SUMMARY

[0005] The present disclosure provides, among other things, chimeric antigen receptors (CARs) modified to improve target specificity in CAR T cell therapy, and related nucleic acids, expression vectors, cells, compositions and methods of treatment of cancer and other conditions amenable to CAR T cell therapy. The present disclosure provides a modification to CARs, the CD45 gate, that at least partially suppresses the modified CAR's activation in the presence of the CAR's antigen, thereby reducing the CAR's activity relative to the CAR not so modified or relative to the CAR not modified. This suppression and reduction in activity is partially or fully relieved when the modified CAR is in the presence of certain proteases, as occurs at the site of a tumor that is the CAR T cell therapy target. Disclosed herein are at least the following aspects and examples of the disclosure. Also provided is an anti-MUC16 CAR, including an anti-MUC16 CAR for use as a component of the protease-activating CD45-gate CAR disclosed herein.

[0006] CD45 is a phosphatase that plays a dynamic role in regulating T cell activation (1). It was found to directly activate TCR signaling by dephosphorylating other kinases, e.g. LCK, which activates these kinases and the downstream TCR signaling (2). CD45 is also able to directly dephosphorylate CD3zeta, which leads to the deactivation of TCR signaling (3).

[0007] Accordingly, in one example, provided herein is a protease-activating CD45-gate effector polypeptide comprising a CD45 recruiting domain connected to an effector entity optionally by a linker. In some examples, the effector entity can be reversibly modulated by CD45. In some examples, the effector entity is activated when the CD45 recruiting domain is released from the protease-activating CD45-gate effector by protease cleavage. In some examples, the CD45 recruiter is released by protease cleavage at the linker. In some examples, the effector entity comprises a chimeric antigen receptor (CAR). In some examples, the effector entity comprises a T cell receptor (TCR). In some examples, the CD45 recruiting domain (e.g. an anti-CD45 scFv) is prepared, using no more than ordinary skill in the art, from the CD45 antibodies disclosed in, for example, WO2005/026210, WO20131048804, WO2017009473, WO2020092654, EP1664122B1, and US Pat. No. 6,106,834.

[0008] In one example, provided herein is a protease-activating CD45-gate chimeric antigen receptor (CD45-gate CAR) comprising an extracellular domain, a transmembrane domain,

and an intracellular domain, wherein the extracellular domain comprises: a CD45 recruiting domain, a linker comprising one or more protease cleavage sites that can be cleaved by at least one protease, and an antigen binding domain. In some examples of the protease-activating CD45-gate CAR, the intracellular domain comprises at least one signaling domain that can be reversibly inactivated by CD45. In an example, the linker is between the CD45 recruiting domain and the antigen binding domain. In an example, the CD45 recruiting domain comprises one or more linkers. In an example, the CD45 recruiting domain comprises an anti-CD45 antibody comprising a heavy chain variable domain (VH) and a light chain variable domain (VL). In some examples, the linker is between the VH and VL of the anti-CD45 antibody. In some examples, the protease-activating CD45-gate CAR comprises additional flexible regions e.g. placed between domains.

[0009] In a further example, provided herein is a protease-activating CD45-gate chimeric antigen receptor (CD45-gate CAR) comprising an extracellular domain, a transmembrane domain, and an intracellular domain, wherein the extracellular domain comprises:

a CD45 recruiting domain,

an antigen binding domain, and

a linker connecting the carboxy terminus of the CD45 recruiting domain to the amino terminus of the antigen binding domain, wherein the linker comprises at least one protease cleavage site that is recognized by a protease,

and further wherein the intracellular domain comprises at least one (e.g. one or more than one) signaling domain that can be reversibly inactivated by CD45.

[0009a] In one aspect, the present disclosure refers to a protease-activating CD45-gate chimeric antigen receptor (CD45-gate CAR) comprising an extracellular domain, a transmembrane domain, and an intracellular domain, wherein the extracellular domain comprises: a CD45 recruiting domain, an antigen binding domain, and a linker connecting the carboxy terminus of the CD45 recruiting domain to the amino terminus of the antigen binding domain, wherein the linker comprises one or more protease cleavage site that is recognized by at least one protease, and optionally wherein the intracellular domain comprises at least one signaling domain that can be reversibly inactivated by CD45.

[0010] In some examples, the CD45 recruiting domain comprises one or more of an anti-CD45 antibody antigen binding domain, an anti-CD45 nanobody, an anti-CD45 scFv, a viral protein binder of CD45, a truncated viral protein binder of CD45, an anti-CD45 Fab, an anti-CD45 camelid VHH, a CD45-binding protein, an endogenous CD45 binder, or a truncated endogenous CD45 binder. In some examples, the CD45 recruiting domain comprises one or more of a peptide or protein that interacts with CD45, for example, full-length or truncated UL11, full-length or truncated sec49K, and full-length or truncated BTN3A1. In some examples, the CD45 recruiting domain comprises a truncated BTN3A1, e.g. a truncated BTN3A1 having the amino acid sequence of SEQ ID NO: 14 or of SEQ ID NO: 15.

[0011] In some examples, the CD45 recruiting domain comprises an anti-CD45 scFv. In some examples, the CD45 recruiting domain comprises an anti-CD45 antibody antigen binding domain. In some examples, the CD45 recruiting domain comprises one or more of truncated UL11, truncated sec49K, and truncated BTN3A1. In some examples, the CD45 recruiting domain comprises the amino acid sequence of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15.

[0012] In some examples, the antigen binding domain specifically binds BCMA, MUC16 (also known as CA125), EGFR, EGFRvIII, MUC1, Flt-3, WT-1, CD20, CD23, CD30, CD38, CD70, CD33, CD133, MHC-WT1 , TSPAN10, MHC-PRAME, MHC-NY-ESO1, HER2 (ERBB2), CAIX (Carbonic anhydrase IX), LIV1, ADAM10, CHRNA2, LeY, NKG2D, CS1, CD44v6, ROR1, CD19, Claudin-18.2 (Claudin-18A2, or Claudin18 isoform 2), PSCA, DLL3 (Delta-like protein 3, Drosophila Delta homolog 3, Delta3), Mud 7 (Mucin17, Muc3, Muc3), FAP alpha (Fibroblast Activation Protein alpha), Ly6G6D (Lymphocyte antigen 6 complex locus protein G6d, c6orf23, G6D, MEGT1, NG25), PSMA, MSLN, or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43).

[0013] In some examples, the antigen binding domain specifically binds MUC16. In some examples, the antigen binding domain specifically binds MUC16 and the protease-activating CD45-gate CAR comprises the amino acid sequence of any of SEQ ID NOs: 11, 56, 75-79, and 121-170, each of which can have or not have the signal sequence of SEQ ID NO:1. In some examples, the antigen binding domain specifically binds MUC16 and the protease-activating CD45-gate CAR comprises the amino acid sequence of SEQ ID NO: 75, with or without the signal sequence of SEQ ID NO:1. In some examples, the antigen binding domain specifically binds MUC16 and the protease-activating CD45-gate CAR comprises the amino acid sequence of SEQ ID NO: 76, with or without the signal sequence of SEQ ID NO:1. In some examples, the extracellular domain comprises the amino acid sequence of any one of SEQ ID NOs: 3, 4, 5, 6, 12, 13, 14, 15, 56, and 56 without the signal sequence of SEQ ID NO:1.

[0014] In some examples, the antigen binding domain specifically binds to an antigen expressed by a certain type of tumor and the protease cleavage sites can be recognized and cleaved by a protease that the same type of tumor expresses. In some examples, the protease-activating CD45-gate CAR both (1) can specifically recognize

and bind to an antigen expressed by a tumor and (2) comprises a CD45 recruiting domain-CAR linker that can be cleaved by a protease that the tumor secretes and/or that is present and/or active in the tumor microenvironment.

[0015] In some examples, the antigen binding domain specifically binds to a breast cancer tumor antigen or a colorectal cancer tumor antigen and one or more of the at least one protease cleavage sites can be recognized and cleaved by uPA. In some examples, the protease-activating CD45-gate CAR's antigen binding domain specifically recognizes and binds to an antigen characteristic of any of cervical, breast, ovarian and colorectal cancers and at least one of its protease cleavage sites can be recognized and cleaved by MMP-2 and/or MMP-9. In some examples, the protease-activating CD45-gate CAR's antigen binding domain specifically recognizes and binds to an antigen characteristic of any of breast and ovarian cancers and at least one of its protease cleavage sites can be recognized and cleaved by matriptase.

