGAAGAC CCCCAGGCAGTGACGTATGCCGAGGTGAAACACTCCAGACCT AGGAGAGAAATGGCCTCTCCTCCTTCCCCACTGTCTGGGGAA TTCCTGGACACAAAGGACAGACAGGCGGAAGAGGACAGGCAG ATGGACACTGAGGCTGTGTCATCTGAAGCCCCCAGGATGTG ACCTACGCCCAGCTGCACAGCTTGACCCCTCAGACGGGAGGCA ACTGAGCCTCCTCCATCCCAGGAAGGGCCCTCTCCAGCTGTG CCCAGCATCTACGCCACTCTGGCCATCCACTAG (SEQ ID NO: 113)</p>
<p>PA2.1.1 4 scFv LIR1 sHTICD</p>	<p>QVQLVQSGAEVKK PGSSVKVSKKASG YTFTSYHIHWVRQ APGQGLEWIGWLY PGNVNTEYNEKFK GKATITADESTNT AYMESSLRSED AVYYCAREEITYA MDYWGQGLTVTVS SGGGSGGGGSGG GGSGGDIQMTQSP STLSASVGDRTI TCRSSQSIVHSNG NTYLEWYQKPKG APKLLIYKVSNRF SGVPARFSGSGS TEFTLTISLQPD DFATYYCFQGS PRTFGQGTKVEVK VVSGPSGGPSSPT TGPTSTSGPEDQP LTPTGSDPQSLG RHLGVVIGILVAV ILLILLILLILLI LRHRRQGHWTST QRKADFQHPAGAV GPEPTDRGLQWRS SPAADAQEENLYA</p>	<p>CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCT GGGTCTCAGTGAAGGTTTCTGCAAGGCTTCTGGATACACC TTCCTAGCTATCATATACATTGGGTGCGCCAGGCCCCCGGA CAAGGGCTTGAGTGGATCGGATGGATCTACCCCTGGCAATGTT AACACAGAATATAATGAGAAGTTCAAGGGCAAAGCCACCATT ACCGCGGACGAATCCACGAACACAGCCTACATGGAGCTGAGC AGCCTGAGATCTGAAGACACGGCTGTGTATTACTGTGCGAGG GAGGAAATTACCTACGCTATGGACTACTGGGGCCAGGGAACC CTGGTCACCGTGTCTCAGGCGGAGGTGGAAGCGGAGGGGGA GGATCTGGCGGCGGAGGAAGCGGAGGCGACATTCAAATGACC CAGAGCCCATCCACCCTGAGCGCATCTGTAGGTGACCGGGTC ACCATCACTTGTAGATCCAGTCAGAGTATTGTACACAGTAAT GGGAACACCTATTTGGAATGGTATCAGCAGAAACCAGGTAAA GCCCCAAAATTGCTCATCTACAAAGTCTCTAACAGATTTAGT GGTGTACCAGCCAGGTTACGCGGTTCCGGAAAGTGGTACTGAA TTCACCCCTACGATCTCCTCTCTCCAGCCAGATGATTTCCGC ACTTATTACTGTTTTCAAGGTTACATGTGCCGCGCACATTTC GGTCAGGGTACTAAAGTAGAAGTCAAAGTGGTCTCAGGACCG TCTGGGGGCCCCAGCTCCCCGACAACAGGCCCCACCTCCACA TCTGGCCCTGAGGACCAGCCCTCACCCCAACCGGTTCGGAT CCCCAGAGTGGTCTGGGAAGGCACCTGGGGGTGTGATCGGC ATCTTGGTGGCCGTCACTCTACTGCTCCTCCTCCTCCTCCTC CTCTTCTCATCCTCCGACATCGAGCTCAGGGCAAACACTGG ACATCGACCCAGAGAAAGGCTGATTTCCAACATCCTGCAGGG GCTGTGGGGCCAGAGCCACAGACAGAGGCTGCACTGGAGG TCCAGCCAGCTGCCGATGCCAGGAAGAAAACCTCTATGCT GCCGTGAAGCACACACAGCCTGAGGATGGGGTGGAGATGGAC ACTCGGAGCCACACGATGAAGACCCCCAGGCAGTGACGTAT</p>

