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(56) Fremdragne publikationer:
WO-A1-2013/123061
WO-A1-2014/172584
WO-A2-2005/097832
WO-A2-2014/164709
US-A1- 2006 251 658
US-A1- 2015 038 684
ZENAN L CHANG ET AL: "Rewiring T-cell responses to soluble factors with chimeric antigen receptors", NATURE CHEMICAL BIOLOGY, vol. 14, no. 3, 29 January 2018 (2018-01-29), pages 317-324, XP055594731, Basingstoke ISSN: 1552-4450, DOI: 10.1038/nchembio.2565
DOTTI et al.: "Design and development of therapies using chimeric antigen receptor-expressing T cells", Immunological Reviews, vol. 257, no. 1, 13 January 2014 (2014-01-13), pages 107-126, XP055177338, DOI: doi:10.1111/imr.12131

Fortsættes ...

HILLERDAL et al.: "Chimeric antigen receptor-engineered T cells for the treatment of metastatic prostate cancer", BioDrugs, vol. 29, 10 April 2015 (2015-04-10), pages 75-89, XP055379652,
CHENG ZHANG ET AL: "Engineering CAR-T cells", BIOMARKER RESEARCH, BIOMED CENTRAL LTD, LONDON, UK, vol. 5, no. 1, 24 June 2017 (2017-06-24), pages 1-6, XP021246561, DOI: 10.1186/S40364-017-0102-Y
Yang Zhi-Zhang ET AL: "Soluble and Membrane-Bound TGF-[beta]-Mediated Regulation of Intratumoral T Cell Differentiation and Function in B-Cell Non-Hodgkin Lymphoma", PLoS ONE, vol. 8, no. 3, 1 January 2013 (2013-01-01) , page e59456, XP055847288, DOI: 10.1371/journal.pone.0059456 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/artic les/PMC3598706/pdf/pone.0059456.pdf>
Evripidis Lanitis ET AL: "Redirected Antitumor Activity of Primary Human Lymphocytes Transduced With a Fully Human Anti-mesothelin Chimeric Receptor", Molecular Therapy, vol. 20, no. 3, 1 March 2012 (2012-03-01), pages 633-643, XP055066976, ISSN: 1525-0016, DOI: 10.1038/mt.2011.256
Ma Q. ET AL: "Carcinoembryonic antigen- immunoglobulin Fc fusion protein (CEA-Fc) for identification and activation of anti-CEA im- munoglobulin-T- cell receptor-modified T cells, representative of a new class of Ig fusion proteins", Cancer Gene Therapy, vol. 11, 1 January 2004 (2004-01-01), pages 297-306, XP055788799,
Westwood: "The Lewis-Y Carbohydrate Antigen is Expressed by Many Human Tumors and Can Serve as a Target for Genetically Redirected T cells Despite the Presence of Soluble Antigen in Serum", Journal of Immunotherapy, 1 April 2009 (2009-04-01), XP55847476,
HOMBACH A ET AL: "An anti-CD30 chimeric receptor that mediates CD3-zeta-independent T-cell activation against Hodgkin's lymphoma cells in the presence of soluble CD30", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 58, no. 6, 15 March 1998 (1998-03-15) , pages 1116-1119, XP002718445, ISSN: 0008-5472
ZENAN L CHANG ET AL: "Rewiring T-cell responses to soluble factors with chimeric antigen receptors", NATURE CHEMICAL BIOLOGY, vol. 14, no. 3, 29 January 2018 (2018-01-29), Basingstoke, pages 317 - 324, XP055594731, ISSN: 1552-4450, DOI: 10.1038/nchembio.2565
CHENG ZHANG ET AL: "Engineering CAR-T cells", BIOMARKER RESEARCH, BIOMED CENTRAL LTD, LONDON, UK, vol. 5, no. 1, 24 June 2017 (2017-06-24), pages 1 - 6, XP021246561, DOI: 10.1186/S40364-017-0102-Y
YANG ZHI-ZHANG ET AL: "Soluble and Membrane-Bound TGF-[beta]-Mediated Regulation of Intratumoral T Cell Differentiation and Function in B-Cell Non-Hodgkin Lymphoma", PLOS ONE, vol. 8, no. 3, 1 January 2013 (2013-01-01), pages e59456, XP055847288, Retrieved from the Internet <URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3598706/pdf/pone.0059456.pdf> DOI: 10.1371/journal.pone.0059456
EVRIPIDIS LANITIS ET AL: "Redirected Antitumor Activity of Primary Human Lymphocytes Transduced With a Fully Human Anti-mesothelin Chimeric Receptor", MOLECULAR THERAPY, vol. 20, no. 3, 1 March 2012 (2012-03-01), pages 633 - 643, XP055066976, ISSN: 1525-0016, DOI: 10.1038/mt.2011.256
MA Q. ET AL: "Carcinoembryonic antigen- immunoglobulin Fc fusion protein (CEA-Fc) for identification and activation of anti-CEA im- munoglobulin-T- cell receptor-modified T cells, representative of a new class of Ig fusion proteins", CANCER GENE THERAPY, vol. 11, 1 January 2004 (2004-01-01), pages 297 - 306, XP055788799
WESTWOOD: "The Lewis-Y Carbohydrate Antigen is Expressed by Many Human Tumors and Can Serve as a Target for Genetically Redirected T cells Despite the Presence of Soluble Antigen in Serum", JOURNAL OF IMMUNOTHERAPY, 1 April 2009 (2009-04-01), XP055847476
HOMBACH A ET AL: "An anti-CD30 chimeric receptor that mediates CD3-zeta-independent T-cell activation against Hodgkin's lymphoma cells in the presence of soluble CD30", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 58, no. 6, 15 March 1998 (1998-03-15), pages 1116 - 1119, XP002718445, ISSN: 0008-5472
DOTTI ET AL.: "Design and development of therapies using chimeric antigen receptor-expressing T cells", IMMUNOLOGICAL REVIEWS, vol. 257, no. 1, 13 January 2014 (2014-01-13), pages 107 - 126, XP055177338, DOI: doi:10.1111/imr.12131
HILLERDAL ET AL.: "Chimeric antigen receptor-engineered T cells for the treatment of metastatic prostate cancer", BIODRUGS, vol. 29, 10 April 2015 (2015-04-10), pages 75 - 89, XP 055379652

DESCRIPTION

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The present invention relates generally to the field of biotechnology and medicine. More particularly, it concerns polypeptides and cells containing the polypeptides useful for stimulating an immune response in the presence of TGF-β.

2. Background

[0002] TGF-β is a pleiotropic cytokine found at high levels in a variety of pathogenic states, including solid tumors, fibrosis, and disregulated wounds. Theraputic design for solid tumors has been directed to neutralizing TGF-β in the tumor microenvironment. While many anti-TGF-β antibodies exist, antibodies as therapeutics have some drawbacks. For example, antibodies can be large compared to other antigen-binding molecules and are multi-chain proteins encoded by multiple genes. Both of these aspects lead to higher production costs. Chemicals that inhibit TGF-β have also been identified, but typically come with toxicity issues stemming from metabolic byproducts in the liver. WO 2013/123061 relates to a bispecific chimeric antigen receptor. WO 2005/097832 relates to a TGF-β antibody. WO 2014/172584 relates to chimeric T cell receptors with an extracellular TGF-β specific domain.

[0003] Strategies using adoptive T-cell therapy with TGF-β-insensitive T cells expressing a dominant-negative TGF-β receptor have been explored. However, the mere neutralization of the TGF-β signal may not be sufficient, and reversing the TGF-β signal from an immunosuppressant to an immunostimulant may provide more promising therapeutic strategies.

[0004] Therefore, there is a need in the art for more effective therapies that counteract the action of TGF-β and also provide the benefit of a more cost-effective production.

SUMMARY OF THE INVENTION

[0005] The invention is defined in the independent claims. Further aspects and preferred embodiments are defined in the dependent claims. Any aspects, embodiments and examples of the present disclosure which do not fall under the scope of the appended claims do not form part of the invention and are merely provided for illustrative purposes. The references to

methods of treatment in the subsequent paragraphs of this description are to be interpreted as references to the compounds, pharmaceutical compositions and medicaments of the present invention for use in a method for treatment of the human (or animal) body by therapy (or for diagnosis).

[0006] The invention provides a chimeric antigen receptor (CAR) comprising a signal peptide, a TGF-β antigen-binding domain with a variable heavy (VH) and variable light (VL) region of an antibody, a peptide spacer, a transmembrane domain, and an endodomain that transmits an activation signal to the T cell after antigen is bound; wherein the TGF-β antigen-binding domain specifically binds to soluble TGF-β and wherein: (a) the VH region comprises SEQ ID NO:5 as HCDR1, SEQ ID NO:6 as HCDR2; and SEQ ID NO:7 as HCDR3 and the VL region comprises SEQ ID NO:8 as LCDR1, SEQ ID NO:9 as LCDR2; and SEQ ID NO:10 as LCDR3; or (b) the VH region comprises SEQ ID NO:11 as HCDR1, SEQ ID NO:12 as HCDR2; and SEQ ID NO:13 as HCDR3 and the VL region comprises SEQ ID NO:14 as LCDR1, SEQ ID NO:15 as LCDR2; and SEQ ID NO:16 as LCDR3; or (c) the VH region comprises SEQ ID NO:21 as HCDR1, SEQ ID NO:22 as HCDR2; and SEQ ID NO:23 as HCDR3 and the VL region comprises SEQ ID NO:24 as LCDR1, SEQ ID NO:25 as LCDR2; and SEQ ID NO:26 as LCDR3; and wherein the CAR has the structure: S-X-PL-Y-PS-T-E or S-Y-PL-X-PS-T-E wherein S is the signal peptide, X is VH, PL is a peptide linker, Y is VL, PS is the peptide spacer, T is the transmembrane domain, and E is the endodomain.

[0007] The present invention also provides an isolated nucleic acid encoding a CAR of the invention.

[0008] The present invention further provides a cell comprising a CAR of the invention or a nucleic acid of the invention, wherein: (A) optionally the cell further comprises a cancer-specific chimeric antigen receptor (CAR), and optionally the cancer-specific CAR specifically binds to: (i) Her2 or (ii) specifically binds to CD19 or CD20, wherein optionally the cell is an immune cell; and (B) optionally: (a) the cell is a T cell, optionally a CD4+ or CD8+ T cell; or T regulatory cell; or (b) the cell is a natural killer cell.

[0009] The present invention additionally provides a cell of the invention for use in a method for stimulating an immune response, said method comprising contacting the cell of the invention with TGF-β, wherein optionally: (i) stimulating an immune response comprises increasing expression and/or secretion of immune stimulating cytokines and/or molecules, wherein optionally the immune stimulating cytokines and/or molecules are one or more of TNF-α, IFN-β, IFN-γ, IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18 and granulocyte-macrophage colony stimulating factor; or (ii) stimulating an immune response comprises increasing proliferation of immune cells, optionally wherein the immune cells are T cells.

[0010] The present invention also provides a cell of the invention, for use in a method of stimulating an immune response, said method comprising contacting the cell of the invention with TGF-β, wherein the cell is *in vivo* in a subject in need of immune stimulation and optionally the TGF-β is endogenous TGF-β produced in the subject in need of immune stimulation,

wherein optionally: (i) the human subject has cancer, fibrosis, or an open wound, wherein optionally the cancer is melanoma; (ii) the human subject has a B-cell malignancy; or (iii) the human subject has a solid tumor; and wherein optionally: (iv) the method further comprises administering the cell to a human subject; and/or (v) the method further comprises administering TGF-β to the subject.

[0011] The present invention further provides an *ex vivo* method for detecting TGF-β in a solution comprising contacting the cells of the invention with the solution and measuring immune stimulation; wherein an increase in immune stimulation indicates the presence of TGF-β and no increase in immune stimulation indicates the absence of TGF-β, wherein optionally: (i) immune stimulation comprises the expression of immune stimulating cytokines and/or molecules, wherein optionally the immune stimulating cytokines and/or molecules are one or more of TNF-α, IFN-β, IFN-γ, IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18 and granulocyte-macrophage colony stimulating factor; or (ii) immune stimulation comprises an increase in the proliferation of immune cells, wherein optionally the immune cells are T cells.

[0012] The present invention additionally provides a method for making the CAR of the invention comprising expressing a nucleotide encoding the CAR in a cell.

[0013] The present invention also provides a method for expanding T cells *in vitro,* the method comprising contacting an *in vitro* T cell of the invention with a composition comprising TGF-β, wherein optionally the composition: (a) comprises 1-50 ng/mL of TGF-β; and/or (b) comprises IL-2 and optionally the composition comprises 20-400 U/mL of IL-2, wherein optionally: (a) (i) the method further comprises contacting the cells with feeder cells, wherein optionally the feeder cells are irradiated; or (ii) the method excludes contact of the T cells with feeder cells; and/or (b) the T cell is a regulatory T cell, wherein optionally the expanded regulatory T cells comprise less than 10% of non-regulatory T cells.

[0014] The present invention further provides a cell of the invention for use in a method for treating a disease or pathological condition in a patient, wherein optionally: (a) the cell is a T regulatory cell, wherein optionally the disease is: an autoimmune disease; or (b) the disease is cancer; wherein optionally the method further comprises expanding the cells in vitro by a method comprising contacting the in vitro cell with a composition comprising TGF-β;, wherein optionally: (a) the composition comprises 1-50 ng/mL of TGF-β; and/or (b) the composition further comprises IL-2, and optionally the composition comprises 20-400 U/mL of IL-2; wherein optionally: (a) the method further comprises contacting the cells with feeder cells, wherein optionally the feeder cells are irradiated; or (b) the method excludes contact of the T cells with feeder cells; wherein optionally the T cell is a regulatory T cell, wherein optionally the expanded regulatory T cells comprise less than 10% of non-regulatory T cells; wherein optionally the autoimmune disease is rheumatoid arthritis; and wherein optionally the method further comprises administration of TGF-β to the patient.

SUMMARY OF TECHNICAL TEACHINGS

[0015] The polypeptides described herein fulfill the need in the art by providing polypeptides that, when expressed in a cell, are capable of not only neutralizing the TGF-β but also specifically triggering T-cell activation in the presence of TGF-β. T-cell activation spurs the immune cell to produce immunostimulatory cytokines and proliferate, thus turning TGF-β from an immunosuppressive signal to an activating stimulus. Accordingly, aspects of the disclosure relate to polypeptides comprising a signal peptide, an antigen-binding domain with a variable heavy (VH) and variable light (VL) region, a peptide spacer, a transmembrane domain, and an endodomain; wherein the antigen-binding domain specifically binds to TGFβ.

[0016] In some aspects the disclosure relates to a polypeptide comprising a signal peptide, an antigen-binding domain with a variable heavy (VH) and variable light (VL) region, a peptide spacer, a transmembrane domain, and an endodomain; wherein the VH region comprises SEQ ID NO:5 (HCDR1), SEQ ID NO:6 (HCDR2); and SEQ ID NO:7 (HCDR3) and the VL region comprises SEQ ID NO:8 (LCDR1), SEQ ID NO:9 (LCDR2); and SEQ ID NO:10 (LCDR3). In some embodiments, the VH comprises SEQ ID NO:1 and the VL comprises SEQ ID NO:2.

[0017] In some aspects the disclosure relates to a polypeptide comprising a signal peptide, an antigen-binding domain with a variable heavy (VH) and variable light (VL) region, a peptide spacer, a transmembrane domain, and an endodomain; wherein the VH region comprises SEQ ID NO:11 (HCDR1), SEQ ID NO:12 (HCDR2); and SEQ ID NO:13 (HCDR3) and the VL region comprises SEQ ID NO:14 (LCDR1), SEQ ID NO:15 (LCDR2); and SEQ ID NO:16 (LCDR3). In some embodiments, the VH comprises SEQ ID NO:3 and the VL comprises SEQ ID NO:4.

[0018] In some aspects the disclosure relates to a polypeptide comprising a signal peptide, an antigen-binding domain with a variable heavy (VH) and variable light (VL) region, a peptide spacer, a transmembrane domain, and an endodomain; wherein the VH region comprises SEQ ID NO:21 (HCDR1), SEQ ID NO:22 (HCDR2); and SEQ ID NO:23 (HCDR3) and the VL region comprises SEQ ID NO:24 (LCDR1), SEQ ID NO:25 (LCDR2); and SEQ ID NO:26 (LCDR3). In some embodiments, the VH comprises SEQ ID NO:19 and the VL comprises SEQ ID NO:20.

[0019] The polypeptides described above and herein are polypeptides that are a continuous, single chain.

[0020] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%--or any range derivable therein) of "sequence identity" or "homology" to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Ausubel et al. eds. (2007) Current Protocols in Molecular Biology.

[0021] The polypeptides of the current disclosure may have a region, domain, linker, spacer, etc. that has at least, at most, or exactly 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93,

94, 95, 96, 97, 98, 99, or 100 % identity (or any range derivable therein) to all or a portion of the amino acid sequences described herein. In certain aspects, polypeptides described throughout this disclosure are isolated, meaning it is not found in the cellular milieu. In some cases, they are purified, which means it is mostly if not completely separated from polypeptides having a different amino acid sequence and/or chemical formula.

[0022] In some aspects, the VH and VL are separated by a peptide linker. It is contemplated that a peptide linker may separate any domain/region described in the polypeptides of the disclosure. In some aspects, the peptide linker is a peptide composed of only glycine and serine residues (a glycine-serine linker). In some aspects, the peptide linker is at least, at most, or exactly 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 100, 125, 150, or 200 amino acids (or any derivable range therein). In some aspects, the peptide linker is one known in the art or described herein.

[0023] The CAR of the present invention has the structure: S-X-PL-Y-PS-T-E or S-Y-PL-X-PS-T-E wherein S is the signal peptide, X is VH, PL is a peptide linker, Y is VL, PS is the peptide spacer, T is the transmembrane domain, and E is the endodomain. In some aspects, a polypeptide has the structure: S-X-Y-PS-T-E or S-Y-X-PS-T-E, wherein S, X, Y, PS, T, and E are defined as above. When referring to peptides and polypeptides herein, the sequences and structures are written and interpreted as proceeding from the N-terminus to the C-terminus, which is standard practice in the art.

[0024] In some aspects, the polypeptide further comprises a co-stimulatory region. In some aspects, the co-stimulatory region is between the transmembrane domain and endodomain. In some aspects, the polypeptides comprise at least, at most, or exactly 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 (or any derivable ranges therein) co-stimulatory regions. In some aspects, the co-stimulatory region is one known in the art or described herein.

[0025] In some aspects, the transmembrane domain comprises a transmembrane domain of CD28. In some aspects the transmembrane domain is all or part of a transmembrane domain known in the art or described herein.

[0026] In some aspects, the endodomain comprises a CD28 or CD3-zeta signaling domain or both. In some aspects the endodomain is all or part of an endodomain known in the art or described herein. In some aspects, the endodomain is a CD3-zeta signaling domain. In some aspects, the endodomain comprises one or more, such as 2, 3, 4, 5, 6, 7, 8, 9, 10 portions of suitable endodomains described herein.

[0027] In some aspects, the peptide spacer comprises a hinge region. In some aspects, the hinge is the hinge region of an IgG molecule. In some aspects, the hinge is a hinge region known in the art or described herein. In some aspects, the peptide spacer comprises or further comprises a CH_2CH_3 region of an IgG molecule. In some aspects, the peptide spacer comprises one or more of a hinge region, CH_1, CH_2, and CH_3 region. In some aspects, the peptide spacer is derived from a hinge, CH_1, CH_2, and/or CH_3 region or other region of an

IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM from human, mouse, rat, dog, donkey, goat, or rabbit. In some aspects, the peptide spacer comprises the hinge and CH_2CH_3 region of an IgG molecule. In some aspects, the CH_2CH_3 region of an IgG molecule has additional L235E/N297Q or L235D/N297Q mutations to prevent Fc receptor binding. In some aspects, the peptide spacer consists of the hinge region of an IgG molecule. In some aspects, the peptide spacer is less than 30, 20, 15, 10, 9, 8, 7, 6, 5, or 4 amino acids. In some aspects, the peptide spacer is less than, more than, or exactly 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 160, 170, 180, 190, 200, 210, 220, 225, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 450, 500, 550, 600, or 700 amino acids (or any derivable range therein). In some aspects, the peptide spacer comprises less than 50 amino acids. In some aspects, the peptide spacer comprises more than 50 amino acids.

[0028] In some aspects, the polypeptide further comprises a detection peptide. In some aspects, the detection peptide is a peptide of SEQ ID NO:17, an HA tag (SEQ ID NO:94), or a cMyc tag (SEQ ID NO:95). In some aspects, the detection peptide is flanked by linkers. In some aspects, a linker (e.g. peptide linker as described herein) is at the amino portion of the detection peptide. In some aspects, a linker (e.g. peptide linker as described herein) is at the carboxy portion of the detection peptide. In some aspects, a linker is at the amino and carboxy portion of the detection peptide. In some aspects, the detection peptide is at the amino portion of the VH and VL regions. In some aspects, the detection peptide is between the signal peptide and the antigen binding domain.

[0029] In some aspects, the signal peptide comprises SEQ ID NO:18. In some aspects, the signal peptide is one know in the art or described herein.

[0030] In some aspects, the polypeptide further comprises a cancer-molecule-specific antigen-binding domain. For example, the polypeptide may be a bi-specific chimeric antigen receptor (CAR) wherein the polypeptide comprises an antigen-binding domain for TGF-β and an antigen-binding domain for a cancer molecule or cancer antigen. The antigen-binding domains may be separated by a peptide spacer/linker. In some aspects, the cancer molecule comprises Her2. In some aspects, the cancer molecule comprises CD19 or CD20. In some aspects, the cancer molecule or cancer antigen is one known in the art or described herein.

[0031] The antigen-binding domain specifically binds to soluble TGF-β. It was previously unknown that polypeptides similar to those of the disclosure could bind to soluble antigens and transduce signals in response to soluble antigens.

[0032] The polypeptides described herein may include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or more variant amino acids within at least, or at most 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28,

29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400, 500, 550, 1000 or more contiguous amino acids, or any range derivable therein, of SEQ ID NO: 1-95.

[0033] Further aspects of the disclosure relate to nucleic acids encoding a polypeptide described herein. In some aspects, the disclosure relates to a cell comprising one or more polypeptides described herein. In some aspects, the cell further comprises a cancer-specific CAR In some aspects, the cancer-specific CAR is a separate polypeptide from the TGF-β CAR. In some aspects, the cancer-specific CAR specifically binds to Her2. In some aspects, the cancer-specific CAR specifically binds to CD19 or CD20. In some aspects, the cancer-specific CAR specifically binds to a cancer molecule or antigen known in the art and/or described herein. In some aspects, the cell is an immune cell. In some aspects, the cell is a progenitor cell or stem cell. In some aspects, the progenitor or stem cell is *in vitro* differentiated into an immune cell. In some aspects, the cell is a T cell. In some aspects, the cell is a CD4+ or CD8+ T cell. In some aspects, the cell is a natural killer cell. In some aspects, the cell is *ex vivo.* The term immune cells includes cells of the immune system that are involved in defending the body against both infectious disease and foreign materials. Immune cells may include, for example, neutrophils, eosinophils, basophils, natural killer cells, lymphocytes such as B cells and T cells, and monocytes. T cells may include, for example, CD4+, CD8+, T helper cells, cytotoxic T cells, γδ T cells, regulatory T cells, suppressor T cells, and natural killer T cells. In a specific aspect, the T cell is a regulatory T cell.

