

CHIMERIC ANTIGEN RECEPTORS TARGETING CD70

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Appl. No. 62/775,246, filed December 4, 2018, U.S. Provisional Patent Appl. No. 62/641,869, filed March 12, 2018, U.S. Provisional Patent Appl. No. 62/641,873, filed March 12, 2018, U.S. Provisional Patent Appl. No. 62/625,009, filed February 1, 2018, and U.S. Provisional Patent Appl. No. 62/625,019, filed February 1, 2018, each of which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] This application is being filed electronically via EFS-Web and includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled “ALGN_014_03WO_SeqList_ST25.txt” created on January 24, 2019 and having a size of ~725 kilobytes. The sequence listing contained in this .txt file is part of the specification and is incorporated herein by reference in its entirety.

FIELD

[0003] The disclosure relates to chimeric antigen receptors (CAR). CARs are able to redirect immune cell specificity and reactivity toward a selected target exploiting the ligand-binding domain properties. In particular, the disclosure relates to CARs that specifically bind to Cluster of Differentiation 70 (CD70-specific CARs). The disclosure further relates to polynucleotides encoding CD70-specific CARs and isolated cells expressing CD70-specific CARs at their surface. The disclosure further relates to methods for engineering immune cells expressing CD70-specific CARs at their surface. The disclosure is particularly useful for the treatment of cancer such as lymphoma, leukemia, glioma or Renal Cell Carcinoma (RCC). The disclosure further relates to immune cells comprising the CD70-specific CARs (CD70-specific CAR-T cells), compositions comprising the CD70-specific CAR-T cells, and methods of using the CD70-specific CAR-T cells for treating conditions associated with malignant cells expressing CD70 (*e.g.*, cancer).

BACKGROUND

[0004] Adoptive transfer of immune cells genetically modified to recognize malignancy-associated antigens is showing promise as a new approach to treating cancer (see, *e.g.*, Brenner et al., *Current Opinion in Immunology*, 22(2): 251-257 (2010); Rosenberg et al., *Nature Reviews Cancer*, 8(4): 299-308 (2008)). T cells can be genetically modified to express chimeric antigen receptors (CARs), fusion proteins comprised of an antigen recognition moiety and T cell activation domains (see, *e.g.*, Eshhar et al., *Proc. Natl. Acad. Sci. USA*, 90(2): 720-724 (1993), and Sadelain et al., *Curr. Opin. Immunol*, 21(2): 215-223 (2009)).

[0005] Cluster of Differentiation 70 (CD70, CD27LG or TNFSF7) is a member of the tumor necrosis factor (TNF) superfamily and the ligand for CD27, a TNF superfamily receptor. The transient interaction between CD27 and CD70 provides T cell costimulation complementary to that provided by CD28. CD70 is expressed on hematological cancers such as Non-Hodgkin's Lymphoma and Hodgkin's disease as well as on solid tumors such as Glioblastoma and Renal Cell Carcinoma; with its expression on ccRCC being nearly uniform (see *e.g.*, Grewal I., et al., *Expert Opinion on Therapeutic Targets*, 12(3): 341-351 (2008)). Adoptive transfer of T cells genetically modified to recognize malignancy-associated antigens is showing promise as a new approach to treating cancer (see, *e.g.*, Brenner et al., *Current Opinion in Immunology*, 22(2): 251-257 (2010); Rosenberg et al., *Nature Reviews Cancer*, 8(4): 299-308 (2008)). T cells can be genetically modified to express chimeric antigen receptors (CARs), which are fusion proteins comprised of an antigen recognition moiety and T cell activation domains (see, *e.g.*, Eshhar et al., *Proc. Natl. Acad. Sci. USA*, 90(2): 720-724 (1993), and Sadelain et al., *Current Opinion in Immunology*, 21(2): 215-223 (2009)). Expression of CD70 on normal tissues is limited to activated T cells, B cells, NK cells, and dendritic cells. However, CD70 expression on activated T cells may pose a concern for production of CAR T cells due to potential target-driven T cell differentiation, exhaustion, and fratricide during the production process.

[0006] Renal Cell Carcinoma (RCC) is a cancer that originates in the renal cortex and accounts for about 90% of cancers in the kidney. Based on histology, RCC can be classified into several sub-types, of which Clear Cell Renal Cell Carcinoma (ccRCC) is the most common and leads to the most deaths. Each year, over 320,000 cases of RCC are reported worldwide leading to roughly 140,000 deaths. The incidence of RCC has risen steadily over the last 10 years and accounts for 2-3% of all adult malignancies. Patients with early stage localized tumors can opt for surgical resection; however, localized disease can undergo early hematogenous

dissemination leading to metastasis. Sites of early metastases include lungs, lymph nodes, liver, bone, and brain; less commonly the adrenal glands, and the contralateral kidney. Patients with advanced disease face high morbidity rates with a 5-year median survival rate of 53% for stage III disease and only 8% for metastatic disease. Current first-line treatment options for advanced disease include small molecule Tyrosine Kinase Inhibitors (TKIs) such as sunitinib and pazopanib that target Vascular Endothelial Growth Factor (VEGF) receptor, monoclonal antibody targeting VEGF such as bevacizumab, mammalian target of Rapamycin (mTOR) inhibitor temsirolimus, as well as high dose IL-2. Although these VEGF-targeted therapies have improved over-all survival, long-term drug resistance leads to disease relapse and treatment for advanced disease still remains an unmet need (see, *e.g.*, Zarrabi, K. et al., Journal of Hematology and Oncology, 10:38 (2017)).

[0007] Accordingly, there is a need for alternative treatments for cancer and in particular malignancies involving aberrant expression of CD70. Novel immunotherapies, such as CAR T therapy, have the potential to significantly improve the outcome for patients with cancer where CD70 is expressed, for example in mRCC. Accordingly, treatment to a cancer (such as, *e.g.*, mRCC) using CD70-specific CARs and CD70-specific CAR-T cells would make a promising therapeutic agent. Provided herein are methods and compositions addressing this need.

SUMMARY

[0008] Chimeric antigen receptors (CARs) that bind to CD70 are provided herein, as well as methods of making and methods of using the same. Also provided herein are immune cells, *e.g.* T-cells comprising such CD70 CARs. It is demonstrated that certain CD70-specific CARs are effective when expressed in T cells to activate T cells upon contact with CD70. Advantageously, the CD70-specific CARs provided herein bind human CD70. Also advantageously, the CD70-specific CAR-T cells provided herein exhibit cytotoxic activity upon contact with CD70-expressing cells. Also provided herein are antibodies that bind to CD70, as well as methods of making and methods of using the same. CD70-specific antibodies provided herein bind human CD70.

[0009] In one aspect, the disclosure provides Cluster of Differentiation 70 (CD70) 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 Fv fragment (scFv) binding to the extracellular domain of CD70.

[0010] In some embodiments, the disclosure provides a CD70-specific CAR wherein the extracellular domain of a CAR provided herein comprises a scFv comprising a heavy chain variable (VH) region comprising three CDRs from the VH region comprising the sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379 or 381; and a light chain variable (VL) region comprising three CDRs from the VL region shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378 or 380. In some embodiments, the VH region comprises the sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379 or 381, 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: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378 or 380 or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR.

[0011] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 49, 50, or 51; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 52 or 53; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 54; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 193; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 194; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 195.

[0012] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 2 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 1.

[0013] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 55, 56, or 57; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 58 or 59; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 60; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 196; a VL CDR2 comprising the amino acid sequence shown in

SEQ ID NO: 197; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 198.

[0014] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 4 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 3.

[0015] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 61, 62, or 63; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 64 or 65; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 66; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 199; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 200; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 201.

[0016] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 6 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 5.

[0017] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 67, 68, or 69; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 70 or 71; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 72; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 202; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 203; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 204.

[0018] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 8 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 7.

[0019] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 73, 74, or 75; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 76 or 77; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 78; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 205; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 206; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 207.

[0020] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 10 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 9

[0021] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 79, 80, or 81; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 82 or 83; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 84; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 208; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 209; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 210.

[0022] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 12 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 11.

[0023] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 85, 86, or 87; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 88 or 89; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 90; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 211; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 212; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 213.

In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 14 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 13.

[0024] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 91, 92, or 93; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 94 or 95; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 96; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 214; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 215; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 216.

[0025] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 16 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 15.

[0026] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 97, 98, or 99; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 100 or 101; and a VH CDR3 comprising the amino acid

sequence shown in SEQ ID NO: 102; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 217; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 218; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 219.

[0027] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 18 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 17.

[0028] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 103, 104, or 105; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 106 or 107; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 108; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 220; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 221; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 222.

[0029] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 20 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 19.

[0030] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 109, 110, or 111; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 112 or 113; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 114; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 223; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 224; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 225.

[0031] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 22 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 21.

[0032] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 115, 116, or 117; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 118 or 119; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 120; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 226; a VL CDR2 comprising the amino acid

sequence shown in SEQ ID NO: 227; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 228.

[0033] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 24 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 23.

[0034] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 121, 122, or 123; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 124 or 125; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 126; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 229; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 230; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 231.

[0035] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 26 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 25.

[0036] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 127, 128, or 129; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 130 or 131; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 132; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 232; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 233; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 234.

[0037] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 28 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 27.

[0038] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 133, 134, or 135; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 136 or 137; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 138; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 235; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 236; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 237.

[0039] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 30 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 29.

[0040] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 139, 140, or 141; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 142 or 143; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 144; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 238; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 239; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 240.

[0041] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 32 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 31.

[0042] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 145, 146, or 147; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 148 or 149; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 150; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 241; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 242; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 243.

[0043] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 34 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 33.

[0044] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 151, 152, or 153; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 154 or 155; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 156; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 244; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 245; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 246.

[0045] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 36 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 35.

[0046] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 157, 158, or 159; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 160 or 161; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 162; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 247; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 248; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 249.

[0047] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 38 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 37.

[0048] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 163, 164, or 165; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 166 or 167; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 168; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 250; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 251; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 252.

[0049] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 40 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 39.

[0050] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 169, 170, or 171; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 172 or 173; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 174; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 253; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 254; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 255.

[0051] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 42 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 41.

[0052] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 175, 176, or 177; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 178 or 179; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 180; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 256; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 257; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 258.

[0053] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 44 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 43.

[0054] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 181, 182, or 183; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 184 or 185; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 186; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 259; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 260; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 261.

[0055] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 46 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 45.

[0056] In some embodiments, the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 187, 188, or 189; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 190 or 191; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 192; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 262; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 263; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 264.

[0057] In some embodiments, the VH region comprises the amino acid sequence shown in SEQ ID NO: 48 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 47.

[0058] In some embodiments, 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.

[0059] In some embodiments, each CDR is defined in accordance with the Kabat definition, the Chothia definition, the extended definition, the combination of the Kabat definition and the Chothia definition, the AbM definition, the contact definition, and/or the conformational definition of CDRs.

[0060] In some embodiments, the intracellular signaling domain comprises a CD3 ζ signalling domain. In some embodiments, the intracellular signaling domain comprises a 4-1BB domain. In some embodiments, the CAR further comprises a second intracellular signaling domain. In some embodiments, the second intracellular signaling domain comprises a 4-1BB domain. In some embodiments, the CAR comprises a first CD3 ζ intracellular signaling domain and a second 4-1BB intracellular signaling domain.

[0061] In some embodiments, the intracellular signaling domain comprises a CD3 ζ signalling domain. In some embodiments, the intracellular signaling domain comprises a 4-1BB domain. In some embodiments, the CAR further comprises two intracellular signaling domains. In some embodiments, the CAR further comprises 3, 4, 5, or 6 intracellular signaling domains. In some embodiments, the CAR comprises a first intracellular signaling domain and a second intracellular signaling domain, wherein the second intracellular signaling domain comprises a 4-1BB domain. In some embodiments, the CAR comprises an CD3 ζ intracellular signaling domain and a 4-1BB intracellular signaling domain.