[0016] In some examples, the linker comprises a GS sequence on either or both ends of each of the one or more protease cleavage sites. In some examples, the GS sequence is a polypeptide that consist of only glycine and serine residues. In some examples, each GS sequence comprises a series of linked GS peptides, a GS peptide being, for example, a peptide having an amino acid sequence comprising only glycine and serine residues, e.g. a peptide having an amino acid sequence comprising one or more glycine residues followed by one or more serine residues, for example, a peptide having an amino acid sequence of GS, GGS, GGGS (SEQ ID NO: 184), or GGGGS (SEQ ID NO:178), wherein the linked GS peptides are linked in any order and in any combination. In some examples, the GS sequence comprises any combination of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 GS peptides or comprises between 1 and 15 GS peptides in any order and combination.

[0017] In some examples, the linker of the protease-activating CD45-gate CAR disclosed herein has a length of 15-100 amino acids, 30-100 amino acids, 15-75 amino acids, 20-50 amino acids, 20 amino acids, 25 amino acids, 30 amino acids, 35 amino acids, 40 amino acids, 45 amino acids, or 50 amino acids, or about 20 amino acids, about 25 amino acids, about 30 amino acids, about 35 amino acids, about 40 amino acids, about 45 amino acids, or about 50 amino acids.

[0018] In some examples of the protease-activating CD45-gate CAR disclosed herein, the linker comprises one or more protease cleavage sites that comprise the amino acid

sequence of SEQ ID NO: 32, 91-98, 103-105 or SEQ ID NO: 106. In some examples, the linker comprises one or more amino acid sequences of SEQ ID NO: 53, 89, or 90. In some examples, the linker comprises one or more amino acid sequences of SEQ ID NO: 8-10, 99-102, 107-120, 172-176 or SEQ ID NO: 177. In some examples, the linker comprises two or more protease cleavage sites and each cleavage site is the same as or different from any of the other cleavage sites. In some examples, the number of protease cleavage sites is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or is between 1 and 15. In some examples, when the linker comprises more than one protease cleavage site, each protease cleavage site is independently the same as or different from one or more of the other protease cleavage sites.

[0019] In some examples, the linker comprises one protease cleavage site (e.g. any of the protease cleavage sites disclosed herein, e.g. a cleavage site having the amino acid sequence of, for example, any of SEQ ID NOs: 32, 89-98 and 103-106) (SEQ ID NOs: 89 and 90 each have two protease cleavage sites, a MMP2/9 site and a MTSP site.). In some examples, the protease cleavage site comprises the amino acid sequence of SEQ ID NO: 32 [LVPRGS]. In some examples, the protease cleavage site comprises the amino acid sequence of SEQ ID NO: 91. In some examples, the protease cleavage site comprises the amino acid sequence of SEQ ID NO: 92. In some examples, the protease cleavage site comprises the amino acid sequence of SEQ ID NO:93. In some examples, the protease cleavage site comprises the amino acid sequence of SEQ ID NO:94. In some examples, the protease cleavage site comprises the amino acid sequence of SEQ ID NO:95. In some examples, the protease cleavage site comprises the amino acid sequence of SEQ ID NO:96. In some examples, the protease cleavage site comprises the amino acid sequence of SEQ ID NO:97. In some examples, the protease cleavage site comprises the amino acid sequence of SEQ ID NO:98. In some examples, the protease cleavage site comprises the amino acid sequence of SEQ ID NO: 103. In some examples, the protease cleavage site comprises the amino acid sequence of SEQ ID NO:104. In some examples, the protease cleavage site comprises the amino acid of any one of SEQ ID NOs: 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 103, 104, 105, and 106. In some examples, the linker comprises multiple protease cleavage sites (for example, any of the protease cleavage sites known and/or disclosed herein, e.g. a cleavage site having the amino acid sequence of any of SEQ ID NOs: 32, 89-98 and 103-104), such as two, three, four, five, six or more protease cleavage sites, e.g. up to ten or up to fifteen protease cleavage

sites, and each cleavage site is the same as or different from any of the other cleavage sites. In some examples, one or more than one of the multiple protease cleavage sites comprises the amino acid sequence of SEQ ID NO: 32 (amino acid sequence LVPRGS). In some examples, the CD45 recruiting domain comprises one or more than one protease cleavage sites. In some examples, the linker amino acid sequence is or comprises the amino acid sequence of any one or more of (e.g. 1, 2, 3, 4, 5, 6 or more) of SEQ ID NOs: 99-102, 172-177, and 108-120. In some examples, the linker comprises one copy or more than one copy (e.g. 1, 2, 3, 4, 5, 6 or more) of the amino acid sequence of any of SEQ ID NOs: 89-98 and 103-106.

[0020] In some examples, the protease cleavage site, or one or more of the multiple protease cleavage sites, is recognized by one or more (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 15, or any range between any two of these values) of thrombin, trypsin, plasmin, prostate-specific antigen (PSA), urokinase plasminogen activator (uPA), urokinase plasminogen activator receptor (uPAR), matrix metalloproteinase (MMP), matriptase (MT-SP1), legumain, a disintegrin and metalloproteinase (ADAM), and transmembrane Serine Protease (TMPRSS). Alternatively stated, in some examples, the protease that recognizes the protease cleavage site, or that recognizes one or more of the multiple protease cleavage sites, is one or more of thrombin, trypsin, plasmin, prostate-specific antigen (PSA), urokinase plasminogen activator (uPA), urokinase plasminogen activator receptor (uPAR), matrix metalloproteinase (MMP), matriptase (MT-SP1), legumain, a disintegrin and metalloproteinase (ADAM), transmembrane Serine Protease (TMPRSS), Granzyme B, activated protein C, Caspase, Cathepsin, Chymase, Elastase, Guanidinobenzoatase, HtrAl, Human Neutrophil Elastase, Lactoferrin, Marapsin, NS3/4A, PACE4, tissue plasminogen activator (tPA), DESC1, DPP-4, FAP, Hepsin, Matriptase-2, secretase, kallikrein-related peptidase (KLK), and tryptase, or a serine protease, a cysteine-type lysosomal protease, a metalloproteinase, a coagulation factor protease, or an aspartyl-type lysosomal protease. In some examples, the protease cleavage site comprises an amino acid sequence that is cleaved by a protease or type of protease listed herein. In some examples, the protease cleavage site or one or more of the multiple protease cleavage sites is recognized by an endogenous protease or by a protease which is present in a tumor microenvironment. In some examples, the protease that recognizes the protease cleavage site, or that recognizes one or more of the multiple protease cleavage sites, is a protease which is present in a tumor microenvironment.

[0021] In some examples, the linker comprises, in any order, including e.g. from the amino to the carboxy terminus and from the carboxy to the amino terminus, any of the following combinations of cleavage sites: for example, uPA and MMP, uPA and matriptase, MMP and matriptase, matriptase and MMP and uPA. In some examples, the linker further comprises one or more spacer sequences at either or both ends of one or more of each protease cleavage site to provide flexibility. In some examples, the linker further comprises one or more of a GS sequence, e.g. a GS sequence as disclosed herein (e.g. any one or more of SEQ ID NOs: 52, 54, 57), at either or both ends of one or more of each protease cleavage site. In some examples, the CD45-gate CAR comprises one or more of the linkers disclosed herein (e.g. SEQ ID NOs: 7-10, 99-102, 172-177, 108-120), e.g. 1, 2, 3, 4, 5, 15, or any intervening integer value or any range between any two such values. The linkers can be placed, e.g., between the CD45 recruiting domain, e.g., an anti-CD45 scFv, and an antigen binding domain, e.g., an antigen binding domain of a CAR. In some examples, the CD45 recruiting domain comprises an anti-CD45 antibody such as scFv, and the linkers can be placed between VL and VH or between VH and VL of the CD45 binding domain. In some examples, the antigen binding domain comprises 53B6 as disclosed herein. In some examples, the GS sequence provides a spacer sequence that creates flexibility between functional domains. In some examples, either region or both regions flanking the protease cleavage site, or the spacer sequence providing flexibility is not a GS sequence, as many flexible sequences that may function equally well are known in the art. See for example, Whitlow M. et al., An improved linker for single-chain Fv with reduced aggregation and enhanced proteolytic stability. Protein Eng. 1993 Nov;6(8):989-95. doi: 10.1093/protein/6.8.989. PMID: 8309948. In some examples, the spacer sequence creates flexibility and does not interfere with the activities of each functional domain. In some examples, the linker can be any suitable length, e.g. from 15-100 amino acids, 30-100 amino acids, 20-80 amino acids, 20-50 amino acids, or 25, 30, 35, 40, 45, 50, or 55 amino acids in length, or about 25, about 30, about 35, about 40, about 45, about 50, or about 55 amino acids in length.