[0034] Further aspects of the disclosure relate to methods for stimulating an immune response comprising contacting a cell of the disclosure (i.e. cell comprising an antigen-binding polypeptide described herein) with TGF-β. In some aspects, stimulating an immune response comprises increasing expression and/or secretion of immune stimulating cytokines and/or molecules. In some aspects, the cytokine and/or molecule is a pro-inflammatory cytokine or molecule. In some aspects, the immune stimulating cytokines and/or molecules are one or more of TNF-α, IFN-β, IFN-γ, IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18 and granulocyte-macrophage colony stimulating factor. In some aspects, stimulating an immune response comprises increasing proliferation of immune cells. In some aspects, the immune cells are T cells. In some aspects, the TGF-β is endogenous TGF-β produced in a human subject in need of immune stimulation. In some aspects, the human subject has cancer, fibrosis, or an open wound. In some aspects the human subject has a B-cell malignancy. In some aspects, the

human subject has a solid tumor. A solid tumor is an abnormal mass of tissue that usually does not contain cysts or liquid areas. Solid tumors may be benign (not cancer), or malignant (cancer). Different types of solid tumors are named for the type of cells that form them. Examples of solid tumors are sarcomas, carcinomas, and lymphomas. In some aspects, the methods are for treating a person with an indication, wherein the indication is characterized by a pathogenic level of expression of TGF- β. In some aspects, the cells and polypeptides described herein can be used for treating cancer, dysregulated wounds, fibrosis, open wounds, solid tumors, etc... where the etiology of the condition, at least in part, is based on the expression of TGF-β. In some aspects, the cell (i.e. cell of the disclosure comprising the antigen-binding polypeptide) is in the human subject in need of immune stimulation. In some aspects, the method further comprises administering a cell described herein comprising the polypeptides or nucleic acids of the disclosure to a human subject.

[0035] Further method aspects relate to a method for detecting TGF-β in a solution comprising contacting the cells of the disclosure and measuring immune stimulation; wherein an increase in immune stimulation indicates the presence of TGF-β and no increase in immune stimulation indicates the absence of TGF-β. In some aspects, immune stimulation comprises the expression of immune stimulating cytokines and/or molecules. In some aspects, the immune stimulating cytokines and/or molecules are one or more of TNF-α, IFN-β, IFN-γ, IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18 and granulocyte-macrophage colony stimulating factor. In some aspects, immune stimulation comprises an increase in the proliferation of immune cells. In some aspects, the immune cells are T cells. In some aspects, the cells are *ex vivo.*

[0036] An increase in expression or proliferation as described herein may be at least, at most, or exactly 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 50, 100, 200, 300, 500, or 1000 fold increase over a base-line expression level such as a control (non-disease, non-TGF-β or non-antigen binding polypeptide control).

[0037] Further aspects of the disclosure relate to methods for making the polypeptides of the disclosure comprising expressing a nucleotide encoding the polypeptide in a cell. Further aspects relate to cultured cells, frozen cells, suspended cells, or adhered cells comprising a polypeptide described herein.

[0038] Aspects of the disclosure relate to a method for treating a disease or pathological condition comprising administering a cell of the disclosure to a patient. In some aspects, the patient is a human patient.

[0039] In some aspects, the cell is a T regulatory cell (*i.e.,* regulatory T cell comprising the TGF-β-binding polypeptides described herein). In some aspects, the disease is an autoimmune disease. In some aspects, the autoimmune disease is rheumatoid arthritis. In some aspects, the autoimmune disease is one described herein.

[0040] In some aspects of the method aspects, the method further comprises administration of TGF-β to the subject.

[0041] In some aspects, the method comprises or further comprises expanding and/or inducing proliferation of T cells *in vitro,* the method comprising contacting the *in vitro* T cell of the disclosure with a composition comprising TGF-β. In some aspects, the T cell is a regulatory cell. In some aspects, the T cell is a T cell described herein. In some aspects, the expanded regulatory T cells comprise less than 10% of non-regulatory T cells. In some aspects, the expanded regulatory T cells comprise less than 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, or 50% or any derivable range therein.

[0042] In some aspects, the composition comprises 1-50 ng/mL of TGF-β. In some aspects, the composition comprises at least, at most, or about 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 ng/mL of TGF-β (or any range derivable therein).

[0043] In some aspects, the composition further comprises IL-2. In some aspects, the composition comprises 20-400 U/mL of IL-2. In some aspects, the composition comprises at least, at most, or about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, 495, 500, 505, 510, 515, 520, 525, 530, 535, 540, 545, 550, 555, 560, 565, 570, 575, 580, 585, 590, 595, 600 U/mL of IL-2 (or any range derivable therein).

[0044] In some aspects, the method further comprises contacting the cells with feeder cells. In some aspects, the feeder cells are irradiated. Feeder cells or support cells can include, for example, fibroblasts, mouse embryonic fibroblasts, JK1 cells, SNL 76/7 cells, human fetal skin cells, human fibroblasts, and human foreskin fibroblasts.

[0045] In some aspects, the method excludes contacting T cells with feeder cells. In some cases, the excluded feeder cells are from a different animal species as the T cells.

[0046] In one aspect of the methods described herein, the subject is a human subject. The terms "individual," "subject," "host," and "patient," used interchangeably herein, refer to a mammal, including, but not limited to, murines (e.g., rats, mice), lagomorphs (e.g., rabbits), non-human primates, humans, canines, felines, ungulates (e.g., equines, bovines, ovines, porcines, caprines), etc.

[0047] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated

range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific aspects presented herein.

FIG. 1 shows that TGF-β scFvs neutralizes human TGF-β. The indicated amounts of TGF-β and anti-TGF-β scFvs were added to HepG2 cells in culture for 30 min. Neutralization of TGF-β is indicated by a loss in the phospho-SMAD2 response, as detected by western blot.

FIG. 2 shows that TGF-β CARs are expressed on the cell surface. A TGF-β CAR was created using scFv#2. Surface staining and flow cytometry show that TGF-β CARs are presented at the cell surface of CD4+ and CD8+ T cells. The receptor's extracellular domain contains the FLAG epitope. EGFRt is a truncated epidermal growth factor receptor that is an indicator of cell transduction.

FIG. 3A-B demonstrates that TGF-β CARs block endogenous TGF-β signaling in CD8+ (A) and CD4+ (B) T cells. TGF-β CAR expression in T cells blocks TGF-β signaling via the SMAD pathway. T cells expressing the indicated receptor were incubated with TGF-β for 30 min and probed for phospho-SMAD2 via western blot. scFv-less refers to a CAR that lacks any ligand (antigen)-binding scFv domain, EGFRt refers to a truncated epidermal growth factor receptor that is irrelevant to the other components here.

FIG. 4A-C. (A) Jurkat cells stably expressing the TGF-β CAR and an NFAT reporter (EGFP expressed from an NFAT-inducible promoter) show increased activation in response to increasing TGF-β concentrations. (B,C) Primary human CD4+ T cells stably expressing the TGF-β CAR upregulate (B) CD69 expression and (C) Th1 cytokine production in response to TGF-β stimulation. CD69 upregulation was monitored by surface staining after a one-day incubation with or without TGF-β. Cytokine production was detected by applying the protein transport inhibitor Brefeldin A and performing intracellular staining after a one-day incubation with or without TGF-β. "Mock" denotes T cells transduced with an irrelevant construct. "scFv-less" denotes T cells expressing a CAR that is identical to the TGF-β CAR except it lacks the scFv domain and thus cannot bind to TGF-β. "DNR" is the dominant-negative TGF-β receptor, which is a truncated TGF-β receptor chain 2 that lacks the cytoplasmic signaling domain. Values shown are the means of triplicates with error bars indicating ± 1 standard deviation (s.t.d.). * $p < 0.05$; ** $p < 0.005$, *** $p < 0.0005$, **** * $p \leq 0.0005$.

FIG. 5 shows that dominant-negative TGF-β receptor is unable to trigger cytokine production. While the dominant-negative TGF-β receptor has been reported to also inhibit TGF-β signaling, it does not trigger immunostimulatory actions such as TNF-α production. Tβ short and Tβ long are two different TGF-β CARs, Dom-Neg refers to the dominant-negative TGF-β receptor, and scFv-less refers to a CAR that lacks any ligand-binding scFv domain. Tβ short refers to the TGF-β CAR polypeptide with a peptide spacer consisting of the IgG4 hinge region only. Tβ long refers to the TGF-β CAR polypeptide with a peptide spacer that contains the IgG4 hinge and CH_2CH_3 regions.

FIG. 6 demonstrates that TGF-β CAR-T cells proliferate in response to TGF-β.

FIG. 7 shows the inhibition of murine TGF-β/SMAD signaling. Indicated amounts of scFv and mouse TGF-β1 were applied to NIH3T3 fibroblasts for 30 min. Cells were lysed and probed for SMAD2 phosphorylation.

FIG. 8. Jurkat cells stably expressing the TGF-β CAR and an NFAT reporter (EGFP expressed from an NFAT-inducible promoter) show increased activation in response to increasing input of murine TGF-β1, indicating that the TGF-β CAR engineered to recognize human TGF-β also cross-reacts with murine TGF-β.

FIG. 9. TGF-β CAR presents to the cell surface more efficiently than the dominant-negative TGF-β receptor.

FIG. 10. TGF-β CAR function can be tuned by co-stimulatory domain choice.

FIG. 11. TGF-β consistently triggers TNF-α production in a dose-dependent manner across cells from different donors.

FIG. 12. TGF-β CAR's peptide spacer length modulates the triggering threshold.

FIG. 13. CAR signaling requires ligand-mediated CAR dimerization, but there is no requirement that the ligand or the CAR itself pre-exists as a dimer. CD69 surface staining was performed on Jurkat cell lines that carry the indicated CAR(s). GFP CAR #1 and GFP CAR #3 both exist predominantly as homodimers, and the two CARs bind to different epitopes on EGFP and can concurrently bind a monomeric EGFP molecule. GFP CAR #1 and GFP CAR #2 bind to the same epitope on EGFP, but CAR #2 exists as a monomer rather than a homodimer.

FIG. 14. Soluble, dimeric antigen molecues can trigger signaling by ligating receptors on different cells.

FIG. 15. TGF-β CAR can be triggered in both cell-cell contact-dependent and - independent manners.

FIG. 16. TGF-β CAR-T cells can be activated in the absence of cell-cell contact.

DESCRIPTION OF ILLUSTRATIVE ASPECTS

[0049] Polypeptides, cells, and methods described herein can be used to neutralize TGF-β and specifically trigger T-cell activation in the presence of TGF-β.

I. Definitions

[0050] The peptides of the disclosure relate to peptides comprising CARs or chimeric antigen receptors. CARs are engineered receptors, which graft an arbitrary specificity onto an immune effector cell. Typically, these receptors are used to graft the specificity of a monoclonal antibody onto a T cell. The receptors are called chimeric because they are composed of parts from different sources.

[0051] The terms "protein," "polypeptide," and "peptide" are used interchangeably herein when referring to a gene product.

[0052] "Homology," "identity," or "similarity" refers to sequence similarity between two peptides or between two nucleic acid molecules. Identity can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules share sequence identity at that position. A degree of identity between sequences is a function of the number of matching or homologous positions shared by the sequences. An "unrelated" or "non-homologous" sequence shares less than 40% identity, or less than 25% identity, with one of the sequences of the current disclosure.

[0053] The terms "amino portion," "N-terminus," "amino terminus," and the like as used herein are used to refer to order of the regions of the polypeptide. Furthermore, when something is N-terminal to a region it is not necessarily at the terminus (or end) of the entire polypeptide, but just at the terminus of the region or domain. Similarily, the terms "carboxy portion," "C-terminus," "carboxy terminus," and the like as used herein is used to refer to order of the regions of the polypeptide, and when something is C-terminal to a region it is not necessarily at the terminus (or end) of the entire polypeptide, but just at the terminus of the region or domain.

[0054] The terms "polynucleotide," "nucleic acid," and "oligonucleotide" are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, dsRNA, siRNA, miRNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications

to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any aspect of this disclosure that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

[0055] The term "subject," "individual," or "patient" is used interchangeably herein and refers to a vertebrate, for example a primate, a mammal or preferably a human. Mammals include, but are not limited to equines, canines, bovines, ovines, murines, rats, simians, humans, farm animals, sport animals and pets.

[0056] The term "xeno-free (XF)" or "animal component-free (ACF)" or "animal free," when used in relation to a medium, an extracellular matrix, or a culture condition, refers to a medium, an extracellular matrix, or a culture condition which is essentially free from heterogeneous animal-derived components. For culturing human cells, any proteins of a non-human animal, such as mouse, would be xeno components. In certain aspects, the xeno-free matrix may be essentially free of any non-human animal-derived components, therefore excluding mouse feeder cells or Matrigel™. Matrigel™ is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in extracellular matrix proteins to include laminin (a major component), collagen IV, heparin sulfate proteoglycans, and entactin/nidogen. In some aspects, the compositions described herein or cells of the disclosure are cultured in and/or prepared in/with xeno-free or animal component-free or animal free medium.

[0057] Cells are "substantially free" of certain reagents or elements, such as serum, signaling inhibitors, animal components or feeder cells, exogenous genetic elements or vector elements, as used herein, when they have less than 10% of the element(s), and are "essentially free" of certain reagents or elements when they have less than 1% of the element(s). However, even more desirable are cell populations wherein less than 0.5% or less than 0.1% of the total cell population comprise exogenous genetic elements or vector elements.

[0058] A culture, matrix or medium are "essentially free" of certain reagents or elements, such as serum, signaling inhibitors, animal components or feeder cells, when the culture, matrix or medium respectively have a level of these reagents lower than a detectable level using conventional detection methods known to a person of ordinary skill in the art or these agents have not been extrinsically added to the culture, matrix or medium. The serum-free medium may be essentially free of serum.

[0059] A "gene," "polynucleotide," "coding region," "sequence," "segment," "fragment," or "transgene" which "encodes" a particular protein, is a nucleic acid molecule which is transcribed and optionally also translated into a gene product, *e.g.,* a polypeptide, *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The coding region

may be present in either a cDNA, genomic DNA, or RNA form. When present in a DNA form, the nucleic acid molecule may be single-stranded (i.e., the sense strand) or double-stranded. The boundaries of a coding region are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A gene can include, but is not limited to, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the gene sequence.

[0060] The term "cell" is herein used in its broadest sense in the art and refers to a living body which is a structural unit of tissue of a multicellular organism, is surrounded by a membrane structure which isolates it from the outside, has the capability of self-replicating, and has genetic information and a mechanism for expressing it. Cells used herein may be naturally-occurring cells or artificially modified cells (e.g., fusion cells, genetically modified cells, etc.).

[0061] As used herein, the term "stem cell" refers to a cell capable of self-replication and pluripotency or multipotency. Typically, stem cells can regenerate an injured tissue. Stem cells herein may be, but are not limited to, embryonic stem (ES) cells, induced pluripotent stem cells or tissue stem cells (also called tissue-specific stem cell, or somatic stem cell).

[0062] "Embryonic stem (ES) cells" are pluripotent stem cells derived from early embryos. An ES cell was first established in 1981, which has also been applied to production of knockout mice since 1989. In 1998, a human ES cell was established, which is currently becoming available for regenerative medicine.

[0063] Unlike ES cells, tissue stem cells have a limited differentiation potential. Tissue stem cells are present at particular locations in tissues and have an undifferentiated intracellular structure. Therefore, the pluripotency of tissue stem cells is typically low. Tissue stem cells have a higher nucleus/cytoplasm ratio and have few intracellular organelles. Most tissue stem cells have low pluripotency, a long cell cycle, and proliferative ability beyond the life of the individual. Tissue stem cells are separated into categories, based on the sites from which the cells are derived, such as the dermal system, the digestive system, the bone marrow system, the nervous system, and the like. Tissue stem cells in the dermal system include epidermal stem cells, hair follicle stem cells, and the like. Tissue stem cells in the digestive system include pancreatic (common) stem cells, liver stem cells, and the like. Tissue stem cells in the bone marrow system include hematopoietic stem cells, mesenchymal stem cells, and the like. Tissue stem cells in the nervous system include neural stem cells, retinal stem cells, and the like.

[0064] "Induced pluripotent stem cells," commonly abbreviated as iPS cells or iPSCs, refer to a type of pluripotent stem cell artificially prepared from a non-pluripotent cell, typically an adult somatic cell, or terminally differentiated cell, such as fibroblast, a hematopoietic cell, a myocyte, a neuron, an epidermal cell, or the like, by introducing certain factors, referred to as reprogramming factors.

[0065] "Pluripotency" refers to a stem cell that has the potential to differentiate into all cells

constituting one or more tissues or organs, or particularly, any of the three germ layers: endoderm (interior stomach lining, gastrointestinal tract, the lungs), mesoderm (muscle, bone, blood, urogenital), or ectoderm (epidermal tissues and nervous system). "Pluripotent stem cells" used herein refer to cells that can differentiate into cells derived from any of the three germ layers, for example, direct descendants of totipotent cells or induced pluripotent cells.

[0066] As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, e.g., in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

[0067] In some aspects, the methods are useful for reducing the size and/or cell number of a solid tumor. In some aspects, the method of the disclosure are useful for inhibiting the growth of tumors, such as solid tumors, in a subject.

[0068] The term "antigen" refers to any substance that causes an immune system to produce antibodies against it, or to which a T cell responds. In some aspects, an antigen is a peptide that is 5-50 amino acids in length or is at least, at most, or exactly 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 250, or 300 amino acids, or any derivable range therein.

[0069] The term "antibody" includes monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies and antibody fragments that may be human, mouse, humanized, chimeric, or derived from another species. A "monoclonal antibody" is an antibody obtained from a population of substantially homogeneous antibodies that is being directed against a specific antigenic site.

[0070] "Antibody or functional fragment thereof means an immunoglobulin molecule that specifically binds to, or is immunologically reactive with a particular antigen or epitope, and includes both polyclonal and monoclonal antibodies. The term antibody includes genetically engineered or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies (e.g., bispecific antibodies, diabodies, triabodies, and tetrabodies). The term functional antibody fragment includes antigen binding fragments of antibodies, including e.g., Fab', $F(ab')_2$, Fab, Fv, rIgG, and scFv fragments. The term scFv refers to a single chain Fv antibody in which the variable domains of the heavy chain and of the light chain of a traditional two chain antibody have been joined to form one chain.

[0071] The use of a single chain variable fragment (scFv) is of particular interest. scFvs are

recombinant molecules in which the variable regions of light and heavy immunoglobulin chains encoding antigen-binding domains are engineered into a single polypeptide. Generally, the V_H and V_L sequences are joined by a linker sequence. See, for example, Ahmad (2012) Clinical and Developmental Immunology Article ID 980250.

[0072] A "therapeutically effective amount" or "efficacious amount" refers to the amount of an agent, or combined amounts of two agents, that, when administered to a mammal or other subject for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on the agent(s), the disease and its severity and the age, weight, etc., of the subject to be treated.

[0073] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one.

[0074] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." As used herein "another" may mean at least a second or more.

[0075] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

II. Polypeptides

A. Signal peptides

[0076] A "signal peptide" refers to a peptide sequence that directs the transport and localization of the protein within a cell, e.g. to a certain cell organelle (such as the endoplasmic reticulum) and/or the cell surface. A signal peptide directs the nascent protein into the endoplasmic reticulum. This is essential if the receptor is to be glycosylated and anchored in the cell membrane. Generally, the signal peptide natively attached to the amino-terminal most component is used (e.g. in a scFv with orientation light chain - linker - heavy chain, the native signal of the light-chain is used). In some aspects the signal peptide is SEQ ID NO:18.

[0077] In some aspects, the signal peptide is cleaved after passage of the endoplasmic reticulum (ER), i.e. is a cleavable signal peptide. In some aspects, a restriction site is at the carboxy end of the signal peptide to facilitate cleavage.

B. Antigen-binding domain

[0078] The antigen-binding domain is a single-chain variable fragment (scFv) based on TGF-β antibodies. "Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. In some aspects, the antigen-binding domain further comprises a peptide linker between the V_H and V_L domains, which enables the scFv to form the desired structure for antigen binding.

[0079] The variable regions of the antigen-binding domains of the polypeptides of the disclosure can be modified by mutating amino acid residues within the VH and/or VL CDR 1, CDR 2 and/or CDR 3 regions to improve one or more binding properties (e.g., affinity) of the antibody. The term "CDR" refers to a complementarity-determining region that is based on a part of the variable chains in immunoglobulins (antibodies) and T-cell receptors, generated by B cells and T cells respectively, where these molecules bind to their specific antigen. Since most sequence variation associated with immunoglobulins and T-cell receptors are found in the CDRs, these regions are sometimes referred to as hypervariable regions. Mutations may be introduced by site-directed mutagenesis or PCR-mediated mutagenesis and the effect on antibody binding, or other functional property of interest, can be evaluated in appropriate *in vitro* or *in vivo* assays. Preferably conservative modifications are introduced and typically no more than one, two, three, four or five residues within a CDR region are altered. The mutations may be amino acid substitutions, additions or deletions.

[0080] Framework modifications can be made to the antibodies to decrease immunogenicity, for example, by "backmutating" one or more framework residues to the corresponding germline sequence.

[0081] It is also contemplated that the antigen binding domain may be multi-specific or multivalent by multimerizing the antigen binding domain with VH and VL region pairs that bind either the same antigen (multi-valent) or a different antigen (multi-specific).

[0082] As used herein, the term "affinity" refers to the equilibrium constant for the reversible binding of two agents and is expressed as a dissociation constant (Kd). Affinity can be at least 1-fold greater, at least 2-fold greater, at least 3-fold greater, at least 4-fold greater, at least 5-fold greater, at least 6-fold greater, at least 7-fold greater, at least 8-fold greater, at least 9-fold greater, at least 10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 60-fold greater, at least 70-fold greater, at least 80-fold greater, at least 90-fold greater, at least 100-fold greater, or at least 1000-fold greater, or more (or any derivable range therein), than the affinity of an antibody for unrelated amino acid sequences. As used herein, the term "avidity" refers to the resistance of a complex of two or more agents to dissociation after dilution. The terms "immunoreactive" and "preferentially binds" are used interchangeably herein with respect to antibodies and/or antigen-binding fragments.

[0083] The term "binding" refers to a direct association between two molecules, due to, for

example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges.

C. Peptide spacer

[0084] A spacer region links the antigen-binding domain to the transmembrane domain. It should be flexible enough to allow the antigen-binding domain to orient in different directions to facilitate antigen recognition. The simplest form is the hinge region from IgG. Alternatives include the CH_2CH_3 region of immunoglobulin and portions of CD3. In some aspects, the CH_2CH_3 region may have L235E/N297Q or L235D/N297Q modifications, or at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% amino acid sequence identity of the CH_2CH_3 region. For most scFv-based constructs, the IgG hinge suffices. However the best spacer often has to be determined empirically. In some aspects, the spacer is from IgG4.