[0062] In some embodiments, the CAR can comprise a stalk domain between the extracellular ligand-binding domain and the first transmembrane domain. In some embodiments, the stalk domain is selected from the group consisting of: a human CD8 α hinge, an IgG1 hinge, and an Fc γ RIII α hinge. In some embodiments, the stalk domain is a human CD8 α hinge, a human IgG1 hinge, or a human Fc γ RIII α hinge.

[0063] In some embodiments, the CAR can comprise a CD20 epitope. In some embodiments, the CD20 epitope comprises the amino acid sequence shown in SEQ ID NO: 293 or SEQ ID NO: 294 or SEQ ID NO: 609.

[0064] In some embodiments, the CAR can comprise the amino acid sequence shown in SEQ ID NO: 311 to 334 listed in Table 5. In some embodiments, the CAR can comprise the amino acid sequence shown in SEQ ID NO: 319 or 327.

[0065] In some embodiments, the first transmembrane domain comprises a CD8 α chain transmembrane domain.

[0066] In some embodiments, the CAR can comprise another extracellular ligand-binding domain which is not specific for CD70.

[0067] In some embodiments, the extracellular ligand-binding domain(s), the first transmembrane domain, and intracellular signaling domain(s) are on a single polypeptide.

[0068] In some embodiments, 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, wherein the first transmembrane domain comprises a transmembrane domain from the α chain of the high-affinity IgE receptor (Fc ϵ RI) and the second transmembrane domain comprises a transmembrane domain from the γ or β chain of Fc ϵ RI.

[0069] In some embodiments, 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 Fc ϵ RI.

[0070] In another aspect, the disclosure provides an isolated polynucleotide comprising a nucleic acid sequence encoding the CD70-specific CAR described herein.

[0071] In another aspect, the disclosure provides an expression vector comprising the polynucleotide encoding the CD70-specific CAR described herein.

[0072] In another aspect, the disclosure provides an engineered immune cell expressing at its cell-surface membrane a CD70-specific CAR described herein. In some embodiments, the engineered immune cell can comprise another CAR which is not specific for CD70. In some embodiments, the engineered immune cell can comprise a polynucleotide encoding a suicide polypeptide. In some embodiments, the suicide polypeptide is RQR8.

[0073] In some embodiments, the engineered immune is derived from an inflammatory T-lymphocyte, a cytotoxic T-lymphocyte, a regulatory T-lymphocyte, or a helper T-lymphocyte.

[0074] In some embodiments, the engineered immune cell can comprise a disruption one or more endogenous genes, wherein the endogenous gene encodes TCR α , TCR β , CD52,

glucocorticoid receptor (GR), deoxycytidine kinase (dCK), CD70 or an immune checkpoint protein such as for example programmed death-1 (PD-1).

[0075] In some embodiments, the engineered immune cell is obtained from a healthy donor. In some embodiments, the engineered immune cell is obtained from a patient.

[0076] In another aspect, the disclosure provides an engineered immune cell expressing at its cell-surface membrane a CD70-specific CAR as described herein for use as a medicament. In some embodiments, the medicament is for use in treatment of a cancer. In some embodiments, the medicament is for treatment of Renal Cell Carcinoma, Glioblastoma, glioma such as low grade glioma, Non-Hodgkin's Lymphoma (NHL), Hodgkin's Disease (HD), Waldenstrom's macroglobulinemia, Acute Myeloid Leukemia, Multiple Myeloma, diffuse large-cell lymphoma, follicular lymphoma or Non-Small Cell Lung Cancer.

[0077] In another aspect, the disclosure provides a method of engineering an immune cell comprising: providing an immune cell; and expressing at the surface of the cell at least one CD70-specific CAR as described herein. In some embodiments, the method comprises: providing an immune cell; introducing into the cell at least one polynucleotide encoding said CD70-specific CAR; and expressing said polynucleotide into the cell.

[0078] In some embodiments, the method comprises providing an immune cell; introducing into the cell at least one polynucleotide encoding said CD70-specific CAR; and introducing at least one other CAR which is not specific for CD70.

[0079] In another aspect, the disclosure provides a method of treating a subject suffering from a condition associated with malignant cells, the method comprising: providing a immune cell expressing at the surface a CD70-specific CAR as described herein; and administering said immune cells to said patient.

[0080] In another aspect, the disclosure provides a pharmaceutical composition comprising an engineered immune cell as described herein.

[0081] In another aspect, the disclosure provides a method of treating a condition associated with malignant cells expressing CD70 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 embodiments, the condition is a cancer. In some embodiments, the cancer is a Renal Cell Carcinoma, Glioblastoma, glioma such as low grade glioma, Non-Hodgkin's Lymphoma (NHL), Hodgkin's Disease (HD), Waldenstrom's

macroglobulinemia, Acute Myeloid Leukemia, Multiple Myeloma, diffuse large-cell lymphoma, follicular lymphoma or Non-Small Cell Lung Cancer.

[0082] In another aspect, the disclosure provides a method of inhibiting tumor growth or progression in a subject who has malignant cells expressing CD70, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition comprising an engineered immune cell as described herein to the subject.

[0083] In another aspect, the disclosure provides a method of inhibiting metastasis of malignant cells expressing CD70 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 to the subject.

[0084] In another aspect, the disclosure provides a method of inducing tumor regression in a subject who has malignant cells expressing CD70, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition comprising an engineered immune cell as described herein to the subject.

[0085] In some embodiments, 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 embodiments, 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 embodiments, any of the above methods further comprises administering a Receptor Tyrosine Kinase inhibitor such as sunitinib or axitinib.

[0086] In some embodiments, the disclosure provides a CD70-specific CAR comprising an extracellular ligand-binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the extracellular domain comprises a single chain Fv fragment (scFv) binding to the extracellular domain of CD70 having a heavy chain variable (VH) region and a light chain variable (VL) region; wherein the VH region comprises an amino acid sequence that shares at least 95%, 96%, 97%, 98%, 99%, or 100% with SEQ ID NO: 18 and the VL region comprises an amino acid sequence that shares at least 95%, 96%, 97%, 98%, 99%, or 100% with SEQ ID NO: 17; or the VH region comprises an amino acid sequence that shares at least 95%, 96%, 97%, 98%, 99%, or 100% with SEQ ID NO: 34 and the VL region comprises an amino acid sequence that shares at least 95%, 96%, 97%, 98%, 99%, or 100% with SEQ ID NO: 33.

[0087] In some embodiments, the extracellular domain comprises an amino acid sequence that shares at least 95%, 96%, 97%, 98%, 99%, or 100% with SEQ ID NO: 319. In some

embodiments, the extracellular domain comprises an amino acid sequence that shares at least 95%, 96%, 97%, 98%, 99%, or 100% with SEQ ID NO: 327.

[0088] In some embodiments, the disclosure provides a polynucleotide encoding a CD70-specific CAR, wherein the polynucleotide comprises a nucleic-acid sequence that shares at least 95%, 96%, 97%, 98%, 99%, or 100% with SEQ ID NO: 297 and shares at least 95%, 96%, 97%, 98%, 99%, or 100% with SEQ ID NO: 298; or shares at least 95%, 96%, 97%, 98%, 99%, or 100% with SEQ ID NO: 307 and shares at least 95%, 96%, 97%, 98%, 99%, or 100% with SEQ ID NO: 308.

[0089] In some embodiments, the disclosure provides CAR comprising an antigen binding molecule that specifically binds to CD70, wherein the antigen binding molecule comprises at least one of: a variable heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 49-51, 55-57, 61-63, 67-69, 73-75, 79-81, 85-87, 91-93, 97-99, 103-105, 109-111, 115-117, 121-123, 127-129, 133-135, 139-141, 145-147, 151-153, 157-159, 163-165, 169-171, 175-177, 181-183, 187-189, 382-384, 388-390, 394-396, 400-402, 406-408, 412-414, 418-420, 424-426, 430-432, 436-438, 442-444, 448-450, 454-456, 460-462, 466-468, 472-474, 478-480, 484-486, 490-492, 496-498, 502-504, and 508-510; a variable heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ NOs 52, 53, 58, 59, 64, 65, 70, 71, 76, 77, 82, 83, 88, 89, 94, 95, 100, 101, 106, 107, 112, 113, 118, 119, 124, 125, 130, 131, 136, 137, 142, 143, 148, 149, 154, 155, 160, 161, 166, 167, 172, 173, 178, 179, 184, 185, 190, 191, 385, 386, 391, 392, 397, 398, 403, 404, 409, 410, 415, 416, 421, 422, 427, 428, 433, 434, 439, 440, 445, 446, 451, 452, 457, 458, 463, 464, 469, 470, 475, 476, 481, 482, 487, 488, 493, 494, 499, 500, 505, 506, 511, and 512; a variable heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120, 126, 132, 138, 144, 150, 156, 162, 168, 174, 180, 186, 192, 387, 393, 399, 405, 411, 417, 423, 429, 435, 441, 447, 453, 459, 465, 471, 477, 483, 489, 495, 501, 507, and 513; a variable light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ NOs: 193, 196, 199, 202, 205, 208, 211, 214, 217, 220, 223, 226, 229, 232, 235, 238, 241, 244, 247, 250, 253, 256, 259, 262, 514, 517, 520, 523, 526, 529, 532, 535, 538, 541, 544, 547, 550, 553, 556, 559, 562, 565, 568, 571, 574, and 577; a variable light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 194, 197, 200, 203, 206, 209, 212, 215, 218, 221, 224, 227, 230, 233, 236, 239, 242, 245, 248, 251, 254, 257, 260, 263, 515, 518, 521, 524, 527, 530, 533, 536,

539, 542, 545, 548, 551, 554, 557, 560, 563, 566, 569, 572, 575, and 578; and a variable light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 195, 198, 201, 204, 207, 210, 213, 216, 219, 222, 225, 228, 231, 234, 237, 240, 243, 246, 249, 252, 255, 258, 261, 264, 516, 519, 522, 525, 528, 531, 534, 537, 540, 543, 546, 549, 552, 555, 558, 561, 564, 567, 570, 573, 576, and 579.

[0090] In some embodiments, the antigen binding molecule comprises a variable heavy chain domain comprising amino acid sequences for CDRH1, CDRH2, and CDRH3, respectively, selected from one of SEQ ID NOs: 49-51, 52-53, 54; 55-57, 58-59, 60; 61-63, 64-65, 66; 67-69, 70-71, 72; 73-75, 76-77, 78; 79-81, 82-83, 84; 85-87, 88-89, 90; 91-93, 94-95, 96; 97-99, 100-101, 102; 103-105, 106-107, 108; 109-111, 112-113, 114; 115-117, 118-119, 120; 121-123, 124-125, 126; 127-129, 130-131, 132; 133-135, 136-137, 138; 139-141, 142-143, 144; 145-147, 148-149, 150; 151-153, 154-155, 156; 157-159, 160-161, 162; 163-165, 166-167, 168; 169-171, 172-173, 174; 175-177, 178-179, 180; 181-183, 184-185, 186; 187-189, 190-191, 192; 382-384, 385-386, 387; 388-390, 391-392, 393; 394-396, 397-398, 399; 400-402, 403-404, 405; 406-408, 409-410, 411; 412-414, 415-416, 417; 418-420, 421-422, 423; 424-426, 427-428, 429; 430-432, 433-434, 435; 436-438, 439-440, 441; 442-444, 445-446, 447; 448-450, 451-452, 453; 454-456, 457-458, 459; 460-462, 463-464, 465; 466-468, 469-470, 471; 472-474, 475-476, 477; 478-480, 481-482, 483; 484-486, 487-488, 489; 490-492, 493-494, 495; 496-498, 499-500, 501; 502-504, 505-506, 507; or 508-510, 511-512, 513; and a variable light chain domain comprising amino acid sequences for CDRL1, CDRL2, and CDRL3, respectively, selected from one of SEQ ID NOs: 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, 251, 252; 253, 254, 255; 256, 257, 258; 259, 260, 261; 262, 263, 264; 514, 515, 516; 517, 518, 519; 520, 521, 522; 523, 524, 525; 526, 527, 528; 529, 530, 531; 532, 533, 534; 535, 536, 537; 538, 539, 540; 541, 542, 543; 544, 545, 546; 547, 548, 549; 550, 551, 552; 553, 554, 555; 556, 557, 558; 559, 560, 561; 562, 563, 564; 565, 566, 567; 568, 569, 570; 571, 572, 573; 574, 575, 576; and 577, 578, 579.