[0022] In some examples, the protease cleavage site or one or more of the multiple protease cleavage sites is recognized by one or more of a serine protease, a cysteine-type lysosomal protease, an aspartyl-type lysosomal protease and a metalloproteinase. In some examples, a protease that recognizes the protease cleavage site, or that recognizes one or

more of the multiple protease cleavage sites, is one or more of a serine protease, a cysteine-type lysosomal protease, an aspartyl-type lysosomal protease and a metalloproteinase.

[0023] In some examples, the linker comprises between 1 and 15 or between 1 and 10 or 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 cleavage sites, each one of which may be the same as or different from one or more of the other cleavage sites, and/or the number of proteases that recognize the protease cleavage site or sites is between 1 and 15 or between 1 and 10 or is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

[0024] In some examples, the linker comprises an amino GS peptide, a peptide comprising a protease cleavage site, and a carboxy GS peptide. In some examples, the linker comprises the amino acid sequence of SEQ ID NO: 9, which comprises an amino GS peptide (SEQ ID NO: 52), a cleavage peptide (e.g. SEQ ID NO: 53) comprising a protease cleavage site, and a carboxy GS peptide (SEQ ID NO: 54). In some examples, the linker comprises the same amino and carboxy GS peptides and a different cleavage peptide comprising the same or a different protease cleavage site.

[0025] In some examples, the linker of the protease-activating CD45-gate CAR disclosed herein comprises the amino acid sequence of any one or more of SEQ ID NOs: 9, 10, 102, 172-177, and 108-120. In some examples, the linker comprises one or more protease cleavage sites having an amino acid sequence selected from the group consisting of SEQ ID NOs: 32, 89-98 and 103-104, optionally wherein the number of protease cleavage sites is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or between 1 and 15, and, when the linker comprises more than one protease cleavage site, each protease cleavage site is independently the same as or different from one or more of the other protease cleavage sites.

[0026] In some examples, the linker of the protease-activating CD45-gate CAR disclosed herein comprises, on either or both ends of, and contiguous with, one or more of the protease cleavage sites, one or more sets of contiguous GS sequences, wherein each GS sequence has an amino acid sequence of GS, GGS, GGGS (SEQ ID NO: 184), or GGGGS (SEQ ID NO:178), and the sets of contiguous GS sequences comprise GS sequences in any combination, optionally wherein a set of contiguous GS sequences comprises any combination of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 GS sequences or comprises between 1 and 15 GS sequences in any combination. Exemplary examples of linkers comprising such sets of contiguous GS sequences include SEQ ID NOs: 9, 10, 102, 172-177, and 107-120.

[0027] In some examples, the protease-activating CD45-gate CAR comprises more than one linker comprising a protease cleavage site, wherein each linker has an amino acid sequence independently selected from SEQ ID NOs: 9, 10, 102, 172-177, and 107-120, and optionally wherein the number of linkers is 2, 3, 4, 5, or an integer value between 2 and 10. Exemplary examples of protease-activating CD45-gate CARs comprising more than one linker include SEQ ID NOs: 125-128 and 142-145.

[0028] In some examples, the extracellular domain comprises a stalk domain between the antigen binding domain and the transmembrane domain.

[0029] In some examples, the intracellular signaling domain comprises an activating domain. In some examples, the intracellular signaling domain comprises a costimulatory domain. In some examples, the intracellular signaling domain comprises an activating domain and a costimulatory domain. In some examples, the intracellular signaling domain comprises an activating domain such as an ITAM-containing domain. In some examples, the intracellular signaling domain comprises the CD3 zeta intracellular domain. In some examples, the intracellular signaling domain comprises a CD3 zeta domain that comprises the amino acid sequence of SEQ ID NO: 34 or a fragment thereof.

[0030] In some examples, an intracellular signaling domain for use in a protease-activating CD45-gate CAR can be the cytoplasmic sequences of, for example without limitation, the T cell receptor and co- receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability.

[0031] In some examples, the intracellular domain comprises or further comprises at least one costimulatory domain. In some examples, the at least one costimulatory domain is a cytoplasmic signaling region or domain of CD28, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, programmed death-1 (PD- 1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1 (CD1 la/CD 18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRFl), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta,

IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD1 Id, ITGAE, CD103, ITGAL, CD1 la, LFA-1, IT GAM, CD1 lb, ITGAX, CD1 lc, ITGB1, CD29, ITGB2, CD 18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAMI (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD 160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD 19a, a ligand that specifically binds with CD83, or any combination thereof.

[0032] In some examples, the protease-activating CD45-gate CAR comprises a signal sequence. In some examples, the signal sequence is the CD8 signal sequence. In some examples, the signal sequence comprises the amino acid sequence of SEQ ID NOs: 1 or 44.

[0033] In some examples, the amino acid sequence of the protease-activating CD45-gate CAR is or comprises the amino acid sequence of any one of SEQ ID NOs: 16-31 and 121-170, or is or comprises the amino acid sequence of any one of SEQ ID NOs: 16-31 and 121-170 but which lacks any one or more of: the signal sequence, hemagglutinin (HA) tag, and/or V5 tag.

[0034] In an alternative example of the protease-activating CD45-gate CAR disclosed herein, the CD45 recruiting domain itself comprises one or more than one protease cleavage sites (e.g. protease cleavage sites as disclosed herein), and the linker that joins the CD45 recruiting domain to the antigen binding domain either comprises no protease cleavage site or comprises one or more than one protease cleavage sites (e.g. protease cleavage sites as disclosed herein). In an example, protease cleavage produces a CAR attached to a fragment of the CD45 protein, and the protease cleavage partially or wholly relieves the CD45-induced inhibition of the CAR's activity.

[0035] Also provided herein is a CAR that specifically recognizes and binds to MUC16. In an example, the anti-MUC16 CAR comprises the amino acid sequence of any of SEQ ID NO: 11, 75, 76, 77, 78, and 79.

[0036] The present disclosure further provides a protease-activating CD45-gate T cell receptor (TCR). In an example, the protease-activating CD45-gate TCR comprises a

modified TCR α, β, γ, or δ polypeptide, wherein the modification comprises a CD45 recruiting domain joined by a protease-cleavable linker peptide to the amino terminus of the TCR α, β, γ, or δ polypeptide. In an example, the protease-activating CD45-gate TCR comprises a modified single-chain TCR, wherein the modification comprises a CD45 recruiting domain joined by a protease-cleavable linker peptide to the amino terminus of a single-chain TCR. In an example, the polypeptide is a CD45-TCR α, β, γ, or δ polypeptide. In an example, the polypeptide is a CD45-single chain TCR. In another example, provided herein is a nucleic acid that encodes the CD45-gate TCR disclosed herein.

[0037] In another example, provided herein is a nucleic acid encoding the extracellular domain of the protease-activating CD45-gate CAR disclosed herein. In some examples, the encoded extracellular domain comprises a CD45 recruiting domain such as but not limited to a CD45 recruiting domain disclosed herein, the extracellular domain of a CAR such as but not limited to a CAR disclosed herein, and a linker connecting the carboxy terminus of the CD45 recruiting domain to the amino terminus of the CAR extracellular domain, such as but not limited to a linker disclosed herein.

[0038] In another example, provided herein is a nucleic acid that encodes the CD45-gate CAR disclosed herein.

[0038a] In another aspect, the present disclosure refers to a nucleic acid encoding the protease-activating CD45-gate CAR as disclosed herein.

[0039] Also provided herein is a nucleic acid that encodes a polypeptide that comprises the amino acid sequence of any of SEQ ID NOs: 11, 56, 75, 76, 77, 78, 79 and 121-170. In some examples, the polypeptide is an anti-MUC16 CAR as disclosed herein. In some examples, the polypeptide is a protease-activating CD45-gate CAR as disclosed herein.

[0040] In another aspect, provided herein is a vector comprising a nucleic acid as disclosed herein. In an example, the vector is an expression vector. In an example, the vector is a viral vector, a retroviral vector, a DNA vector, a plasmid, a RNA vector, an adenoviral vector, an adenovirus associated vector, a lentiviral vector, or any combination thereof.

[0041] In a further example, provided herein is an engineered cell e.g. an engineered immune cell comprising a protease-activating CD45-gate CAR as disclosed herein.

[0042] In a further example, provided herein is an engineered immune cell comprising a nucleic acid as disclosed herein.

[0043] In another example, provided herein is an engineered immune cell comprising a vector as disclosed herein.

[0043a] In another aspect, the present disclosure refers to an engineered immune cell comprising the nucleic acid as disclosed herein or the vector as disclosed herein, or comprising or functionally expressing the protease-activating CD45-gate CAR as disclosed herein.