[0085] As used herein, the term "hinge" refers to a flexible polypeptide connector region (also referred to herein as "hinge region" or "spacer") providing structural flexibility and spacing to flanking polypeptide regions and can consist of natural or synthetic polypeptides. A "hinge" derived from an immunoglobulin (e.g., IgGl) is generally defined as stretching from Glu216 to Pro230 of human IgGl (Burton (1985) Molec. Immunol., 22: 161- 206). Hinge regions of other IgG isotypes may be aligned with the IgGl sequence by placing the first and last cysteine residues forming inter-heavy chain disulfide (S-S) bonds in the same positions. The hinge region may be of natural occurrence or non-natural occurrence, including but not limited to an altered hinge region as described in U.S. Pat. No. 5,677,425. The hinge region can include complete hinge region derived from an antibody of a different class or subclass from that of the CH_1 domain. The term "hinge" can also include regions derived from CD8 and other receptors that provide a similar function in providing flexibility and spacing to flanking regions.

[0086] The peptide spacer can have a length of at least, at most, or exactly 4, 5, 6, 7, 8, 9, 10, 15, 16, 17, 18, 19, 20, 20, 25, 30, 35, 40, 45, 50, 75, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 260, 270, 280, 290, 300, 325, 350, or 400 amino acids (or any derivable range therein). In some aspects, the peptide spacer consists of or comprises a hinge region from an immunoglobulin. Immunoglobulin hinge region amino acid sequences are known in the art; see, e.g., Tan et al. (1990) Proc. Natl. Acad. Sci. USA 87: 162; and Huck et al. (1986) Nucl. Acids Res.

[0087] The length of a peptide spacer may have effects on the response to TGF-β and/or expansion properties. In some aspects, a shorter spacer such as less than 50, 45, 40, 30, 35, 30, 25, 20, 15, or 10 amino acids may have the advantage of a decrease in the concentration of TGF-β required for an effective activation response. In some aspects, a longer spacer, such

as one that is at least 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 260, 270, 280, or 290 amino acids may have the advantage of increased expansion in vivo or in vitro.

[0088] As non-limiting examples, an immunoglobulin hinge region can include one of the following amino acid sequences: DKTHT (SEQ ID NO:27); CPPC (SEQ ID NO:28); CPEPKSCDTPPPCPR (SEQ ID NO:29); ELKTPLGDTTHT (SEQ ID NO:30); KSCDKTHTCP (SEQ ID NO:31); KCCVDCP (SEQ ID NO:32); KYGPPCP (SEQ ID NO:33); EPKSCDKTHTCPPCP (SEQ ID NO:34) (human IgGI hinge); ERKCCVECPPCP (SEQ ID NO:35) (human IgG2 hinge); ELKTPLGDTTHTCPRCP (SEQ ID NO:36) (human IgG3 hinge); SPNMVPHAHHAQ (SEQ ID NO:37); ESKYGPPCPPCP (SEQ ID NO:98) or ESKYGPPCPSCP (SEQ ID NO:99) (human IgG4 hinge-based) and the like. In some aspects, the hinge is SEQ ID NO:98 or SEQ ID NO:99. In some aspects, the hinge is SEQ ID NO:99.

[0089] The hinge region can comprise an amino acid sequence of a human IgGI, IgG2, IgG3, or IgG4, hinge region. The hinge region can include one or more amino acid substitutions and/or insertions and/or deletions compared to a wild-type (naturally-occurring) hinge region. For example, His229 of human IgGI hinge can be substituted with Tyr, so that the hinge region comprises the sequence EPKSCDKTYTCPPCP (SEQ ID NO:38).

[0090] The hinge region can comprise an amino acid sequence derived from human CD8; e.g., the hinge region can comprise the amino acid sequence: TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:39), or a variant thereof.

D. Transmembrane domain

[0091] The transmembrane domain is a hydrophobic alpha helix that spans the membrane. Generally, the transmembrane domain from the most membrane proximal component of the endodomain is used. Different transmembrane domains result in different receptor stability.

[0092] The transmembrane domain is interposed between the peptide spacer and the endodomain. In some aspects, the transmembrane domain is interposed between the peptide spacer and a co-stimulatory region. In some aspects, a linker is between the transmembrane domain and a co-stimulatory region or endodomain.

[0093] Any transmembrane domain that provides for insertion of a polypeptide into the cell membrane of a eukaryotic (e.g., mammalian) cell is suitable for use. As one non-limiting example, the transmembrane sequence IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO:48) can be used. In some aspects, the transmembrane domain is CD8 beta derived: LGLLVAGVLVLLVSLGVAIHLCC (SEQ ID NO:49); CD4 derived:

ALIVLGGVAGLLLFIGLGIFFCVRC (SEQ ID NO:50); CD3 zeta derived: LCYLLDGILFIYGVILTALFLRV (SEQ ID NO:51); CD28 derived: WVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:52); CD134 (OX40) derived: VAAILGLGLVLGLLGPLAILLALYLL (SEQ ID NO:53); or CD7 derived: ALPAALAVISFLLGLGLGVACVLA (SEQ ID NO:54).

E. Endodomain

[0094] After antigen recognition, receptors cluster and a signal is transmitted to the cell through the endodomain and/or co-stimulatory domain. In some aspects, the co-stimulatory domains described herein are part of the endodomain. The most commonly used endodomain component is CD3-zeta, which contains 3 ITAMs. This transmits an activation signal to the T cell after antigen is bound. CD3-zeta may not provide a fully competent activation signal and additional co-stimulatory signaling is needed. For example, chimeric CD28 and OX40 can be used with CD3-Zeta to transmit a proliferative/survival signal, or all three can be used together.

[0095] Further endodomains suitable for use in the polypeptides of the disclosure include any desired signaling domain that provides a distinct and detectable signal (e.g., increased production of one or more cytokines by the cell; change in transcription of a target gene; change in activity of a protein; change in cell behavior, e.g., cell death; cellular proliferation; cellular differentiation; cell survival; modulation of cellular signaling responses; etc.) in response to activation by way of binding of the antigen to the antigen binding domain. In some aspects, the endodomain includes at least one (e.g., one, two, three, four, five, six, etc.) ITAM motif as described herein. In some aspects, the endodomain includes DAP10/CD28 type signaling chains.

[0096] Endodomains suitable for use in the polypeptides of the disclosure include immunoreceptor tyrosine-based activation motif (ITAM)-containing intracellular signaling polypeptides. An ITAM motif is $YX_1X_2(L/I)$, where X_1 and X_2 are independently any amino acid (SEQ ID NO:64). In some cases, the endodomain comprises 1, 2, 3, 4, or 5 ITAM motifs. In some cases, an ITAM motif is repeated twice in an endodomain, where the first and second instances of the ITAM motif are separated from one another by 6 to 8 amino acids, e.g., $(YX_1X_2(L/I))(X3)_n(YX_1X_2(L/I))$, where n is an integer from 6 to 8, and each of the 6-8 X_3 can be any amino acid (SEQ ID NO:65).

[0097] A suitable endodomain may be an ΓΓAM motif-containing portion that is derived from a polypeptide that contains an ITAM motif. For example, a suitable endodomain can be an ITAM motif-containing domain from any ITAM motif-containing protein. Thus, a suitable endodomain need not contain the entire sequence of the entire protein from which it is derived. Examples of suitable ITAM motif-containing polypeptides include, but are not limited to: DAP12; FCER1G (Fc epsilon receptor I gamma chain); CD3D (CD3 delta); CD3E (CD3 epsilon); CD3G (CD3 gamma); CD3Z (CD3 zeta); and CD79A (antigen receptor complex-associated protein alpha

chain).

[0098] In some cases, the endodomain is derived from DAP12 (also known as TYROBP; TYRO protein tyrosine kinase binding protein; KARAP; PLOSL; DN AX- activation protein 12; KAR-associated protein; TYRO protein tyrosine kinase- binding protein; killer activating receptor associated protein; killer- activating receptor- associated protein; etc.). For example, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to

MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSPGVLAGIVMGDLVLTVLI ALAVYFLGRLVPRGRGAAEAATRKORITETESPYOELOGORSDVYSDLNTQRPYYK (SEQ ID NO:66);

MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSPGVLAGIVMGDLVLTVLI ALAVYFLGRLVPRGRGAAEATRKORITETESPYOELOGORSDVYSDLNTQRPYYK (SEQ ID NO:67);

MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFLGRLVP RGRGAAEAATRKORITETESPYOELOGORSDVYSDLNTQRPYYK (SEQ ID NO:68); or MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFLGRLVP RGRGAAEATRKORITETESPYOELOGORSDVYSDLNTQRPYYK (SEQ ID NO:69).

[0099] In some aspects, a suitable endodomain polypeptide can comprise an ITAM motif-containing portion of the full length DAP12 amino acid sequence. Thus, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to ESPYOELOGORSDVYSDLNTO (SEQ ID NO: 70).

[0100] In some aspects, the endodomain is derived from FCER1G (also known as FCRG; Fc epsilon receptor I gamma chain; Fc receptor gamma-chain; fc-epsilon RI-gamma; fcRgamma; fceRI gamma; high affinity immunoglobulin epsilon receptor subunit gamma; immunoglobulin E receptor, high affinity, gamma chain; etc.). For example, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% amino acid sequence identity to

MIPAVVLLLLLLVEQAAALGEPQLCYILDAILFLYGIVLTLLYCRLKIQVRKAAITSYE KSDGVYTGLSTRNQETYETLKHEKPPQ (SEQ ID NO: 71).

[0101] In some aspects, a suitable endodomain polypeptide can comprise an ITAM motif-containing portion of the full length FCERIG amino acid sequence. Thus, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to DGVYTGLSTRNOETYETLKHE (SEQ ID NO: 72).

[0102] In some aspects, the endodomain is derived from T-cell surface glycoprotein CD3 delta chain (also known as CD3D; CD3-DELTA; T3D; CD3 antigen, delta subunit; CD3 delta; CD3d antigen, delta polypeptide (TiT3 complex); OKT3, delta chain; T-cell receptor T3 delta chain; T-cell surface glycoprotein CD3 delta chain; etc.). For example, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 170 aa, of either of the following amino acid sequences (2 isoforms): MEHSTFLSGLVLATLLSQVSPFKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLD LGKRILDPRGIYRCNGTDIYKDKESTVQVHYRMCQSCVELDPATVAGIIVTDVIATLL LALGVFCFAGHETGRLSGAADTQALLRNDQVYQPLRDRDDAQYSHLGGNWARNK (SEQ ID NO:73) or MEHSTFLSGLVLATLLSQVSPFKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLD LGKRILDPRGIYRCNGTDIYKDKESTVQVHYRTADTQALLRNDQVYQPLRDRDDAQ YSHLGGNWARNK (SEQ ID NO: 74).

[0103] In some aspects, a suitable endodomain polypeptide can comprise an ITAM motif-containing portion of the full length CD3 delta amino acid sequence. Thus, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to DQVYQPLRDRDDAQYSHLGGN (SEQ ID NO:75).

[0104] In some aspects, the endodomain is derived from T-cell surface glycoprotein CD3 epsilon chain (also known as CD3e, T-cell surface antigen T3/Leu-4 epsilon chain, T-cell surface glycoprotein CD3 epsilon chain, AI504783, CD3, CD3epsilon, T3e, etc.). For example, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 205 aa, of the following amino acid sequence:
MQSGTHWRVLGLCLLSVGVWGQDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEI
LWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLY
LRARVCENCMEMDVMSVATIVIVDICITGGLLLLVYYWSKNRKAKAKPVTRGAGAG
GRQRGQNKERPPPVPNPDYEPIRKGQRDLYSGLNQRRI (SEQ ID NO:76).

[0105] In some aspects, a suitable endodomain polypeptide can comprise an ITAM motif-containing portion of the full length CD3 epsilon amino acid sequence. Thus, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to NPDYEPIRKGQRDLYSGLNQR (SEQ ID NO:77).

[0106] In some aspects, the endodomain is derived from T-cell surface glycoprotein CD3 gamma chain (also known as CD3G, T-cell receptor T3 gamma chain, CD3-GAMMA, T3G, gamma polypeptide (TiT3 complex), etc.). For example, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 180 aa, of the following amino acid sequence:
MEQGKGLAVLILAIILLQGTLAQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNITWFK
DGKMIGFLTEDKKKWNLGSNAKDPRGMYQCKGSQNKSKPLQVYYRMCQNCIELNA
ATISGFLFAEIVSIFVLAVGVYFIAGQDGVRQSRASDKQTLLPNDQLYQPLKDREDDQ
YSHLQGNQLRRN (SEQ ID NO:78).

[0107] In some aspects, a suitable endodomain polypeptide can comprise an ITAM motif-containing portion of the full length CD3 gamma amino acid sequence. Thus, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to DQLYQPLKDREDDQYSHLQGN (SEQ ID NO:79).

[0108] In some aspects, the endodomain is derived from T-cell surface glycoprotein CD3 zeta chain (also known as CD3Z, T-cell receptor T3 zeta chain, CD247, CD3-ZETA, CD3H, CD3Q, T3Z, TCRZ, etc.). For example, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 160 aa, of either of the following amino acid sequences (2 isoforms):
MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSA
DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELO
KDKMAEAYSEIGMKGERRRGKGHDGLYOGLSTATKDTYDALHMQALPPR (SEQ ID NO: 80) or
MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSA
DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNEL
OKDKMAEAYSEIGMKGERRRGKGHDGLYOGLSTATKDTYDALHMQALPPR(SEQ ID NO: 81).

[0109] In some aspects, a suitable endodomain polypeptide can comprise an ITAM motif-containing portion of the full length CD3 zeta amino acid sequence. Thus, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to any of the following amino acid sequences:
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ

EGLYNELOKDKMAEAYSEIGMKGERRRGKGHDGLYOGLSTATKDTYDALHMQALP PR (SEQ ID NO: 82); NOLYNELNLGRREEYDVLDKR (SEQ ID NO:83); EGLYNELQKDKMAEAYSEIGMK (SEQ ID NO:84); or DGLYOGLSTATKDTYDALHMO (SEQ ID NO:85).

[0110] In some aspects, the endodomain is derived from CD79A (also known as B-cell antigen receptor complex-associated protein alpha chain; CD79a antigen (immunoglobulin-associated alpha); MB-1 membrane glycoprotein; ig-alpha; membrane- bound immunoglobulin-associated protein; surface IgM-associated protein; etc.). For example, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 150 aa, from about 150 aa to about 200 aa, or from about 200 aa to about 220 aa, of either of the following amino acid sequences (2 isoforms): MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPH NSSNNANVTWWRVLHGNYTWPPEFLGPGEDPNGTLIIQNVNKSHGGIYVCRVQEGN ESYQQSCGTYLRVRQPPPRPFLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFRKRWO NEKLGLDAGDEYEDENLYEGLNLDDCSMYEDISRGLOGTYQDVGSLNIGDVQLEKP (SEQ ID NO:86); or MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPH NSSNNANVTWWRVLHGNYTWPPEFLGPGEDPNEPPPRPFLDMGEGTKNRIITAEGIIL LFCAVVPGTLLLFRKRWQNEKLGLDAGDEYEDENLYEGLNLDDCSMYE DISRGLQGTYQDVGSLNIGDVQLEKP (SEQ ID NO:87).

[0111] In some aspects, a suitable endodomain polypeptide can comprise an ITAM motif-containing portion of the full length CD79A amino acid sequence. Thus, a suitable endodomain polypeptide can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100%, amino acid sequence identity to the following amino acid sequence: ENLYEGLNLDDCSMYEDISRG (SEQ ID NO:88).

[0112] In some aspects, suitable endodomains can comprise a DAP10/CD28 type signaling chain. An example of a DAP 10 signaling chain is the amino acid sequence: RPRRSPAQDGKVYINMPGRG (SEQ ID NO:89). In some aspects, a suitable endodomain comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the entire length of the amino acid sequence RPRRSPAQDGKVYINMPGRG (SEQ ID NO:90).

[0113] An example of a CD28 signaling chain is the amino acid sequence FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQP YAPPRDFAAYRS (SEQ ID NO:91). In some aspects, a suitable endodomain comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the entire length of the amino acid sequence FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQP YAPPRDFAAYRS (SEQ ID NO: 92).

[0114] Further endodomains suitable for use in the polypeptides of the disclosure include a ZAP70 polypeptide, e.g., a polypeptide comprising an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids to about 400 amino acids, from about 400 amino acids to about 500 amino acids, or from about 500 amino acids to 619 amino acids, of the following amino acid sequence: MPDPAAHLPFFYGSIS RAEAEEHLKLAGMADGLFLLRQCLRSLGGYVLSLVHDVRFHHFPIERQLNGTYAIAG GKAHCGPAELCEFYSRDPDGLPCNLRKPCNRPSGLEPQPGVFDCLRDAMVRDYVRQ TWKLEGEALEQAIISQAPQVEKLIATTAHERMPWYHSSLTREEAERKLYSGAQTDGK FLLRPRKEQGTYALSLIYGKTVYHYLISQDKAGKYCIPEGTKFDTLWQLVEYLKLKA DGLIYCLKEACPNSSASNASGAAAPTLPAHPSTLTHPQRRIDTLNSDGYTPEPARITSP DKPRPMPMDTSVYESPYSDPEELKDKKLFLKRDNLLIADIELGCGNFGSVRQGVYRM RKKQIDVAIKVLKQGTEKADTEEMMREAQIMHQLDNPYIVRLIGVCQAEALMLVME MAGGGPLHKFLVGKREEIPVSNVAELLHQVSMGMKYLEEKNFVHRDLAARNVLLV NRHYAKISDFGLSKALGADDSYYTARSAGKWPLKWYAPECINFRKFSSRSDVWSYG VTMWEALSYGQKPYKKMKGPEVMAFIEQGKRMECPPECPPELYALMSDCWIYKWE DRPDFLTVEQRMRACYYSLASKVEGPPGSTQKAEAACA (SEQ ID NO:93).

F. Detection peptides

[0115] Suitable detection peptides include hemagglutinin (HA; e.g., YPYDVPDYA (SEQ ID NO:94); FLAG (e.g., DYKDDDDK (SEQ ID NO:17); c-myc (e.g., EQKLISEEDL; SEQ ID NO:95), and the like. Other suitable detection peptides are known in the art.

G. Peptide linkers

[0116] In some aspects, the polypeptides of the disclosure include peptide linkers (sometimes referred to as a linker). A peptide linker may be separating any of the peptide domain/regions described herein. As an example, a linker may be between the signal peptide and the antigen binding domain, between the VH and VL of the antigen binding domain, between the antigen binding domain and the peptide spacer, between the peptide spacer and the transmembrane domain, flanking the co-stimulatory region or on the N- or C- region of the co-stimulatory region, and/or between the transmembrane domain and the endodomain. The peptide linker may have any of a variety of amino acid sequences. Domains and regions can be joined by a peptide linker that is generally of a flexible nature, although other chemical linkages are not excluded. A linker can be a peptide of between about 6 and about 40 amino acids in length, or between about 6 and about 25 amino acids in length. These linkers can be produced by using synthetic, linker-encoding oligonucleotides to couple the proteins.

[0117] Peptide linkers with a degree of flexibility can be used. The peptide linkers may have virtually any amino acid sequence, bearing in mind that suitable peptide linkers will have a

sequence that results in a generally flexible peptide. The use of small amino acids, such as glycine and alanine, are of use in creating a flexible peptide. The creation of such sequences is routine to those of skill in the art.

[0118] Suitable linkers can be readily selected and can be of any of a suitable of different lengths, such as from 1 amino acid (e.g., Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and may be 1, 2, 3, 4, 5, 6, or 7 amino acids.

[0119] Exemplary flexible linkers include glycine polymers $(G)_n$, glycine- serine polymers (including, for example, (GS)n, $(GSGGS)_n$ (SEQ ID NO:40) and $(GGGS)_n$ (SEQ ID NO:41), where n is an integer of at least one, glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers can be used; both Gly and Ser are relatively unstructured, and therefore can serve as a neutral tether between components. Glycine polymers can be used; glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains. Exemplary spacers can comprise amino acid sequences including, but not limited to, GGSG (SEQ ID NO:42), GGSGG (SEQ ID NO:43), GSGSG (SEQ ID NO:44), GSGGG (SEQ ID NO:45), GGGSG (SEQ ID NO:46), GSSSG (SEQ ID NO:47), and the like.

H. Co-stimulatory region

[0120] Non-limiting examples of suitable co-stimulatory regions include, but are not limited to, polpeptides from 4-1BB (CD137), CD28, ICOS, OX-40, BTLA, CD27, CD30, GITR, and HVEM.

[0121] A co- stimulatory region may have a length of at least, at most, or exactly 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or 300 amino acids or any range derivable therein. In some aspects, the co-stimulatory region is derived from an intracellular portion of the transmembrane protein 4-1BB (also known as TNFRSF9; CD137; 4-1BB; CDwl37; ILA; etc.). For example, a suitable co-stimulatory region can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% amino acid sequence identity to KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO:55).

[0122] In some aspects, the co-stimulatory region is derived from an intracellular portion of the transmembrane protein CD28 (also known as Tp44). For example, a suitable co-stimulatory region can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% amino acid sequence identity to FWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO:56).

[0123] In some aspects, the co-stimulatory region is derived from an intracellular portion of the

transmembrane protein ICOS (also known as AILIM, CD278, and CVID1). For example, a suitable co-stimulatory region can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% amino acid sequence identity to TKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL (SEQ ID NO:57).

[0124] In some aspects, the co-stimulatory region is derived from an intracellular portion of the transmembrane protein OX-40 (also known as TNFRSF4, RP5-902P8.3, ACT35, CD134, OX40, TXGP1L). For example, a suitable co- stimulatory region can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% amino acid sequence identity to RRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI (SEQ ID NO:58).

[0125] In some aspects, the co-stimulatory region is derived from an intracellular portion of the transmembrane protein BTLA (also known as BTLA1 and CD272). For example, a suitable co-stimulatory region can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% amino acid sequence identity to
CCLRRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYDNDPDL
CFRMQEGSEVYSNPCLEENKPGIVYASLNHSVIGPNSRLARNVKEAPTEYASICVRS
(SEQ ID NO:59).

[0126] In some aspects, the co-stimulatory region is derived from an intracellular portion of the transmembrane protein CD27 (also known as S 152, T14, TNFRSF7, and Tp55). For example, a suitable co-stimulatory region can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% amino acid sequence identity to HQRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP (SEQ ID NO:60).

[0127] In some aspects, the co-stimulatory region is derived from an intracellular portion of the transmembrane protein CD30 (also known as TNFRSF8, D1S166E, and Ki-1). For example, a suitable co-stimulatory region can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% amino acid sequence identity to
RRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGLMS
QPLMETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKAD
TVIVGTVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEE
EGKEDPLPTAASGK (SEQ ID NO:61).

[0128] In some aspects, the co-stimulatory region is derived from an intracellular portion of the transmembrane protein GITR (also known as TNFRSF18, RP5-902P8.2, AITR, CD357, and GITR-D). For example, a suitable co- stimulatory region can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% amino acid sequence identity to

HIWQLRSQCMWPRETQLLLEVPPSTEDARSCQFPEEERGERSAEEKGRLGDLWV (SEQ ID NO:62).