	<p>AVKHTQPEDGVEM DTRSPHDEDPQAV TYAEVKHSRPRRE MASPPSPLSGEFL DTKDRQAEEDRQM DTEAAASEAPQDV TYAQLHSLTLRRE ATEPPPSQEGPSP AVPSIYATLAIH (SEQ ID NO: 90)</p>	<p>GCCGAGGTGAAACACTCCAGACCTAGGAGAGAAATGGCCTCT CCTCCTTCCCCACTGTCTGGGGAATTCCTGGACACAAAGGAC AGACAGGCGGAAGAGGACAGGCAGATGGACACTGAGGCTGCT GCATCTGAAGCCCCCAGGATGTGACCTACGCCAGCTGCAC AGCTTGACCCCTCAGACGGGAGGCAACTGAGCCTCCTCCATCC CAGGAAGGGCCCTCTCCAGCTGTGCCCAGCATCTACGCCACT CTGGCCATCCACTAG (SEQ ID NO: 112)</p>
<p>PA2.1.1 4 scFv LIR1 HTICD</p>	<p>QVQLVQSGAEVKK PGSSVKVSKASG YTFTSYHIHWVRQ APGQGLEWIGWIY PGNVNTEYNEKFK GKATITADESTNT AYMELSSLRSED AVYYCAREEITYA MDYWGQGTLLTVS SGGGSGGGSGSG GGSGGDIQMTQSP STLSASVGDRTI TCRSSQSIVHSNG NTYLEWYQKPKG APKLLIYKVSNRF SGVPARFSGSGS TEFTLTISSLQPD DFATYYCFQGS PRTFGQGTKEVK YGSQSSKPYLLTH PSDPLELVVSGPS GGPSSPTTGPTST SGPEDQPLTPTGS DPQSGGLGRHLGV IGILVAVILLLLL LLLLFLILRHRRQ GKHWTSTQRKADF QHPAGAVGPEPTD RGLQWRSSPAADA QEENLYAAVKHTQ PEDGVEMDTRSPH DEDPAVITYAEVK HSRPRREMASPPS PLSGEFLDTKDRQ AEEDRQMDTEAAA SEAPQDVITYAQLH SLTLRREATEPPP SQEGPSPAVPSIY ATLAIH (SEQ ID NO: 89)</p>	<p>CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCT GGGTCCCTCAGTGAAGGTTTCCTGCAAGGCTTCTGGATACACC TTCACTAGCTATCATATACATTGGGTGCCCCAGGCCCCCGGA CAAGGGCTTGAGTGGATCGGATGGATCTACCCTGGCAATGTT AACACAGAATATAATGAGAAGTTCAAGGGCAAAGCCACCATT ACCGCGGACGAATCCACGAACACAGCCTACATGGAGCTGAGC AGCCTGAGATCTGAAGACACGGCTGTGTATTACTGTGCGAGG GAGGAAATTACCTACGCTATGGACTACTGGGGCCAGGGAACC CTGGTCACCGTGTCTCAGGCGGAGGTGAAGCGGAGGGGGA GGATCTGGCGGCGGAGGAAGCGGAGGCGACATTCAAATGACC CAGAGCCCATCCACCCTGAGCGCATCTGTAGGTGACCGGGTC ACCATCACTTGTAGATCCAGTCAGAGTATTGTACACAGTAAT GGGAACACCTATTTGGAATGGTATCAGCAGAAACCAGGTAAA GCCCCAAAATTGCTCATCTACAAAGTCTCTAACAGATTTAGT GGTGTACCAGCCAGGTTTCCGGAAGTGGTACTGAA TTCACCCCTCACGATCTCCTCTCTCCAGCCAGATGATTTCCGC ACTTATTACTGTTTTCAAGGTTTACATGTGCCGCGCACATTC GGTCAGGGTACTAAAGTAGAAGTCAAATACGGCTCACAGAGC TCCAAACCCTACCTGCTGACTCACCCAGTGACCCCTGGAG CTCGTGGTCTCAGGACCGTCTGGGGGCCCCAGCTCCCCGACA ACAGGCCCCACCTCCACATCTGGCCCTGAGGACCAGCCCCCTC ACCCCCACCGGGTCGGATCCCCAGAGTGGTCTGGGAAGGCAC CTGGGGGTGTGATCGGCATCTTGGTGGCCGTCTCCTACTG CTCCTCCTCCTCCTCCTCCTCTTCTCCTCATCCTCCGACATCGA CGTCAGGGCAAACACTGGACATCGACCCAGAGAAAGGCTGAT TTCCAACATCCTGCAGGGGCTGTGGGGCCAGAGCCACAGAC AGAGGCCTGCAGTGGAGGTCCAGCCAGCTGCCGATGCCCAG GAAGAAAACCTCTATGCTGCCGTGAAGCACACACAGCCTGAG GATGGGGTGGAGATGGACACTCGGAGCCACACGATGAAGAC CCCCAGGCAGTGACGTATGCCGAGGTGAAACACTCCAGACCT AGGAGAGAAATGGCCTCTCCTCCTTCCCCACTGTCTGGGAA TTCCTGGACACAAAGGACAGACAGGCGGAAGAGGACAGGCAG ATGGACACTGAGGCTGCTGCATCTGAAGCCCCCAGGATGTG ACCTACGCCCAGCTGCACAGCTTGACCCTCAGACGGGAGGCA ACTGAGCCTCCTCCATCCAGGAAGGGCCCTCTCCAGCTGTG CCCAGCATCTACGCCACTCTGGCCATCCACTAG (SEQ ID NO: 111)</p>

Example 7: Comparison of LIR-1, CTLA-4 and PD-1 Inhibitory Receptors

[0283] An NY-ESO-1-responsive inhibitory construct was created by fusing the NY-ESO-1 scFv LBD to domains of receptors including hinge, transmembrane region, and/or intracellular domain of leukocyte immunoglobulin-like receptor subfamily B member 1, LILRB1 (LIR-1), programmed cell death protein 1, PDCD1 (PD-1), or cytotoxic T-lymphocyte protein 4, CTLA4 (CTLA-4). MAGE-A3 activating CAR constructs contained an scFv fused to the CD8 α hinge, CD28 TM, and CD28, 4-1BB and CD 3zeta intracellular domains (ICDs). Gene segments were combined using Golden Gate cloning and inserted downstream of a human EF1 α promoter contained in a lentiviral expression plasmid.