[0044] In various examples, any of the engineered cells e.g. any of the engineered immune cells disclosed herein functionally express a protease-activating CD45-gate CAR as disclosed herein. In various examples, any of the engineered cells e.g. any of the engineered immune cells disclosed herein functionally express a protease-activating CD45-gate CAR that comprises the amino acid sequence of any of SEQ ID NOs: 11, 56, 75, 76, 77, 78, 79, and 121-170. Also provided herein are engineered cells e.g. engineered immune cells that functionally express a MUC16-specific CAR that comprises the amino acid sequence of any of SEQ ID NOs: 11, 56, 75, 76, 77, 78, 79 and 121-170. In various examples, any of the engineered cells e.g. any of the engineered immune cells disclosed herein is an isolated cell.

[0045] In various examples, the engineered cell e.g. engineered immune cell disclosed herein functionally expresses the protease-activating CD45-gate CAR disclosed herein from a nucleic acid encoding the protease-activating CD45-gate CAR. In various examples, the engineered cell e.g. engineered immune cell disclosed herein functionally expresses CD45, e.g. functionally expresses the cell's endogenous CD45 gene or functionally expresses CD45, or a fragment or variant of CD45, from an exogenous nucleic acid that encodes CD45 and that was introduced into the cell. In various examples, the engineered cell e.g. engineered immune cell disclosed herein functionally expresses the anti-MUC16 CAR disclosed herein from a nucleic acid encoding the anti-MUC16 CAR. In some examples, the anti-MUC16 CAR is a protease-activating CD45-gate MUC16CAR.

[0046] In various examples, the engineered immune cell disclosed herein is an engineered B cell, mast cell, myeloic-derived phagocyte, T cell e.g. an alpha/beta and/or gamma/delta T cell, tumor infiltrating lymphocyte (TIL), NK cell, TCR-expressing cell, dendritic cell, or NK-T cell. In some examples, the cell is and/or is derived from an autologous T cell. In some examples, the cell is and/or is derived from an allogeneic T cell or an iPSC (induced pluripotent stem cell). In some examples, the cell is and/or is derived from an iPSC-derived T cell.

[0047] In an example, an engineered immune cell as disclosed herein comprises or further comprises one or more genomic modifications of one or more of the endogenous CD52 gene and the endogenous TCR genes, e.g. the TCRα gene. In an example, an engineered immune cell as disclosed herein is a T cell.

[0048] In another example, provided herein is a population of immune cells comprising one or more of the engineered immune cells disclosed herein. In an example, the population comprises about or at least about $1x10^4$, about or at least about $1x10^5$, about or at least about $1x10^6$, about or at least about $1x10^7$, or about or at least about $1x10^8$ engineered cells, e.g. engineered immune cells, as disclosed herein, optionally wherein the population does not comprise more than about $1x10^{10}$ or more than about $1x10^9$ or more than about $5x10^9$ engineered cells, e.g. engineered immune cells, as disclosed herein. In an example, a population of immune cells as disclosed herein is enriched for the engineered immune cell as disclosed herein. In various examples, the population of immune cells is at least 50%, e.g. 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more than 95% engineered cells that are T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and/or myeloic-derived phagocytes. In various examples, the population of immune cells is at least 50%, e.g. 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more than 95% engineered T cells.

[0048a] In another aspect, the present disclosure refers to a population of cells comprising at least about $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$ or $1x10^8$ cells as disclosed herein.

[0049] In another example, provided herein is a composition comprising an engineered cell e.g. an engineered immune cell as disclosed herein or a population of cells as disclosed herein, and a pharmaceutically acceptable carrier.

[0049a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the cell as disclosed herein or the population of cells as disclosed herein and a pharmaceutically acceptable carrier.

[0050] Also provided herein is the use of any of the engineered immune cells e.g. engineered T cells provided herein in the manufacture of a medicament for the treatment of cancer or for inhibiting tumor growth or progression in a subject in need thereof. Also provided herein is any of the engineered immune cells e.g. engineered T cells provided herein for use as a medicament and for use in treating any disease or condition amenable to treatment by CAR T cell therapy. In some examples, the disease or condition is a cancer, autoimmune disease, or infection. In some examples, the disease or condition is a form of cancer.

[0050a] In another aspect, the present disclosure refers to use of the cell as disclosed herein, the population of cells as disclosed herein, or the composition as disclosed herein, in the manufacture of a medicament for treating a disease or condition in a patient.

[0050b] In another aspect, the present disclosure refers to use of the cells as disclosed herein, the population of cells as disclosed herein or the composition as disclosed herein, in the manufacture of a medicament for reducing on-target off-tumor toxicity of CAR T cells or reducing the incidence of side effects in CAR T cell therapy in a patient, wherein the on-target, off-tumor toxicity or incidence of side effects is lower than the on-target, off-tumor

toxicity of control cells, a population of control cells or a composition of control cells, respectively, to be administered under the same conditions, wherein the control cells, population of control cells, or control cells of the composition comprise a CAR that lacks at least one of a CD45 recruiting domain and a linker that comprises a protease cleavage site.

[0050c] In another aspect, the present disclosure refers to a method of dissociating CD45 and a protease-activating CD45-gate CAR in a CAR T cell, the method comprising the steps of: (a) providing a T cell that expresses CD45, (b) introducing a nucleic acid encoding a protease-activating CD45-gate CAR as disclosed here into the T cell to generate a CAR T cell, (c) maintaining the CAR T cell under conditions in which both CD45 and the CAR are functionally expressed at the cell surface, and (d) subjecting the CAR T cell to a protease capable of cleaving the linker, wherein cleavage of the linker by the protease dissociates CD45 from the protease-activating CD45-gate CAR in the CAR T cell.

[0051] In another example, provided herein is a method of treating a condition in a patient comprising administering to the patient an engineered immune cell as disclosed herein. In an example of the method, the engineered immune cell is an allogeneic engineered immune cell derived from a donor other than the patient.

[0052] In another example, provided herein is a method of treating a condition in a patient comprising administering to the patient a population of engineered immune cells as disclosed herein. In an example of the method, the engineered immune cells of the population are derived from one or more allogeneic immune cells from a donor other than the patient.

[0053] In another example, provided herein is a method of treating a condition in a patient comprising administering to the patient a pharmaceutical composition as disclosed herein. In an example of the method, the composition comprises one or more engineered allogeneic immune cells derived from a donor other than the patient.

[0054] In examples of the methods of treating a condition in a patient disclosed herein, the condition may be any disease or condition amenable to treatment by CAR T cell therapy. In some examples, the disease or condition is a cancer, autoimmune disease, or infection. In some examples, the disease or condition is a form of cancer. In some examples, the cancer is a hematological malignancy or non-solid tumor. In some examples, the cancer is a solid cancer or solid tumor. In some examples, the cancer is a hematological malignancy that is acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic eosinophilic leukemia (CEL), myelodysplasia syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM). In some examples, the cancer is a solid cancer that is biliary cancer, bladder cancer, bone or soft tissue carcinoma, brain tumor, breast cancer, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, lung cancer, malignant melanoma, osteosarcoma, ovarian cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, rhabdomyosarcoma, skin cancer,

soft tissue sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, or uterine cancer.

[0055] In another example, provided herein is a method of reducing on-target off-tumor toxicity of CAR T cells comprising administering to a patient a cell as disclosed herein, a population of cells as disclosed herein, or a composition as disclosed herein, wherein the on-target, off-tumor toxicity is lower than the on-target, off-tumor toxicity of control cells, a population of control cells or a composition of control cells, respectively, administered under the same conditions, wherein the control cells, population of control cells, or control cells of the composition comprise a CAR that lacks at least one of a CD45 recruiting domain and a linker that comprises a protease cleavage site.

[0056] In another example, provided herein is a method of increasing the efficiency of CAR T cells or CAR T cell therapy comprising administering to a patient a cell as disclosed herein, a population of cells as disclosed herein, or a composition as disclosed herein, wherein the efficiency is greater than the efficiency of control cells, a population of control cells or a composition of control cells, respectively, administered under the same conditions, wherein the control cells, population of control cells, or control cells of the composition comprise a CAR that lacks at least one of a CD45 recruiting domain and a linker that comprises a protease cleavage site.

[0057] In another example, provided herein is a method of increasing the efficacy of CAR T cell therapy comprising administering to a patient a cell as disclosed herein, a population of cells as disclosed herein, or a composition as disclosed herein, wherein the efficacy is greater than the efficacy of control cells, a population of control cells or a composition of control cells, respectively, administered under the same conditions, wherein the control cells, population of control cells, or control cells of the composition comprise a CAR that lacks at least one of a CD45 recruiting domain and a linker that comprises a protease cleavage site.

[0058] In another example, provided herein is a method of increasing the efficacy of CAR T cell therapy against a solid tumor comprising administering to a patient having a solid tumor a cell as disclosed herein, a population of cells as disclosed herein, or a composition as disclosed herein, wherein the efficacy against the solid tumor is greater than the efficacy against the solid tumor of control cells, a population of control cells or a composition of

control cells, respectively, administered under the same conditions, wherein the control cells, population of control cells, or control cells of the composition comprise a CAR that lacks at least one of a CD45 recruiting domain and a linker that comprises a protease cleavage site.