[0129] In some aspects, the co-stimulatory region derived from an intracellular portion of the transmembrane protein HVEM (also known as TNFRSF14, RP3-395M20.6, ATAR, CD270, HVEA, HVEM, LIGHTR, and TR2). For example, a suitable co-stimulatory region can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% amino acid sequence identity to CVKRRKPRGDVVKVIVSVQRKRQEAEGEATVIEALQAPPDVTTVAVEETIPSFTGRSP NH (SEQ ID NO:63).

I. Additional Modifications

[0130] Additionally, the polypeptides of the disclosure may be chemically modified. Glycosylation of the polypeptides can be altered, for example, by modifying one or more sites of glycosylation within the polypeptide sequence to increase the affinity of the polypeptide for antigen (U.S. Pat. Nos. 5,714,350 and 6,350,861).

[0131] The polypeptides of the disclosure can be pegylated to increase biological half-life by reacting the polypeptide with polyethylene glycol (PEG) or a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the polypeptide. Polypeptide pegylation may be carried out by an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive watersoluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. Methods for pegylating proteins are known in the art and can be applied to the polypeptides of the disclosure (EP 0 154 316 and EP 0 401 384).

[0132] Additionally, polypeptides may be chemically modified by conjugating or fusing the polypeptide to serum protein, such as human serum albumin, to increase half-life of the resulting molecule. Such approach is for example described in EP 0322094 and EP 0 486 525.

[0133] The polypeptides of the disclosure may be conjugated to a diagnostic or therapeutic agent and used diagnostically, for example, to monitor the development or progression of a disease and determine the efficacy of a given treatment regimen. The polypeptides may also be conjugated to a therapeutic agent to provide a therapy in combination with the immunostimulating effect of the polypeptide. Examples of diagnostic agents include enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the polypeptide, or indirectly, through a linker using techniques

known in the art. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase. Examples of suitable prosthetic group complexes include streptavidin/ biotin and avidin/biotin. Examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin. An example of a luminescent material includes luminol. Examples of bioluminescent materials include luciferase, luciferin, and aequorin. Examples of suitable radioactive material include .sup.125I, .sup.131I, Indium-111, Lutetium-171, Bismuth-212, Bismuth-213, Astatine-211, Copper-62, Copper-64, Copper-67, Yttrium-90, Iodine-125, Iodine-131, Phosphorus-32, Phosphorus-33, Scandium-47, Silver-111, Gallium-67, Praseodymium-142, Samarium-153, Terbium-161, Dysprosium-166, Holmium-166, Rhenium-186, Ithenium-188, Rhenium-189, Lead-212, Radium-223, Actinium-225, Iron-59, Selenium-75, Arsenic-77, Strontium-89, Molybdenum-99, Rhodium-1105, Palladium-109, Praseodymium-143, Promethium-149, Erbium-169, Iridium-194, Gold-198, Gold-199, and Lead-211. Chelating agents may be attached through amities (Meares et al., 1984 Anal. Biochem. 142: 68-78); sulfhydral groups (Koyama 1994 Chem. Abstr. 120: 217262t) of amino acid residues and carbohydrate groups (Rodwell et al. 1986 PNAS USA 83: 2632-2636; Quadri et al. 1993 Nucl. Med. Biol. 20: 559-570).

[0134] The polypeptides may also be conjugated to a therapeutic agent to provide a therapy in combination with the immunostimulating effect of the polypeptide.

[0135] Additional suitable conjugated molecules include ribonuclease (RNase), DNase I, an antisense nucleic acid, an inhibitory RNA molecule such as a siRNA molecule, an immunostimulatory nucleic acid, aptamers, ribozymes, triplex forming molecules, and external guide sequences. Aptamers are small nucleic acids ranging from 15-50 bases in length that fold into defined secondary and tertiary structures, such as stern-loops or G-quartets, and can bind small molecules, such as ATP (U.S. Pat. No. 5,631,146) and theophiline (U.S. Pat. No. 5,580,737), as well as large molecules, such as reverse transcriptase (U.S. Pat. No. 5,786,462) and thrombin (U.S. Pat. No. 5,543,293). Ribozymes are nucleic acid molecules that are capable of catalyzing a chemical reaction, either intramolecularly or intermolecularly. Ribozymes typically cleave nucleic acid substrates through recognition and binding of the target substrate with subsequent cleavage. Triplex forming function nucleic acid molecules can interact with double-stranded or single-stranded nucleic acid by forming a triplex, in which three strands of DNA form a complex dependant on both Watson-Crick and Hoogsteen base-pairing. Triplex molecules can bind target regions with high affinity and specificity.

[0136] The functional nucleic acid molecules may act as effectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional nucleic acid molecules may possess a de novo activity independent of any other molecules.

J. Cancer-specific Chimeric Antigen Receptors

[0137] In some aspects, the cells may further comprise a cancer-specific chimeric antigen

receptor (CAR). The term "cancer-specific" in the context of CARs refers to CARs that have an antigen binding specificity for a cancer-specific molecule, such as a cancer-specific antigen. In some aspects, the cancer specific CAR is in a cell with a TGF-β CAR. In some aspects, the cancer-specific CAR and TGF-β care are on separate polypeptides. In some aspects, the CAR is a bi-specific CAR that has antigen binding for a cancer-specific molecule and for TGF-β. For exampole, a bi-specific CAR may have a signaling peptide, a cancer molecule-specific scFv, optionally a peptide linker/spacer, followed by a TGF-β scFv, followed by a spaer, a transmembrane domain, and a costimulatory domain. In some aspects, the bi-specific CAR comprises one or more additional peptide segments described herein.

[0138] In some aspects, polypeptides of the disclosure may comprise a CD20 scFv. An exemplary CD20 scFv comprises the following:
DIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWIYATSNLASGV
PARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPTFGGGTKLEIKGSTSGGGSG
GGSGGGGSSEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMHVWKQTPGQGLE
WIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYY
GSSYWFFDVWGAGTTVTVSS (SEQ ID NO:100).

[0139] In some aspects, polypeptides of the disclosure may comprise a CD19 scFv. An exemplary CD19 scFv comprises the following:
DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV
PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPG
SGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG
VIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYA
MD YWGQGTSVTVSS (SEQ ID NO:101)

[0140] Other cancer-specific molecules (in addition to CD19 and CD20) can include CAIX, CD33, CD44v7/8, CEA, EGP-2, EGP-40, erb-B2, erb-B3, erb-B4, FBP, fetal acetycholine receptor, GD2, GD3, Her2/neu, IL-13R-a2, KDR, k-light chain, LeY, L1 cell adhesion molecule, MAGE-A1, mesothelin, MUC1, NKG2D ligands, oncofetal antigen (h5T4), PSCA, PSMA, TAA targeted by mAb IgE, TAG-72, and VEGF-R2. In some aspects, the cancer-specific molecule comprises Her2.

III. Cells

[0141] Certain aspects relate to cells comprising polypeptides or nucleic acids of the disclosure. In some aspects the cell is an immune cell or a T cell. "T cell" includes all types of immune cells expressing CD3 including T-helper cells ($CD4^+$ cells), cytotoxic T-cells ($CD8^+$ cells), T-regulatory cells (Treg) and gamma-delta T cells. A "cytotoxic cell" includes $CD8^+$ T cells, natural-killer (NK) cells, and neutrophils, which cells are capable of mediating cytotoxicity

responses.

[0142] Suitable mammalian cells include primary cells and immortalized cell lines. Suitable mammalian cell lines include human cell lines, non-human primate cell lines, rodent (e.g., mouse, rat) cell lines, and the like. Suitable mammalian cell lines include, but are not limited to, HeLa cells (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos. CRL9618, CCL61, CRL9096), human embryonic kidney (HEK) 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Huh-7 cells, BHK cells (e.g., ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RATI cells, mouse L cells (ATCC No. CCLI.3), HLHepG2 cells, Hut-78, Jurkat, HL-60, NK cell lines (e.g., NKL, NK92, and YTS), and the like.

[0143] In some instances, the cell is not an immortalized cell line, but is instead a cell (e.g., a primary cell) obtained from an individual. For example, in some cases, the cell is an immune cell obtained from an individual. As an example, the cell is a T lymphocyte obtained from an individual. As another example, the cell is a cytotoxic cell obtained from an individual. As another example, the cell is a stem cell or progenitor cell obtained from an individual.

IV. METHODS

[0144] Aspects of the current disclosure relate to methods for stimulating an immune response. The immune response stimulation may be done *in vitro, in vivo,* or *ex vivo.* In some aspects, the methods relate to cells capable of stimulating an immune response in the presence of TGF-β. The method generally involves genetically modifying a mammalian cell with an expression vector, or an RNA (e.g., in vitro transcribed RNA), comprising nucleotide sequences encoding a polypeptide of the disclosure or directly tranferring the polypeptide to the cell. The cell can be an immune cell (e.g., a T lymphocyte or NK cell), a stem cell, a progenitor cell, etc. In some aspects, the cell is a cell described herein.

[0145] In some aspects, the genetic modification is carried out *ex vivo.* For example, a T lymphocyte, a stem cell, or an NK cell (or cell described herein) is obtained from an individual; and the cell obtained from the individual is genetically modified to express a polypeptide of the disclosure. In some cases, the genetically modified cell is activated ex *vivo* (i.e., TGF-β is contacted with the cells *ex vivo*). In other cases, the genetically modified cell is introduced into an individual (e.g., the individual from whom the cell was obtained); and the genetically modified cell is activated *in vivo* (i.e., by endogenously produced TGF-β).

[0146] In some aspects, the methods further comprise the administration of additional therapeutic agents, such as bi-specific T cell engagers (BITE). Such therapeutic agents may be administered in peptide form to the patient or expressed in cells of the disclosure, such as those that that comprise the TGF-β CAR. The BITE may have antigen specificity for a cancer antigen/cancer molecule known in the art and/or described herein and may also have antigen specificity for a T cell molecule such as CD3.

[0147] In some aspects, the methods relate to administration of the cells or peptides described herein for the treatment of a cancer or administation to a person with a cancer. In some aspects the cancer is adrenal cancer, anal cancer, bile duct cancer, bladder cancer, bone cancer, brain/CNS tumors in children or adults, breast cancer, cervical cancer, colon/rectum cancer, endometrial cancer, esophagus cancer, ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumor (GIST), gestation trophoblastic disease, hodgkin disease, kaposi sarcoma, kidney cancer, laryngeal and hypopharyngeal cancer, leukemia, acute lymphocytic leuckemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myelomonocytic leukemia, liver cancer, lung cancer, non-small cell lung cancer, small cell lung cancer, lung carcinoid tumor, lymphoma, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, nasal cavity and paranasal sinum cancer, nasopharyngeal cancer, neuroblastoma, non-hodgkin lymphoma, oral cavity or oropharyngeal cancer, osteosarcoa, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, skin cancer, sarcoma, basal skin cancer, squamous cell skin cancer, melanoma, merkel cell skin cancer, small intestine cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, waldenstrom macroglobulinemia, or wilms tumor.

[0148] Aspects can be used to treat or ameliorate a number of immune-mediated, inflammatory, or autoimmune-inflammatory diseases, e.g., allergies, asthma, diabetes (e.g. type 1 diabetes), graft rejection, etc. Examples of such diseases or disorders also include, but are not limited to arthritis (rheumatoid arthritis such as acute arthritis, chronic rheumatoid arthritis, gout or gouty arthritis, acute gouty arthritis, acute immunological arthritis, chronic inflammatory arthritis, degenerative arthritis, type II collagen-induced arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, Still's disease, vertebral arthritis, and systemic juvenile-onset rheumatoid arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, and ankylosing spondylitis), inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, gutatte psoriasis, pustular psoriasis, and psoriasis of the nails, atopy including atopic diseases such as hay fever and Job's syndrome, dermatitis including contact dermatitis, chronic contact dermatitis, exfoliative dermatitis, allergic dermatitis, allergic contact dermatitis, dermatitis herpetiformis, nummular dermatitis, seborrheic dermatitis, nonspecific dermatitis, primary irritant contact dermatitis, and atopic dermatitis, x-linked hyper IgM syndrome, allergic intraocular inflammatory diseases, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria, myositis, polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, scleroderma (including systemic scleroderma), sclerosis such as systemic sclerosis, multiple sclerosis (MS) such as spino-optical MS, primary progressive MS (PPMS), and relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, ataxic sclerosis, neuromyelitis optica (NMO), inflammatory bowel disease (IBD) (for example, Crohn's disease, autoimmune-mediated gastrointestinal diseases, colitis such as ulcerative colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis

polyposa, necrotizing enterocolitis, and transmural colitis, and autoimmune inflammatory bowel disease), bowel inflammation, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, respiratory distress syndrome, including adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological disorder, rheumatoid spondylitis, rheumatoid synovitis, hereditary angioedema, cranial nerve damage as in meningitis, herpes gestationis, pemphigoid gestationis, pruritis scroti, autoimmune premature ovarian failure, sudden hearing loss due to an autoimmune condition, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis such as Rasmussen's encephalitis and limbic and/or brainstem encephalitis, uveitis, such as anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior uveitis, or autoimmune uveitis, glomerulonephritis (GN) with and without nephrotic syndrome such as chronic or acute glomerulonephritis such as primary GN, immune-mediated GN, membranous GN (membranous nephropathy), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous proliferative GN (MPGN), including Type I and Type II, and rapidly progressive GN, proliferative nephritis, autoimmune polyglandular endocrine failure, balanitis including balanitis circumscripta plasmacellularis, balanoposthitis, erythema annulare centrifugum, erythema dyschromicum perstans, eythema multiform, granuloma annulare, lichen nitidus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, lichen planus, lamellar ichthyosis, epidermolytic hyperkeratosis, premalignant keratosis, pyoderma gangrenosum, allergic conditions and responses, allergic reaction, eczema including allergic or atopic eczema, asteatotic eczema, dyshidrotic eczema, and vesicular palmoplantar eczema, asthma such as asthma bronchiale, bronchial asthma, and auto-immune asthma, conditions involving infiltration of T cells and chronic inflammatory responses, immune reactions against foreign antigens such as fetal A-B-O blood groups during pregnancy, chronic pulmonary inflammatory disease, autoimmune myocarditis, leukocyte adhesion deficiency, lupus, including lupus nephritis, lupus cerebritis, pediatric lupus, non-renal lupus, extra-renal lupus, discoid lupus and discoid lupus erythematosus, alopecia lupus, systemic lupus erythematosus (SLE) such as cutaneous SLE or subacute cutaneous SLE, neonatal lupus syndrome (NLE), and lupus erythematosus disseminatus, juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), and adult onset diabetes mellitus (Type II diabetes) and autoimmune diabetes. Also contemplated are immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, sarcoidosis, granulomatosis including lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitides, including vasculitis, large-vessel vasculitis (including polymyalgia rheumatica and gianT cell (Takayasu's) arteritis), medium-vessel vasculitis (including Kawasaki's disease and polyarteritis nodosa/periarteritis nodosa), microscopic polyarteritis, immunovasculitis, CNS vasculitis, cutaneous vasculitis, hypersensitivity vasculitis, necrotizing vasculitis such as systemic necrotizing vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS) and ANCA-associated small-vessel vasculitis, temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), Addison's disease, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis,

CNS inflammatory disorders, Alzheimer's disease, Parkinson's disease, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex-mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Behcet's disease/syndrome, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous and skin pemphigoid, pemphigus (including pemphigus vulgaris, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, and pemphigus erythematosus), autoimmune polyendocrinopathies, Reiter's disease or syndrome, thermal injury, preeclampsia, an immune complex disorder such as immune complex nephritis, antibody-mediated nephritis, polyneuropathies, chronic neuropathy such as IgM polyneuropathies or IgM-mediated neuropathy, autoimmune or immune-mediated thrombocytopenia such as idiopathic thrombocytopenic purpura (ITP) including chronic or acute ITP, scleritis such as idiopathic cerato-scleritis, episcleritis, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune endocrine diseases including thyroiditis such as autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis (Hashimoto's thyroiditis), or subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis such as allergic encephalomyelitis or encephalomyelitis allergica and experimental allergic encephalomyelitis (EAE), experimental autoimmune encephalomyelitis, myasthenia gravis such as thymoma-associated myasthenia gravis, cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, gianT cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis (LIP), bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barre syndrome, Berger's disease (IgA nephropathy), idiopathic IgA nephropathy, linear IgA dermatosis, acute febrile neutrophilic dermatosis, subcorneal pustular dermatosis, transient acantholytic dermatosis, cirrhosis such as primary biliary cirrhosis and pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac or Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AIED), autoimmune hearing loss, polychondritis such as refractory or relapsed or relapsing polychondritis, pulmonary alveolar proteinosis, Cogan's syndrome/nonsyphilitic interstitial keratitis, Bell's palsy, Sweet's disease/syndrome, rosacea autoimmune, zoster-associated pain, amyloidosis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis (e.g., benign monoclonal gammopathy and monoclonal gammopathy of undetermined significance, MGUS), peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal or segmental or focal segmental glomerulosclerosis (FSGS), endocrine opthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple

endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases and chronic inflammatory demyelinating polyneuropathy, Dressler's syndrome, alopecia greata, alopecia totalis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyl), and telangiectasia), male and female autoimmune infertility, e.g., due to anti-spermatozoan antibodies, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, parasitic diseases such as leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis (acute or chronic), or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, SCID, acquired immune deficiency syndrome (AIDS), echovirus infection, sepsis, endotoxemia, pancreatitis, thyroxicosis, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, chorioiditis, gianT cell polymyalgia, chronic hypersensitivity pneumonitis, keratoconjunctivitis sicca, epidemic keratoconjunctivitis, idiopathic nephritic syndrome, minimal change nephropathy, benign familial and ischemia-reperfusion injury, transplant organ reperfusion, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway/pulmonary disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders, asperniogenese, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmia phacoanaphylactica, enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensoneural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, leucopenia, mononucleosis infectiosa, traverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia symphatica, orchitis granulomatosa, pancreatitis, polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyreoiditis, acquired spenic atrophy, non-malignant thymoma, vitiligo, toxic-shock syndrome, food poisoning, conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease, allergic neuritis, autoimmune polyendocrinopathies, oophoritis, primary myxedema, autoimmune atrophic gastritis, sympathetic ophthalmia, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulitis, polyendocrine failure, autoimmune polyglandular syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), cardiomyopathy such as dilated cardiomyopathy, epidermolisis bullosa acquisita (EBA), hemochromatosis, myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent sinusitis, acute or chronic sinusitis,

ethmoid, frontal, maxillary, or sphenoid sinusitis, an eosinophil-related disorder such as eosinophilia, pulmonary infiltration eosinophilia, eosinophilia-myalgia syndrome, Loffler's syndrome, chronic eosinophilic pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma, or granulomas containing eosinophils, anaphylaxis, seronegative spondyloarthritides, polyendocrine autoimmune disease, sclerosing cholangitis, sclera, episclera, chronic mucocutaneous candidiasis, Bruton's syndrome, transient hypogammaglobulinemia of infancy, Wiskott-Aldrich syndrome, ataxia telangiectasia syndrome, angiectasis, autoimmune disorders associated with collagen disease, rheumatism, neurological disease, lymphadenitis, reduction in blood pressure response, vascular dysfunction, tissue injury, cardiovascular ischemia, hyperalgesia, renal ischemia, cerebral ischemia, and disease accompanying vascularization, allergic hypersensitivity disorders, glomerulonephritides, reperfusion injury, ischemic re-perfusion disorder, reperfusion injury of myocardial or other tissues, lymphomatous tracheobronchitis, inflammatory dermatoses, dermatoses with acute inflammatory components, multiple organ failure, bullous diseases, renal cortical necrosis, acute purulent meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, narcolepsy, acute serious inflammation, chronic intractable inflammation, pyelitis, endarterial hyperplasia, peptic ulcer, valvulitis, graft versus host disease, contact hypersensitivity, asthmatic airway hyperreaction, and endometriosis.

V. Pharmaceutical Compositions

[0149] The present disclosure includes methods for modulating immune responses in a subject in need thereof. The disclosure includes cells that may be in the form of a pharmaceutical composition that can be used to induce or modify an immune response.

[0150] Administration of the compositions according to the current disclosure will typically be via any common route. This includes, but is not limited to parenteral, orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal, intranasal, or intravenous injection.

[0151] Typically, compositions of the disclosure are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immune modifying. The quantity to be administered depends on the subject to be treated. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner.

[0152] The manner of application may be varied widely. Any of the conventional methods for administration of pharmaceutical compositions comprising cellular components are applicable. The dosage of the pharmaceutical composition will depend on the route of administration and will vary according to the size and health of the subject.

[0153] In many instances, it will be desirable to have multiple administrations of at most about or at least about 3, 4, 5, 6, 7, 8, 9, 10 or more. The administrations may range from 2-day to

12-week intervals, more usually from one to two week intervals. The course of the administrations may be followed by assays for alloreactive immune responses and T cell activity.

[0154] The phrases "pharmaceutically acceptable" or "pharmacologically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal, or human. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients, its use in immunogenic and therapeutic compositions is contemplated. The pharmaceutical compositions of the current disclosure are pharamaceutically acceptable compositions.

[0155] The compositions of the disclosure can be formulated for parenteral administration, e.g., formulated for injection via the intravenous, intramuscular, subcutaneous, or even intraperitoneal routes. Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and, the preparations can also be emulsified.

[0156] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil, or aqueous propylene glycol. It also should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0157] Sterile injectable solutions are prepared by incorporating the active ingredients (i.e. cells of the disclosure) in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above.

[0158] An effective amount of a composition is determined based on the intended goal. The term "unit dose" or "dosage" refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the composition calculated to produce the desired responses discussed herein in association with its administration, i.e., the appropriate route and regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the result and/or protection desired. Precise amounts of the composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the subject, route of administration, intended goal of treatment (alleviation of symptoms versus cure), and potency, stability, and toxicity of the particular composition. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is

therapeutically or prophylactically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above.

VI. Sequences

[0159] Antigen binding domains of the disclosure include the following VH (variable heavy) and VL (variable light) regions:

scFv#1 VH:
EVQLVESGGGLVQPGGSLRLSCAASGYAFTNYLIEWVRQAPGKGLEWVGVINPGSG
GSNYNEKFKGRATISADNSKNTLYLQMNSLRAEDTAVYYCARSGGFYFDYWGQGT
LVTVSSASTKGPS (SEQ ID NO:1)

scFv#1 VL:
DIQMTQSPSSLSASVGDRVTITCRASQSVLYSSNQKNYLAWYQQKPGKAPKLLIYWA
STRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYLSSDTFGQGTKVEIKRTVA
(SEQ ID NO:2)

scFv#2 VH:
QVQLVQSGAEVKKPGSSVKVSCKASGYTFSSNVISWVRQAPGQGLEWMGGVIPIVDI
ANYAQRFKGRVTITADESTSTTYMELSSLRSEDTAVYYCALPRAFVLDAMDYWGQG
TLVTVSS (SEQ ID NO:3)

scFv#2 VL:
ETVLTQSPGTLSLSPGERATLSCRASQSLGSSYLAWYQQKPGQAPRLLIYGASSRAPG
IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYADSPITFGQGTRLEIK (SEQ ID
NO:4)

scFv#3 VH:
EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVISYDGS
IKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTGEYSGYDTDPQYS
WGQGTTVTVSS (SEQ ID NO:19)

scFv#3 VL:
EIVLTQSPSSLSASVGDRVTITCRSSQGIGDDLGWYQQKPGKAPILLIYGTSTLQSGVP
SRFSGSGSGTDFTLTINSLQPEDFATYYCLQDSNYPLTFGGGTRLEIK (SEQ ID NO:20)

[0160] The corresponding CDRs of the VH and VL regions of scFv#1 are the following amino acid sequences: scFv#1 HCDR1: GYAFTNYLIE (SEQ ID NO:5); scFv#1 HCDR2: VINPGSGGSNYNEKFKG (SEQ ID NO:6); scFv#1 HCDR3: SGGFYFDY (SEQ ID NO:7); scFv#1 LCDR1: RASQSVLYSSNQKNYLA (SEQ ID NO:8); scFv#1 LCDR2: WASTRES (SEQ ID NO:9); scFv#1 LCDR3: HQYLSSDT (SEQ ID NO:10).