[0284] Initially, peptide-MHC (pMHC) targets for both the activator and blocker receptors were used, because pMHCs allow convenient quantification of the pharmacology of the system (FIG. 14A). Specifically, a single-chain fragment variable (scFv) that binds HLA-A*02-NY-ESO-1_(SLLMWITQC/V) was used as the inhibitor receptor ligand-binding domain (LBD), and a second scFv against HLA-A*02-MAGE-A3_(FLWGPRALV) pMHC (Gallo, unpublished) was as part of an activator receptor third-generation CAR. Jurkat effector cells that express luciferase upon NFAT activation were used to readout activator sensitivity, with EC₅₀ values reporting the half-maximum activator peptide concentration required for a response. Both PD-1 and CTLA-4 intracellular domains (ICD) mediated a shift in EC₅₀ of activation in Jurkat cells of less than ~10x, measured by titration of peptides loaded on T2 cells as stimulus (FIG. 14B).

[0285] A variety of potential inhibitor (blocker) receptor constructs were screened, and the LIR-1 blocker constructed was discovered to have stronger blocking properties than PD-1 and CTLA-4. This blocker receptor includes the intracellular, transmembrane (TM) and hinge domains of the LIR-1 (LILRB1) receptor, one of several LIR-family molecules encoded by the human genome. The LIR-1 blocker (henceforth referred to as LIR-1) fused to the NY-ESO-1 LBD mediated an EC₅₀ shift of >5,000x (FIG. 14B, FIGS. 17A-17D). A control, titration of unrelated HLA-A*02-binding peptides provided an estimate of the shift caused by competition of loaded peptides on T2 cells for available HLA molecules, a contribution to the total shift typically less than ~10x (FIG. 15). For the EC₅₀ shift values reported here, comparisons were typically to EC₅₀s of activator-only constructs. Further, for a given pair of activator/blocker receptors, the mid-point of titration for inhibition was approximately constant and depended on the ratio of activating to

blocking peptide, presumably directly correlated with the target-antigen ratio (FIGS. 16A-16D). The target concentration explored in the majority of these experiments is estimated to have ranged from ~1,000-10,000 copies/cell.

Example 8: LIR-1 Inhibitory Receptors with Multiple scFv Ligand Binding Domains

[0286] The activity of the LIR-1 inhibitory receptor was tested with a variety of antigen-binding domains specific to other pMHC targets. For four different pMHC targets, a total of six different scFvs grafted onto the LIR-1 mediated dramatic shifts in EC50, ranging from 10 to 1,000x (FIG. 14C). With respect to its interaction with activator receptors, the LIR-1 receptor was also robust; its blocking behavior was applicable to multiple targets and scFvs (FIG. 14D). The blockade was ligand-dependent (FIG. 17A), although many LIR-1 constructs produced lower basal/tonic signaling when paired with specific activator receptors. The EC50 shifts depended on the presence of a fused ICD, as LIR-1 constructs completely lacking ICD, or containing mutations in key elements of the ICD, had no effect (FIG. 17B). The ligand-independent blocker activity, however, had little effect on the activation EC50 absent ligand (FIG. 16A-16D). LIR-1 inhibitory receptors are a modular, adaptable, ligand-gated system that functions across multiple targets and antigen-binding domains.

Example 9: LIR-1 Inhibitory Domains Fused to TCR alpha and TCR beta

[0287] The LIR-1 inhibitory receptor was tested when fused to TCRalpha and TCR beta subunits, or when in combination with a TCR activator receptor. TCRs directed against 3 different pMHC targets, 2 from MAGE-A3 and one from HPV (see Methods, *supra*). In every case, LIR-1 shifted the activation EC50 by large amounts, estimated to range >1,000x (FIG. 14E; FIG. 19A). Furthermore, an NY-ESO-1 TCR LBD, when fused to LIR-1, also produced substantial EC50 shifts (Fig. 14F; FIG. 19B). Indeed, all combinations of a MAGE-A3_(FLWGPRALV) CAR or TCR with an NY-ESO-1_(SLLMWITQV) scFv or TCR displayed large shifts. Thus, the LIR-1 inhibitory receptor exhibited modularity that encompassed both CARs and TCRs.

Example 10: LIR-1 Inhibitory Receptors Respond to Target Antigen Present in *cis* to Activator Receptor Target Antigen

[0288] The ability of the LIR-1 blocker receptor to inhibit activation by an activator receptor when activator and inhibitor targets were presented in *cis* was assayed. In a first assay, a simplified stimulus consisting of target-loaded beads roughly the size of cells ($d \sim 2.8 \mu\text{m}$) was used. Jurkat cells expressing activator and blocker receptors were activated only by beads that contained the A (activator) target, not by beads with dual A/B (activator/blocker) targets (FIG. 18A). Interestingly, the effector cells were activated by a mixture of A+ and B+ beads, even when the A+ beads comprised only 20% of the total. This demonstrated that the cells expressing the activator and blocker receptors are: (i) blocked from activation by the blocker receptor when the targets are present in *cis* on the same surface; and, (ii) activated by individual A+ beads among an excess of B+ beads.