[0091] In some embodiments, the antigen binding molecule comprises amino acid sequences for CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, selected from one of SEQ ID NOs 49-51, 52-53, 54, 193, 194, 195; 55-57, 58-59, 60, 196, 197, 198; 61-63, 64-65, 66, 199, 200, 201; 67-69, 70-71, 72, 202, 203, 204; 73-75, 76-77, 78, 205, 206, 207; 79-81, 82-

83, 84, 208, 209, 210; 85-87, 88-89, 90, 211, 212, 213; 91-93, 94-95, 96, 214, 215, 216; 97-99, 100-101, 102, 217, 218, 219; 103-105, 106-107, 108, 220, 221, 222; 109-111, 112-113, 114, 223, 224, 225; 115-117, 118-119, 120, 226, 227, 228; 121-123, 124-125, 126, 229, 230, 231; 127-129, 130-131, 132, 232, 233, 234; 133-135, 136-137, 138, 235, 236, 237; 139-141, 142-143, 144, 238, 239, 240; 145-147, 148-149, 150, 241, 242, 243; 151-153, 154-155, 156, 244, 245, 246; 157-159, 160-161, 162, 247, 248, 249; 163-165, 166-167, 168, 250, 251, 252; 169-171, 172-173, 174, 253, 254, 255; 175-177, 178-179, 180, 256, 257, 258; 181-183, 184-185, 186, 259, 260, 261; 187-189, 190-191, 192, 262, 263, 264; 382-384, 385-386, 387, 514, 515, 516; 388-390, 391-392, 393, 517, 518, 519; 394-396, 397-398, 399, 520, 521, 522; 400-402, 403-404, 405, 523, 524, 525; 406-408, 409-410, 411, 526, 527, 528; 412-414, 415-416, 417, 529, 530, 531; 418-420, 421-422, 423, 532, 533, 534; 424-426, 427-428, 429, 535, 536, 537; 430-432, 433-434, 435, 538, 539, 540; 436-438, 439-440, 441, 541, 542, 543; 442-444, 445-446, 447, 544, 545, 546; 448-450, 451-452, 453, 547, 548, 549; 454-456, 457-458, 459, 550, 551, 552; 460-462, 463-464, 465, 553, 554, 555; 466-468, 469-470, 471, 556, 557, 558; 472-474, 475-476, 477, 559, 560, 561; 478-480, 481-482, 483, 562, 563, 564; 484-486, 487-488, 489, 565, 566, 567; 490-492, 493-494, 495, 568, 569, 570; 496-498, 499-500, 501, 571, 572, 573; 502-504, 505-506, 507, 574, 575, 576; and 508-510, 511-512, 513, 577, 578, 579.

[0092] In some embodiments, the antigen binding molecule comprises a variable light chain domain comprising an amino acid sequences for CDRH1, CDRH2, and CDRH3, respectively, selected from one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, and 380; and a variable heavy chain domain comprising amino acid sequences for CDRL1, CDRL2, and CDRH3, respectively, selected from one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, and 381.

[0093] In some embodiments, the antigen binding molecule comprises amino acid sequences for light chain variable domain and heavy chain variable domain, respectively, selected from one of SEQ ID NOs: 1 and 2; 3 and 4; 5 and 6; 7 and 8; 9 and 10; 11 and 12; 13 and 14; 15 and 16; 17 and 18; 19 and 20; 21 and 22; 23 and 24; 25 and 26; 27 and 28; 29 and 30; 31 and 32; 33 and 34; 35 and 36; 37 and 38; 39 and 40; 41 and 42; 43 and 44; 45 and 46; 47 and 48; 338 and 339; 340 and 341; 342 and 343; 344 and 345; 346 and 347; 348 and 349; 350

and 351; 352 and 353; 354 and 355; 356 and 357; 358 and 359; 360 and 361; 362 and 363; 364 and 365; 366 and 367; 368 and 369; 370 and 371; 372 and 373; 374 and 375; 376 and 377; 378 and 379; and 380 and 381.

[0094] In another aspect, the disclosure provides antibodies, which specifically bind to Cluster of Differentiation 70 (CD70).

[0095] In some embodiments, the antibody comprises a VH region shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 339, 341, 343, 345, 347, 349, 351, 353, 355, 662, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, or 381; and/or a VL region shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 338, 340, 342, 344, 346, 348, 350, 352, 354, 661, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, or 380.

[0096] In some embodiments, the antibody comprises a heavy chain variable (VH) region comprising (i) a VH CDR1 comprising the sequence shown in SEQ ID NO: 49, 50, 51, 55, 56, 57, 61, 62, 63, 67, 68, 69, 73, 74, 75, 79, 80, 81, 85, 86, 87, 91, 92, 93, 97, 98, 99, 103, 104, 105, 109, 110, 111, 115, 116, 117, 121, 122, 123, 127, 128, 129, 133, 134, 135, 139, 140, 141, 145, 146, 147, 151, 152, 153, 157, 158, 159, 163, 164, 165, 169, 170, 171, 175, 176, 177, 181, 182, 183, 187, 188, 189, 382, 383, 384, 388, 389, 390, 394, 395, 396, 400, 401, 402, 406, 407, 408, 412, 413, 414, 418, 419, 420, 424, 425, 426, 430, 431, 432, 663, 664, 665, 436, 437, 438, 442, 443, 444, 448, 449, 450, 454, 455, 456, 460, 461, 462, 466, 467, 468, 472, 473, 474, 478, 479, 480, 484, 485, 486, 490, 491, 492, 496, 497, 498, 502, 503, 504, 508, 509, or 510; (ii) a VH CDR2 comprising the sequence shown in SEQ ID NO: 52, 53, 58, 59, 64, 65, 70, 71, 76, 77, 82, 83, 88, 89, 94, 95, 100, 101, 106, 107, 112, 113, 118, 119, 124, 125, 130, 131, 136, 137, 142, 143, 148, 149, 154, 155, 160, 161, 166, 167, 172, 173, 178, 179, 184, 185, 190, 191, 385, 386, 391, 392, 397, 398, 403, 404, 409, 410, 415, 416, 421, 422, 427, 428, 433, 434, 666, 667, 439, 440, 445, 446, 451, 452, 457, 458, 463, 464, 469, 470, 475, 476, 481, 482, 487, 488, 493, 494, 499, 500, 505, 506, 511, or 512; and iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120, 126, 132, 138, 144, 150, 156, 162, 168, 174, 180, 186, 192, 387, 393, 399, 405, 411, 417, 423, 429, 435, 668, 441, 447, 453, 459, 465, 471, 477, 483, 489, 495, 501, 507, or 513; and/or a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 193, 196, 199, 202, 205, 208, 211, 214, 217, 220, 223, 226, 229, 232, 235, 238, 241, 244, 247, 250, 253, 256, 259, 262, 514, 517, 520, 523, 526, 529, 532, 535, 538, 669, 541, 544, 547, 550, 553, 556, 559, 562, 565, 568,

571, 574, or 577; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 194, 197, 200, 203, 206, 209, 212, 215, 218, 221, 224, 227, 230, 233, 236, 239, 242, 245, 248, 251, 254, 257, 260, 263, 515, 518, 521, 524, 527, 530, 533, 536, 539, 670, 542, 545, 548, 551, 554, 557, 560, 563, 566, 569, 572, 575, or 578; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 195, 198, 201, 204, 207, 210, 213, 216, 219, 222, 225, 228, 231, 234, 237, 240, 243, 246, 249, 252, 255, 258, 261, 264, 516, 519, 522, 525, 528, 531, 534, 537, 540, 671, 543, 546, 549, 552, 555, 558, 561, 564, 567, 570, 573, 576, or 579.

[0097] In some embodiments, the antibody comprises a VH region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 339, 341, 343, 345, 347, 349, 351, 353, 355, 662, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, or 381; and/or a VL region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 338, 340, 342, 344, 346, 348, 350, 352, 354, 661, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, or 380.

[0098] In yet further aspects, the disclosure provides nucleic acids, vectors, host cells, pharmaceutical compositions, methods of making, and method of treating conditions with the antibodies disclosed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0099] FIG. 1 is a plot showing. 1 tumor volumes of mice treated with different doses of 4F11 CAR T-cells in a subcutaneous xenograft model.

[0100] FIG. 2 is a plot showing body weights of mice treated with different doses of 4F11 CAR T-cells in a subcutaneous xenograft model.

[0101] FIG. 3 is a plot showing tumor volumes of mice treated with 4F11 and P08F08 CAR T with or without CD70 KO in a subcutaneous xenograft model.

[0102] FIG. 4 is a plot showing body weights of mice treated with 4F11 and P08F08 CAR T with or without CD70 KO in a subcutaneous xenograft model.

[0103] FIGs. 5A-5C is a series of plots showing tumor flux values of mice treated with control cells, cells expressing the CAR 4F11 with or without CD70 KO and with or without TCRa KO, and mice treated with cells expressing the CAR P08F08 with or without CD70 KO and with or without TCRa KO, across 3 donors in the ACHN lung metastasis model.

[0104] FIG. 5A shows results obtained with control cells, cells expressing the 4F11 CAR with CD70, TCRA or both CD70 and TCR KO, and cells expressing the P08F08 CAR with TCR KO; cells were obtained from Donor D419.

[0105] FIG. 5B shows results obtained with control cells, cells expressing the 4F11 CAR with or without a CD70 KO, and cells expressing the P08F08 CAR; cells were obtained from Donor D710.

[0106] FIG. 5C shows results obtained with control cells, cells expressing the 4F11 CAR with or without a CD70 KO, and cells expressing the P08F08 CAR; cells were obtained from Donor D503.

[0107] FIGs. 6A-6F show five exemplary, non-limiting CAR designs. In each of FIGs. 6A-6F an approximate distance of the antigen-binding fragment of the CD70-specific domain to the cell membrane is indicated by a double arrow, labeled with the number of amino-acid residues (aa) between the scFv and the transmembrane domain.

[0108] FIG. 6A shows a second-generation CAR design including an extracellular domain comprising an scFv specific for CD70, a hinge, a transmembrane domain, a first intracellular domain (a 4-1BB domain), and a second intracellular domain (a CD3 ζ signaling domain).

[0109] FIG. 6B shows the SR2 CAR format in which a suicide switch is created by the insertion of two RTX epitopes (*i.e.* CD20 epitopes) between the hinge and the scFv.

[0110] FIG. 6C shows the RSRQR CAR format, which adds a third RTX epitope and the CD34 epitope.

[0111] FIG. 6D shows the RSR format, which two RTX epitopes flanking the scFv.

[0112] FIG. 6E shows a modification of the RSR CAR format in which the hinge domain is shortened (termed RSR-short).

[0113] FIG. 6F shows the R2S CAR format, in which the two RTX epitopes of the R2 CAR format are moved to N-terminal to the scFv.

[0114] FIG. 7 shows viability of target cells after exposure to the CD70-specific CARs in four formats or non-transduced (NTD) control cells.