[0161] The corresponding CDRs of the VH and VL regions of scFv#2 are the following amino acid sequences: scFv#2 HCDR1: SNVIS (SEQ ID NO:11); scFv#2 HCDR2: GVIPIVDIANYAQRFKG (SEQ ID NO:12); scFv#2 HCDR3: PRAFVLDAMDY (SEQ ID NO:13); scFv#2 LCDR1: RASQSLGSSYLA (SEQ ID NO:14); scFv#2 LCDR2: GASSRAP (SEQ ID NO:15); and scFv#2 LCDR3: QQYADSPIT (SEQ ID NO:16)

[0162] The corresponding CDRs of the VH and VL regions of scFv#3 are the following amino acid sequences: scFv#3 HCDR1: SYGMH (SEQ ID NO:21); scFv#3 HCDR2: VISYDGSIKYYADSVKG (SEQ ID NO:22); scFv#3 HCDR3: TGEYSGYDTDPQYS (SEQ ID NO:23); scFv#3 LCDR1: RSSQGIGDDLG (SEQ ID NO:24); scFv#3 LCDR2: GTSTLQS (SEQ ID NO:25); and scFv#3 LCDR3: LQDSNYPLT (SEQ ID NO:26).

[0163] Detection peptides of the disclosure can include, for example, HA: YPYDVPDYA (SEQ ID NO:94); FLAG: DYKDDDDK (SEQ ID NO:17); and c-myc: EQKLISEEDL; SEQ ID NO:95).

[0164] An exemplary signal peptide includes: METDTLLLWVLLLWVPGSTG (SEQ ID NO:18). Other exemplary signal peptides include: MLLVTSLLLCELPHPAFLLIPDT (SEQ ID NO:94) or MGTSLLCWMALCLLGADHADG (SEQ ID NO:95).

[0165] Exemplary peptide spacer hinge regions include: DKTHT (SEQ ID NO:27), CPPC (SEQ ID NO:28), CPEPKSCDTPPPCPR (SEQ ID NO:29), ELKTPLGDTTHT (SEQ ID NO:30), KSCDKTHTCP (SEQ ID NO:31), KCCVDCP (SEQ ID NO:32), KYGPPCP (SEQ ID NO:33), EPKSCDKTHTCPPCP (SEQ ID NO:34; human IgGI hinge), ERKCCVECPPCP (SEQ ID NO:35; human IgG2 hinge), ELKTPLGDTTHTCPRCP (SEQ ID NO:36; human IgG3 hinge), SPNMVPHAHHAQ (SEQ ID NO:37); ESKYGPPCPPCP (SEQ ID NO:98), ESKYGPPCPSCP (SEQ ID NO:99) (human IgG4 hinge-based), EPKSCDKTYTCPPCP (SEQ ID NO:38), and TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:39)

[0166] Exemplary peptide linkers include, for example, (GSGGS)n (SEQ ID NO:40); $(GGGS)_n$ (SEQ ID NO:41); GGSG (SEQ ID NO:42); GGSGG (SEQ ID NO:43); GSGSG (SEQ ID NO:44); GSGGG (SEQ ID NO:45); GGGSG (SEQ ID NO:46); and GSSSG (SEQ ID NO:47).

[0167] Exemplary transmembrane domains include IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO:48), CD8 beta derived: LGLLVAGVLVLLVSLGVAIHLCC (SEQ ID NO:49), CD4 derived: ALIVLGGVAGLLLFIGLGIFFCVRC (SEQ ID NO:50), CD3 zeta derived: LCYLLDGILFIYGVILTALFLRV (SEQ ID NO:51), CD28 derived: WVLVWGGVLACYSLLVTVAFIIFWV (SEQ ID NO:52), CD134 (OX40) derived: VAAILGLGLVLGLLGPLAILLALYLL (SEQ ID NO:53), and CD7 derived: ALPAALAVISFLLGLGLGVACVLA (SEQ ID NO:54).

[0168] Exemplary co-stimulatory regions include: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO:55), FWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO:56), TKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL (SEQ ID NO:57),

RRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI (SEQ ID NO:58), CCLRRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYDNDPDL CFRMQEGSEVYSNPCLEENKPGIVYASLNHSVIGPNSRLARNVKEAPTEYASICVRS (SEQ ID NO:59), HQRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP (SEQ ID NO:60), RRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGLMS QPLMETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKAD TVIVGTVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEE EGKEDPLPTAASGK (SEQ ID NO:61), HIWQLRSQCMWPRETQLLLEVPPSTEDARSCQFPEEERGERSAEEKGRLGDLWV (SEQ ID NO:62), and CVKRRKPRGDVVKVIVSVQRKRQEAEGEATVIEALQAPPDVTTVAVEETIPSFTGRSP NH (SEQ ID NO:63).

[0169] In some aspects, the endodomain comprises an ITAM motif. An ITAM motif is $YX_1X_2(L/I)$, where X_1 and X_2 are independently any amino acid (SEQ ID NO:64). In some cases, an ITAM motif is repeated twice in an endodomain, where the first and second instances of the ITAM motif are separated from one another by 6 to 8 amino acids, e.g., $(YX_1X_2(L/I))(X_3)_n(YX_1X_2(L/I))$, where n is an integer from 6 to 8, and each of the 6-8 X_3 can be any amino acid (SEQ ID NO:65).

[0170] Exemplary endodomains include polypeptides from: MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSPGVLAGIVMGDLVLTVLI ALAVYFLGRLVPRGRGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK (SEQ ID NO:66), MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSPGVLAGIVMGDLVLTVLI ALAVYFLGRLVPRGRGAAEATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK (SEQ ID NO:67); MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFLGRLVP RGRGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK (SEQ ID NO:68); MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFLGRLVP RGRGAAEATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK (SEQ ID NO:69); ESPYQELQGQRSDVYSDLNTO (SEQ ID NO: 70); MIPAVVLLLLLLVEQAAALGEPQLCYILDAILFLYGIVLTLLYCRLKIQVRKAAITSYE KSDGVYTGLSTRNQETYETLKHEKPPQ (SEQ ID NO: 71); DGVYTGLSTRNQETYETLKHE (SEQ ID NO: 72); MEHSTFLSGLVLATLLSQVSPFKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLD LGKRILDPRGIYRCNGTDIYKDKESTVQVHYRMCQSCVELDPATVAGIIVTDVIATLL LALGVFCFAGHETGRLSGAADTQALLRNDQVYQPLRDRDDAQYSHLGGNWAR NK (SEQ ID NO:73); MEHSTFLSGLVLATLLSQVSPFKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLD LGKRILDPRGIYRCNGTDIYKDKESTVQVHYRTADTQALLRNDQVYQPLRDRDDAQ YSHLGGNWARNK (SEQ ID NO:74); DQVYQPLRDRDDAQYSHLGGN (SEQ ID NO:75); MQSGTHWRVLGLCLLSVGVWGQDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEI LWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLY LRARVCENCMEMDVMSVATIVIVDICITGGLLLLVYYWSKNRKAKAKPVTRGAGAG GRQRGQNKERPPPVPNPDYEPIRKGQRDLYSGLNQRRI (SEQ ID NO:76); NPDYEPIRKGQRDLYSGLNQR (SEQ ID NO:77);

MEQGKGLAVLILAIILLQGTLAQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNITWFK DGKMIGFLTEDKKKWNLGSNAKDPRGMYQCKGSQNKSKPLQVYYRMCQNCIELNA ATISGFLFAEIVSIFVLAVGVYFIAGQDGVRQSRASDKQTLLPNDQLYQPLKDREDDQ YSHLQGNQLRRN (SEQ ID NO:78); DQLYQPLKDREDDQYSHLQGN (SEQ ID NO:79); MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 80); MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR(SEQ ID NO: 81); RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP PR (SEQ ID NO: 82); NQLYNELNLGRREEYDVLDKR (SEQ ID NO:83); EGLYNELQKDKMAEAYSEIGMK (SEQ ID NO:84); DGLYQGLSTATKDTYDALHMQ (SEQ ID NO:85); MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPH NSSNNANVTWWRVLHGNYTWPPEFLGPGEDPNGTLIIQNVNKSHGGIYVCRVQEGN ESYQQSCGTYLRVRQPPPRPFLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFRKRWQ NEKLGLDAGDEYEDENLYEGLNLDDCSMYEDISRGLQGTYQDVGSLNIGDVQLEKP (SEQ ID NO:86); MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPH NSSNNANVTWWRVLHGNYTWPPEFLGPGEDPNEPPPRPFLDMGEGTKNRIITAEGIIL LFCAVVPGTLLLFRKRWQNEKLGLDAGDEYEDENLYEGLNLDDCSMYE DISRGLQGTYQDVGSLNIGDVQLEKP (SEQ ID NO:87); ENLYEGLNLDDCSMYEDISRG (SEQ ID NO:88); RPRRSPAQDGKVYINMPGRG (SEQ ID NO:89); RPRRSPAQDGKVYINMPGRG (SEQ ID NO:90); FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQP YAPPRDFAAYRS (SEQ ID NO:91); FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQP YAPPRDFAAYRS (SEQ ID NO: 92); and MPDPAAHLPFFYGSIS RAEAEEHLKLAGMADGLFLLRQCLRSLGGYVLSLVHDVRFHHFPIERQLNGTYAIAG GKAHCGPAELCEFYSRDPDGLPCNLRKPCNRPSGLEPQPGVFDCLRDAMVRDYVRQ TWKLEGEALEQAIISQAPQVEKLIATTAHERMPWYHSSLTREEAERKLYSGAQTDGK FLLRPRKEQGTYALSLIYGKTVYHYLISQDKAGKYCIPEGTKFDTLWQLVEYLKLKA DGLIYCLKEACPNSSASNASGAAAPTLPAHPSTLTHPQRRIDTLNSDGYTPEPARITSP DKPRPMPMDTSVYESPYSDPEELKDKKLFLKRDNLLIADIELGCGNFGSVRQGVYRM RKKQIDVAIKVLKQGTEKADTEEMMREAQIMHQLDNPYIVRLIGVCQAEALMLVME MAGGGPLHKFLVGKREEIPVSNVAELLHQVSMGMKYLEEKNFVHRDLAARNVLLV NRHYAKISDFGLSKALGADDSYYTARSAGKWPLKWYAPECINFRKFSSRSDVWSYG VTMWEALSYGQKPYKKMKGPEVMAFIEQGKRMECPPECPPELYALMSDCWIYKWE DRPDFLTVEQRMRACYYSLASKVEGPPGSTQKAEAACA (SEQ ID NO:93) or portions thereof.

VII. Examples

Example 1

[0171] This disclosure describes single-chain variable fragments (scFvs) that neutralize human and mouse TGF-β, as well as chimeric antigen receptors (CARs) that are responsive to human and mouse TGF-β. The level of TGF-β conservation across mammals suggests that the scFvs and CARs described can be used to bind most mammalian TGF-β. Two scFvs are constructed from anti-TGF-β antibodies by connecting the heavy-chain variable domain (VH) to the light-chain variable domain (VL) with a (G4S)3 linker such that the final orientation is N-terminus-VH- $(G_4S)_3$-VL-C-terminus. These scFvs can be produced by transfecting eukaryotic cells with DNA sequences encoding the scFv amino acid sequences indicated in the table below:

Single Chain Variable Fragment	Amino Acid Sequence (Single Letter Abbreviations)
scFv #1	METDTLLLWVLLLWVPGSTGAGGSDYKDDDDKGGSEVQLVESGGGL VQPGGSLRLSCAASGYAFTNYLIEWVRQAPGKGLEWVGVINPGSGGSN YNEKFKGRATISADNSKNTLYLQMNSLRAEDTAVYYCARSGGFYFDY WGQGTLVTVSSASTKGPSGGGGSGGGGSGGGGSDIQMTQSPSSLSASV GDRVTITCRASQSVLYSSNQKNYLAWYQQKPGKAPKLLIYWASTRESG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYLSSDTFGQGTKVEIKR TVA (SEQ ID NO:96)
scFv #2	METDTLLLWVLLLWVPGSTGAGGSDYKDDDDKGGSQVQLVQSGAEV KKPGSSVKVSCKASGYTFSSNVISWVRQAPGQGLEWMGGVIPIVDIAN YAQRFKGRVTITADESTSTTYMELSSLRSEDTAVYYCALPRAFVLDAM DYWGQGTLVTVSSGGGGSGGGGSGGGGSETVLTQSPGTLSLSPGERAT LSCRASQSLGSSYLAWYQQKPGQAPRLLIYGASSRAPGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQQYADSPITFGQGTRLEIK (SEQ ID NO:97)

[0172] Each scFv can be tagged with the leader (i.e. signal) peptide from the murine kappa light chain so that scFvs are secreted and can be directly collected from the media in which the producer cells are cultured. The scFvs can also be tagged at the N-terminal end (after the leader peptide but before the VH sequence) with markers such as the DYKDDDDK (SEQ ID NO:17) epitope, HA tag, or cMyc tag flanked by GGS linkers. Each scFv can be directly administered to neutralize human or mouse TGF-β. scFv#1 and #2 are superior to scFv#3 (FIG. 1 and 7). Furthermore, each scFv can be used to construct a CAR responsive to TGF-β. CARs are fusion proteins composed of an extracellular antigen-binding domain, an extracellular spacer, a transmembrane domain, costimulatory signaling regions (the number of which varies depending on the specific CAR design), and a CD3-zeta signaling domain/endodomain. TGF-β CARs can be constructed using the scFvs described above as the extracellular antigen-binding domain. Immune cells, including T cells and natural killer (NK) cells, can be engineered to express TGF-β CARs by a variety of methods, including viral

transduction, DNA nucleofection, and RNA nucleofection. TGF-β binding to the TGF-β CAR can activate human T cells, thereby redirecting TGF-β signaling from an immunosuppressive response to an immunostimulatory response. The TGF-β CAR can also be used as an accessory receptor to counter immunosuppression and boost T-cell-mediated responses in all adoptive T-cell therapies, whether based on tumor infiltrating lymphocytes, T-cell receptor engineering, or other CARs.

[0173] As shown in FIG. 2, TGF-β CARs are efficiently expressed on the surface of primary human T cells. A TGF-β CAR was created using scFv #2. Surface staining and flow cytometry show that TGF-β CARs are presented on the surface of primary human CD4+ and CD8+ T cells. The receptor's extracellular domain contains an N-terminal FLAG epitope. EGFRt is a truncated epidermal growth factor receptor that is an indicator of cell transduction. It was further found that TGF-β CAR presents to the cell surface more efficiently than the dominant-negative TGF-β receptor (FIG. 9). A dominant-negative TGF-β receptor (DNR) comprising a truncated TGF-β receptor chain II that is missing the intracellular signaling domain has been reported to inhibit TGF-β signaling and improve T-cell effector function. However, the DNR is not efficiently expressed on the cell surface whereas the TGF-β CAR is. The data shown in FIG. 9 are from the transduction of primary human CD4+ T cells with lentiviruses encoding either FLAG-tagged TGF-β CARs or FLAG-tagged DNR. Each receptor is tagged via a T2A cleavage peptide to truncated epidermal growth factor receptor (EGFRt), such that transduced cells can be identified by EGFRt staining whereas receptor surface expression can be identified by FLAG staining. The results of FIG. 9 indicate that TGF-β CARs are much more efficiently expressed on T cell surfaces than the DNR

[0174] FIG. 3 shows that TGF-β CARs block endogenous TGF-β signaling. TGF-β CAR expression in primary human T cells blocks TGF-β signaling via the SMAD pathway. T cells expressing the indicated receptor were incubated with TGF-β at the indicated concentration for 30 min and probed for phospho-SMAD2 via Western Blot. EGFRt refers to T cells expressing a truncated epidermal growth factor receptor and serves as a "no CAR" control; scFv-less refers to T cells expressing a CAR that lacks any ligand-binding scFv domain but is otherwise identical to the TGF-β CAR long. "Long" and "short" labels behind TGF-β CARs refer to the length of their extracellular peptide spacers. As shown in FIG. 6, TGF- β CAR-T cells proliferate in response to TGF-β. T cells expressing TGF-β CARs convert TGF-β from a growth-inhibitory cytokine to a growth-promoting cytokine.

[0175] It was also found that TGF-β CARs activate T cells and trigger cytokine production (FIG. 4). As shown in FIG. 4, TGF-β CAR-T cells upregulate expression of the CD69 activation marker and produce the immunostimulatory cytokines IFN-γ and TNF-α in response to TGF-β exposure. CD69 upregulation was monitored by surfaced staining after a 24-hour incubation with or without TGF-β. Cytokine production was detected by applying the protein transport inhibitor Brefeldin A and performing intracellular staining after a 24-hour incubation with or without TGF-β. "scFv-less" refers to a CAR lacking any ligand-binding scFv domain.

[0176] Unlike TGF-β CARs, the dominant-negative TGF-β receptor cannot trigger cytokine

production. While the dominant-negative TGF-β receptor has been reported to also inhibit TGF-β signaling, it does not trigger immunostimulatory actions such as TNF-α production (FIG. 5). "Tβ short" and "Tβ long" are two different TGF-β CARs, "Dom-Neg" refers to the dominant-negative TGF-β receptor, and "scFv-less" refers to a CAR that lacks any ligand-binding scFv domain.

[0177] It was further found that TGF-β CAR function can be tuned by co-stimulatory domain choice. As shown in FIG. 10, switching between CD28 and 4-1BB co-stimulatory domains alters the cytokine production levels in response to TGF-β. Furthermore, it was found that TGF-β consistently triggers TNF-α production in a dose-dependent manner across cells with the TGF-β CAR from different donors, suggesting that the performance of the CAR is robust enough to be relied upon for clinical applications (FIG. 11).

[0178] It was further found that the TGF-β CAR spacer length modulates the triggering threshold. Increasing the extracellular spacer length increases the TGF-β threshold of CAR triggering (FIG. 12). This suggests that one can customize the CAR responsiveness to the needs of the application by altering the coupling between the ligand binding domain and the intracellular signaling domains. "Short spacer" comprises the hinge portion of human IgG4; "long spacer" comprises IgG4 hinge-CH2-CH3.

[0179] Next, it was discovered that soluble ligand-mediated CAR dimerization triggers CAR signaling. Shown in FIG. 13 are Jurkat cell lines carrying the indicated CAR(s). GFP CAR #1 and GFP CAR #3 both exist predominantly in homodimer form, and the two CARs bind to different epitopes on EGFP and can concurrently bind an individual EGFP molecule. GFP CAR #1 and GFP CAR #2 bind to the same epitope on EGFP, but CAR #2 exists as a monomer rather than a homodimer. Results indicate that CAR signaling can be facilitated by ligand-mediated CAR dimerization, but there is no requirement that the ligand or the CAR itself pre-exist as a dimer (FIG. 13). One possible mechanism by which soluble ligands can trigger CAR signaling is by ligating receptors on two different cells, thereby forming an immunological synapse. This mechanism is consistent with the observation that Jurkat cells expressing a single type of GFP CAR can be activated by dimeric but not monomeric EGFP. It is also consistent with the observation that a mixture of two Jurkat cell lines, each expressing a different GFP CAR, can be activated by both dimeric and monomeric EGFP. In this instance, monomeric EGFP can also trigger cell-cell ligation since the CARs on the two Jurkat cell lines bind to two different epitopes on the same EGFP molecule (FIG. 14).

[0180] It was found that TGF-β CAR can be triggered in both cell-cell contact-dependent and -independent manners. Although cell-cell contact is one possible mechanism by which soluble ligands such as EGFP and TGF-β can trigger CAR signaling, CAR-T cell activation can also be triggered by soluble ligand in the absence of cell-cell contact. In FIG. 15, Jurkat cells stably expressing the TGF-β CAR and an EGFP reporter expressed from an NFAT promoter are seeded at various cell densities and incubated with or without 5 ng/mL TGF-β. Even at very low cell densities where cells exist predominantly as single-cell isolates, clear EGFP signal is observed in the presence of TGF-β. Furthermore, for a 10-fold range in cell density (from 500

to 5000 cells/cm^2), there is no increase in EGFP output with cell density (FIG. 15). These results indicate that soluble TGF-β can trigger T-cell activation independently of cell-cell contact. However, EGFP output increases significantly beyond a threshold cell density, confirming the contribution of cell-cell contact at higher cell density levels. To further test this, primary human CD4+ T cells expressing TGF-β CAR were labeled with the calcium indicator Fluo-4-AM and imaged by fluorescence microscopy. Fluo-4-AM signals observed after TGF-β addition in the absence of cell-cell contact confirms that TGF-β CAR-T cells can be activated by soluble ligands without cell-cell ligation (FIG. 16).

[0181] The TGF-β CAR addresses the need for immune cells to counteract TGF-β's role as a driver of immunosuppression in the tumor microenvironment. While CAR-T-cell therapy has yielded remarkable clinical outcomes against B-cell malignancies, its efficacy against solid tumors has been significantly more limited. Solid tumors are known to generate a highly immunosuppressive microenvironment through the overproduction of TGF-β and other cytokines, ultimately resulting in the inactivation of T cells. The TGF-β CAR endows T cells with the ability to not only counter immunosuppression by reducing signaling through the endogenous TGF-β pathway, but also specifically trigger T-cell activation in the presence of TGF-β. T-cell activation spurs the immune cell to produce immunostimulatory cytokines and proliferate, thus turning TGF-β from an immunosuppressive signal to an activating stimulus that invigorates the anti-tumor immune response.

Example 2: Engineering Multi-functional Regulatory T-Cell Therapy for Autoimmune Diseases

[0182] This example describes methods that can be used to engineer chimeric antigen receptor (CAR)-expressing regulatory T cells (Tregs) that can be activated by tumor growth factor beta (TGF-β) to selectively expand *ex vivo,* maintain robust suppressive function *in vivo,* and secrete anti-interleukin 6 receptor alpha (IL-6Rα) single-chain variable fragments (scFvs) to effectively reduce inflammation in a mouse model of rheumatoid arthritis (RA). It is contemplated that other autoimmune diseases, such as those known in the art and/or described herein, can also be treated by methods described in this example and disclosure.