Example 11: LIR-1 Inhibitory Receptors and Cell Surface Antigens

[0289] The ability of the LIR-1 inhibitor receptor to block activation in response to non-pMHC targets, representing surface antigens that can extend into the realm of 100,000 epitopes/cell, was assayed. scFvs that bind either the B-cell marker CD19, the solid-tumor antigen mesothelin (MSLN), or HLA-A*02 in a peptide-independent fashion were tested. In these cases, the target antigen concentration was not controlled, as with exogenous peptide as with pMHCs. Instead, the ratio of activator to blocker expression was varied using different DNA concentrations in transient transfection assays. Though assay sensitivity prevented exploration of the full range of EC50 shifts, shifts in Emax over 10x were observed. These experiments showed that the properties of the LIR-1 receptor in a dual receptor system were generally the same for high-density targets (FIG. 18B; FIGS. 16-16D, FIG. 21A) and that the blocker receptor blocked activation of the A receptor to an extent reflected by its relative surface level on the effector cells (FIG. 18C). The LIR-1 receptor also behaved in a modular manner in this high-antigen-density setting. An scFv against HLA-A*02 acted either as activator (FIG. 18D) or blocker when fused to either an activator or blocker receptor, respectively. The LIR-1 receptor is flexible enough to accommodate low and high target densities, in principle allowing optimization for pMHC targets as well as non-pMHC surface antigens.

Example 12: LIR-1 Inhibitory Receptors in Primary T Cells

[0290] The ability of the LIR-1 receptor to block activation of primary T cells was assayed. pMHC targets were used initially. After enrichment for transduced T cells via physical selection, engineered T cells expressing activator and blocker receptors were assayed using target cells engineered to express luciferase as the readout for viable cells. With an HPV TCR as activator, the NY-ESO-1 scFv fused to LIR-1 shifted the cell-count vs. peptide-concentration curve in peptide-loaded MCF7 tumor cells by ~25x (FIG. 20A; FIG. 23). Thus, the behavior of LIR-1 receptor demonstrated in Jurkat cell activation assays was extended to primary T cell functions, including cytotoxicity.

[0291] To establish proof of concept, the HLA-A*02 LIR-1 construct was shown to function as a blocker in the presence of pMHC-dependent activators in Jurkat cells with T2 target cells (FIG. 20B). For the activator, a CD19 scFv was used as a CAR. This activator/blocker pair was shown to function together as robustly as other pairs previously tested in Jurkat cells (FIG. 18B). T model tumor cells that differentially express the LIR-1 receptor ligand, CD19-positive, HLA-A*02-negative Raji cells were used. To model the corresponding normal cells, the same cell line stably expressing the HLA-A*02 gene was used (FIG. 24A). The cell lines activated Jurkat cells if the target cells expressed CD19 only, but not if they expressed both CD19 and HLA-A*02; i.e., the HLA-A*02 blocker receptor blocked activation by CD19-CAR in a ligand-dependent way (FIG. 24B).

[0292] The CD19/HLA-A*02 receptor pair also worked in primary T cells (FIG. 21). Engineered T cells killed CD19-expressing Raji cells in the absence of HLA-A*02 expression. Raji cells that expressed both CD19 and HLA-A*02 were blocked from cytotoxicity. Importantly, primary T cells bearing the receptor pairs distinguished CD19+ “tumor” from CD19+/HLA-A*02+ “normal” cells in a mixed culture. These findings mirrored results from the above-mentioned bead experiment, but in a more complex cellular setting where the effector cells’ cytotoxicity focused on “tumor” targets, even when surrounded by “normal” cells. The degree of selectivity was impressive given that neither receptor had been subjected to deliberate optimization for maximal selectivity. This behavior was confirmed with a second antigen, MSLN (FIG. 22B).

Example 13: Reversibility of Inhibition by LIR-1 Inhibitory Receptors

[0293] The LIR-1 inhibitory receptor was tested for its ability to function reversibly; that is, to cycle from a state of blockade to activation and back to blockade. Effector T cells expressing the LIR-1 receptor and activator receptor were tested to see if they could function reversibly and iteratively. The effector cells were co-cultured with Raji cells, either to mimic tumor (CD19+) or normal (CD19+/HLA-A*02+) cell encounter. After each round of exposure to target cells, the Raji cells were removed from the culture and a new population of target cells was introduced. Cytotoxicity and gamma-interferon (IFN γ) were measured at the end of each round. In both permutations, block-kill-block and kill-block-kill, the T cells functioned as required by a cell therapeutic of this type (FIGS. 25A-25D). They reversibly cycled from a state of block to cytotoxicity and back, depending on the target cells to which they were exposed. This result demonstrates that a T cell expressing an activator receptor and the LIR-1 inhibitory receptor do not get stuck in one state (blockade or activation), but rather can switch back and forth as it integrates signals from normal and tumor cells. Additionally, these experiments were reproduced in primary T cells from multiple donors (FIGS. 21A-D, FIGS. 25A-25D; FIGS. 26A-26B), despite their complexity, heterogeneity and donor-to-donor variability, demonstrating the robust functions of the LIR-1 inhibitory receptor.