[0115] FIGs. 8A-8D is a series of plots showing cell killing of 786-0, ACHN, or REH cells using CD70-specific CAR T cells where the CAR extracellular domain comprises the scFvs indicated in the legend in FIG. 8D.

[0116] FIG. 8A shows cell killing of 786-0, where the CAR extracellular domain comprises the scFvs indicated in the legend shown in FIG. 8D.

[0117] FIG. 8B shows cell killing of ACHN, where the CAR extracellular domain comprises the scFvs indicated in the legend shown in FIG. 8D.

[0118] FIG. 8C shows cell killing of REH, where the CAR extracellular domain comprises the scFvs indicated in the legend shown in FIG. 8D.

[0119] FIGs. 9A-9D are a series of plots showing serial killing of 786-O, ACHN, or REH cells using CD70-specific CAR T cells where the CAR extracellular domain comprises the scFvs indicated in the legend in Figure 9D.

[0120] FIG. 9A is a plot showing the efficacy of CD70-specific CARs upon repeated exposure to luciferase-labeled 786-O target cells (CAR T cells were transferred to a 96-well plate containing fresh targets every 2-3 days). The E:T ratio was 3:1. The CARs were expressed in cells from donor D503.

[0121] FIG. 9B is a plot showing the efficacy of CD70-specific CARs upon repeated exposure to luciferase-labeled ACHN target cells (CAR T cells were transferred to a 96-well plate containing fresh targets every 2-3 days). The E:T ratio was 10:1. The CARs were expressed in cells from donor D503.

[0122] FIG. 9C is a plot showing the efficacy of CD70-specific CARs upon repeated exposure to luciferase-labeled REH target cells (2×10^6 cells added at indicated time-points). The E:T ratio was 1:5. The CARs were expressed in cells from donor D503.

[0123] FIG. 10 is a series of plots showing the efficacy of CD70-specific CARs in either R2S, SR2, or RSRQR format upon repeated exposure to luciferase-labeled REH target cells (2×10^6 cells added at indicated time-points). The E:T ratio was 1:5. The CARs were expressed in cells from donor D772.

[0124] FIG. 11 is a plot showing tumor flux values of mice treated with control cells or cells expressing various CAR scFvs in the ACHN lung metastasis model.

[0125] FIGs. 12A-12C is a series of plots showing the quantification of CD70 expression in terms of CD70 antibody binding capacity (ABC) on various tested cell lines or cell lines and RCC patient-derived cells. The data from RCC patient-derived cells is also shown.

[0126] FIG. 12A shows the quantification of CD70 expression in terms of CD70 antibody binding capacity (ABC) on various tested cell lines.

[0127] FIG. 12B shows the quantification of CD70 expression in terms of CD70 antibody binding capacity (ABC) on RCC patient-derived cells.

[0128] FIG. 12C shows the data from RCC patient-derived cells.

[0129] FIGs. 13A-13C is a series of plots showing killing of target cells from RCC patient WD-59279, patient-derived cell lines, or ACHN and antibody binding capacity is indicated in each panel.

[0130] FIG. 13A-B show killing of target cells from RCC patients, and antibody binding capacity.

[0131] FIG. 13C shows killing of target ACHN cells, and antibody binding capacity.

[0132] FIGs. 14A-14B is a series of bar graphs showing quantification of CD70 receptor numbers and heme tumor-cell killing by 4F11 CAR in the QR3 format at 1:1 E:T for further cell lines expressing CD70 at varied levels.

[0133] FIG. 14A is a bar graph showing quantification of CD70 receptor numbers of the 4F11 CAR in the QR3 format at 1:1 E:T for further cell lines expressing CD70 at varied levels.

[0134] FIG. 14B is a bar graph showing heme tumor-cell killing by 4F11 CAR in the QR3 format at 1:1 E:T for further cell lines expressing CD70 at varied levels.

[0135] FIGs. 15A-15B show is a series of plots showing tumor volumes and body weights of mice treated with 4F11 and P08F08 CAR T at 10×10^6 cell or 5×10^6 cell dose in a subcutaneous xenograft model.

[0136] FIG. 15A is a plot showing tumor volumes of mice treated with 4F11 and P08F08 CAR T at 10×10^6 cell or 5×10^6 cell dose in a subcutaneous xenograft model.

[0137] FIG. 15B is a plot showing body weights of mice treated with 4F11 and P08F08 CAR T at 10×10^6 cell or 5×10^6 cell dose in a subcutaneous xenograft model.

DETAILED DESCRIPTION

[0138] The disclosure disclosed herein provides chimeric antigen receptors (CARs) and immune cells comprising CARs (*e.g.* CAR-T cells) that specifically bind to CD70 (*e.g.*, human CD70). The disclosure also provides polynucleotides encoding these CARs, compositions comprising these CAR-T cells, and methods of making and using these CARs and CAR-T cells. The disclosure also provides methods for treating a condition associated with malignant CD70 expression in a subject, such as cancer.

General Techniques

[0139] The compositions and methods of the disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques),

microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995).

Definitions

[0140] The term “extracellular ligand-binding domain” as used herein refers to an oligo- or polypeptide that is capable of binding a ligand. In some exemplary embodiments, the domain will be capable of interacting with a cell surface molecule. For example, the extracellular ligand-binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state.

[0141] The term “stalk domain” or “hinge domain” are used interchangeably herein to refer to any oligo- or polypeptide that functions to link the transmembrane domain to the extracellular ligand-binding domain in a CAR. In particular, stalk domains are used to provide more flexibility and accessibility for the extracellular ligand-binding domain.

[0142] The term “intracellular signaling domain” refers to the portion of a protein which transduces the effector signal function signal and directs the cell to perform a specialized function.

[0143] A “co-stimulatory molecule” as used herein refers to the cognate binding partner on an immune cell, *e.g.*, a T cell, that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the cell, such as, but not limited to proliferation. Co-stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and Toll ligand receptor. Examples of costimulatory molecules include CD27, CD28, CD8, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3 and a ligand that specifically binds with CD83 and the like.

[0144] A “co-stimulatory ligand” refers to a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory signal molecule on an immune cell, *e.g.*, a T cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation activation, differentiation and the like. A co-stimulatory ligand can include but is not limited to CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, M1CB, HVEM, lymphotoxin β receptor, 3/TR6, ILT3, ILT4, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, *inter alia*, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LTGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83.

[0145] An “antibody” is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂, Fv), single chain (scFv) and domain antibodies (including, for example, shark and camelid antibodies), and fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. An antibody includes an antibody of any class, such as IgG, IgA, IgE, IgD, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA,

IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0146] The term “antigen binding fragment” or “antigen binding portion” of an antibody, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to a given antigen (*e.g.*, CD70). Antigen binding functions of an antibody can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term “antigen binding fragment” of an antibody include Fab; Fab’; F(ab’)₂; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment (Ward et al., Nature 341:544-546, 1989), and an isolated complementarity determining region (CDR).

[0147] An antibody, an antigen-binding fragment, an antibody conjugate, or a polypeptide that “preferentially binds” or “specifically binds” (used interchangeably herein) to a target (*e.g.*, CD70 protein) is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit “specific binding” or “preferential binding” if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody “specifically binds” or “preferentially binds” to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to a CD70 epitope is an antibody that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other CD70 epitopes or non-CD70 epitopes. It is also understood that by reading this definition, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

[0148] A “variable region” of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four

framework regions (FR) connected by three complementarity determining regions (CDRs) also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (*i.e.*, Kabat et al. Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda MD); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-lazikani et al., 1997, J. Molec. Biol. 273:927-948). As used herein, a CDR may refer to CDRs defined by either approach or by a combination of both approaches.

[0149] A “CDR” of a variable domain are amino acid residues within the variable region that are identified in accordance with the definitions of the Kabat, Chothia, the accumulation of both Kabat and Chothia, AbM, contact, and/or conformational definitions or any method of CDR determination well known in the art. Antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. See, *e.g.*, Kabat et al., 1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C. The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others. See, *e.g.*, Chothia et al., Nature 342:877-883, 1989. Other approaches to CDR identification include the “AbM definition,” which is a compromise between Kabat and Chothia and is derived using Oxford Molecular’s AbM antibody modeling software (now Accelrys®), or the “contact definition” of CDRs based on observed antigen contacts, set forth in MacCallum et al., J. Mol. Biol., 262:732-745, 1996. In another approach, referred to herein as the “conformational definition” of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, *e.g.*, Makabe et al., Journal of Biological Chemistry, 283:1156-1166, 2008. Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, AbM, contact, and/or conformational definitions.

[0150] As used herein, “monoclonal antibody” refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the disclosure may be made by the hybridoma method first described by Kohler and Milstein, *Nature* 256:495, 1975, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., *Nature* 348:552-554, 1990, for example.

[0151] As used herein, “humanized” antibody refers to forms of non-human (*e.g.* murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. In some exemplary embodiments, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Exemplary embodiments are antibodies having

Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (CDR L1, CDR L2, CDR L3, CDR H1, CDR H2, or CDR H3) which are altered with respect to the original antibody, which are also termed one or more CDRs “derived from” one or more CDRs from the original antibody.

[0152] As used herein, “human antibody” means an antibody having an amino acid sequence corresponding to that of an antibody produced by a human and/or which has been made using any of the techniques for making human antibodies known to those skilled in the art or disclosed herein. This definition of a human antibody includes antibodies comprising at least one human heavy chain polypeptide or at least one human light chain polypeptide. One such example is an antibody comprising murine light chain and human heavy chain polypeptides. Human antibodies can be produced using various techniques known in the art. In some embodiments, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., *Nature Biotechnology*, 14:309-314, 1996; Sheets et al., *Proc. Natl. Acad. Sci. (USA)* 95:6157-6162, 1998; Hoogenboom and Winter, *J. Mol. Biol.*, 227:381, 1991; Marks et al., *J. Mol. Biol.*, 222:581, 1991). Human antibodies can also be made by immunization of animals into which human immunoglobulin loci have been transgenically introduced in place of the endogenous loci, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely disrupted or inactivated. This approach is described in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016.

Alternatively, the human antibody may be prepared by immortalizing human B lymphocytes that produce an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or from single cell cloning of the cDNA, or may have been immunized *in vitro*). See, *e.g.*, Cole et al. *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77, 1985; Boerner et al., *J. Immunol.*, 147 (1):86-95, 1991; and U.S. Pat. No. 5,750,373.

[0153] The term “chimeric antibody” is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

[0154] The terms “polypeptide”, “oligopeptide”, “peptide,” and “protein” are used interchangeably herein to refer to chains of amino acids of any length—in some embodiments, relatively short (*e.g.*, 10-100 amino acids). The chain may be linear or branched, it may comprise modified amino acids, and/or may be interrupted by non-amino acids. The terms also

encompass an amino acid chain that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that the polypeptides can occur as single chains or associated chains.

[0155] A “monovalent antibody” comprises one antigen binding site per molecule (*e.g.*, IgG or Fab). In some instances, a monovalent antibody can have more than one antigen binding sites, but the binding sites are from different antigens.

[0156] A “bivalent antibody” comprises two antigen binding sites per molecule (*e.g.*, IgG). In some instances, the two binding sites have the same antigen specificities. However, bivalent antibodies may be bispecific.

[0157] Antibodies of the disclosure can be produced using techniques well known in the art, *e.g.*, recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art (see, for example, Jayasena, S.D., Clin. Chem., 45: 1628-50, 1999 and Fellouse, F.A., et al, J. Mol. Biol., 373(4):924-40, 2007).