[0183] Adoptive T-cell therapy using conventional T cells (Tconvs) expressing chimeric antigen receptors (CARs) have demonstrated remarkable clinical efficacy against refractory cancers, particularly B-cell malignancies. However, the application of CAR-T-cell therapy to the treatment of autoimmune diseases is still in its infancy.

[0184] Tregs suppress Tconv function through multiple mechanisms, one of which is the secretion of TGF-β, a potent immunosuppressive cytokine that inhibits both effector T-cell and natural killer cell functions. The inventors have developed a TGF-β CAR that specifically activates Tconvs in the presence of TGF-β (FIG. 4), and have confirmed that TGF-β CAR-T cells respond to TGF-β in both soluble and immobilized forms. Human CD4+ and CD8+ Tconvs expressing the TGF-β CAR trigger robust NFAT signaling and produce Th2 cytokines in the

presence of TGF-β, despite the fact that TGF-β is normally a highly immunosuppressive agent (FIG. 4).

[0185] It is contemplated that the TGF-β CAR would be uniquely suited for Treg therapy for the following reasons: 1) TGF-β is known to promote Treg differentiation, thus TGF-β-mediated expansion of CAR-expressing T cells present a method to selectively expand Tregs while preventing the outgrowth of contaminating Tconvs. We have observed that TGF-β drives robust proliferation of TGF-β CAR-expressing Tconvs, but only in the presence of irradiated feeder cells. In the absence of feeder cells, the proliferation of TGF-β CAR-Tconvs is specifically inhibited by the presence of TGF-β, and the inhibition is significantly stronger against TGF-β CAR-Tconvs compared to unmodified Tconvs or Tconvs expressing non-TGFP CARs. The inventor's experimental results suggest that TGF-β, which exists as a natural homodimer, can cause conjugation between two T cells that both express the TGF-β CAR. This cell-cell conjugation may result in fratricidal toxicity among Tconvs. This toxicity can be more than compensated by the proliferative response that results from CAR signaling, but only with the support of irradiated feeder cells. Unlike Tconvs, Tregs do not exhibit granzyme-mediated cytotoxicity and they are not suppressed by endogenous TGF-β signaling. Therefore, it is contemplated that-in the absence of feeder-cell support-TGF-β CAR-expressing Tregs can be selectively expanded over contaminating Tconvs in a TGF-β-driven *ex vivo* expansion protocol, thereby addressing one of the major obstacles in the production of therapeutic Tregs. 2) Activated Tregs naturally produce TGF-β, thus providing a mechanism for self-sustaining activation of TGF-β CAR-Tregs *in vitro* and *in vivo.* It has been shown that antigen-specific Tregs are more effective than polyclonal Tregs in immunosuppression. On the other hand, it has also been shown that once activated, Tregs can exert suppressor function in an antigen-nonspecific manner. Furthermore, pre-activated, polyclonal Tregs have been demonstrated to inhibit collagen-induced arthritis in mice. Taken together, these results suggest that antigen-specific Tregs may be more effective because they are more likely to be activated than non-antigen-specific Tregs, and that specificity toward the target cell-though likely advantageous-is not essential for therapeutic function. Naturally antigen-specific Tregs are difficult to isolate and expand to large enough quantities for therapeutic applications. Although the introduction of transgenic T-cell receptors (TCRs) provide an appealing alternative, each disease would require its own specific TCR and the availability of a suitable antigen target, the latter of which has been recognized as a major bottleneck in the development of T-cell therapies. Since TGF-β production is a natural output of Tregs regardless of TCR specificity, the TGF-β CAR presents a generalizable strategy to enable self-sustaining Treg activation, which could support Treg-mediated suppression against a wide variety of disease targets without the need for disease-specific receptors.

[0186] This example provides novel methodologies and specific Treg products for cell-based immunotherapy against autoimmune diseases, with RA as the disease model for initial studies. The overall objective is to establish a generalizable approach to the generation of therapeutic Tregs with sustained therapeutic function, and to demonstrate the utility of engineered Tregs in a collagen-induced arthritis model in mice.

A. Develop a TGF-β-mediated *ex vivo* expansion protocol for robust Treg propagation

[0187] Primary human Tregs can be isolated from healthy donor blood samples using a RosetteSep CD4+ T-Cell Enrichment Kit followed by magnetic bead-based enrichment of CD127- and CD25+ cells. Isolated cells can be activated with CD3/CD28 Dynabeads and cultured in complete media (RPMI + 10% heat-inactivated fetal bovine serum) supplemented with 300 U/ml IL-2 for 2 days prior to lentiviral transduction. Lentiviral vectors encoding the TGF-β CAR tagged (via a T2A cleavage peptide) with a truncated epidermal growth factor receptor (EGFRt) have already been constructed and validated by transduction into primary human Tconvs. CAR-expressing cells can be isolated by magnetic bead-based sorting for EGFRt+ cells. This sorting scheme avoids the need for direct antibody binding to the CAR and reduces the likelihood for unproductive T-cell activation. Sorted CAR-Tregs can be expanded in 96-well plates under a variety of culture conditions: (a) 300 U/ml IL-2 only, (b) TGF-β only at a gradient of concentrations (1 - 20 ng/ml), (c) a gradient of IL-2 (50 - 300 U/ml) plus TGF-β (1 - 20 ng/ml), and (d) a gradient of IL-2 and TGF-β concentrations plus irradiated feeder cells (TM-LCLs) at a 1:7 T-cell:TM-LCL ratio. Viable cell counts can be quantified by flow cytometry through a 3-week period. Furthermore, Foxp3 expression levels can be quantified by intracellular staining throughout the cell-preparation process, beginning with freshly isolated CD4+/CD25+/CD127- cells. A "scFv-less" CAR that is identical to the TGF-β CAR except it lacks the TGF-β -binding scFv domain has been constructed and can be included as a negative control.

[0188] Through the studies described in this example, one may determine the following: (1) whether TGF-β CAR expression confers Tregs with the ability to proliferate specifically in response to TGF-β addition and (2) identify the optimal combination of IL-2, TGF-β, and/or feeder cells for *ex vivo* Treg expansion with high efficiency (defined by large fold-expansion) and high purity (defined by minimal presence of contaminating Tconvs). The inventors have developed multiple TGF-β CARs with differing structural properties, and have observed distinct signaling thresholds of these CARs in response to TGF-β. Two different TGF-β CARs, one with a short (12-amino acid) extracellular spacer and another with a long (229-amino acid) extracellular spacer can be evaluated to determine the optimal construct for Treg applications.

B. Optimize the suppressor function of TGF-β CAR-Tregs *in vitro* and *in vivo*

[0189] The suppressor functions of Tregs that stably express the TGF-β CAR can be further investigated. Tregs will be isolated, transduced, and sorted as described above. Both the long-spacer and short-spacer TGF-β CARs can be evaluated. As a negative control, a fraction of the CD4+ T cells (prior to enrichment for CD127-/CD25+ phenotype) can be transduced and sorted to provide a TGF-β CAR-Tconv comparison against the engineered Tregs. As a second negative control, Tregs transduced with the scFv-less CAR described above can be included in the study. Sorted CAR-Tregs can be expanded in complete media supplemented with 300 U/ml

IL-2, as this IL-2 concentration has been reported to support Treg survival and proliferation *ex vivo* and is an intermediate concentration among those reported in published studies. The expansion procedure can be updated to the condition determined to support optimal Treg expansion. Tconvs will be expanded in complete media supplemented with 50 U/ml IL-2 and 1 ng/ml IL-15.

[0190] The suppressor function of the TGF-β CAR-Tregs can be evaluated in co-cultures with target Tconvs. Target Tconvs will be CD4+ T cells lentivirally transduced to express the CD19 CAR. To assay Treg function, TGF-β CAR-Tregs can be co-incubated with both CFSE-labeled CD19 CAR-Tconvs and CD19+ Raji lymphoma cells in the presence or absence of soluble TGF-β. Proliferation of the CD19 CAR-Tconvs can be evaluated by CFSE dilution as well as viable cell counting via flow cytometry. CFSE-based proliferation assay can be performed instead of the more widely used 3H-thymidine incorporation assay in order to clearly distinguish Treg vs. Tconv proliferation in this co-culture setting. CFSE-labeled cells can be accurately quantified by flow cytometry starting from early time points, while CFSE dilution peaks will reveal cell-division dynamics over a 7-day period. As negative controls, TGF-β CAR-Tconvs or scFv-less CAR-Tregs can replace TGF-β CAR-Tregs in the co-incubation samples. It is expected that the scFv-less CAR-Tregs will show some suppressive function, but that TGF-β CAR-Tregs will show enhanced suppression due to TGF-β production and subsequent autocrine Treg activation through the TGF-β CAR. Furthermore, it is anticipated that the addition of exogenous TGF-β will further strengthen the TGF-β CAR-Tregs' suppressor function, resulting in minimal CD19 CAR-Tconv expansion despite the presence of CD19+ target cells.

[0191] Upon verification of the TGF-β CAR-Tregs' suppressor function *in vitro,* the engineered Tregs' ability to execute suppressor function *in vivo* can be evaluated. For animal studies, murine CD4+/CD25+ Tregs will be isolated from the lymph nodes and spleens of DBA/1 mice by magnetic bead-based cell sorting. Sorted cells can be activated by murine CD3/CD28 Dynabeads and transduced, expanded, and sorted for TGF-β CAR expression as previously described for human Tregs. Although the TGF-β CAR is constructed with an scFv that targets human TGF-β, inventors have confirmed that this receptor cross-reacts with murine TGF-β with high efficiency (FIG. 8). To trigger collagen-induced arthritis (CIA), DBA/1 mice can be injected at the base of the tail with a 100-μl emulsion containing complete Freund adjuvant mixed at 1:1 ratio with 2 mg/ml chicken type II collagen dissolved in PBS with 0.1 M acetic acid. One million Tregs can be administered via tail-vein injection either one day before CIA immunization or two weeks after CIA immunization in order to evaluate the performance of Tregs at different stages of disease progression. TGF-β CAR-Tregs can be compared against scFv-less CAR-Tregs and a "no-Treg" control (i.e., no Treg administration after CIA immunization) for their ability to prevent or ameliorate arthritis. Mice can be assessed for clinical arthritis in the paws based on a 4-point scale as previously described by Kelchtermans, H. et al. (Arthritis Res Ther 7, R402-415 (2005)). Ten animals can be included for each test condition (40 animals total). The n = 10 sample size will provide 92% power to detect a difference of 1.5 standard deviations in arthritis scoring in a two-sided *t*-test with $\alpha = 0.05$. Based on available literature, it is anticipated that the scFv-less CAR-Tregs will achieve

noticeable suppression of arthritic symptoms in the CIA model. However, it is anticipated that addition of the TGF-β CAR will result in enhanced Treg functionality, leading to more effective inhibition of CIA. It remains to be see whether the long-spacer or the short-spacer CAR will exhibit superior *in vivo* functionality in the CIA model.

REFERENCES

[0192]

US20110008364;

US7151169;

Yingling et al, Nat Rev Drug Discov 2004;

US8012482;

US20060135517;

US 2004026871;

US20070142376;

Gorelik and Flavell, Nat Med 2001;

Bollard et al, Blood 2002;

Zhang et al, Cancer Res 2005;

Foster et al, J Immunother 2008;

Zhang et al, Gene Ther 2012;

Quatromoni et al, J Transl Med 2012;

Bendle et al, J Immunol 2013

Brentjens, R.J. et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med 5, 177ra138 (2013).

Kalos, M. et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med 3, 95ra73 (2011).

Kochenderfer, J.N. et al. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood 119, 2709-2720 (2012).

Porter, D.L., Levine, B.L., Kalos, M., Bagg, A. & June, C.H. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 365, 725-733 (2011).

Davila, M.L. et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med 6, 224ra225 (2014).

Ellebrecht, C.T. et al. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. Science 353, 179-184 (2016).

Wright, G.P., Ehrenstein, M.R. & Stauss, H.J. Regulatory T-cell adoptive immunotherapy: potential for treatment of autoimmunity. Expert Rev Clin Immunol 7, 213-225 (2011).

Wright, G.P. et al. Adoptive therapy with redirected primary regulatory T cells results in antigen-specific suppression of arthritis. Proc Natl Acad Sci USA 106, 19078-19083 (2009).

Kelchtermans, H. et al. Activated CD4+CD25+ regulatory T cells inhibit osteoclastogenesis and collagen-induced arthritis. Ann Rheum Dis 68, 744-750 (2009).

Morgan, M.E. et al. Effective treatment of collagen-induced arthritis by adoptive transfer of CD25+ regulatory T cells. Arthritis Rheum 52, 2212-2221 (2005).Blat, D., Zigmond, E., Alteber, Z., Waks, T. & Eshhar, Z. Suppression of murine colitis and its associated cancer by carcinoembryonic antigen-specific regulatory T cells. Mol Ther 22, 1018-1028 (2014).

Tang, Q. & Bluestone, J.A. Regulatory T-cell therapy in transplantation: moving to the clinic. Cold Spring Harb Perspect Med 3 (2013).

Nakamura, K. et al. TGF-beta 1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice. J Immunol 172, 834-842 (2004).

Thornton, A.M. & Shevach, E.M. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. J Immunol 164, 183-190 (2000).

Brusko, T.M. et al. Human antigen-specific regulatory T cells generated by T cell receptor gene transfer. PLoS One 5, e11726 (2010).

Rosenberg, S.A. Finding suitable targets is the major obstacle to cancer gene therapy. Cancer Gene Ther 21, 45-47 (2014).

Myasoedova, E., Crowson, C.S., Kremers, H.M., Therneau, T.M. & Gabriel, S.E. Is the incidence of rheumatoid arthritis rising?: results from Olmsted County, Minnesota, 1955-2007. Arthritis Rheum 62, 1576-1582 (2010).

Widdifield, J. et al. The epidemiology of rheumatoid arthritis in Ontario, Canada. Arthritis Rheumatol 66, 786-793 (2014).

Lee, D.W. et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood 124, 188-195 (2014).

Brudno, J.N. & Kochenderfer, J.N. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood 127, 3321-3330 (2016).

Brunstein, C.G. et al. Umbilical cord blood-derived T regulatory cells to prevent GVHD: kinetics, toxicity profile, and clinical effect. Blood 127, 1044-1051 (2016).

Kelchtermans, H. et al. Defective CD4+CD25+ regulatory T cell functioning in collagen-induced arthritis: an important factor in pathogenesis, counter-regulated by endogenous IFN-gamma. Arthritis Res Ther 7, R402-415 (2005).

Wu, Y. et al. FOXP3 controls regulatory T cell function through cooperation with NFAT. Cell 126, 375-387 (2006).

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patent documents cited in the description

- WO2013123061A [0002]
- WO2005097832A [0002]
- WO2014172584A [0002]
- US5677425A [0085]
- US5714350A [0130]
- US6350861B [0130]
- EP0154316A [0131]
- EP0401384A [0131]
- EP0322094A [0132]
- EP0486525A [0132]
- US5631146A [0135]
- US5580737A [0135]
- US5786462A [0135]
- US5543293A [0135]
- US20110008364A [0192]
- US7151169B [0192]

- US8012482B [0192]
- US20060135517A [0192]
- US20040266671A [0192]
- US20070142376A [0192]

Non-patent literature cited in the description

- Current Protocols in Molecular Biology20070000 [0020]
- **AHMAD**Clinical and Developmental Immunology, 2012, [0071]
- **BURTON**Molec. Immunol., 1985, vol. 22, 161-206 [0085]
- **TAN et al.**Proc. Natl. Acad. Sci. USA, 1990, vol. 87, 162- [0086]
- **HUCK et al.**Nucl. Acids Res., 1986, [0086]
- **MEARES et al.**Anal. Biochem., 1984, vol. 142, 68-78 [0133]
- **KOYAMA**Chem. Abstr., 1994, vol. 120, 217262t- [0133]
- **RODWELL et al.**PNAS USA, 1986, vol. 83, 2632-2636 [0133]
- **QUADRI et al.**Nucl. Med. Biol., 1993, vol. 20, 559-570 [0133]
- **KELCHTERMANS, H. et al.**Arthritis Res Ther, 2005, vol. 7, R402-415 [0191]
- **YINGLING et al.**Nat Rev Drug Discov, 2004, [0192]
- **GORELIKFLAVELL**Nat Med, 2001, [0192]
- **BOLLARD et al.**Blood, 2002, [0192]
- **ZHANG et al.**Cancer Res, 2005, [0192]
- **FOSTER et al.**J Immunother, 2008, [0192]
- **ZHANG et al.**Gene Ther, 2012, [0192]
- **QUATROMONI et al.**J Transl Med, 2012, [0192]
- **BENDLE et al.**J Immunol, 2013, [0192]
- **BRENTJENS, R.J. et al.**CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemiaSci Transl Med, 2013, vol. 5, 177ra138- [0192]
- **KALOS, M. et al.**T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemiaSci Transl Med, 2011, vol. 3, 95ra73- [0192]
- **KOCHENDERFER, J.N. et al.**B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cellsBlood, 2012, vol. 119, 2709-2720 [0192]
- **PORTER, D.L.LEVINE, B.L.KALOS, M.BAGG, A.JUNE, C.H.**Chimeric antigen receptor-modified T cells in chronic lymphoid leukemiaN Engl J Med, 2011, vol. 365, 725-733 [0192]
- **DAVILA, M.L. et al.**Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemiaSci Transl Med, 2014, vol. 6, 224ra225- [0192]
- **ELLEBRECHT, C.T. et al.**Reengineering chimeric antigen receptor T cells for targeted

therapy of autoimmune diseaseScience, 2016, vol. 353, 179-184 [0192]

- **WRIGHT, G.P.EHRENSTEIN, M.R.STAUSS, H.J.**Regulatory T-cell adoptive immunotherapy: potential for treatment of autoimmunityExpert Rev Clin Immunol, 2011, vol. 7, 213-225 [0192]
- **WRIGHT, G.P. et al.**Adoptive therapy with redirected primary regulatory T cells results in antigen-specific suppression of arthritisProc Natl Acad Sci USA, 2009, vol. 106, 19078-19083 [0192]
- **KELCHTERMANS, H. et al.**Activated CD4+CD25+ regulatory T cells inhibit osteoclastogenesis and collagen-induced arthritisAnn Rheum Dis, 2009, vol. 68, 744-750 [0192]
- **MORGAN, M.E. et al.**Effective treatment of collagen-induced arthritis by adoptive transfer of CD25+ regulatory T cellsArthritis Rheum, 2005, vol. 52, 2212-2221 [0192]
- **BLAT, D.ZIGMOND, E.ALTEBER, Z.WAKS, T.ESHHAR, Z.**Suppression of murine colitis and its associated cancer by carcinoembryonic antigen-specific regulatory T cellsMol Ther, 2014, vol. 22, 1018-1028 [0192]
- **TANG, Q.BLUESTONE, J.A.**Regulatory T-cell therapy in transplantation: moving to the clinicCold Spring Harb Perspect Med, 2013, vol. 3, [0192]
- **NAKAMURA, K. et al.**TGF-beta 1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and miceJ Immunol, 2004, vol. 172, 834-842 [0192]
- **THORNTON, A.M.SHEVACH, E.M.**Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecificJ Immunol, 2000, vol. 164, 183-190 [0192]
- **BRUSKO, T.M. et al.**Human antigen-specific regulatory T cells generated by T cell receptor gene transferPLoS One, 2010, vol. 5, e11726- [0192]
- **ROSENBERG, S.A.**Finding suitable targets is the major obstacle to cancer gene therapyCancer Gene Ther, 2014, vol. 21, 45-47 [0192]
- **MYASOEDOVA, E.CROWSON, C.S.KREMERS, H.M.THERNEAU, T.M.GABRIEL, S.E.**Is the incidence of rheumatoid arthritis rising?: results from Olmsted County, Minnesota, 1955-2007Arthritis Rheum, 2010, vol. 62, 1576-1582 [0192]
- **WIDDIFIELD, J. et al.**The epidemiology of rheumatoid arthritis in Ontario, CanadaArthritis Rheumatol, 2014, vol. 66, 786-793 [0192]
- **LEE, D.W. et al.**Current concepts in the diagnosis and management of cytokine release syndromeBlood, 2014, vol. 124, 188-195 [0192]
- **BRUDNO, J.N.KOCHENDERFER, J.N.**Toxicities of chimeric antigen receptor T cells: recognition and managementBlood, 2016, vol. 127, 3321-3330 [0192]
- **BRUNSTEIN, C.G. et al.**Umbilical cord blood-derived T regulatory cells to prevent GVHD: kinetics, toxicity profile, and clinical effectBlood, 2016, vol. 127, 1044-1051 [0192]
- **KELCHTERMANS, H. et al.**Defective CD4+CD25+ regulatory T cell functioning in collagen-induced arthritis: an important factor in pathogenesis, counter-regulated by endogenous IFN-gammaArthritis Res Ther, 2005, vol. 7, R402-415 [0192]
- **WU, Y. et al.**FOXP3 controls regulatory T cell function through cooperation with NFATCell, 2006, vol. 126, 375-387 [0192]

PATENTKRAV

1. Kimærisk antigenreceptor (CAR), der omfatter et signalpeptid, et TGF-β-antigen-bindingsdomæne med et variabelt tungt (VH) og variabelt let (VL) område af et antistof, en peptid-spacer, et transmembrandomæne og et endodomæne, der transmitterer et aktiveringssignal til T-celle, efter antigenet er bundet; hvor TGF-β-antigenbindingsdomænet specifikt binder til opløseligt TGF-β, og hvor:

(a) VH-området omfatter SEQ ID NO: 5 som HCDR1, SEQ ID NO: 6 som HCDR2; og SEQ ID NO: 7 som HCDR3 og VL-området omfatter SEQ ID NO: 8 som LCDR1, SEQ ID NO: 9 som LCDR2; og SEQ ID NO: 10 som LCDR3; eller

(b) VH-området omfatter SEQ ID NO: 11 som HCDR1, SEQ ID NO: 12 som HCDR2; og SEQ ID NO: 13 som HCDR3 og VL-området omfatter SEQ ID NO: 14 som LCDR1, SEQ ID NO: 15 som LCDR2; og SEQ ID NO: 16 som LCDR3; eller

(c) VH-området omfatter SEQ ID NO: 21 som HCDR1, SEQ ID NO: 22 som HCDR2; og SEQ ID NO: 23 som HCDR3 og VL-området omfatter SEQ ID NO: 24 som LCDR1, SEQ ID NO: 25 som LCDR2; og SEQ ID NO: 26 som LCDR3; og

hvor CAR'en har strukturen: S-X-PL-Y-PS-T-E eller S-Y-PL-X-PS-T-E, hvor S er signalpeptidet, X er VH, PL er en peptidlinker, Y er VL, PS er peptid-spaceren, T er transmembrandomænet, og E er endodomænet.

2. CAR ifølge krav 1(a), hvor VH-området omfatter SEQ ID NO: 1 og VL-området omfatter SEQ ID NO: 2.

3. CAR ifølge krav 1, hvor:

(a) VH-området ifølge krav 1(b) omfatter SEQ ID NO: 3, og VL-området omfatter SEQ ID NO: 4; eller

(b) VH-området ifølge krav 1(c) omfatter SEQ ID NO: 19, og VL-området omfatter SEQ ID NO: 20.