Example 14: Human T Cells expressing an Activator Receptor and LIR-1 Blocker Receptor Selectively Target Cancer Cells in a Mouse Model

[0294] The ability of the CD19/HLA-A*02 activator/blocker pair engineered in primary T cells to allow expansion of the T cells *in vitro* to large numbers using standard CD3/CD28 stimulation was assayed (FIG. 27A; FIG. 28A). Thus, T cells expressing the activator and LIR-1 receptors can be scaled up to sufficient numbers for animal experiments and ultimately for patients.

[0295] The CD19/HLA-A*02 activator/blocker combination was tested *in vitro* to demonstrate selective killing of CD19+ tumor cells, while sparing CD19+/HLA-A*02+ cells in a mouse xenograft cancer model (FIG. 27B). The same two Raji cell lines described above, one CD19+ and the other CD19+/HLA-A*02+, were injected into the flanks of immunocompromised (NGS-HLA-A2.1) mice. T cells engineered with the test constructs were injected at two doses, 2e6

(not shown) or 1×10^7 T cells. The growth of the tumor, as well as the persistence of the implanted T cells, were analyzed over time. Only the CD19⁺ tumor cells were killed in the mouse and the tumor control tracked with transferred T cell numbers, promoting survival of the host mice (FIGS. 27C-27E; FIGS. 28B-28C). The CD19⁺/HLA-A*02⁺ cells designed to model normal cells were unaffected. Human CD4⁺/CD8⁺ cell ratios in the blood of mice bearing engineered cells tracked with their control counterpart; i.e., in “tumor”-grafted mice, CD4⁺ > CD8⁺ cells, as in the CD19 CAR positive control mice; and, in “normal”-grafted mice, CD4⁺ < CD8⁺ cells, similar to mice harboring untransduced T cells (FIG. 28D). Moreover, huCD3⁺ cells in the tumor (FIGS. 29A-29B) correlated inversely with tumor volume, and engineered T cells expressing the two receptors behaved like untransduced T cells in “normal” grafts (low infiltrate), and like the CD19 CAR in “tumor” grafts (high infiltrate). Finally, the mice appeared normal with regard to typical clinical observations (Table 10). Together, these results demonstrated that the LIR-1 inhibitory receptor can function *in vivo* to inhibit an activator receptor, in a simplified setting devised to mimic the different normal and tumor cell types that will be encountered in patients.

Table 10. Clinical observations of mice during *in vivo* experiment

	Study Day:	6	8	10	13	15	17	20	22	24	28	29
Group	Animal ID	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO
Tumor Untreated	59	N	N	N	N	N	N	N	N	N	N	N, EUT
	100	N	N	N	N	N	N	N	N	N	N	N
	61	N	N	N	N	N	N	N	N	N	19AN(1)	19AN(1)
	285	N	N	N	N	N	N	N	N	N	N	N, EUT
	289	N	N	N	N	N	N	N	N	19AN(1)	N	N, EUT
	87	N	N	N	N	N	N	N	N	N	N	N, EUT
	60	N	N	N	N	N	N	N	N	N	N	N
Tumor Untransduced	286	N	N	N	N	N	N	N	N	N	19AN(1)	19AN(1), EUT
	78	N	N	N	N	N	N	N	N	N	N	N
	76	N	N	N	N	N	N	N	N	N	N	N
	89	N	N	N	N	N	N	N	N	N	N	N, EUT
	90	N	N	N	N	N	N	N	N	N	N	N

	275	N	N	N	N	N	N	N	N	N	N	N
	83	N	N	N	N	N	N	N	N	N	19AN(1)	19AN(1), EUT
Tumor CD19-CAR only	280	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1)	19AN(1)	N	N
	99	N	N	N	N	N	N	N	N	N	N	N
	284	N	N	N	N	N	N	N	N	N	N	N
	71	N	N	N	N	N	N	N	N	N	N	N
	279	N	N	N	N	N	N	N	N	N	N	N
	77	N	N	N	N	N	N	N	N	N	N	N
	94	N	N	N	N	N	N	N	N	N	N	N
Tumor CD19-CAR + A2-LIR1	64	N	N	N	N	N	N	N	N	N	N	N
	69	N	N	N	N	N	N	N	N	N	N	N
	274	N	N	N	N	N	N	N	N	N	N	N
	96	N	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1)
	277	N	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1)
	97	N	N	N	N	N	N	N	N	N	N	N
“Normal” Untreated	66	N	N	N	N	N	N	N	N	19AN(1)	19AN(2)	19AN(2), EUT
	294	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(2)	19AN(2), EUT
	293	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(2)	19AN(2), EUT
	91	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(2)	19AN(2), EUT
	287	N	N	N	N	N	N	N	N	19AN(1)	19AN(2)	19AN(2), EUT
	281	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(2)	19AN(2), EUT
	282	N	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1), EUT
“Normal” Untransduced	290	N	N	N	N	N	N	N	N	N	19AN(1)	19AN(1), EUT