[0158] As known in the art, “polynucleotide” or “nucleic acid,” as used interchangeably herein, refer to chains of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a chain by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the chain. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications include, for example, “caps”, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (*e.g.*, nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (*e.g.*, acridine, psoralen, etc.), those containing chelators (*e.g.*, metals,

radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (*e.g.*, alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, alpha- or beta-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S("thioate"), P(S)S ("dithioate"), (O)NR₂ ("amidate"), P(O)R, P(O)OR', CO or CH₂ ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0159] As known in the art a "constant region" of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

[0160] As used herein, "substantially pure" refers to material which is at least 50% pure (*i.e.*, free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

[0161] A "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected *in vivo* with a polynucleotide(s) of this disclosure.

[0162] As used herein, “immune cell” refers to a cell of hematopoietic origin functionally involved in the initiation and/or execution of innate and/or adaptative immune response.

[0163] As known in the art, the term “Fc region” is used to define a C-terminal region of an immunoglobulin heavy chain. The “Fc region” may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991. The Fc region of an immunoglobulin generally comprises two constant regions, CH2 and CH3.

[0164] As used in the art, “Fc receptor” and “FcR” describe a receptor that binds to the Fc region of an antibody. In some embodiments, the FcR is a native sequence human FcR. Moreover, in some embodiments, the FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. FcRs are reviewed in Ravetch and Kinet, *Ann. Rev. Immunol.*, 9:457-92, 1991; Capel et al., *Immunomethods*, 4:25-34, 1994; and de Haas et al., *J. Lab. Clin. Med.*, 126:330-41, 1995. “FcR” also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.*, 117:587, 1976; and Kim et al., *J. Immunol.*, 24:249, 1994).

[0165] The term “compete”, as used herein with regard to an antibody, means that a first antibody, or an antigen binding fragment (or portion) thereof, binds to an epitope in a manner sufficiently similar to the binding of a second antibody, or an antigen binding portion thereof, such that the result of binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody

with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to “cross-compete” with each other for binding of their respective epitope(s). Both competing and cross-competing antibodies are encompassed by the disclosure. Regardless of the mechanism by which such competition or cross-competition occurs (*e.g.*, steric hindrance, conformational change, or binding to a common epitope, or portion thereof), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

[0166] As used herein “autologous” means that cells, a cell line, or population of cells used for treating patients are originating from said patient.

[0167] As used herein “allogeneic” means that cells or population of cells used for treating patients are not originating from said patient but from a donor.

[0168] As used herein, “treatment” is an approach for obtaining beneficial or desired clinical results. For purposes of this disclosure, beneficial or desired clinical results include, but are not limited to, one or more of the following: reducing the proliferation of (or destroying) neoplastic or cancerous cells, inhibiting metastasis of neoplastic cells, shrinking or decreasing the size of CD70 expressing tumor such as a renal cell carcinoma (RCC) lymphoma, leukemia, or glioma, remission of a CD70 associated disease (*e.g.*, cancer), decreasing symptoms resulting from a CD70 associated disease (*e.g.*, cancer), increasing the quality of life of those suffering from a CD70 associated disease (*e.g.*, cancer), decreasing the dose of other medications required to treat a CD70 associated disease (*e.g.*, cancer), delaying the progression of a CD70 associated disease (*e.g.*, cancer), curing a CD70 associated disease (*e.g.*, cancer), and/or prolong survival of patients having a CD70 associated disease (*e.g.*, cancer).

[0169] “Ameliorating” means a lessening or improvement of one or more symptoms as compared to not administering a CD70-specific CAR or a CD70-specific CAR-T-cell.

“Ameliorating” also includes shortening or reduction in duration of a symptom.

[0170] As used herein, an “effective dosage” or “effective amount” of drug, compound, or pharmaceutical composition is an amount sufficient to effect any one or more beneficial or desired results. For prophylactic use, beneficial or desired results include eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as reducing incidence or

amelioration of one or more symptoms of various CD70 associated diseases or conditions (such as for example multiple myeloma), decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication, and/or delaying the progression of the CD70 associated disease of patients. An effective dosage can be administered in one or more administrations. For purposes of this disclosure, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an “effective dosage” may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0171] An “individual,” “patient,” or a “subject” is a mammal—in some embodiments, a human. Mammals include, but are not limited to, humans, monkeys, pigs, and other farm animals, sport animals, pets, primates, horses, dogs, cats, rodents including mice, rats, guinea pigs, etc. A subject is a mammal and the terms are used interchangeably herein. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human primate. In some embodiments, the subject is a human or a monkey, *e.g.*, a cynomolgus monkey.

[0172] As used herein, “vector” means a construct, which is capable of delivering, and, in some embodiments, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

[0173] As used herein, “expression control sequence” means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

[0174] As used herein, “pharmaceutically acceptable carrier” or “pharmaceutical acceptable excipient” includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject’s immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a

phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Exemplary diluents for aerosol or parenteral administration are phosphate buffered saline (PBS) or normal (0.9%) saline. Compositions comprising such carriers are formulated by well known conventional methods (see, for example, Remington's Pharmaceutical Sciences, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990; and Remington, The Science and Practice of Pharmacy 21st Ed. Mack Publishing, 2005).

[0175] The term " k_{on} ", as used herein, refers to the rate constant for association of an antibody or scFv or CAR to an antigen.

[0176] The term " k_{off} ", as used herein, refers to the rate constant for dissociation of an antibody or scFv or CAR from the antibody/antigen complex.

[0177] The term " K_D ", as used herein, refers to the equilibrium dissociation constant of an antibody-antigen or scFv-antigen or CAR-antigen interaction.

[0178] Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X." Numeric ranges are inclusive of the numbers defining the range.

[0179] It is understood that wherever embodiments are described herein with the language "comprising," otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0180] Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the invention encompasses not only the entire group listed as a whole, but each member of the group individually and all possible subgroups of the main group, but also the main group absent one or more of the group members. The invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

[0181] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In case of conflict, the present specification, including definitions, will control. Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless

otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0182] Exemplary methods and materials are described herein, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the invention. The materials, methods, and examples are illustrative only and not intended to be limiting.

CD70-SPECIFIC CARS AND METHODS OF MAKING THEREOF

[0183] The instant disclosure provides CARs that bind to CD70 (*e.g.*, human CD70 (*e.g.*, SEQ ID NO: 335), such as those deposited under the provisions of the Budapest Treaty and assigned accession number: P32970-1. CD70-specific CARs provided herein include single chain CARs and multichain CARs. In some embodiments, the CARs have the ability to redirect T cell specificity and reactivity toward CD70 in a non-MHC-restricted manner, exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T cells expressing CARs the ability to recognize an antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape.

[0184] In some embodiments, CARs provided herein comprise an extracellular ligand-binding domain (*e.g.*, a single chain variable fragment (scFv)), a transmembrane domain, and an intracellular signaling domain. In some embodiments, the CARs provided herein further comprises a “hinge” or “stalk” domain, which can be situated between the extracellular ligand-binding domain and the transmembrane domain. In some embodiments, the extracellular ligand-binding domain, transmembrane domain, and intracellular signaling domain are in one polypeptide, *i.e.*, in a single chain. Multichain CARs and polypeptides are also provided herein. In some embodiments, the multichain CARs comprise: a first polypeptide comprising a transmembrane domain and at least one extracellular ligand-binding domain, and a second polypeptide comprising a transmembrane domain and at least one intracellular signaling domain, wherein the polypeptides assemble together to form a multichain CAR. In some embodiments, the CARs are inducible, such as by small molecule (*e.g.*, AP1903) or protein (*e.g.*, Epo, Tpo, or PD-1). In some embodiments, a CD70-specific multichain CAR is based on the high affinity receptor for IgE (FcεRI). The FcεRI expressed on mast cells and basophiles triggers allergic reactions. FcεRI is a tetrameric complex composed of a single α subunit, a single β subunit, and two disulfide-linked γ subunits. The α subunit contains the IgE-binding domain. The β and γ subunits contain ITAMs that mediate signal transduction. In some embodiments, the

extracellular domain of the FcR α chain is deleted and replaced by a CD70-specific extracellular ligand-binding domain. In some embodiments, the multichain CD70-specific CAR comprises an scFv that binds specifically to CD70, the CD8 α hinge, and the ITAM of the FcR β chain. In some embodiments, the CAR may or may not comprise the FcR γ chain.

[0185] In some embodiments, the extracellular ligand-binding domain comprises an scFv comprising the light chain variable (VL) region and the heavy chain variable (VH) region of a target antigen (i.e., CD70) specific monoclonal antibody joined by a flexible linker. Single chain variable region fragments are made by linking light and/or heavy chain variable regions by using a short linking peptide (Bird et al., Science 242:423-426, 1988). An example of a linking peptide is the GS linker having the amino acid sequence (GGGGS)₃ (SEQ ID NO: 296), which bridges approximately 3.5 nm between the carboxy terminus of one variable region and the amino terminus of the other variable region. Linkers of other sequences have been designed and used (Bird et al., 1988, supra). Other exemplary linkers can generally include other GS linkers can generally include (GGGGS)_x, where x is 1, 2, 3, 4, 5 (SEQ ID NO: 613). In some embodiments, x is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or any integer less than about 20. In some embodiments, the linker is (GGGGS)₄ (SEQ ID NO: 602). In some embodiments the linker is GSTSGSGKPGSGEGSTKG (SEQ ID NO: 612), as described in Whitlow et al, *Protein Eng.* (1993) 6(8): 989-995. In general, linkers can be short, flexible polypeptides, which in some embodiments are comprised of about 20 or fewer amino acid residues. Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0186] In another aspect, provided is a CAR, which specifically binds to CD70, wherein the CAR comprises an extracellular ligand-binding domain comprising: a VH region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46 or 48; and/or a VL region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 1,

3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 or 47. In some embodiments, the VH and VL are linked together by a flexible linker. In some embodiments a flexible linker comprises the amino acid sequence shown in SEQ ID NO: 296.

[0187] In some embodiments, a CAR of the disclosure comprises an extracellular ligand-binding domain having any one of partial light chain sequence as listed in Table 1 and/or any one of partial heavy chain sequence as listed in Table 1. In Table 1, the underlined sequences are CDR sequences according to Kabat and in bold according to Chothia.

Table 1

mAb	Light Chain	Heavy Chain
31H1	DIVMTQNPLSSPVTLGQPASISCRSS <u>QSLVHSDGNTYLS</u> WLQQRPGQSPR LLIYKISNRFSGVPDRFSGSGAGTDF TLKISRVEAEDVGVYYC MQATQFP LT IGGGSKVEIK (SEQ ID NO: 1)	QVQLVQSGAEVKKPGSSVKVSCKAS GGTFSSYGF SWVRQAPGQGLEWMG <u>GIPIFGS</u> ANYAQKFQGRVTITADKS TSTVYMELISLRSEDTAVYYCARG GG SSPFAY WGQGTLLTVSS (SEQ ID NO: 2)
63B2	DIVMTQTPLSSPVTLGQPASISCRSS <u>QSLVHSDGNTYLS</u> WLQQRPGQSPR LLIYKISNRFSGVPDRFSGSGAGTDF TLKISRVEAEDVGVYYC MQATQFP LT IGGGSKVEIK (SEQ ID NO: 3)	QVQLVQSGAEVKKPGSSVKVSCKAS GGTFSSYGF SWVRQAPGQGLEWMG <u>GIPIFGT</u> ANYAQKFQGRVTITADKS TSTVFMELISLRSEYTAVYYCARG GG SSPFAY WGQGTLLTVSS (SEQ ID NO: 4)
40E3	DIQMTQSPSSLSASVGDRVTITCRAS <u>QGISNYLA</u> WFQKPGKAPKSLIYA <u>ASSLQSG</u> VPSKFSGSGSGTDFLTIS SLQPEDFATYYC QQYNSYPLT FGG GTKVEIK (SEQ ID NO: 5)	QVQLQESGPGLVKPSSETLSLTCTVSG GSISSYYWN WIRQPPGKGLEWIGYIY <u>YSGSTN</u> YNPSLKSRVTISVDTSKNQF SLKLRSVTAADTAVYYCARD DIRTW GQGTLLTVSS (SEQ ID NO: 6)