[0059] In another example, provided herein is a method of reducing the incidence of side effects in CAR T cell therapy comprising administering to a patient a cell as disclosed herein, a population of cells as disclosed herein, or a composition as disclosed herein, wherein the incidence of side effects is lower than the incidence of side effects when control cells, a population of control cells or a composition of control cells, respectively, are administered under the same conditions, wherein the control cells, population of control cells, or control cells of the composition comprise a CAR that lacks at least one of a CD45 recruiting domain and a linker that comprises a protease cleavage site.

[0060] In another example, provided herein is a method of effecting reversible colocalization of CD45 and a CD45-gate CAR in a CAR T cell, the reversible colocalization comprising a CD45-gate CAR recruiting domain binding to a CD45 protein on a CAR T cell surface, resulting in colocalization, which binding and colocalization can be disrupted by a protease cleaving a protease cleavage site in the CD45-gate CAR's linker, thereby reversing the colocalization, the method comprising the steps of:

(a) providing a T cell that expresses CD45,

(b) introducing a nucleic acid encoding a protease-activating CD45-gate CAR as disclosed herein into the T cell,

(c) maintaining the T cell under conditions in which both CD45 and the CAR are functionally expressed at the cell surface,

resulting in a CD45-gate CAR recruiting domain binding to a CD45 protein on the CAR T cell surface and colocalization of the CAR and the CD45 protein, which colocalization can be reversed by a protease cleaving a protease cleavage site in the CD45-gate CAR's linker, wherein the cleaving produces a functional CAR no longer connected by the linker to the CD45-gate CAR recruiting domain.

[0061] In another example, provided herein is a method of effecting reversible reduction of CAR activity by a CD45 protein in a CAR T cell, the reversible reduction of CAR activity comprising a CD45-gate CAR recruiting domain binding to a CD45 protein on a CAR T cell surface, resulting in inactivation of the chimeric antigen receptor by the CD45, which inactivation can be disrupted by a protease cleaving a protease cleavage site in the CD45-gate CAR's linker, resulting in the disassociation of the chimeric antigen receptor and the CD45 protein and ending the inactivation of the chimeric antigen receptor by the CD45, the method comprising the steps of:

(a) providing a T cell that expresses CD45,

(b) introducing a nucleic acid encoding a CD45-gate CAR of any one of claims 1-14 into the T cell,

(c) maintaining the T cell under conditions in which both CD45 and the CAR are functionally expressed at the cell surface,

resulting in a CD45-gate CAR recruiting domain binding to a CD45 protein on the CAR T cell surface, association of the CAR and the CD45 protein, and reduction of the CAR's activity by the CD45 protein, which activity reduction can be reversed by a protease cleaving a protease cleavage site in the CD45-gate CAR's linker, thereby ending the association of the CAR and the CD45 protein, and ending the CD45 protein's reduction of or inhibition of CAR activity, wherein the cleaving produces a functional CAR no longer connected by the linker to the CD45-gate CAR recruiting domain.

[0062] In another example, provided herein is a method of treating with CAR T cell therapy a patient who has a tumor characterized by a protease-rich tumor microenvironment, comprising administering to the patient a cell as disclosed herein, a population of cells as disclosed herein, or a composition as disclosed herein, wherein CAR T cell activity is lower outside the protease-rich tumor microenvironment than in the protease-rich tumor microenvironment.

[0063] In another example, provided herein is a method of regulating cytotoxic activity of a CAR T cell that comprises a CAR as disclosed herein, the method comprising inhibiting the cytotoxic activity of the CAR T cell by associating the CAR with CD45 of the CAR T

cell, and activating the cytotoxic activity of the CAR T cell by subjecting and/or exposing the CAR T cell to a protease that recognizes and cleaves the protease cleavage site.

[0063a] In another aspect, the present disclosure refers to a method of regulating cytotoxic activity of a CAR T cell that comprises the CAR as disclosed herein, the method comprising inhibiting the cytotoxic activity of the CAR T cell by associating the CAR with CD45 of the CAR T cell, and activating the cytotoxic activity of the CAR T cell by subjecting the CAR T cell to a protease that recognizes the protease cleavage site, optionally wherein the method is an in vitro method.

[0064] In examples of the methods of treating a condition in a patient disclosed herein, the cell, population of cells or composition can be administered to the subject on one occasion or can be administered to the subject on two or more occasions spaced at least about 1, 2, 3, 4, 5, 6, 7, or more days apart. In some examples, the disorder can be a viral disease, a bacterial disease, a cancer, an inflammatory disease, an immune disease, or an aging-associated disease.

[0065] Also provided herein are chimeric antigen receptors (CARs) that bind to MUC16 ("MUC16-specific CARs" or "anti-MUC16 CARs" or "MUC16 CARs"). It is demonstrated that the expression of MUC16 specific CARs in T cells is effective to activate the T cells upon contact with MUC16. The MUC16 specific CARs provided herein bind human MUC16 and exhibit cytotoxic activity upon contact with MUC16-expressing cells.

[0066] Accordingly, in another example, provided herein is a MUC16 specific chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular domain comprises a single chain variable fragment (scFv) binding to the extracellular domain of MUC16.

[0065a] In another aspect, the present disclosure refers to a MUC16-specific chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular ligand-binding domain comprises a single chain variable fragment (scFv) having binding specificity for the extracellular domain of MUC16.

[0067] The extracellular domain of MUC16 specific CARs comprises a scFv, wherein the scFv comprises a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions each comprising three complementarity determining regions (CDRs) specific for MUC16.

[0068] In some examples, the VH region comprises (i) a VH complementarity determining region one (CDR1) having the amino acid sequence shown in SEQ ID NO: 60 or 63; (ii) a VH complementarity determining region two (CDR2) having the amino acid sequence shown in SEQ ID NO: 61 or 64; and (iii) a VH complementarity determining region three (CDR3) having the amino acid sequence shown in SEQ ID NO: 62 or 65; and/or the VL region comprises (i) a VL complementarity determining region one (CDR1) having the amino acid sequence shown in SEQ ID NO: 66 or 69; (ii) a VL complementarity determining region two (CDR2) having the amino acid sequence shown in SEQ ID NO: 67

or 70; and (iii) a VL complementarity determining region three (CDR3) having the amino acid sequence shown in SEQ ID NO: 68 or 71.

[0069] In some examples, provided are MUC16 specific CARs comprising a scFv, wherein the scFv comprises a VH region having the sequence shown in SEQ ID NO: 58.

[0070] In some examples, provided are MUC16 specific CARs comprising a scFV, wherein the scFv comprises a VL region having the sequence shown in SEQ ID NO: 59.

[0071] In some examples, provided are MUC16 specific CARs comprising a scFV comprising a VH region and a VL region, wherein the VH region has the sequence shown in SEQ ID NO: 58, and the VL region has the sequence shown in SEQ ID NO: 59.

[0072] In some examples, the VH region comprises the sequence shown in SEQ ID NO: 58, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR and/or the VL region comprises the amino acid sequence shown in SEQ ID NO: 59, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR.

[0073] In some examples, the VH region comprises the amino acid sequence shown in SEQ ID NO: 58 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 59.

[0074] In some examples, each CDR is defined in accordance with the Kabat definition, the Chothia definition, the combination of the Kabat definition and the Chothia definition, the AbM definition, or the contact definition of CDR.

[0075] In some examples, the MUC16 specific CAR intracellular signaling domain comprises a CD3ζ signalling domain. In some examples, the intracellular signaling domain comprises a 4-1BB domain. In some examples, the MUC16 specific CAR comprises a second intracellular signaling domain. In some examples, the second intracellular signaling domain comprises a 4-1BB domain.

[0076] In some examples, the MUC16 specific CARs disclosed herein may comprise a stalk domain between the extracellular ligand-binding domain and the first transmembrane domain. In some examples, the stalk domain is selected from the group consisting of: a CD8α hinge, an IgG1 hinge, and an FcγRIIIα hinge. In some examples, the stalk domain is a human CD8α hinge, a human IgG1 hinge, or a human FcγRIIIα hinge.

[0077] In some examples, the MUC16 specific CARs disclosed herein may comprise one or more epitopes or mimotopes specific for one or more monoclonal antibodies. In some examples, the one or more epitopes or mimotopes specific for a monoclonal antibody includes a CD20 epitope or mimotope. In some examples, the CD20 epitope or mimotope comprises the amino acid sequence shown in SEQ ID NO: 50 or SEQ ID NO: 51.