	25	N	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1), EUT
	93	N	N	N	N	N	N	N	N	N	N	N, EUT
	84	N	N	N	N	N	N	N	N	N	N	N, EUT
	292	N	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1)
	65	N	N	N	N	N	N	N	N	N	19AN(1)	19AN(1), EUT
	79	N	N	N	N	N	N	N	N	N	N	N
“Normal” CD19-CAR only	270	N	N	N	N	N	N	N	N	N	N	N
	283	N	N	N	N	N	N	N	N	N	N	N
	82	N	N	N	N	N	N	N	N	N	N	N
	92	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1)	19AN(1)	19AN(1)	19AN(1)
	95	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1)	19AN(1)
	80	N	N	N	N	N	N	N	N	N	N	N
“Normal” CD19-CAR + A2-LIR1	297	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1)	19AN(1)
	86	N	N	N	N	N	N	N	N	N	N	N
	74	N	N	N	N	N	N	N	N	N	N	N
	288	N	N	N	N	N	N	N	N	N	N	N
	273	N	N	N	N	N	N	N	N	N	N	N
	70	N	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1)
	73	N	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1)
	72	N	N	N	N	N	N	N	N	19AN(1)	19AN(1)	19AN(1), EUT

[0296] Tumor and “normal” cells were injected on study day 0 and treatment started on study day 10. Clinical observation (CO) were performed 3x/week focusing on poor health, stress and pain. Per IACUC guidelines, mice were euthanized if tumors reached $> 2000 \text{ mm}^3$. Codes: N = normal; 19A = Abnormal Tumor [N = Necrotic, O = Open], EUT = Euthanized. Severity codes: 0 = Not present, 1 = Moderate, 2 = Severe.

CLAIMS

What is claimed is:

1. A chimeric antigen receptor comprising a polypeptide, wherein the polypeptide comprises one or more of:
 - a) an LILRB1 hinge domain or functional fragment or variant thereof;
 - b) an LILRB1 transmembrane domain or a functional variant thereof; or
 - c) an LILRB1 intracellular domain or an intracellular domain comprising at least one immunoreceptor tyrosine-based inhibitory motif (ITIM), wherein the ITIM is selected from NLYAAV (SEQ ID NO: 8), VTYA EV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
2. The receptor of claim 1, wherein the polypeptide comprises an LILRB1 intracellular domain and/or an intracellular domain comprising at least two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTYA EV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
3. The receptor of claim 2, wherein the intracellular domain comprises both ITIMs NLYAAV (SEQ ID NO: 8) and VTYA EV (SEQ ID NO: 9).
4. The receptor of claim 3, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 12.
5. The receptor of claim 2, wherein the intracellular domain comprises both ITIMs VTYA EV (SEQ ID NO: 9) and VTYAQL (SEQ ID NO: 10).
6. The receptor of claim 5, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 13.
7. The receptor of claim 2, wherein the intracellular domain comprises both ITIMs VTYAQL (SEQ ID NO: 10) and SIYATL (SEQ ID NO: 11).
8. The receptor of claim 5, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 14.
9. The receptor of claim 1, wherein the polypeptide comprises an intracellular domain comprising at least three immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8),

- VTYAEV (SEQ ID NO: 9) , VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
10. The receptor of claim 2, wherein the intracellular domain comprises the ITIMs NLYAAV (SEQ ID NO: 8), VTYAEV (SEQ ID NO: 9), and VTYAQL (SEQ ID NO: 10).
 11. The receptor of claim 10, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 15.
 12. The receptor of claim 2, wherein the intracellular domain comprises the ITIMs VTYAEV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
 13. The receptor of claim 12, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 16.
 14. The receptor of claim 2, wherein the intracellular domain comprises the ITIMs NLYAAV (SEQ ID NO: 8), VTYAEV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
 15. The receptor of claim 14, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 17.
 16. The receptor of claim 15, wherein the intracellular domain comprises a sequence at least 95% identical to the LILRB1 intracellular domain (SEQ ID NO: 7).
 17. The receptor of claim 1, wherein the intracellular domain comprises a sequence of SEQ ID NOS: 12-17.
 18. The receptor of any one of claim 1-17, wherein the polypeptide comprises the LILRB1 transmembrane domain or a functional variant thereof.
 19. The receptor of claim 18, wherein the LILRB1 transmembrane domain or a functional variant thereof comprises a sequence at least 95% identical to SEQ ID NO: 5.
 20. The receptor of claim 19, wherein the LILRB1 transmembrane domain comprises SEQ ID NO: 5.
 21. The receptor of any one of claim 1-20, wherein the polypeptide comprises the LILRB1 hinge domain or functional fragment or variant thereof.
 22. The receptor of claim 21, wherein the LILRB1 hinge domain or functional fragment or variant thereof comprises a sequence at least 95% identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84 or SEQ ID NO: 93.