mAb	Light Chain	Heavy Chain
42C3	DVVMTQSPSLSPVTLGQPASISCR <u>SS</u> <u>QSLVYSDENTYLN</u> WFQQRPQGSLR RLIY <u>QVSNRDS</u> GVDPDRFSGSGSGTD FTLKISRVEAEDVGVYFC <u>MOGTYW</u> <u>PPT</u> FGGGTKVEIK (SEQ ID NO: 7)	EVQLVESGGGLVQPGGSLRLSCAAS <u>GFTFRNSWMSWVR</u> QAPGKGLEWV <u>ANIKRDGSEKYYVDSVKGR</u> TISR D NAKNSLYLQMNSLRAEDTAVYYCA <u>RDQTGSFDY</u> WGQGTLLTVSS (SEQ ID NO: 8)
45F11	EIVMTQSPATLSMSLGERATLSC <u>RA</u> <u>SQSVSSSLAWYQQKPGQAPRLLIYG</u> <u>ASTRAT</u> GIPARFGSGSGSGTEFTLTIS SLQSEDFAVYYC <u>QQYINWPH</u> FGGG TKVEIK (SEQ ID NO: 9)	QVQLRGSGPGLVKPSETLSLTCTVSD <u>DSISVYYWSWIRQ</u> PAGKGLEWIGRV <u>YSSGNINYNPSLES</u> RVTMSVDTSKSR FSLNLSVTAADTAVYYCARG <u>GLDAF</u> <u>DIW</u> GQGTMTVTVSS (SEQ ID NO: 10)
64F9	DIQMTQSPSSLSASVGDRVTITC <u>QAS</u> <u>QDISNYLNWYQQKPGKAPKILIIYG</u> <u>ASNLET</u> GVPSRFSGSGSGTDFTFAIS SLQPEDVATYYC <u>QQYDNFPIT</u> FGQ GTRLEIK (SEQ ID NO: 11)	EVQLLES GGGLVQPGESLRLSCEVSG <u>FTFTSYAMS</u> WVRQVPGKGLEWVSII <u>SGVAFTTY</u> YADSVKGRFTISRDRHSK NTLYLQMNGLRADTAVYYCVK <u>VD</u> <u>GEVY</u> WGQGTLLTVSS (SEQ ID NO: 12)
72C2	EIVMTQSPDTLSVSPGERAILSC <u>RAS</u> <u>QSVSSNLAWYQQKPGQAPRLLIYS</u> <u>ASTRAS</u> GIPARFSGSGSGTEFTLSISS LQSEDFAVYYC <u>QQYDNWPPLT</u> FG GGTKVEIK (SEQ ID NO: 13)	QVQLVQSGAEVKKPGSSVKVSCEAS <u>GGTFITYAIS</u> WVRQAPGQGLEWMG <u>GIIPFFGT</u> ANYAQKFQGRVTITADKS TSTASMELRSLRSEDAMYYCAQW <u>ELFFDF</u> WGQGTPTVTVSS (SEQ ID NO: 14)
2F10	EIVLTQSPGTLSPGERATLSC <u>RAS</u> <u>QSVSSSYLAWYQQQPGQAPRLLIY</u> <u>GASSRAT</u> GIPDRFSGSGSGTDFTLTI SRLEPEDFAIYYC <u>QQYGSSPLT</u> FGG GTKVEIK (SEQ ID NO: 15)	AVQLVESGGGLVQPGGSLRLSCAAS <u>GFTFTYYSMN</u> WVRQAPGKGLEWVS <u>HISIRSSTIYFADSAKGR</u> TISRDN AK NSLYLQMNSLRDEDTAVYYCARG <u>S</u> <u>GWYGDYFDYWG</u> QGTLLTVSS (SEQ ID NO: 16)

mAb	Light Chain	Heavy Chain
4F11	DIQMTQSPSAMSASVGDRVITITC <u>RA</u> <u>SQDISNYLA</u> WFQQKPGKVPKRLIYA <u>ASSLQSG</u> VPSRFSGSGSGTEFTLTIS LLPEDFATYYC <u>LQLNSFPFT</u> FGGGT KVEIN (SEQ ID NO: 17)	QVTLKESGPVLVKPTETLTCTVSG <u>FSLSNARMGVT</u> WIRQPPGKALEWL AH <u>IFSNDEKSYSTSLK</u> SRLTISKDTSK TQVVLTMNMDPVDATYYCAR <u>IR</u> <u>DYYDISSYYDY</u> WGQGTLVSVSS (SEQ ID NO: 18)
10H10	DIQMTQSPSSVSASVGDRVITITC <u>RAS</u> <u>QGISSWLA</u> WYQQKPGKAPKVLIIYA <u>ASSLQSG</u> VPSRFSGSGSGTDFTLTIS SLQPEDFATYYC <u>QQAFSFPFT</u> FGPG TKVDIK (SEQ ID NO: 19)	EVQLVESGGGLVQPGGSLRLSCAVS <u>GFTFSNHNIH</u> WVRQAPGKGLEWISY <u>ISRSSSTIYYADSVKGR</u> FTISRDN SLYLQMNSLRDEDTAVYYCARD <u>HHA</u> <u>QWYGMDV</u> WGQGTTVTVSS (SEQ ID NO: 20)
17G6	DIVMTQSPDSLAVSLGERATINC <u>KSS</u> <u>QSVLYSYNNKNYVA</u> WYQQKPGQP PNLLIF <u>WASTRES</u> GVPDRFSGSGSG TDFTLTISSLQAEDVAVYYC <u>QOQYYS</u> <u>TLTF</u> GGGGTKVEIK (SEQ ID NO: 21)	EVQLVESGGGLVQPGGSLRLSCVAS <u>GFTFSSYWMSW</u> VVRQAPGKGLEWV AS <u>IKQDGSEKYYVDSVKGR</u> FTISRDN NAKNSVYLQMNSLRDEDTGVYYCA <u>REGVNWGWRLYWHFDL</u> WGRGTL VTVSS (SEQ ID NO: 22)
65E11	EIVLTQSPGTLSPGERVTLSCT <u>RAS</u> <u>QSVSSSYLA</u> WYQQKPGQAPRLIIY <u>DASSRAT</u> GIPDRFSGSGSGTDFTLT SRLEPEDFAVYYC <u>QOYGSSPLT</u> FGG GTKVEIK (SEQ ID NO: 23)	EVQVVESGGGLVQPGGSLRLSCAAS <u>GFTFSSYSMNW</u> VVRQAPGKGLEWVS <u>HSSISRGNIFYADSVKGR</u> FTISRDN KNSLYLQMNSLRDEDTAVYYCARG <u>SGWYGDFDY</u> WGQGTLVTVSS (SEQ ID NO: 24)
P02B10	ELQSVLTQPPSASGTPGQRVTISCS <u>G</u> <u>SSSNIGSNYVY</u> WYQQLPGTAPKLLI Y <u>RNNQRPS</u> GVPDRFSGSGSGTSASL AISGLRSEDEADYYCA <u>AAWDDSLSG</u> <u>VV</u> FGGGTKLTVL (SEQ ID NO: 25)	EVQLLES GGGLVQPGGSLRLSCAAS <u>GFAFSNYAMS</u> WVRQAPGKGLEWVS <u>AIRGGGGSTYYADSVKGR</u> FTISRDN SKNTLYLQMNSLRDEDTAVYYCAR <u>DFISGTWYDPDY</u> WGQGTLVTVSS (SEQ ID NO: 26)

mAb	Light Chain	Heavy Chain
P07D03	ELQSVLTQPPSASGTPGQRVTISC <u>SG</u> <u>SRSNIGSNYVY</u> WYQQLPGTAPKLLI Y <u>RNNQRPS</u> GVPDRFSGSGSGTSASL AISGLRSEDEADYYC <u>ASWDGSLSA</u> <u>VV</u> FGTGTKLTVL (SEQ ID NO: 27)	EVQLVQSGAEVKKPGESLKISCKGS <u>GYRFTSYWIG</u> WVRQMPGKGLEWM <u>GSIIYPDDSD</u> TRYSPSFQGGQVTISADK SISTAYLQWSSLKASDTAMYYCASS <u>TVDYPGYSYFDY</u> WGQGTLVTVSS (SEQ ID NO: 28)
P08A02	ELQSVLTQPPSASGTPGQRVTISC <u>SG</u> <u>SSSNIGSNYVY</u> WYQQLPGTAPKLLI Y <u>RNNQRPS</u> GVPDRFSGSGSGTSASL AISGLRSEDEADYYC <u>ATWDDSLGS</u> <u>PV</u> FGTGTKLTVL (SEQ ID NO: 29)	EVQLVQSGAEVKKPGESLKISCKGS <u>GYTFTNYWIA</u> WVRQMPGKGLEWM <u>GIIYPDGS</u> TRYSPSFQGGQVTISADKS ISTAYLQWSSLKASDTAMYYCARDI <u>TSWYYGEPAFDI</u> WGQGTLVTVSS (SEQ ID NO: 30)
P08E02	ELDIQMTQSPSSLSASVGDRVITITC <u>R</u> <u>ASQISIRYLN</u> WYQQKPGKAPKLLIY <u>AASILQ</u> TGVPDRFSGSGSGTDFTLTI SSLQPEDFATYYC <u>QOSYSTTMWTF</u> GQGTVKVEIK (SEQ ID NO: 31)	EVQLVQSGAEVKKPGESLKISCKGS <u>GYSFTSSWIG</u> WVRQMPGKGLEWMG <u>IIYPGDS</u> TRYSPSFQGGQVTISADKSI STAYLQWSSLKASDTAMYYCAK <u>GL</u> <u>SQAMTGFGFDY</u> WGQGTLVTVSS (SEQ ID NO: 32)
P08F08	ELQSVLTQPPSASGTPGQRVTISC <u>SG</u> <u>SSSNIGSNYVN</u> WYQQLPGTAPKLLI Y <u>GDIYORPS</u> GVPDRFSGSGSGTSASL AISGLRSEDEADYYC <u>ATRDDSLSGS</u> <u>VV</u> FGTGTKLTVL (SEQ ID NO: 33)	EVQLVQSGAEVKKPGESLKISCKGS <u>GYGFTSYWIG</u> WVRQMPGKGLEWM <u>GIIHPDDSD</u> TKYSPSFQGGQVTISADKS ISTAYLQWSSLKASDTAMYYCASSY <u>LRGLWGGYFDY</u> WGQGTLVTVSS (SEQ ID NO: 34)
P08G02	ELDIQMTQSPSSLSASVGDRVITITC <u>R</u> <u>ASQSIYDYLH</u> WYQQKPGKAPKLLI Y <u>DASNLO</u> SGVPSRFSGSGSGTDFTL TISSLQPEDFATYYC <u>QOSYTTPLFTF</u> GQGTVKVEIK (SEQ ID NO: 35)	EVQLVQSGAEVKKPGESLKISCKGS <u>GYTFPSSWIG</u> WVRQMPGKGLEWM <u>GIIYPDTSH</u> TRYSPSFQGGQVTISADKS ISTAYLQWSSLKASDTAMYYCARAS <u>YFDRGTGYSSWWMDV</u> WGQGTLVT VSS (SEQ ID NO: 36)