4. CAR ifølge et hvilket som helst af kravene 1-3, hvor:

(a) VH-område og VL-område er adskilt af en peptidlinker; og/eller

(b) CAR'en endvidere omfatter et costimulerende område, eventuelt hvor det costimulerende område er mellem transmembrandomænet og endodomænet; og/eller

(c) transmembrandomænet omfatter et transmembrandomæne af CD28; og/eller

(d) endodomænet omfatter et CD28- eller CD3-zeta-signaleringsdomæne;

hvor eventuelt peptidlinkeren er en glycin-serinlinker.

5. CAR ifølge et hvilket som helst af kravene 1(b), 1(c), 2, 3 eller 4, hvor peptidlinkeren er mindst 4 aminosyrer.

6. CAR ifølge et hvilket som helst af kravene 1-5, hvor:

(a) endodomænet er et CD3-zeta-signaleringsdomæne; og/eller

(b) peptid-spaceren omfatter: (i) færre end 50 aminosyrer; eller (ii) flere end 50 aminosyrer; og/eller

(c) peptid-spaceren omfatter hængselsområdet af et IgG-molekyle; og/eller

(d) peptid-spaceren omfatter hænglet og CH_2CH_3-området af et IgG-molekyle, hvor eventuelt peptid-spaceren består af hængselsområdet af et IgG-molekyle; og/eller

(e) CAR'en endvidere omfatter et detekteringspeptid, hvor eventuelt detekteringspeptidet er et peptid af SEQ ID NO: 17, 94, eller 95: og eventuelt: (i) detekteringspeptidet er flankeret af linkere; og/eller (ii) detekteringspeptidet er N-terminalt til VH- og VL-områder; og/eller (iii) detekteringspeptidet er mellem signalpeptidet og antigen-bindingsdomænet.

7. CAR ifølge et hvilket som helst af kravene 1-6, hvor:

(a) signalpeptidet omfatter SEQ ID NO: 18; og/eller

(b) CAR'en endvidere omfatter et cancermolekylespecifikt antigenbindingsdomæne, hvor eventuelt cancermolekylet omfatter Her2 eller omfatter CD19 eller CD20; eller

(c) CAR'en endvidere omfatter et cancermolekylespecifikt antigenbindingsdomæne, hvor eventuelt det cancermolekylespecifikke antigenbindingsdomæne binder til CAIX, CD33, CD44v7/8, CEA, EGP-2, EGP-40, erb-B2, erb-B3, erb-B4, FBP, føtal acetycholinreceptor, GD2, GD3, Her2/neu, IL-13Ralpha2, KDR, k-letkæde, LeY, L1-celleadhæsionsmolekyle, MAGE-A1, mesothelin, MUC1, NKG2D-ligander, oncofetal antigen (h5T4), PSCA, PSMA, TAA targeteret af mAb IgE, TAG-72 eller VEGF-R2.

8. Isoleret nukleinsyre, der koder for CAR'en ifølge et hvilket som helst af kravene 1-7.

9. Celle, der omfatter CAR'en ifølge et hvilket som helst af kravene 1-7 eller nukleinsyren ifølge krav 8, hvor:

(A) eventuelt cellen endvidere omfatter en cancerspecifik kimærisk antigenreceptor (CAR), og eventuelt den cancerspecifikke CAR specifikt binder til: (i) Her2 eller (ii) specifikt binder til CD19 eller CD20, hvor eventuelt cellen er en immun celle; og

(B) eventuelt: (a) cellen er en T-celle, eventuelt en CD4+- eller CD8+-T-celle; eller regulatorisk T-celle; eller (b) cellen er en naturlig dræbercelle.

10. Celle ifølge krav 9 til anvendelse i en fremgangsmåde til stimulering af et immunrespons, hvilken fremgangsmåde omfatter at bringe cellen ifølge krav 9 i kontakt med TGF-β, hvor eventuelt:

(i) stimulering af et immunrespons omfatter øgning af ekspression og/eller sekretion af immunstimulerende cytokiner og/eller molekyler, hvor eventuelt de immune stimulerende cytokiner og/eller molekyler er én eller flere af TNF-α, IFN-β, IFN-γ, IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18 og granulocyt-makrophag-kolonistimulerende faktor; eller

(ii) stimulering af et immunrespons omfatter øgning af proliferation af immune celler, eventuelt hvor de immune celler er T-celler.

11. Celle ifølge krav 9 til anvendelse i en fremgangsmåde til stimulering af et immunrespons, hvilken fremgangsmåde omfatter et bringe cellen ifølge krav 9 eller

krav 10 i kontakt med TGF-β, hvor cellen er *in vivo* hos et individ med behov for immunstimulation, og eventuelt TGF-β er endogent TGF-β produceret i individet med behov for immunstimulation, hvor eventuelt:

(i) det humane individ har cancer, fibrose eller et åbent sår, hvor eventuelt canceren er melanom;

(ii) det humane individ har en B-cellemalignitet; eller

(iii) det humane individ has a solid tumor; og hvor eventuelt:

(iv) fremgangsmåden endvidere omfatter indgivelse af cellen til et humant individ; og/eller

(v) fremgangsmåden endvidere omfatter indgivelse af TGF-β til individet.

12. Fremgangsmåde *ex vivo* til detektering af TGF-β i en opløsning omfattende at bringe celler ifølge krav 9 i kontakt med opløsningen og måling af immunstimulering; hvor en stigning i immunstimulering indikerer tilstedeværelse af TGF-β, og ingen stigning i immunstimulering indikerer fravær af TGF-β, hvor eventuelt:

(i) immunstimulering omfatter ekspressionen af immune stimulerende cytokinet og/eller molekyler, hvor eventuelt de immune stimulerende cytokiner og/eller molekyler er ét eller flere af TNF-α, IFN-β, IFN-γ, IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18 og granulocyt-makrofag-kolonistimulerende faktor; eller

(ii) immunstimulering omfatter en stigning i proliferationen af immune celler, hvor eventuelt de immune celler er T-celler.

13. Fremgangsmåde til fremstilling af CAR ifølge et hvilket som helst af kravene 1-7 omfattende ekspression af et nukleotid, der koder for CAR'en i en celle.

14. Fremgangsmåde til ekspansion af T-celler *in vitro,* hvilken fremgangsmåde omfatter at bringe T-cellen ifølge krav 9B i kontakt med en sammensætning omfattende TGF-β, hvor eventuelt sammensætningen omfatter: (a) omfatter 1-50 ng/mL TGF-β; og/eller (b) omfatter IL-2 og eventuelt sammensætningen omfatter 20-400 U/mL IL-2,

hvor eventuelt:

(a) (i) fremgangsmåden endvidere omfatter at bringe cellerne i kontakt med feeder-celler, hvor eventuelt feeder-cellerne er bestrålet; eller (ii) fremgangsmåden omfatter kontakt mellem T-cellerne og feeder-celler; og/eller

(b) T-cellen er en regulatorisk T-celle, hvor eventuelt de ekspanderede regulatoriske T-celler omfatter færre end 10 % af ikke-regulatoriske T-celler.

15. Celle ifølge krav 9B til anvendelse i en fremgangsmåde til behandling af en sygdom eller patologisk tilstand hos en patient,

hvor eventuelt: (a) cellen er en regulatorisk T-celle, hvor eventuelt sygdommen er: en autoimmun sygdom; eller (b) sygdommen er cancer;

hvor eventuelt fremgangsmåden endvidere omfatter ekspansion af cellerne *in vitro* ved hjælp af en fremgangsmåde omfattende at bringe cellen *in vitro* i kontakt med en sammensætning omfattende TGF-β; hvor eventuelt: (a) sammensætningen omfatter 1-50 ng/mL af TGF-β; og/eller (b) sammensætningen endvidere omfatter IL-2, og eventuelt sammensætningen omfatter 20-400 U/mL af IL-2;

hvor eventuelt: (a) fremgangsmåden endvidere omfatter at bringe cellerne I kontakt med feeder-celler, hvor eventuelt feeder-cellerne er bestrålet; eller (b) fremgangsmåden ikke omfatter kontakt mellem T-cellerne og feeder-celler;

hvor eventuelt T-cellen er en regulatorisk T-celle, hvor eventuelt de ekspanderede regulatoriske T-celler omfatter færre end 10 % af ikke-regulatoriske T-celler;

hvor eventuelt den autoimmune sygdom er rheumatoid arthritis; og

hvor eventuelt fremgangsmåden endvidere omfatter indgivelse af TGF-β til patienten.

DRAWINGS

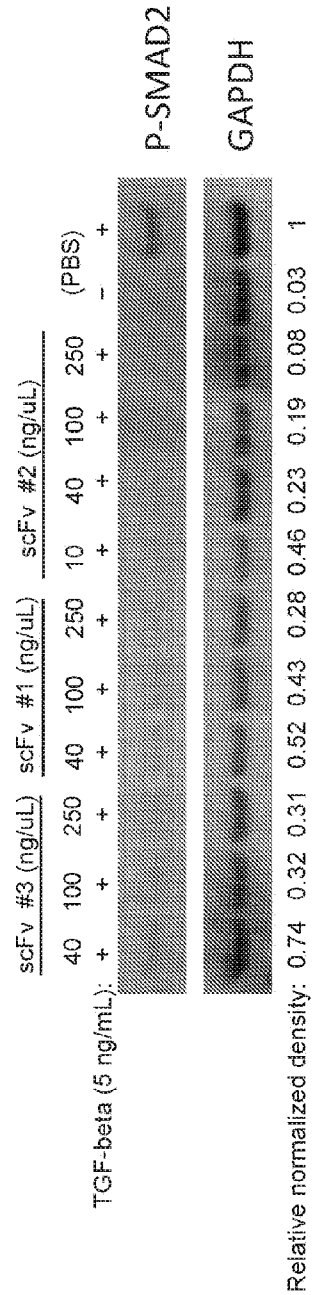

FIG. 1

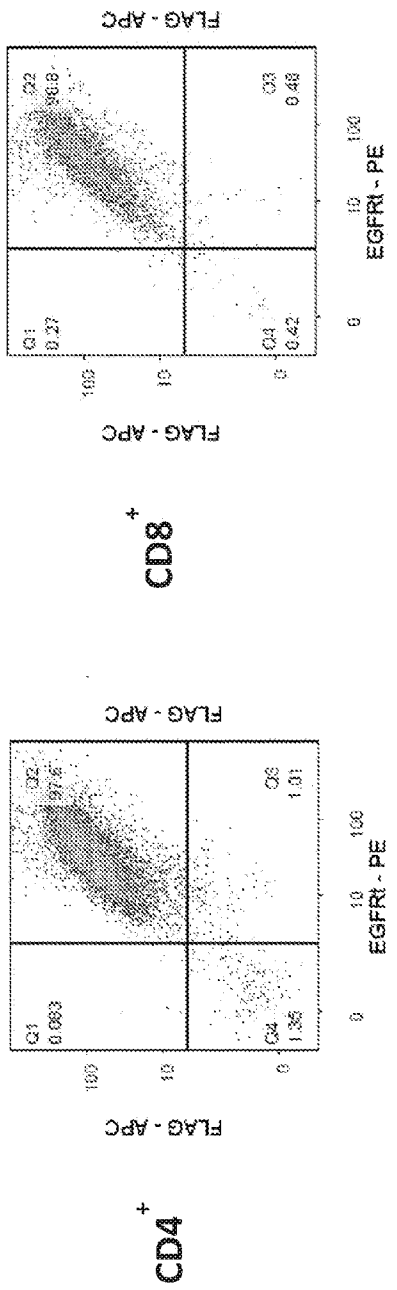
CD8+
FLAG - APC
EGFRt - PE
Q1 0.27
Q2 99.6
Q3 0.48
Q4 0.42
CD4+
FLAG - APC
EGFRt - PE
Q1 0.883
Q2 97.6
Q3 1.01
Q4 1.36

FIG. 2

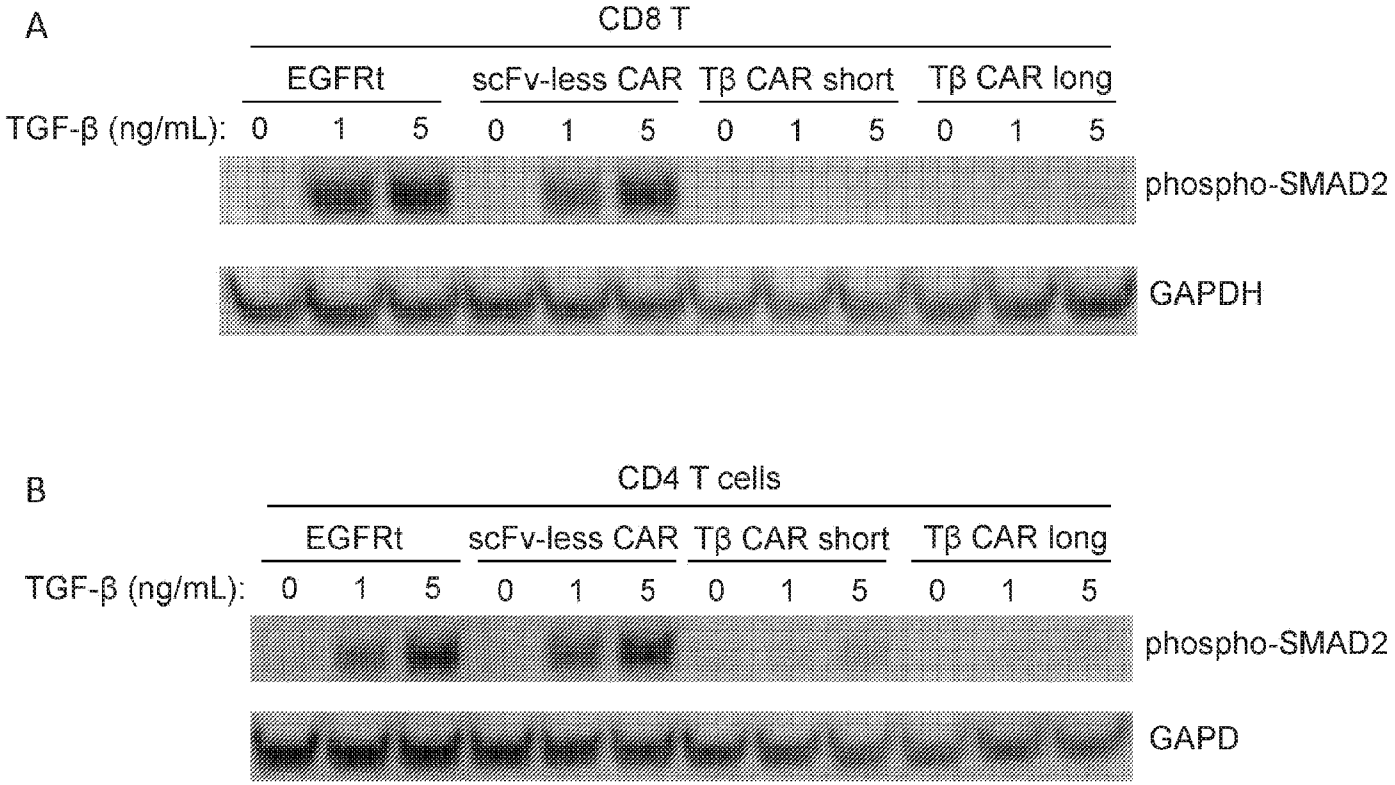

FIG. 3A-B

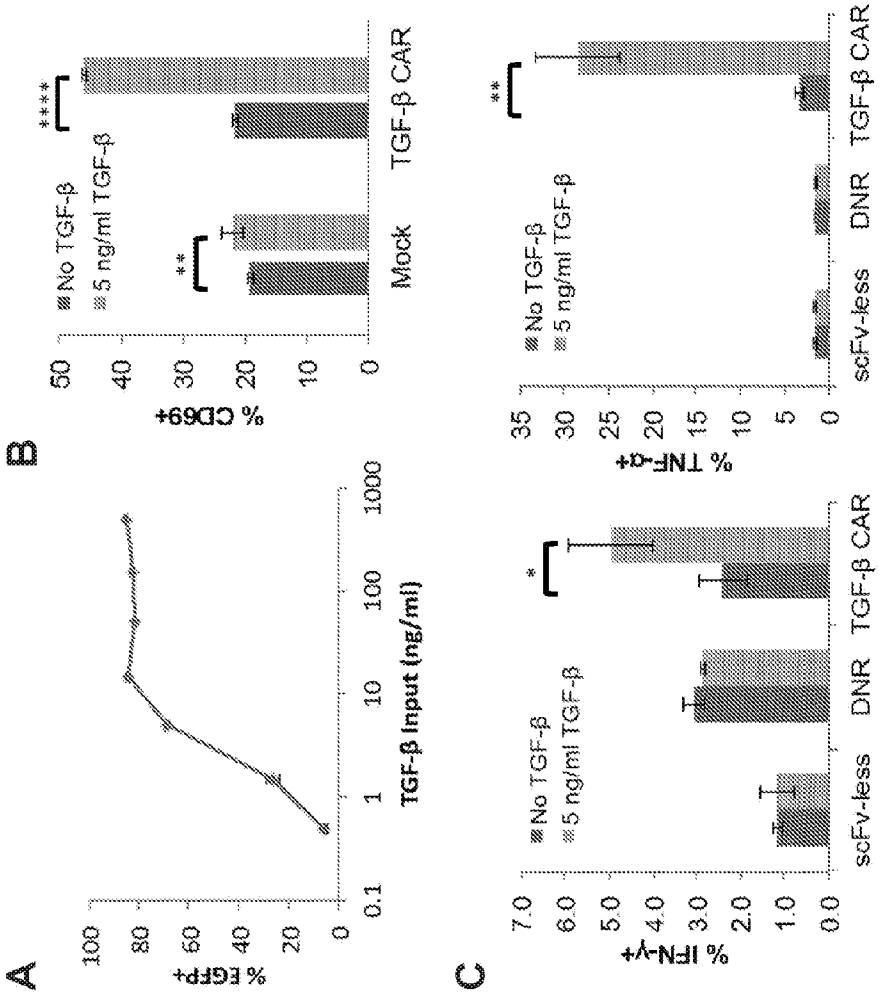
A
% EGFP+
TGF-β Input (ng/ml)
B
% CD69+
No TGF-β
5 ng/ml TGF-β
Mock
TGF-β CAR
C
% IFN-γ+
% TNF-α+
scFv-less
DNR
TGF-β CAR

FIG. 4A-C

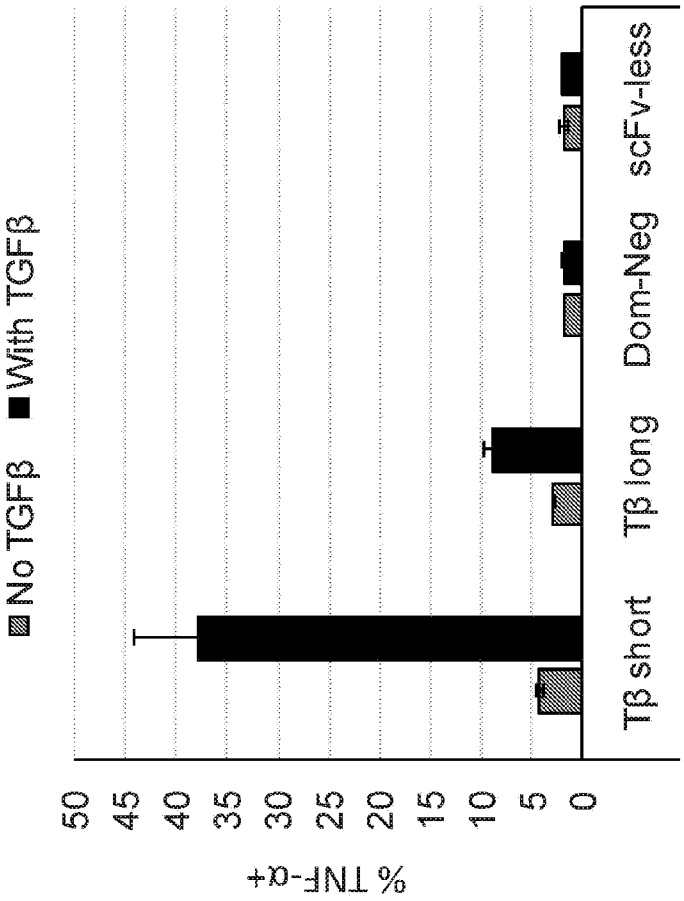
No TGFβ
With TGFβ
% TNF-α+
50
45
40
35
30
25
20
15
10
5
0
Tβ short
Tβ long
Dom-Neg
scFv-less

FIG. 5

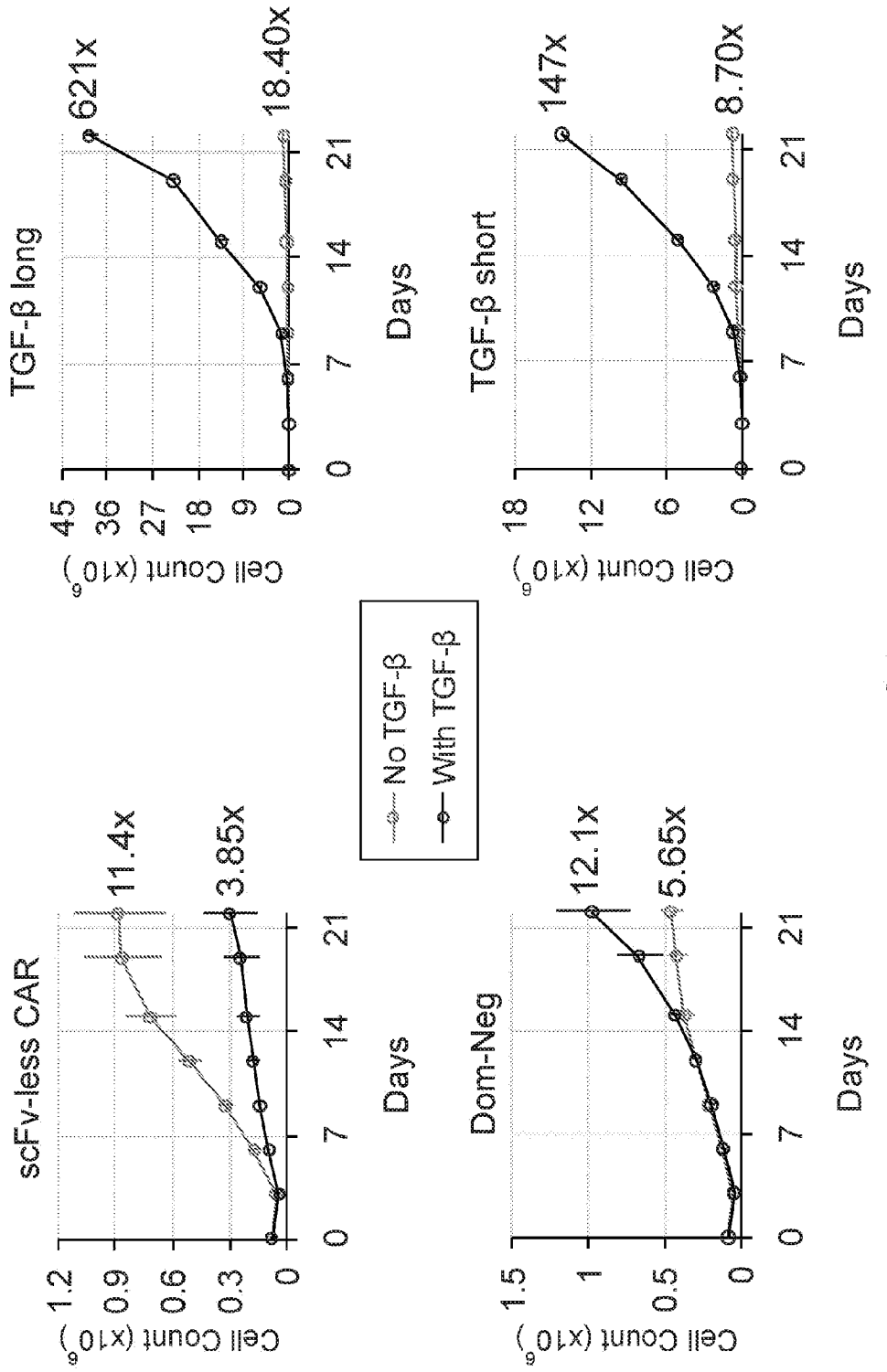
TGF-β long
621x
18.40x
Cell Count (x10^6)
Days
TGF-β short
147x
8.70x
scFv-less CAR
11.4x
3.85x
Dom-Neg
12.1x
5.65x
No TGF-β
With TGF-β