23. The receptor of claim 21, wherein the LILRB1 hinge domain comprises a sequence identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84 or SEQ ID NO: 93.
24. The receptor of claim 21, wherein the LILRB1 hinge domain or functional fragment or variant thereof comprises a sequence at least 95% identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83 or SEQ ID NO: 84.
25. The receptor of claim 21, wherein the LILRB1 hinge domain comprises a sequence identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83 or SEQ ID NO: 84.
26. The receptor of any one of claims 1-25, wherein the polypeptide comprises:
- a) an LILRB1 hinge domain or functional fragment or variant thereof, and
 - b) the LILRB1 transmembrane domain or a functional variant thereof.
27. The receptor of claim 26, wherein the polypeptide comprises a sequence at least 95% identical to SEQ ID NO: 20.
28. The receptor of any one of claims 1-27, wherein the polypeptide comprises:
- a) the LILRB1 transmembrane domain or a functional variant thereof, and
 - b) an LILRB1 intracellular domain and/or an intracellular domain comprising at least two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTAAEV (SEQ ID NO: 9), VTAAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
29. The receptor of claim 28, wherein the polypeptide comprises a sequence at least 95% identical to SEQ ID NO: 21.
30. The receptor of claim 28, wherein the polypeptide comprises a sequence of SEQ ID NO: 21.
31. The receptor of claim 1, wherein the polypeptide comprises:
- a) an LILRB1 hinge domain or functional fragment or variant thereof;
 - b) an LILRB1 transmembrane domain or a functional variant thereof; and
 - c) an LILRB1 intracellular domain and/or an intracellular domain comprising at least two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each

ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTYAEV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

32. The receptor of claim 1, wherein the polypeptide comprises a sequence at least 95% identical to SEQ ID NO: 2 or SEQ ID NO: 3.
33. The receptor of claim 1, wherein the polypeptide comprises a sequence at least 99% identical to SEQ ID NO: 20.
34. The receptor of claim 1, wherein the polypeptide comprises a sequence at least 99% identical to SEQ ID NO: 21.
35. The receptor of claim 1, wherein the polypeptide comprises a sequence at least 99% identical to SEQ ID NO: 2 or SEQ ID NO: 3.
36. The receptor of claim 1, wherein the polypeptide comprises a sequence identical to SEQ ID NO: 20.
37. The receptor of claim 1, wherein the polypeptide comprises a sequence identical to SEQ ID NO: 21.
38. The receptor of claim 1, wherein the polypeptide comprises a sequence identical to SEQ ID NO: 2 or SEQ ID NO: 3.
39. The receptor of claim 1, wherein the polypeptide comprises antigen-binding domain.
40. The receptor of claim 39, wherein the antigen-binding domain is an antigen-binding domain other than the LILRB1 extracellular ligand binding protein.
41. The receptor of claim 39, wherein the polypeptide comprises two or more antigen-binding domains
42. The receptor of any one of claims 1-41, wherein the receptor is an inhibitory receptor.
43. The receptor of claim 39, wherein the antigen-binding domain comprises a single chain variable fragment (scFv).
44. The receptor of claim 1, wherein the receptor comprises a second polypeptide.
45. The receptor of claim 44, wherein the first polypeptide comprises a first chain of an antibody and the second polypeptide comprise a second chain of said antibody.
46. The receptor of claim 44, wherein the receptor comprises a Fab fragment of an antibody.
47. The receptor of claim 44, wherein:
 - a) the first polypeptide comprises an antigen-binding fragment of the heavy chain of the antibody, and

- b) the second polypeptide comprises an antigen-binding fragment of the light chain of the antibody.
48. The receptor of claim 44, wherein:
- a) the first polypeptide comprises an antigen-binding fragment of the light chain of the antibody, and
 - b) the second polypeptide comprises an antigen-binding fragment of the heavy chain of the antibody.
49. The receptor of claim 44, wherein the first polypeptide comprises a first chain of a T-cell receptor (TCR) and the second polypeptide comprises a second chain of said TCR.
50. The receptor of claim 44, wherein the receptor comprises an extracellular fragment of a T cell receptor (TCR).
51. The receptor of claim 50, wherein:
- a) the first polypeptide comprises an antigen-binding fragment of an alpha chain of the TCR, and
 - b) the second polypeptide comprises an antigen-binding fragment of the beta chain of the TCR.
52. The receptor of claim 50, wherein:
- a) the first polypeptide comprises an antigen-binding fragment of the beta chain of the TCR, and
 - b) the second polypeptide comprises an antigen-binding fragment of the alpha chain of the TCR.
53. The receptor of claim 1, wherein the receptor comprises a single-chain TCR.
54. The receptor of claim 43, wherein the scFv comprises the complementarity determined regions (CDRs) of any one of SEQ ID NOS: 22-33.
55. The receptor of claim 43, wherein the scFv comprises a sequence at least 95% identical to any one of SEQ ID NOS: 35-46 or 125.
56. The receptor of claim 43, wherein the scFv comprises a sequence identical to any one of SEQ ID NOS: 35-46 or 125.
57. The receptor of claim 47 or claim 48, wherein the heavy chain of the antibody comprises the heavy chain CDRs of any one of SEQ ID NOS: 25-27 or 31-33, and wherein the light