mAb	Light Chain	Heavy Chain
P12B09	ELDIQMTQSPSSLSASVGDRVITITCR <u>ASQYIGRYLN</u> WYQQKRGKAPKLLI H <u>GATSLAS</u> GVPSRFSGSGSGTDFTL TISSLQPEDFATYYC <u>QOSYSTTSPTF</u> GQGTKVEIK (SEQ ID NO: 37)	EVQLLES GGGLVQP GGSLRLSCAAS <u>GFTFSQYMS</u> WVRQAPGKGLEWVS <u>AISGGGVSTYYADSVKGR</u> FTISRDN KNTLYLQMNSLRAEDTAVYYCAS <u>DI</u> <u>SDSGGSHWYFDY</u> WGQGTLVTVSS (SEQ ID NO: 38)
P12F02	ELQSVLTQPPSASGTPGQRVTISC <u>SG</u> <u>STSNIGRNYVY</u> WYQQLPGTAPKLLI Y <u>RTNQRP</u> SGVPDRFSGSKSGTSASL AISGLRSEDEADYYCA <u>AWDDSLSG</u> <u>RV</u> FGTGKLTVL (SEQ ID NO: 39)	EVQLLES GGGLVQP GGSLRLSCAAS <u>GFTFSSYAMS</u> WVRQAPGKGLEWVS <u>TISGTGGTTYADSVKGR</u> FTISRDN KNTLYLQMNSLRAEDTAVYYCAK <u>V</u> <u>RAGIDPTASDV</u> WGQGTLVTVSS (SEQ ID NO: 40)
P12G07	ELQSVLTQPPSASGTPGQRVTISC <u>SG</u> <u>SSSNIGSNYVY</u> WYQQLPGTAPKPLI Y <u>MNNQRP</u> SGVPDRFSGSKSGTSAS LAISGLRSEDEADYYCA <u>AWDDSL</u> <u>AVV</u> FGTGKLTVL (SEQ ID NO: 41)	EVQLLES GGGLVQP GGSLRLSCAAS <u>GFTFN NFAMS</u> WVRQAPGKGLEWVS <u>GISGSGDNTYYADSVKGR</u> FTISRDN KNTLYLQMNSLRAEDTAVYYCAK <u>D</u> <u>RDIGLGWYSY YLDV</u> WGQGTLVTVS S (SEQ ID NO: 42)
P13F04	ELQSVLTQPPSASGTPGQRVTISC <u>SG</u> <u>SNSNIGTNYVS</u> WYQQLPGTAPKLLI Y <u>RSSRRP</u> SGVPDRFSGSKSGTSASL AISGLRSEDEADYYCA <u>AWDGSLSG</u> <u>HWV</u> FGTGKLTVL (SEQ ID NO: 43)	QVQLVQSGAEVKKPGSSVKVSCAS <u>GGTFSSY AIS</u> WVRQAPGQGLEWMG <u>EIIPFGTASYAQKFQGR</u> VTITADEST STAYMELSSLRSEDTAVYYCAR <u>AG</u> <u>WDDSWFDY</u> WGQGTLVTVSS (SEQ ID NO: 44)
P15D02	ELDIQMTQSPSSLSASVGDRVITITCR <u>ASQSIDTYLN</u> WYQQKPGKAPKLLI Y <u>SASSLHSG</u> GVPSRFSGSGSGTDFTLT ISSLQPEDFATYYC <u>QOSYSTTAWTF</u> GQGTKVEIK (SEQ ID NO: 45)	EVQLVQSGAEVKKPGESLKISCKGS <u>GYSFASYWIG</u> WVRQMPGKGLEWM <u>GVIYPGTSETRYSPSFQGQ</u> VTISADK SISTAYLQWSSLKASDTAMYYCAK <u>G</u> <u>LSASASGYSEFQY</u> WGQGTLVTVSS (SEQ ID NO: 46)

mAb	Light Chain	Heavy Chain
P16C05	ELDIQMTQSPSSLSASVGDRVITITC <u>R</u> <u>ASQSIGQSLN</u> WYQQKPGKAPKLLI Y <u>GASSLQSG</u> VPSRFSGSGSGTDFTL TISSLQPEDFATYYC <u>QOQSYSTPIT</u> FG QGTVKEIK (SEQ ID NO: 47)	EVQLVQSGAEVKKPGESLKISCKGS <u>GYSFTDYWIG</u> WVRQMPGKGLEWM <u>GMISPGGSTTI</u> YRPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARE <u>MYTGGYGGSWYFDY</u> WGQGTLLTV SS(SEQ ID NO: 48)
10A1	DIQMTQSPSTLSASVGDRVITITC <u>RAS</u> <u>QSISTWLA</u> WYQQKPGKAPKVLII <u>K</u> <u>ASSLES</u> GVPSRFSGSGSGTEFILTINS LQPDDFASYC <u>QOQKSYSH</u> TFGQG TKLEIK (SEQ ID NO: 338)	QVQLQESGPGLVKPSETLSLTCTVSG <u>GSISYYYWTWIR</u> QPPGKGLEWIGHI <u>YVSGSTNYP</u> SLKSRVTISIDTSKNLF SLKLSSVTAADTAVYYCAR <u>AEGSID</u> <u>AFDF</u> WGQGTMTVTVSS (SEQ ID NO: 339)
10E2	DIQMTQSPSTLSASVGDRVITITC <u>RAS</u> <u>QSISSWLA</u> WYQQKPGKAPKVLII <u>K</u> <u>ASSLES</u> GVPSRFSGSGSGTEFTLTINS LQPDDFATYYC <u>QOQKSFSL</u> TFGQG TKLEIK (SEQ ID NO: 340)	EVQLVESGGGLIQPGSLRLSCAASG <u>FTVSSNYMTWVR</u> QAPGKGLEWVSV <u>IYSGGSTYYADSVKGR</u> FTISRDNSKN TLYLQMNSLR AEDTAVYYCAR <u>NWG</u> <u>DYW</u> GQGTLLTVTVSS (SEQ ID NO: 341)
11A1	DIQMTQSPSTLSASVGDRVITITC <u>RAS</u> <u>QSISSWLA</u> WYQQKPGKAPKVLII <u>K</u> <u>ASTLES</u> GVPSRFSGSGSGTEFTLTIS LQPDDFATYYC <u>QOQNSYSY</u> TFGHG TKLEIK (SEQ ID NO: 342)	QVQLQESGPGLVKPSGTLSTCTVSG <u>GSIDYYFWNWFR</u> QSPVKGLEWIGHI <u>VYDIGNTKYN</u> PSLKSRVTISIDTSEN QFSLKLNSVTAADTAVYYCARG <u>GEG</u> <u>AIDAFDI</u> WGQGTMTVTVSS (SEQ ID NO: 343)
11C1	DIQMTQSPSILSASVGDRVITITC <u>RAS</u> <u>QSVSSWLA</u> WYQQKPGKAPKVLII <u>K</u> <u>ASSLES</u> GVPSRFSGTGSGTEFTLTIS LQSDDFATYYC <u>QOQNTYSHT</u> TFGQG TKLEIK (SEQ ID NO: 344)	QVQLQESGPGLVKPSETLSLNCTVSG <u>GSISYYYWTWIR</u> QPPGKGLEWIGHV <u>IYSGTTNYP</u> PSLKSRVTISVDTSKNQ FSLKLNSVTAADTAVYYCVRA <u>EGSI</u> <u>DAFDL</u> WGQGTMTVTVSS (SEQ ID NO: 345)

mAb	Light Chain	Heavy Chain
11D1	AIQMTQSPSSLSASVGDRVITITC <u>RAS</u> <u>QGIRNDLG</u> WYQQKPGKAPKLLIYA <u>ASSLQSG</u> VPSRFSGSGSGTDFTLTIS SLQPEDFATYYC <u>LQDYNYPFT</u> FGPG TKVDIK (SEQ ID NO: 346)	QVQLVESGGGVVQPGRSLRLSCVAS <u>GFTFSDYGIH</u> WVRQAPGMGQEWVA <u>VIWYDGSi</u> KKYSDSVKGRFIISRDNS ENTVYLQMNSLRGEDTAIYYCAR <u>DE</u> <u>VGtfGA</u> <u>FD</u> FWGQGTKVTVSS (SEQ ID NO: 347)
11E1	DIQMTQSPSSLSASVGDSITITC <u>RAS</u> <u>QDIDNYLA</u> WYQQKTGKVPKVLIIYA <u>ASALQSG</u> VPSRFSGSGSGTDFTLTIS SLQPEDVATYYC <u>QNYNSGPRT</u> FGQ GTKVEIK (SEQ ID NO: 348)	QVQLQESGPGLVKPLQTLSTCTVS <u>GGSISSdgYY</u> WSWIRQNPGKGLEWI <u>GYMYYS</u> GSTYYNPSLKSRVTISVDT SKNQFSLKLRSVTAADTAVYYCTR <u>D</u> <u>FGWYFDL</u> WGRGTLVTVSS (SEQ ID NO: 349)
12A2	DIQMTQSPSSLSASVGDRVITITC <u>RAS</u> <u>QDISNYLT</u> WYQQKPGRVPEVLIYA <u>ASALQSG</u> VPSRFSGSGSGTDFTLTIS SLQPEDVATYYC <u>QNYNSAPRT</u> FGQ GTKVEIK (SEQ ID NO: 350)	QVQLQESGPGLVKPSQSLSTCSVSG <u>GSVSSdgYY</u> WSWIRQHHPGKGLEWIG <u>YIYYRRITDYN</u> PSLKSRVNISLDTSK NQFSLKLSSVTAADTAVYYCAR <u>DFG</u> <u>WYFDL</u> WGRGTLVAVSS (SEQ ID NO: 351)
12C4	DIVMTQSPLSLPVTTPGEPASISC <u>RSS</u> <u>QSLHNSGYN</u> LDWYLQKPGQSP QVLIL <u>LGSNRAS</u> GVPDRVSASGSGT DFTLKISRMQAEDVGIYYC <u>MQTLQ</u> <u>TPFT</u> FGQGTKLEIK (SEQ ID NO: 352)	QVQLVQSGAEVKKPGASVKVSCKA SGYTFT <u>GYYLH</u> WVRQAPGQGLEW MGWIN <u>pNS</u> GGTNYAQKFQGRVTMT RDTSTTAYMELSRLRIDDTAVYYCA <u>RDRGVtmivDG</u> MDDWGQGTTVTVS S (SEQ ID NO: 353)
12C5	DIQLTQSPSFLSASVGDRVITITC <u>RAS</u> <u>QGINSHLA</u> WYQQKPGKAPKLLIYY <u>ASTLPSG</u> VPSRFSGSGSGTEFTLTVT SLQPEDFATYYC <u>QQLNHYPIT</u> FGQ GTRLIDIN (SEQ ID NO: 354)	EVELVESGGGMVQPGRSLRLSCAAS <u>GFTFSDYGMH</u> WVRQAPGMGLEWV <u>TVIWYDGSn</u> KYYADSVKGRFTISR NSKNTVFLQMNSLRAEDTAVYYCA <u>RDEVGfvGA</u> FDIWGQGTMTVTVSS (SEQ ID NO: 355)