FIG. 6

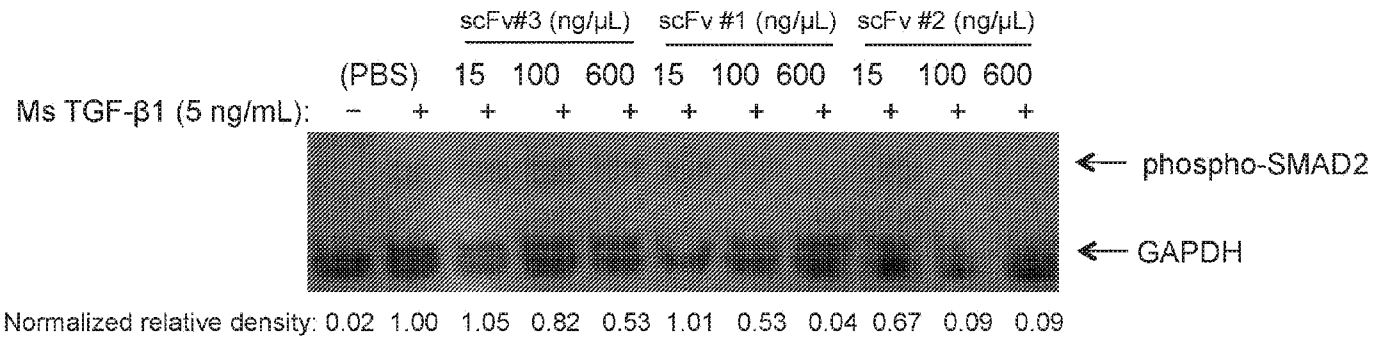

FIG. 7

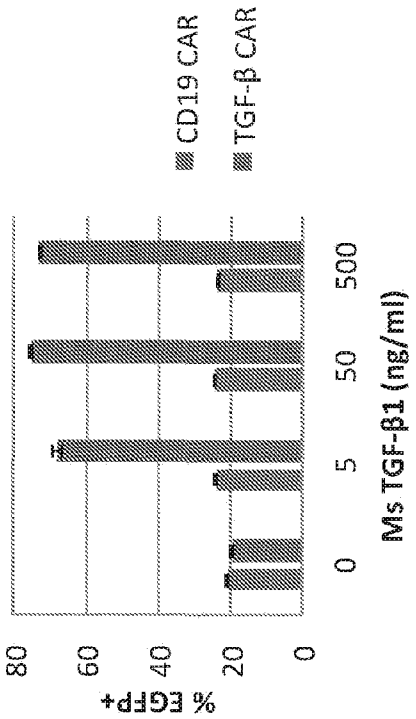

FIG. 8

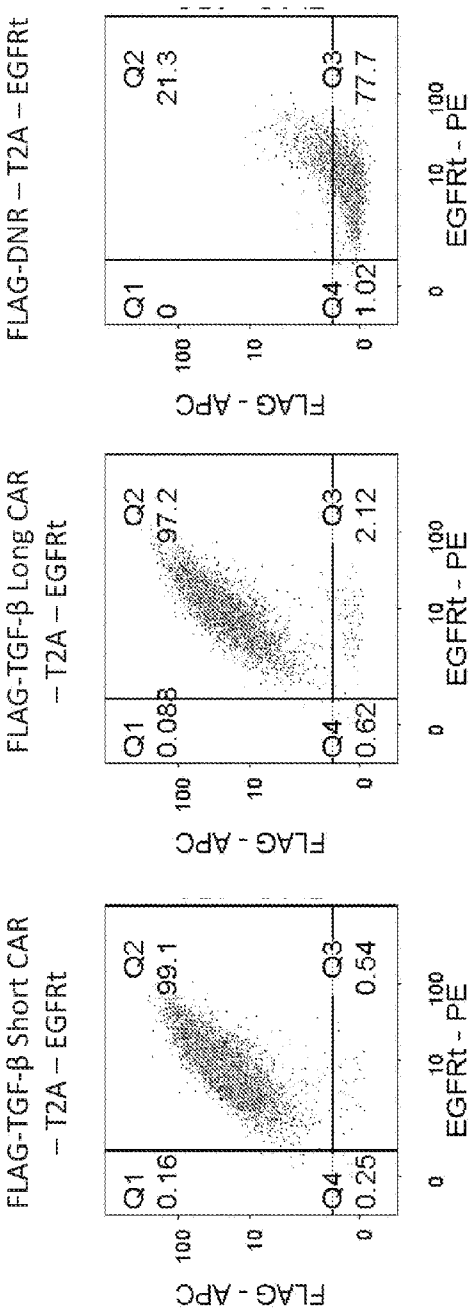
FLAG-TGF-β Short CAR – T2A – EGFRt
FLAG-TGF-β Long CAR – T2A – EGFRt
FLAG-DNR – T2A – EGFRt
Q1
Q2
Q3
Q4
0.16
99.1
0.54
0.25
0.088
97.2
2.12
0.62
0
21.3
77.7
1.02
FLAG - APC
EGFRt - PE
0
10
100

FIG. 9

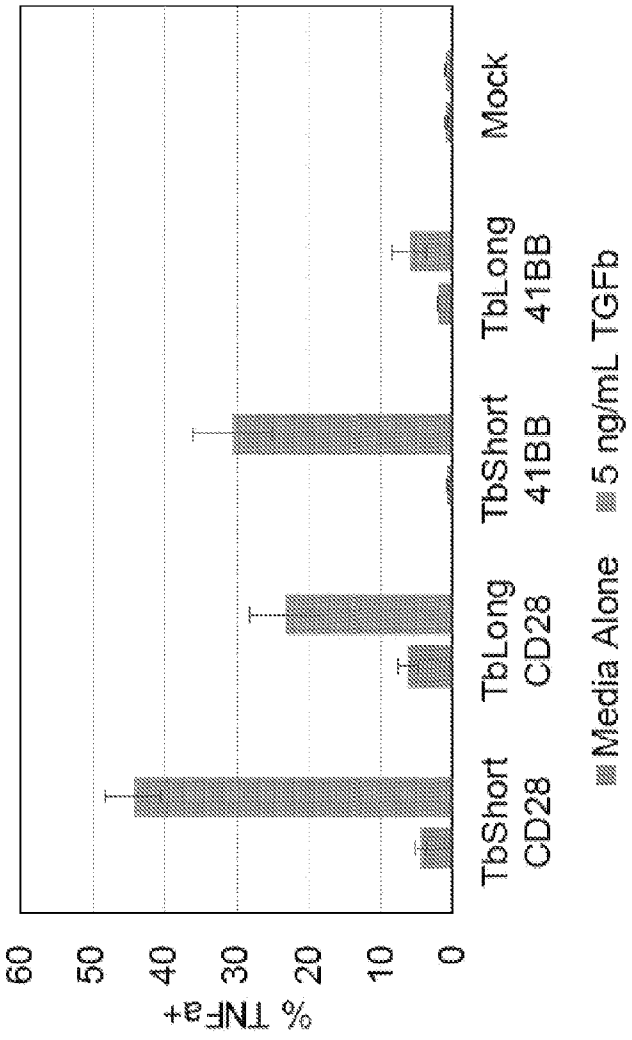
% TNFa+
60
50
40
30
20
10
0
TbShort CD28
TbLong CD28
TbShort 41BB
TbLong 41BB
Mock
Media Alone
5 ng/mL TGFb

FIG. 10

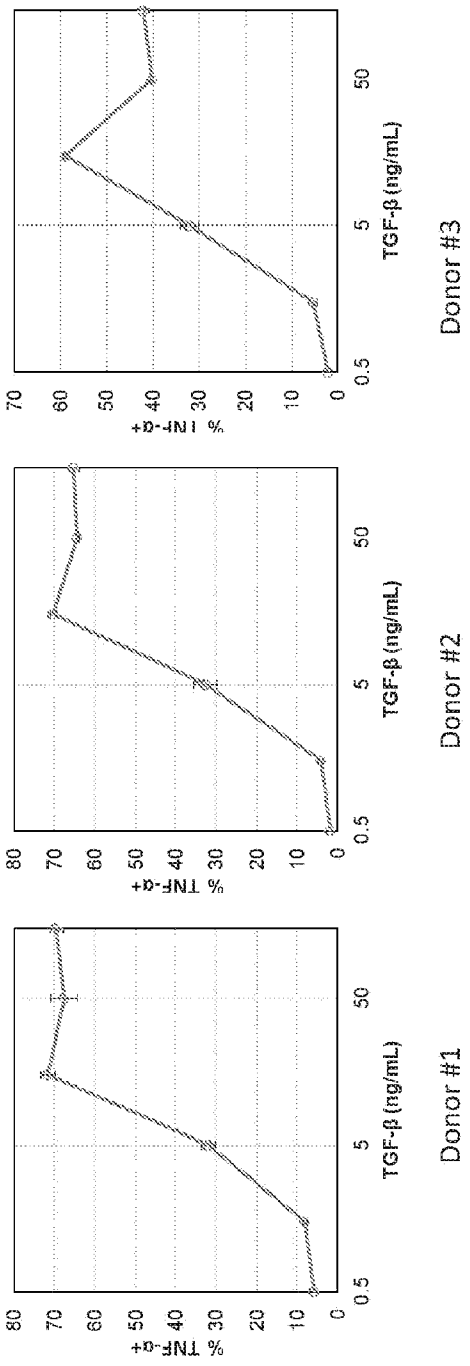
% TNF-α+
TGF-β (ng/mL)
0
10
20
30
40
50
60
70
80
0.5
5
50
Donor #1
Donor #2
Donor #3

FIG. 11

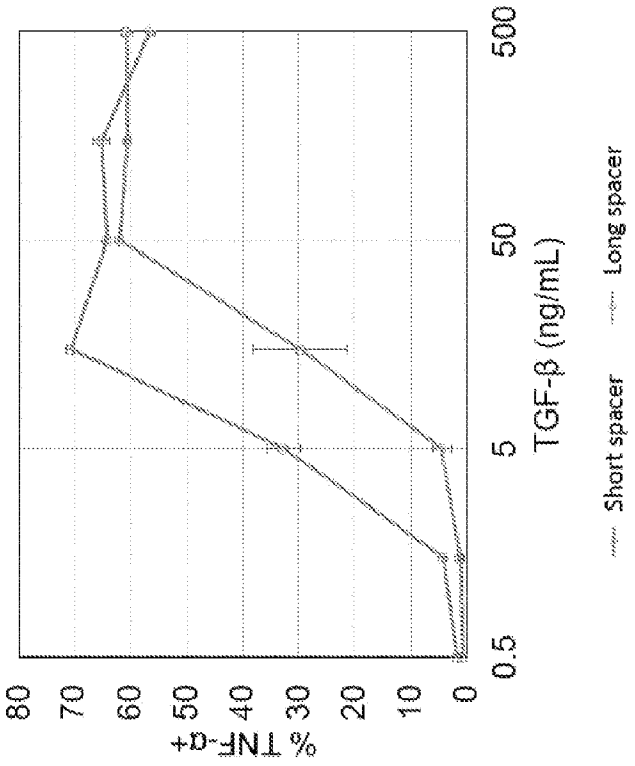
% TNF-α+
80
70
60
50
40
30
20
10
0
0.5
5
50
500
TGF-β (ng/mL)
Short spacer
Long spacer

FIG. 12

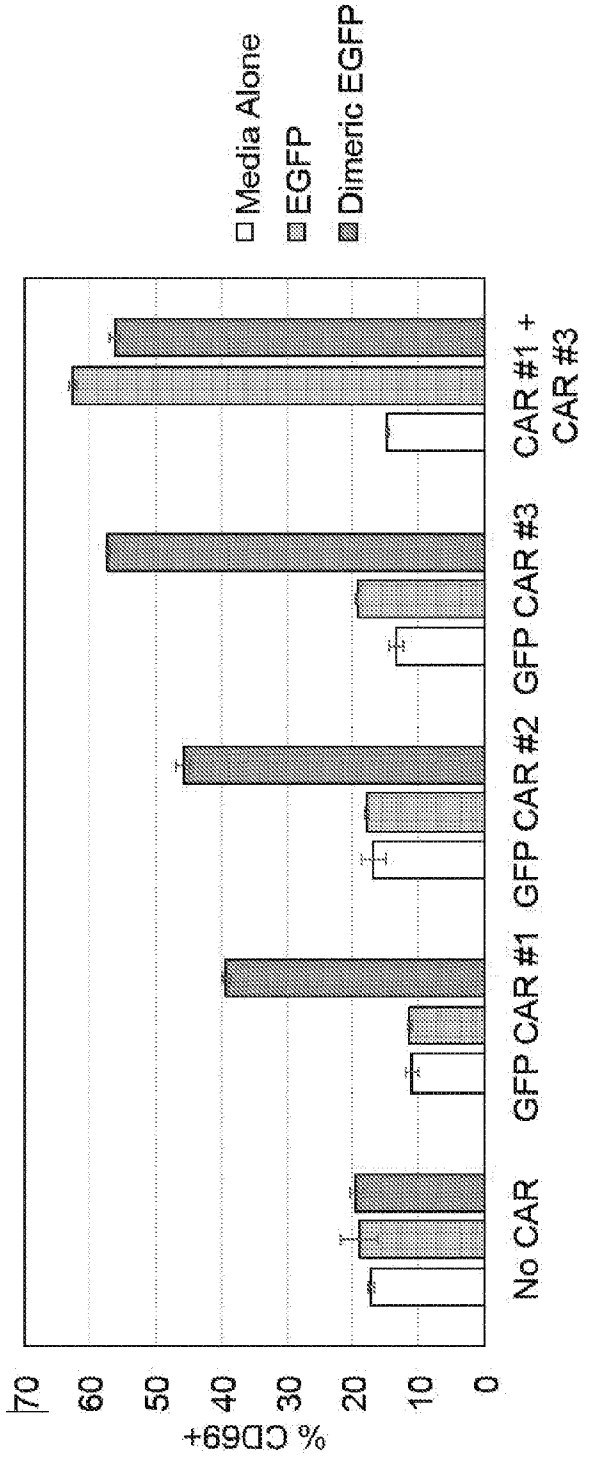
Media Alone
EGFP
Dimeric EGFP
% CD69+
70
60
50
40
30
20
10
0
No CAR
GFP
CAR #1
GFP
CAR #2
GFP
CAR #3
CAR #1 + CAR #3

FIG. 13

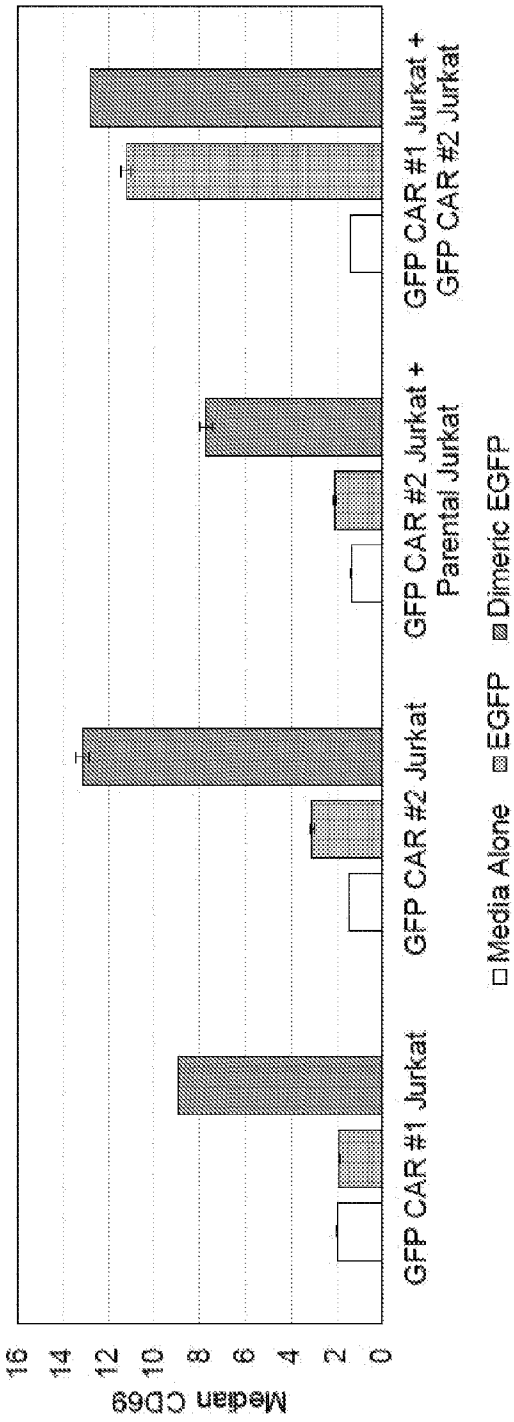
Median CD69
16
14
12
10
8
6
4
2
0
GFP CAR #1 Jurkat
GFP CAR #2 Jurkat
GFP CAR #2 Jurkat + Parental Jurkat
GFP CAR #1 Jurkat + GFP CAR #2 Jurkat
Media Alone
EGFP
Dimeric EGFP

FIG. 14

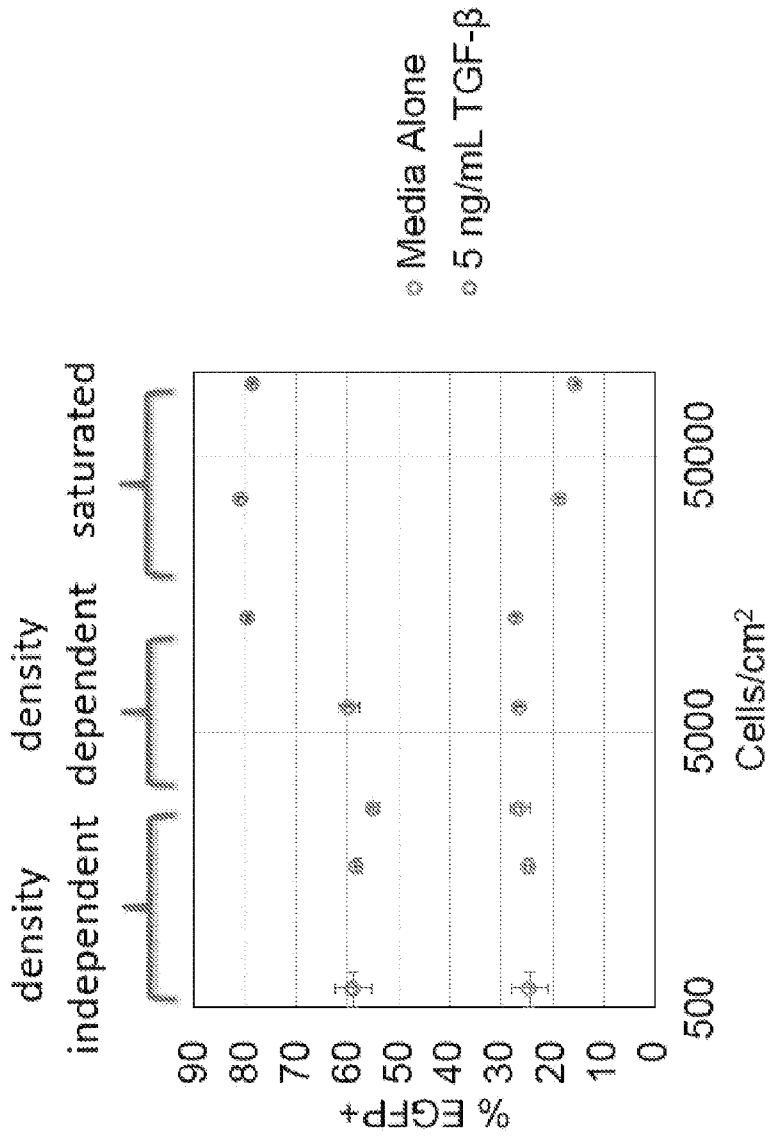
density independent
density dependent
saturated
Media Alone
5 ng/mL TGF-β
% EGFP+
90
80
70
60
50
40
30
20
10
0
500
5000
50000
Cells/cm2

FIG. 15

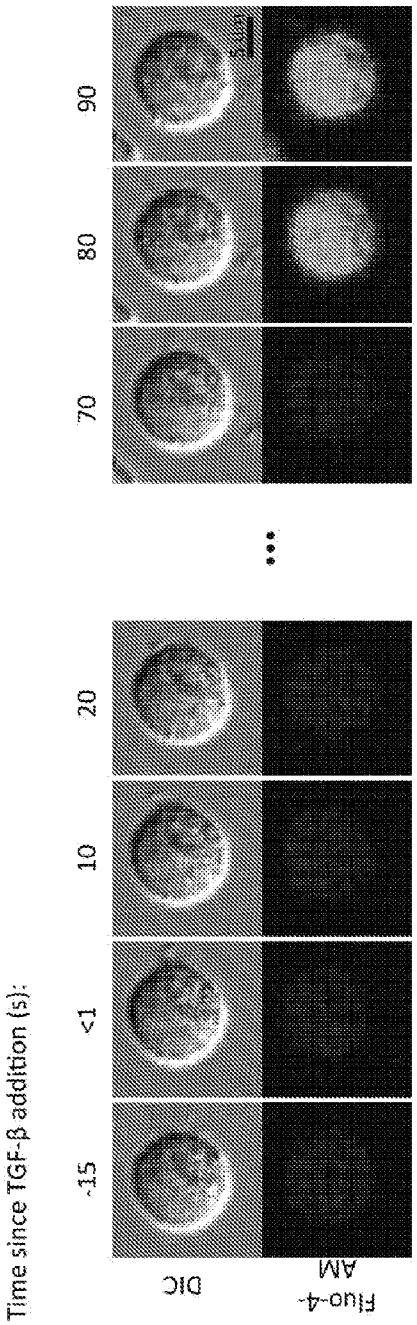

FIG. 16

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.