[0078] In some examples, the MUC16 specific CAR comprises the amino acid sequence shown in any of SEQ ID NO: 11, 75, 76, 77, 78, and 79, with or without a signal sequence. In some examples, the MUC16 specific CAR comprises the amino acid sequence shown in SEQ ID NO: 75, with or without a signal sequence. In some examples, the MUC16 specific CAR comprises the amino acid sequence shown in SEQ ID NO: 76, with or without a signal sequence.

[0079] In some examples, the first transmembrane domain comprises a CD8α chain transmembrane domain.

[0080] In some examples, the MUC16 specific CAR can comprise another extracellular ligand-binding domain which is not specific for MUC16.

[0081] In some examples, the extracellular ligand-binding domain(s), the first transmembrane domain, and intracellular signaling domain(s) are on a single polypeptide.

[0082] In some examples, the CAR can comprise a second transmembrane domain, wherein the first transmembrane domain and the extracellular ligand-binding domain(s) are on a first polypeptide, and wherein the second transmembrane domain and the intracellular signaling domain(s) are on a second polypeptide. In an exemplary example, the first transmembrane domain comprises a transmembrane domain from the α chain of the high-affinity IgE receptor (FcεRI) and the second transmembrane domain comprises a transmembrane domain from the γ or β chain of FcεRI.

[0083] In some examples, the CAR can comprise a third polypeptide comprising a third transmembrane domain fused to an intracellular signaling domain from a co-stimulatory molecule, wherein the third transmembrane domain comprises a transmembrane domain from the γ or β chain of FcεRI.

[0084] In another aspect, provided herein is an isolated polynucleotide comprising a nucleic acid sequence encoding the MUC16 specific CAR described herein.

[0085] In another example, provided herein is an expression vector comprising the polynucleotide encoding the MUC16 specific CAR described herein.

[0085a] In another aspect, the present disclosure refers to an expression vector comprising the polynucleotide as disclosed herein.

[0086] In another example, provided herein is an engineered immune cell expressing at its cell surface membrane a MUC16 specific CAR described herein. In some examples, the engineered immune cell can comprise another CAR which is not specific for MUC16. In some examples, the engineered immune cell can comprise a polynucleotide encoding a suicide polypeptide. In some examples, the suicide polypeptide is RQR8.

[0086a] In another aspect, the present disclosure refers to an engineered immune cell comprising the polynucleotide as disclosed herein or the vector as disclosed herein.

[0086b] In another aspect, the present disclosure refers to an engineered immune cell comprising or functionally expressing the MUC16 specific CAR as disclosed herein.

[0087] In some examples, the engineered immune cell is derived from an inflammatory T-lymphocyte, a cytotoxic T-lymphocyte, a regulatory T- lymphocyte, or a helper T-lymphocyte. In some examples, the engineered immune cell is an inflammatory T-lymphocyte, a cytotoxic T-lymphocyte, a regulatory T- lymphocyte, or a helper T-lymphocyte.

[0088] In some examples, the engineered immune cell can comprise a disruption of one or more endogenous genes, wherein the endogenous gene encodes TCRα, TCRβ, CD52, glucocorticoid receptor (GR), deoxycytidine kinase (dCK), or an immune checkpoint protein such as for example programmed death-1 (PD-1).

[0089] In some examples, the engineered immune cell is obtained from a healthy donor. In some examples, the engineered immune cell is obtained from an individual afflicted with a disease or disorder.

[0090] In another example, provided herein is an engineered immune cell expressing at its cell surface membrane a MUC16 specific CAR as described herein for use as a medicament. In another example, provided herein is a MUC16 specific antibody for use as a medicament. In some examples, the medicament comprising the MUC16 specific CAR expressing immune cells or MUC16 specific antibodies is for use in treatment of a MUC16 associated disease or disorder.

[0091] In one example, provided herein is a population of cells comprising engineered immune cells expressing MUC16 specific CARs described herein, wherein (ii) said population of cells comprises a percentage of stem cell memory and central memory cells greater than 20%, 30% or 40%, and/or (iii) said population of cells achieves a percentage lysis of MUC16 expressing cells greater than 10%, 20%, 30% or 40%.

[0091a] In another aspect, the present disclosure refers to a population of cells comprising between about $1x10^3$ and about $1x10^{10}$ or between about $1x10^4$ and about $1x10^9$ engineered immune cells as disclosed herein.

[0092] In another example, provided herein is a method of engineering an immune cell expressing any one of the MUC16 specific CARs described herein, the method comprising: providing an immune cell; and introducing into the cell at least one polynucleotide encoding said MUC16 specific CAR; whereby said immune cell expresses said MUC16 specific CAR.

[0093] In some examples, the method comprises providing an immune cell; introducing into the cell at least one polynucleotide encoding said MUC16 specific CAR; and introducing at least one polynucleotide encoding a CAR which is not specific for MUC16.

[0094] In another example, provided herein is a method of treating a subject suffering from a MUC16 associated disease or disorder, the method comprising: providing an immune cell expressing at the surface a MUC16 specific CAR as described herein; and administering said immune cells to said subject. The disclosure also provides methods of treating subjects suffering from a MUC16 associated disease or disorder, the method comprising providing MUC16 specific antibodies described herein and administering said antibodies to said subject.

[0095] In some examples, provided herein is a pharmaceutical composition comprising an engineered immune cell expressing MUC16 specific CARs as described herein. In other examples, the disclosure provides pharmaceutical compositions comprising any of the MUC16 specific antibodies described herein.

[0095a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the engineered immune cell as disclosed herein or the population of cells as disclosed herein and a pharmaceutically acceptable carrier.

[0096] In another example, provided herein is a method of treating a condition associated with malignant cells expressing MUC16 in a subject comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition comprising an engineered immune cell as described herein. In some examples, the condition is a cancer.

[0096a] In another aspect, the present disclosure refers to use of the engineered immune cell as disclosed herein, the population of cells as disclosed herein or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a condition in a patient, optionally wherein the condition is a cancer, autoimmune disease or infection.

[0096b] In another aspect, the present disclosure refers to a method of engineering an immune cell expressing the MUC16 specific CAR as disclosed herein, wherein the method comprises: providing an immune cell; and introducing into the cell at least one polynucleotide encoding the MUC16 specific CAR; whereby said immune cell expresses said MUC16 specific CAR.

[0097] In another example, provided herein is a method of inhibiting tumor growth or progression in a subject who has malignant cells expressing MUC16, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition comprising an engineered immune cell as described herein or a MUC16 specific antibody as described herein to the subject.

[0098] In another example, provided herein is a method of inhibiting metastasis of malignant cells expressing MUC16 in a subject, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition comprising an engineered immune cell as described herein or a MUC16 specific antibody as described herein to the subject.

[0099] In another example, provided herein is a method of inducing tumor regression in a subject who has malignant cells expressing MUC16, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition comprising an engineered immune cell as described herein or a MUC16 specific antibody as described herein to the subject.

[0100] In some examples, any of the above methods further comprises administering one or more additional therapies, such as for example, a monoclonal antibody and/or a chemotherapeutic. In some examples, the monoclonal antibody can be, for example, an antibody that binds to a checkpoint inhibitor such as, for example, an anti-PD-1 antibody or an anti-PD-L1 antibody. In some examples, any of the above methods further comprises administering a Receptor Tyrosine Kinase inhibitor, an mTOR inhibitor, an epigenetic modulator, a proteasome inhibitor, an immunomodulatory agent such as lenalidomide, a Hedgehog inhibitor or an Isocitrate Dehydrogenase (IDH) inhibitors, to the subject.

[00100a] In another aspect, the present disclosure refers to an isolated antibody specific for MUC16 comprising a heavy chain variable (VH) region and light chain variable (VL) region, wherein the heavy chain variable (VH) region comprises: (i) a VH complementarity determining region one (VHCDR1) comprising the amino acid sequence shown in SEQ ID NO: 60 or 63; (ii) a VH complementarity determining region two (VHCDR2) comprising the amino acid sequence shown in SEQ ID NO: 61 or 64; and (iii) a VH complementarity determining region three (VHCDR3) comprising the amino acid sequence shown in SEQ ID NO: 62 or 65; and/or the VL region comprises (i) a VL complementarity determining region one (VLCDR1) comprising the amino acid sequence shown in SEQ ID NO: 66; (ii) a VL complementarity determining region two (VLCDR2) comprising the amino acid sequence shown in SEQ ID NO: 67; and (iii) a VL complementarity determining region three (VL CDR3) comprising the amino acid sequence shown in SEQ ID NO: 68.

[00100b] In another aspect, the present disclosure refers to an isolated polynucleotide encoding the antibody specific for MUC16 as disclosed herein.

[00100c] In another aspect, the present disclosure refers to a vector comprising the polynucleotide as disclosed herein.