- chain of the antibody comprises the light chain CDRs of any one of SEQ ID NOS: 22-24 or 28-30.
58. The receptor of claim 47 or claim 48, wherein the heavy chain of the antibody comprises a sequence at least 95% identical to the heavy chain portion of any one of SEQ ID NOS: 35-46 or 125, and wherein the light chain of the antibody comprises a sequence at least 95% identical to the light chain portion of any one of SEQ ID NOS: 35-46 or 125.
59. The receptor of claim 47 or claim 48, wherein the heavy chain of the antibody comprises a sequence identical to the heavy chain portion of any one of SEQ ID NOS: 35-46 or 125, and wherein the light chain of the antibody comprises a sequence identical to the light chain portion of any one of SEQ ID NOS: 35-46 or 125.
60. The receptor of claim 1, wherein the receptor comprises an amino acid sequence at least 95% identical to any one of SEQ ID NOS: 47-71, 77-79, 89-92, 120 or 122.
61. The receptor of claim 1, wherein the receptor comprises an amino acid sequence of SEQ ID NOS: 47-71, 77-79, 89-92, 120 or 122.
62. A polynucleotide comprising a nucleic acid sequence encoding the receptor of any one of claim 1-61.
63. A vector comprising the polynucleotide of claim 62.
64. The vector of claim 63, further comprising a sequence encoding a promoter operably linked to the polynucleotide.
65. An immune cell comprising the receptor of any one of claims 1-61, the polynucleotide of claim 62, or the vector of claim 63 or 64.
66. The immune cell of claim 65, wherein immune cell activation is reduced when the cell is contacted with the antigen or a cell expressing the antigen on its surface.
67. The immune cell of claim 65 or claim 66, wherein immune cell activation comprises expression of a gene operatively linked to an NFAT promoter.
68. The immune cell of any one of claims 65-67, wherein the immune cell is a T cell.
69. The immune cell of any one of claims 65-68, further comprising an activator receptor.
70. The immune cell of claim 69, wherein the activator receptor is a chimeric antigen receptor or a T cell receptor.
71. A method of making an immune cell, comprising introducing the polynucleotide of claim 62, or the vector of claim 63 or 64 into the immune cell.

72. The method of claim 69, wherein the immune cell expresses the receptor.
73. The method of claim 69 or claim 70, wherein the cell is an immune cell.
74. The method of claim 71, wherein the immune cell is a T cell.
75. The method of any one of claims 69-72, wherein immune cell activation is reduced when the cell is contacted with an antigen specific to the chimeric antigen receptor, or a cell expressing the antigen on its surface.
76. The method of claim 73, wherein immune cell activation comprises expression of a gene operatively linked to an NFAT promoter.
77. A method of treating a subject with a disease or a disorder, comprising administering to the subject a plurality of the immune cells of any one of claims 65-70.
78. The method of claim 77, wherein the disease or disorder is cancer.
79. A kit, comprising the receptor of any one of claims 1-61, the polynucleotide of claim 62, the vector of claim 63 or 64, or the immune cell of any one of claims 65-68.
80. An immune cell comprising a chimeric antigen receptor comprising a polypeptide, wherein the polypeptide sequence shares at least 95% identity or 100% identity to SEQ ID NO: 21.
81. The immune cell of claim 80, wherein the polypeptide sequence shares at least 95% identity or 100% identity to SEQ ID NO: 3.
82. The immune cell of claim 80, wherein the polypeptide sequence shares at least 95% identity or 100% identity to SEQ ID NO: 2.
83. The immune cell of claim 81, wherein the chimeric antigen receptor comprises an antigen-binding domain comprising CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, CDR-H3 sequences according to SEQ ID NO: 22-27, respectively.
84. The immune cell of claim 81, wherein the chimeric antigen receptor comprises an antigen-binding domain comprising CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, CDR-H3 sequences according to SEQ ID NO: 28-33, respectively.
85. The immune cell of claim 80, wherein the polypeptide sequence shares at least 95% identity or 100% identity to SEQ ID NO: 122.
86. The immune cell of any one of claims 80-83, wherein the immune cell is a T cell.
87. The immune cell of claim 84, wherein the T cell comprises a chimeric antigen receptor or T cell receptor that specifically binds to a target expressed on tumor cells.

88. The immune cell of claim 87, wherein the T cell comprises a chimeric antigen receptor or T cell receptor that specifically binds to a target selected from etiolate receptor, $\alpha\text{v}\beta\beta$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD19, CD20, CD22, CD30, CD33, CD37, CD44, CD44v6, CD44v7/8, CD70, CD123, CD138, CD171, CEA, DLL4, EGP-2, EGP-40, CSPG4, EGFR, EGFR family including ErbB2 (HER2), EGFRvIII, EPCAM, EphA2, EpCAM, FAP, FBP, fetal acetylcholine receptor, Fzd7, GD2, GD3, Glypican-3 (GPC3), h5T4, IL-11R, IL13R-a2, KDR, κ light chain, λ light chain, LeY, L1 CAM, MAGE-A1, mesothelin, MHC presented peptides, MUC1, MUC16, NCAM, NKG2D ligands, Notch1, Notch2/3, NY-ESO-1, PRAME, PSCA, PSMA, Survivin, TAG-72, TEMs, TERT, VEGFR2, and ROR1.
89. A method of treating and/or preventing cancer in a subject in need thereof, comprising administering to the subject the immune cells of claim 87.
90. A method of treating and/or preventing cancer in a subject in need thereof, comprising administering to the subject the immune cells of claim 88.