

CLAIMS

What is claimed is:

1. An isolated polynucleotide encoding a chimeric antigen receptor (CAR) comprising (i) an antigen binding molecule, (ii) a costimulatory domain, and (iii) an activating domain,

wherein the costimulatory domain comprises an extracellular domain with a hinge, a transmembrane domain, and an intracellular domain, characterized in that the said hinge is a truncated hinge domain (THD) consisting of:

(a) amino acids 123-152 of SEQ ID NO: 1, or

(b) a variant of (a) with one to six amino acids added to or deleted from the C-terminus of the 123-152 segment of SEQ ID NO: 1, or the N-terminus, or both the N-terminus and C-terminus; or

(c) a variant of (a) or (b), having the same length but with an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the amino acid sequence of (a) or (b).

2. The polynucleotide of claim 1, wherein the one to six amino acids are heterologous amino acids in that they are not part of the naturally occurring costimulatory protein sequence of SEQ ID NO: 1.

3. The polynucleotide of claim 1 or 2, wherein the transmembrane domain is a transmembrane domain of 4-1BB/CD137, an alpha chain of a T cell receptor, a beta chain of a T cell receptor, CD3 epsilon, CD4, CD5, CD8 alpha, CD9, CD16, CD19, CD22, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD154, or a zeta chain of a T cell receptor, or any combination thereof or wherein the transmembrane domain comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 5.

4. The polynucleotide of claim 1 or 2, wherein the intracellular domain comprises a signaling region of 4-1BB/CD137, activating NK cell receptors, B7-H3, BAFRR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-H3), CD29, CD3 delta, CD3 epsilon, CD3 gamma, CD30, CD4,

CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8alpha, CD8beta, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, cytokine receptors, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, ICAM-1, Ig alpha (CD79a), IL2R beta, IL2R gamma, IL7R alpha, Immunoglobulin-like proteins, inducible T cell costimulator (ICOS), integrins, ITGA4, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, LFA-1, a ligand that specifically binds with CD83, LIGHT, LIGHT (tumor necrosis factor superfamily member 14; TNFSF14), LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1 (CD11a/CD18)), MHC class I molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), signaling lymphocytic activation molecules (SLAM proteins), SLAM (SLAMF1; CD150; IPO-3), SLAMF4 (CD244; 2B4), SLAMF6 (NTB-A; Lyl08), SLAMF7, SLP-76, TNF receptor proteins, TNFR2, a Toll ligand receptor, TRANCE/RANKL, VLA1, or VLA-6, or a combination thereof, or wherein the intracellular domain comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 7.

5. The polynucleotide of claim 1 or 2, wherein the antigen binding molecule comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises 3 complementarity determining regions (CDRs) and the VL comprises 3 CDRs.

6. The polynucleotide of claim 1 or 2, wherein the antigen binding molecule specifically binds an antigen selected from the group consisting of 5T4, alphafetoprotein, B cell maturation antigen (BCMA), CA-125, carcinoembryonic antigen, CD19, CD20, CD22, CD23, CD30, CD33, CD56, CD123, CD138, c-Met, CSPG4, C-type lectin-like molecule 1 (CLL-1), EGFRvIII, epithelial tumor antigen, ERBB2, FLT3, folate binding protein, GD2, GD3, HER1-HER2 in combination, HER2-HER3 in combination, HER2/Neu, HERV-K, HIV-1 envelope glycoprotein gp41, HIV-1 envelope glycoprotein gp120, IL-11Ralpha, kappa chain, lambda chain, melanoma-associated antigen, mesothelin, MUC-1, mutated p53, mutated ras, prostate-specific antigen, ROR1, or VEGFR2, or a combination thereof.

7. The polynucleotide of claim 1 or 2, wherein the activation domain comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 9 or SEQ ID NO: 251.

8. The polynucleotide of any one of claims 1 to 7, wherein the CAR further comprises a leader peptide, wherein the leader peptide preferably comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 11.

9. A vector comprising the polynucleotide of any one of claims 1 to 8, wherein the vector is preferably an adenoviral vector, an adenovirus-associated vector, a DNA vector, a lentiviral vector, a plasmid, a retroviral vector, or an RNA vector, or any combination thereof.

10. A cell comprising the polynucleotide of any one of claims 1 to 8, the vector of claim 9, or any combination thereof wherein the cell is preferably a T cell.

11. A composition comprising the polynucleotide of any one of claims 1 to 8, the vector of claim 9, or the cell of claim 10 which is preferably formulated to be delivered to a subject, optionally, comprising at least one pharmaceutically-acceptable excipient.

12. An *in vitro* method of making a cell expressing a CAR comprising transducing a cell with the polynucleotide of any one of claims 1 to 8 under suitable conditions optionally comprising a further step of isolating the cell.

13. A polynucleotide of any one of claims 1 to 8, a vector of claim 9, a cell of claim 10, or a composition of claim 11 for use in therapy, preferably for inducing an immunity against a tumor or for treating a cancer, wherein the cancer is preferably a hematologic cancer, a cancer of the white blood cells or a cancer of the plasma cells..

14. The polynucleotide for use of claim 13, the vector for use of claim 13, the cell for use of claim 13 or the composition for use of claim 13, wherein the cancer is leukemia, lymphoma, or myeloma or wherein the cancer is acute lymphoblastic leukemia (ALL) (including non T cell ALL), acute myeloid leukemia, B cell prolymphocytic leukemia, B-cell acute lymphoid leukemia ("BALL"), blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloid leukemia, chronic or acute leukemia, diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), hairy cell leukemia, Hodgkin's Disease, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma (NHL), plasma cell proliferative

disorder (including asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, plasmacytomas (including plasma cell dyscrasia; solitary myeloma; solitary plasmacytoma; extramedullary plasmacytoma; and multiple plasmacytoma), POEMS syndrome (also known as Crow-Fukase syndrome; Takatsuki disease; and PEP syndrome), primary mediastinal large B cell lymphoma (PMBC), small cell- or a large cell-follicular lymphoma, splenic marginal zone lymphoma (SMZL), systemic amyloid light chain amyloidosis, T-cell acute lymphoid leukemia (“TALL”), T-cell lymphoma, transformed follicular lymphoma, or Waldenstrom macroglobulinemia, or a combination thereof.