[00100d] In another aspect, the present disclosure refers to a host cell comprising the polynucleotide as disclosed herein or the vector as disclosed herein. In another aspect, the present disclosure refers to a host cell expressing the antibody as disclosed herein.[00100e] In another aspect, the present disclosure refers to use of the antibody as disclosed herein in the manufacture of a medicament for treating a MUC16 associated disease or condition in a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0101] FIGs. 1A-1B. FIG. 1A (left side) illustrates a potential mechanism of action for an exemplary protease-activating CD45-gate CAR. In the cell membrane of a T cell, for example, a linker peptide tethers (i.e. joins or connects) a CD45 recruiter domain to a CAR. The linker peptide comprises at least one protease site. The recruiter recognizes and binds to a CD45 protein, thus holding the CAR and CD45 protein in relatively close proximity to each other. This close proximity permits the CD45 to inactivate the CD3zeta domain. Thus, the CAR activity is reduced even when it binds to its target antigen, so long as the linker peptide is intact. When a protease cleaves the linker peptide (Fig. 1A, right side) (e.g. when the CAR T cell is in the environment of a tumor that expresses the protease that recognizes the protease site in the linker), the CAR is "released" from the CD45 and ceases to be inactivated by the CD45. The CAR T cell thus selectively becomes active primarily in the vicinity of its target tumor, thereby reducing on-target off-tumor toxicity. Fig. 1B

WHAT IS CLAIMED IS

1. A protease-activating CD45-gate chimeric antigen receptor (CD45-gate CAR) comprising an extracellular domain, a transmembrane domain, and an intracellular domain, wherein the extracellular domain comprises:

a CD45 recruiting domain,

an antigen binding domain, and

a linker connecting the carboxy terminus of the CD45 recruiting domain to the amino terminus of the antigen binding domain, wherein the linker comprises one or more protease cleavage site that is recognized by at least one protease,

and optionally wherein the intracellular domain comprises at least one signaling domain that can be reversibly inactivated by CD45.

2. The protease-activating CD45-gate CAR of claim 1, wherein the CD45 recruiting domain comprises one or more of an anti-CD45 scFv, an anti-CD45 antibody antigen binding domain, an anti-CD45 nanobody, a viral protein binder of CD45, a truncated viral protein binder of CD45, an anti-CD45 Fab, an anti-CD45 camelid VHH, a CD45-binding protein, an endogenous CD45 binder, a truncated endogenous CD45 binder, a peptide or protein that interacts with CD45, full-length or truncated UL11, full-length or truncated sec49K, and full-length or truncated BTN3A1.

3. The protease-activating CD45-gate CAR of claim 1 or 2, wherein the CD45 recruiting domain comprises an anti-CD45 antibody antigen binding domain, optionally wherein the anti-CD45 antibody antigen binding domain comprises an anti-CD45 scFv.

4. The protease-activating CD45-gate CAR of any one of claims 1-3, wherein the CD45 recruiting domain comprises one or more of truncated UL11, truncated sec49K, and truncated BTN3A1.

5. The protease-activating CD45-gate CAR of any one of claims 1-4, wherein the CD45 recruiting domain comprises the amino acid sequence of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15, or SEQ ID NO:56 or SEQ ID NO:56 without the signal sequence of SEQ ID NO:1.

6. The protease-activating CD45-gate CAR of any one of the preceding claims, wherein the antigen binding domain specifically binds BCMA, MUC16 (also known as CA125), EGFR, EGFRvIII, MUC1, Flt-3, WT-1, CD20, CD23, CD30, CD38, CD70, CD33, CD133, MHC-WT1 , TSPAN10, MHC-PRAME, MHC-NY-ESO1, HER2 (ERBB2), CAIX (Carbonic anhydrase IX), LIV1, ADAM10, CHRNA2, LeY, NKG2D, CS1, CD44v6, ROR1, CD19, Claudin-18.2 (Claudin-18A2, or Claudin18 isoform 2), PSCA, DLL3 (Delta-like protein 3, Drosophila Delta homolog 3, Delta3), Mud 7 (Mucin17, Muc3, Muc3), FAP alpha (Fibroblast Activation Protein alpha), Ly6G6D (Lymphocyte antigen 6 complex locus protein G6d, c6orf23, G6D, MEGT1, NG25), PSMA, MSLN, or RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43).

7. The protease-activating CD45-gate CAR of any one of the preceding claims, wherein the linker comprises a GS sequence on either or both ends of each of the one or more protease cleavage sites.

8. The protease-activating CD45-gate CAR of claim 7, wherein each GS sequence comprises a series of linked GS peptides each of which has an amino acid sequence of GS, GGS, GGGS (SEQ ID NO: 184), or GGGGS (SEQ ID NO:178), wherein the linked GS peptides are linked in any order and in any combination, optionally wherein a GS sequence comprises any combination of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 GS peptides or comprises between 1 and 15 GS peptides in any order and combination.

9. The protease-activating CD45 gate CAR of any one of the preceding claims, wherein the linker comprising at least one protease cleavage site has a length of 15-100 amino acids, 30-100 amino acids, 15-75 amino acids, 20-50 amino acids, 20 amino acids, 25 amino acids, 30 amino acids, 35 amino acids, 40 amino acids, 45 amino acids, or 50 amino acids, or about 20 amino acids, about 25 amino acids, about 30 amino acids, about 35 amino acids, about 40 amino acids, about 45 amino acids, or about 50 amino acids.

10. The protease-activating CD45-gate CAR of any one of the preceding claims, wherein the one or more protease cleavage site can be cleaved by at least one protease which is present in a tumor microenvironment.

11. The protease-activating CD45-gate CAR of any one of the preceding claims, wherein the linker comprises at least one protease cleavage site that can be cleaved by a serine protease, a cysteine-type lysosomal protease, a metalloproteinase, a coagulation factor protease, an aspartyl-type lysosomal protease, matrix metalloproteinase (MMP),

matriptase (MT-SP1), trypsin, plasmin, prostate-specific antigen (PSA), urokinase plasminogen activator (uPA), urokinase plasminogen activator receptor (uPAR), legumain, a disintegrin and metalloproteinase (ADAM), a transmembrane Serine Protease (TMPRSS), Granzyme B, activated protein C, Caspase, Cathepsin, Chymase, Elastase, Guanidinobenzoatase, HtrAl, Human Neutrophil Elastase, Lactoferrin, Marapsin, NS3/4A, PACE4, tissue plasminogen activator (tPA), thrombin, DESC1, DPP-4, FAP, Hepsin, Matriptase-2, secretase, kallikrein-related peptidase (KLK), or tryptase.

12. The protease-activating CD45-gate CAR of any one of the preceding claims, wherein the linker comprises one or more protease cleavage sites that comprises the amino acid sequence of SEQ ID NO: 32, 91-98, 103-105 or SEQ ID NO: 106.

13. The protease-activating CD45-gate CAR of any one of the preceding claims, wherein the linker comprises one or more amino acid sequences of SEQ ID NO: 8-10, 53, 89, 90, 99-102, 107-120, 172-176 or SEQ ID NO: 177.

14. The protease-activating CD45-gate CAR of any one of the preceding claims, wherein the linker comprises two or more protease cleavage sites and each cleavage site is the same as or different from any of the other cleavage sites.

15. The protease-activating CD45-gate CAR of any one of the preceding claims, wherein the extracellular domain further comprises a stalk domain between the antigen binding domain and the transmembrane domain.

16. The protease-activating CD45-gate CAR of any one of the preceding claims, wherein the intracellular domain comprises the cytoplasmic signaling domain of one or more of CD3 zeta, CD28, PD1, and CD2, optionally wherein the intracellular domain comprises a CD3 zeta domain comprising the amino acid sequence of SEQ ID NO: 34 or a fragment thereof.

17. The protease-activating CD45-gate CAR of any one of the preceding claims, wherein the intracellular domain comprises at least one costimulatory domain, wherein the at least one costimulatory domain is a signaling region of CD28, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, programmed death-1 (PD- 1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1 (CD1 la/CD 18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF

receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRFl), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD1 Id, ITGAE, CD103, ITGAL, CD1 la, LFA-1, IT GAM, CD1 lb, ITGAX, CD1 lc, ITGB1, CD29, ITGB2, CD 18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAMI (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD 160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD 19a, a ligand that specifically binds with CD83, or any combination thereof.

18. The protease-activating CD45-gate CAR of any one of the preceding claims, wherein the protease-activating CD45-gate CAR comprises the amino acid sequence of any one of SEQ ID NOs: 11, 16-31, 56, 58, 59, 75, 76, 77, 78, 79, and 121-170, or a vaiant thereof.

19. The protease-activating CD45-gate CAR of claim 18, wherein the variant does not comprise the amino acid sequence of the HA tag of SEQ ID NO: 2 and/or does not comprise the amino acid sequence of the V5 peptide motif of SEQ ID NO: 55 and/or does not comprise the signal sequence of SEQ ID NO: 1.

20. A nucleic acid encoding the protease-activating CD45-gate CAR of any one of claims 1-19.

21. A vector comprising the nucleic acid of claim 20.

22. The vector of claim 21, wherein the vector is a retroviral vector, a DNA vector, a plasmid, a RNA vector, an adenoviral vector, an adenovirus associated vector, a lentiviral vector, or any combination thereof, optionally wherein the vector is an expression vector.

23. An engineered immune cell comprising the nucleic acid of claim 20 or the vector of claim 21 or 22, or comprising or functionally expressing the protease-activating CD45-gate CAR of any one of claims 1-19.

24. The engineered immune cell of claim 23, wherein the immune cell is a T cell, tumor infiltrating lymphocyte (TIL), iPSC-derived T cell, NK cell, TCR-expressing cell, dendritic cell, or NK-T cell.

25. The engineered immune cell of claim 24, wherein the cell is an autologous T cell or an allogeneic T cell.

26. A population of cells comprising at least about $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$ or $1x10^8$ cells of any one of claims 23-25.

27. A pharmaceutical composition comprising the cell of any one of claims 23-25 or the population of cells of claim 26 and a pharmaceutically acceptable carrier.

28. Use of the cell of any one of claims 23-25, the population of cells of claim 26, or the composition of claim 27, in the manufacture of a medicament for treating a disease or condition in a patient.

29. The use of claim 28, wherein the disease or condition is cancer, optionally wherein the cancer is solid tumor cancer or liquid tumor cancer.

30. Use of the cells of any one of claims 23-25, the population of cells of claim 26 or the composition of claim 27, in the manufacture of a medicament for reducing on-target off-tumor toxicity of CAR T cells or reducing the incidence of side effects in CAR T cell therapy in a patient, wherein the on-target, off-tumor toxicity or incidence of side effects is lower than the on-target, off-tumor toxicity of control cells, a population of control cells or a composition of control cells, respectively, to be administered under the same conditions, wherein the control cells, population of control cells, or control cells of the composition comprise a CAR that lacks at least one of a CD45 recruiting domain and a linker that comprises a protease cleavage site.

31. A method of dissociating CD45 and a protease-activating CD45-gate CAR in a CAR T cell, the method comprising the steps of:

(a) providing a T cell that expresses CD45,

(b) introducing a nucleic acid encoding a protease-activating CD45-gate CAR of any one of claims 1-19 into the T cell to generate a CAR T cell,

(c) maintaining the CAR T cell under conditions in which both CD45 and the CAR are functionally expressed at the cell surface, and

(d) subjecting the CAR T cell to a protease capable of cleaving the linker,

wherein cleavage of the linker by the protease dissociates CD45 from the protease-activating CD45-gate CAR in the CAR T cell.

32. A method of regulating cytotoxic activity of a CAR T cell that comprises the CAR of any one of claims 1-19, the method comprising inhibiting the cytotoxic activity of the CAR T cell by associating the CAR with CD45 of the CAR T cell, and activating the cytotoxic activity of the CAR T cell by subjecting the CAR T cell to a protease that recognizes the protease cleavage site, optionally wherein the method is an in vitro method.

33. A MUC16-specific chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular ligand-binding domain comprises a single chain variable fragment (scFv) having binding specificity for the extracellular domain of MUC16.

34. The MUC16-specific chimeric antigen receptor (CAR) of claim 33, the extracellular ligand-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the heavy chain variable (VH) region comprises:

(i) a VH complementarity determining region one (VHCDR1) comprising the amino acid sequence shown in SEQ ID NO: 60 or 63; (ii) a VH complementarity determining region two (VHCDR2) comprising the amino acid sequence shown in SEQ ID NO: 61 or 64; and (iii) a VH complementarity determining region three (VHCDR3) comprising the amino acid sequence shown in SEQ ID NO: 62 or 65; and/or the VL region comprises

(i) a VL complementarity determining region one (VLCDR1) comprising the amino acid sequence shown in SEQ ID NO: 66; (ii) a VL complementarity determining region two (VLCDR2) comprising the amino acid sequence shown in SEQ ID NO: 67; and (iii) a VL complementarity determining region three (VL CDR3) comprising the amino acid sequence shown in SEQ ID NO: 68.

35. The MUC16-specific chimeric antigen receptor (CAR) of claim 33 or 34, wherein the extracellular ligand-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the heavy chain variable (VH) region comprises the amino acid sequence shown in SEQ ID NO: 58 and/or the light chain variable (VL) region comprises the amino acid sequence shown in SEQ ID NO: 59.

36. The MUC16-specific chimeric antigen receptor of any one of claims 33-37, further comprising a rituximab mimotope sequence of SEQ ID NO: 50.

37. The MUC16-specific chimeric antigen receptor (CAR) of any one of claims 33-36, wherein the MUC16 specific CAR comprises the amino acid sequence shown in any one of SEQ ID NOs: 11, 75, 76, 77, 78, and 79, with or without a signal sequence.

38. An isolated polynucleotide comprising a nucleic acid sequence encoding the MUC16 specific CAR of any one of claims 33-37.

39. An expression vector comprising the polynucleotide of claim 38.

40. An engineered immune cell comprising the polynucleotide of claim 38 or the vector of claim 39.

41. An engineered immune cell comprising or functionally expressing the MUC16 specific CAR of any one of claims 33-37.

42. A population of cells comprising between about $1x10^3$ and about $1x10^{10}$ or between about $1x10^4$ and about $1x10^9$ engineered immune cells of claim 40 or 41.

43. A pharmaceutical composition comprising the engineered immune cell of any one of claims 40-41 or the population of cells of claim 42 and a pharmaceutically acceptable carrier.

44. Use of the engineered immune cell of any one of claims 40-41, the population of cells of claim 42 or the pharmaceutical composition of claim 43, in the manufacture of a medicament for treating a condition in a patient, optionally wherein the condition is a cancer, autoimmune disease or infection.

45. The use of claim 44, wherein the condition is a cancer selected from acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic eosinophilic leukemia (CEL), myelodysplasia syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM), biliary cancer, bladder cancer, bone or soft tissue carcinoma, brain tumor, breast cancer, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, lung cancer, malignant melanoma, osteosarcoma, ovarian cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid

cancer, prostate cancer, renal cancer, renal cell carcinoma, rhabdomyosarcoma, skin cancer, soft tissue sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, and uterine cancer.

46. The use of any one of claims 44-45, wherein the engineered immune cells, the engineered immune cells of the population or the engineered immune cells of the pharmaceutical composition are derived from one or more immune cells from the patient or from one or more allogeneic immune cells from a donor other than the patient.

47. A method of engineering an immune cell expressing the MUC16 specific CAR of any one of claims 33-37, wherein the method comprises: providing an immune cell; and introducing into the cell at least one polynucleotide encoding the MUC16 specific CAR; whereby said immune cell expresses said MUC16 specific CAR.

48. An isolated antibody specific for MUC16 comprising a heavy chain variable (VH) region and light chain variable (VL) region, wherein the heavy chain variable (VH) region comprises:

(i) a VH complementarity determining region one (VHCDR1) comprising the amino acid sequence shown in SEQ ID NO: 60 or 63; (ii) a VH complementarity determining region two (VHCDR2) comprising the amino acid sequence shown in SEQ ID NO: 61 or 64; and (iii) a VH complementarity determining region three (VHCDR3) comprising the amino acid sequence shown in SEQ ID NO: 62 or 65; and/or the VL region comprises (i) a VL complementarity determining region one (VLCDR1) comprising the amino acid sequence shown in SEQ ID NO: 66; (ii) a VL complementarity determining region two (VLCDR2) comprising the amino acid sequence shown in SEQ ID NO: 67; and (iii) a VL complementarity determining region three (VL CDR3) comprising the amino acid sequence shown in SEQ ID NO: 68.

49. The antibody of claim 48, wherein the heavy chain variable (VH) region comprises the amino acid sequence shown in SEQ ID NO: 58 and/or the light chain variable (VL) region comprises the amino acid sequence shown in SEQ ID NO: 59.

50. An isolated polynucleotide encoding the antibody specific for MUC16 of claim 48 or 49.

51. A vector comprising the polynucleotide of claim 50.

52. A host cell comprising the polynucleotide of claim 50 or the vector of claim 51.

53. A host cell expressing the antibody of claim 48 or 49.

54. Use of the antibody of claim 48 or 49 in the manufacture of a medicament for treating a MUC16 associated disease or condition in a subject in need thereof.