mAb	Light Chain	Heavy Chain
12C6	DIQLTQSPSFLSASVGDRVIITC <u>RAS</u> <u>QGINSHLA</u> WYQQKPGKAPKLLIY <u>Y</u> <u>ASTLPS</u> GVPSRFSGSGSGTEFTLTVT SLQPEDFATYYC <u>QQLNHYPIT</u> FGQ GTRLEIK (SEQ ID NO: 661)	EVELVESGGGMVQPGRSLRLSCAAS <u>GFTFSDYGMH</u> WVRQAPGMGLEWV <u>TVIWDGS_nKYY</u> ADSVKGRFTISRDN SKNTVFLQMNSLRAEDTAVYYCA <u>RDEVGfvGAFDI</u> WGQGTMTVTVSS (SEQ ID NO: 662)
12D3	DIQMTQSPSSLASVGDRVTITC <u>RAS</u> <u>QGISNYLA</u> WYQQKPGKVPKLLIYA <u>ASTLHS</u> GVPSRFSGSGSGTDFTLTIS SLQPEDVATYYC <u>QKYNSAPRT</u> FGQ GTKVEIK (SEQ ID NO: 356)	QVQLQESGPGLVKPSQTLSTCTVSG <u>GSISSdgYYWS</u> WIRQHPGKGLEWIGY <u>MYYSGITYHNPSLKS</u> RVTISVDTSKN QFSLRLSSVTAADTAVYYCAR <u>DFG</u> <u>WYFDL</u> WGRGTLTVTVSS (SEQ ID NO: 357)
12D6	DIQMTQSPSSLASVGDRVTITC <u>RAS</u> <u>QDISNYLA</u> WYQQKPGKVPKLLIYA <u>ASTLHS</u> GVPSRFSGSGSGTDFTLTIS SLQPDDFAAYYC <u>QKYNSAPRT</u> FGQ GTKVEIK (SEQ ID NO: 358)	QVQLQESGPGLVKPSQTLSTCTVSG <u>GSISSdaYYWS</u> WIRQHPGKGLEWIGY <u>MYYSGITYYNPSLKS</u> RVTISVDTSKN QFSLKLSSVTAADTAVYYCAR <u>DFG</u> <u>WYFDL</u> WGRGTLTVTVSS (SEQ ID NO: 359)
12D7	DIQLTQSPSFLSASVGDRVSITC <u>RAS</u> <u>QDISSFLA</u> WYQQKPGKAPVLLIY <u>YA</u> <u>STLQSG</u> VPSPRFSGSGSGTEFTLTVSS LQPEDFATYYC <u>QQLHVYPIT</u> FGQG TRLEIR (SEQ ID NO: 360)	QVQLVESGGGVVQPGRSLRLSCVAS <u>GFTFSDYGIH</u> WVRQAPGMGQEWVA <u>VIWYDGSiKKYSDSVKGR</u> FIISRDN ENTVYLQMNSLRGEDTAIYYCAR <u>DE</u> <u>VGtfGAFD</u> FWGQGTKVTVSS (SEQ ID NO: 361)
12F5	DIVMTQTPLSLPVTGPGEPAISCR <u>RSS</u> <u>QSLDSDDGn_tYLD</u> WYLQKPGQSP QLLIY <u>TLSYRAS</u> GVDPDRFSGSGSGT DFTLKISRVEAEDVGVYYC <u>MQRIEF</u> <u>PFT</u> FGPGTKVDIK (SEQ ID NO: 362)	EVQLVESGGGLVKPGGSLRLSCAAS <u>GFTFSNAWMS</u> WVRQAPGKGLEWV <u>GRIKsktGGG</u> TTDYAAPVKGRFTISR DDSKNTLYLQMNSLKTEDTAVYYC <u>TSLIVGaiSLFDY</u> WGQGTMTVTVSS (SEQ ID NO: 363)

mAb	Light Chain	Heavy Chain
12H4	DIQMTQSPSALSASVGDRVAITCRA <u>SQTISTWLA</u> WYQQKPGKAPKVLIY <u>KASNLES</u> GVPSRFSGSGSGTEFTLTI NSLQPDDFATYYC <u>QQYQTFST</u> FTG QGTKLEIK (SEQ ID NO: 364)	QVQLRESGPGLVKPSETLSLTCTISG <u>GSISYYFWT</u> WIRQPPGRGLEWIGQIY <u>YSGNTNSNPSLKS</u> SRVTISIDTSKNQFS LKLTSVTVADTAVYYCVRA <u>AEGSIDA</u> <u>FDI</u> WGQGTMTAVSS (SEQ ID NO: 365)
8C8	DMQMTQSPSSLASVGDRVTLTCR <u>ASQGISNYLA</u> WFQLKPGKVPKLLIY <u>AASTLOS</u> GVPSRFSGSGSGTDFALTI SSLQPEDVATYYC <u>QKYN</u> SAPLTFG GGTKVEIK (SEQ ID NO: 366)	EVQLVESGGGLVKPGGSLRLSCVAS <u>GFTFSSYS</u> MNWVRQFPKGLEWVS <u>SIS</u> tSSNYIH Y ADSLQGRFTISRDNAL NSLYLQMSSLRVEDTAVYYCVR <u>DK</u> <u>GT</u> ltnWYFDLWGRGTLTVSS (SEQ ID NO: 367)
8F7	DIVMTQSPLSLPVTGPGEPAISCRSS <u>QTLVHSNGYNYLN</u> WYLQKPGQSP QLLIY <u>LGSNRAS</u> GVDPDRFSGSGSGS DFTLKISRMEAEDVGYYC <u>MQAIO</u> <u>TPY</u> TFGQGTNVEIK (SEQ ID NO: 368)	QVQLVESGGGVVQPGSRSLRLSCGAS <u>GFTFSSYGMH</u> WVRQAPKGLEWV A <u>VIWYDGS</u> nKYYADSLKGRFTISRDN NSKNTLYLQMNSLRAEDTAVYYCA <u>RDGYS</u> gssDAFDIWGQGTMTVTVSS (SEQ ID NO: 369)
8F8	DIQMTQSPSTLSASVGDRVTITCRAS <u>QSISSWLA</u> WYQQKPGKAPKVLIYK <u>ASNLES</u> GVPSRFSGSGSGTEFTLTISS LQPDDFATYYC <u>QQYNSYSCT</u> FGQG TKLEIK (SEQ ID NO: 370)	QVQLQESGPGLVQPSETLSLTCTVSG <u>GSISYYYWS</u> WIRQPPKGLEWIGNIN <u>YMGNTIYNPSLKS</u> SRVTISVDTSKDQF SLKLTSVSAADTAVYYCVRA <u>AEGSID</u> <u>AFDF</u> WGQGTLVAVSL (SEQ ID NO: 371)
9D8	DIQMTQSPSSLASVGDRIFTCAQAS <u>QDINNYLH</u> WYQQKPGKAPKLLIYD <u>ASDWET</u> GVPSRFSGSGSGTDFTFITIS SLQPEDIATYYC <u>QQYDHL</u> PITFGQG TRVEIK (SEQ ID NO: 372)	QVQLVQSGAEVTKPGASVKVSCKAS <u>GYIFTGYIY</u> WVRQAPGQGLEWMG <u>WINpSSG</u> GTNYAQKFQGRVTMARD TSISTAYMELSSLRSDDTAVYYCARD <u>RKRevvynFGMDV</u> WGQGTTVTVST (SEQ ID NO: 373)

mAb	Light Chain	Heavy Chain
9E10	DIQMTQSPSSLSASVGDRVILTC <u>QAS</u> <u>QDISNYLHWYQQKPGKAPKLLIYD</u> <u>ASDLET</u> GVPSRFSGSGSGADFTFTIS NLQPEDFATYYC <u>QQYDHLPI</u> TFGQ GTRLEIK (SEQ ID NO: 374)	QVQLVQSGAEVTKPGASVKVSCCKAS <u>GYTFTSHYIYWVRQAPGQGLEWMG</u> <u>WINpNSGGTNYAQKFQDRVTMARD</u> TSISTAYMELSRLRSDDTAVYYCAK <u>DRKReyyynFGMDV</u> WGQGTTVTVSA (SEQ ID NO: 375)
9E5	DIQMTQSPSSLSASVGDRVILTC <u>QAS</u> <u>QDISNYLHWYQQKPGKAPKLLIYD</u> <u>ASDLET</u> GVPSRFSGSGSGADFTFTIS NLQPEDFATYYC <u>QQYDHLPI</u> TFGQ GTRLEIK (SEQ ID NO: 376)	QVQLVQFGVEVRKPGASVKVSCCKVS <u>GFTFTSHYIYWVRQAPGQGLEWMG</u> <u>WINpNSGGTKYAQKFQDRVTMARD</u> TSISTAYMELSRLRSDDTSVYYCVK <u>D</u> <u>RKReyyynFGMDV</u> WGQGTTVTVSS (SEQ ID NO: 377)
9F4	DIQMTQSPSSLSASVGDRVITITC <u>QAS</u> <u>QDISNYLNWYQQKPGKAPKLLIYD</u> <u>ASNLET</u> GVPSRFSGSGSGTDFFTFTIS SLQPEDIATYYC <u>QQYDNLPTY</u> TFGQ GTKLEIK (SEQ ID NO: 378)	EVQMLESGGGLIQPGGSLRLSCKTSG <u>FTLSIYAIHWVRQAPGRGLEWVSSE</u> <u>GgRGSSTYFADSVKGRFTISRDA</u> SEN SLYLHMNSLRAEDTAVYYCAKE <u>ED</u> <u>WgRGFDY</u> WGQGTTLTVSS (SEQ ID NO: 379)
9F8	DIVMTQSPLSLPVTGPGEPAISCR <u>RSS</u> <u>QSLLYSNGYNYLD</u> WYLQKPGQSPQ LLIFL <u>NSNRAS</u> GVDPDRFSGSGSGTDF TLKISRVEAEDVGVYFC <u>MQALQTP</u> <u>LT</u> FGGGTKVEIK (SEQ ID NO: 380)	EVQLVESGGGLVKPGGSLRLSCAAS <u>GFTFSNYSMNWVRQAPGKGLEWVS</u> <u>SISsSTIYIYYADSVKGRFTISR</u> DN AKSLYLQMNSLRAEDTAVYYCARD <u>DIG</u> <u>WevftLGFDY</u> WGQGTQVTVSS (SEQ ID NO: 381)

[0188] Also provided herein are CDR portions of extracellular ligand-binding domains of CARs to CD70 (including Chothia, Kabat CDRs, and CDR contact regions). Determination of CDR regions is well within the skill of the art. It is understood that in some embodiments, CDRs can be a combination of the Kabat and Chothia CDR (also termed “combined CRs” or “extended CDRs”). In some embodiments, the CDRs are the Kabat CDRs. In other embodiments, the CDRs are the Chothia CDRs. In other words, in embodiments with more than

one CDR, the CDRs may be any of Kabat, Chothia, combination CDRs, or combinations thereof. Tables 2A-2B provide examples of CDR sequences provided herein.

Table 2A

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
31H1	SYGFS (SEQ ID NO: 49) (Kabat); GGTFSSY (SEQ ID NO: 50) (Chothia); GGTFSSYGFS (SEQ ID NO: 51) (Extended)	GIPIFGSANYAQK FQG (SEQ ID NO: 52) (Kabat); IPIFGS (SEQ ID NO: 53) (Chothia)	GGSSSPFAY (SEQ ID NO: 54)
63B2	SYGFS (SEQ ID NO: 55) (Kabat); GGTFSSY (SEQ ID NO: 56) (Chothia) GGTFSSYGFS (Extended) (SEQ ID NO: 57)	GIPIFGTANYAQK FQG (SEQ ID NO: 58) (Kabat); IPIFGT (SEQ ID NO: 59) (Chothia)	GGSSSPFAY (SEQ ID NO: 60)
40E3	SYIWN (SEQ ID NO: 61) (Kabat); GGSISSY (SEQ ID NO: 62) (Chothia); GGSISSYIWN (SEQ ID NO: 63) (Extended)	YIYSGSTNYPNS LKS (SEQ ID NO: 64) (Kabat); YYSGS (SEQ ID NO: 65) (Chothia)	DIRTW (SEQ ID NO: 66)
42C3	NSWMS (SEQ ID NO: 67) (Kabat); GFTFRNS (SEQ ID NO: 68) (Chothia); GFTFRNSWMS (SEQ ID NO: 69) (Extended)	NIKRDGSEKYYV DSVKG (SEQ ID NO: 70) (Kabat); KRDGSE (SEQ ID NO: 71) (Chothia)	DQTGSFDY (SEQ ID NO: 72)

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
45F11	VYYWS (SEQ ID NO: 73) (Kabat); DD SISVY (SEQ ID NO: 74) (Chothia); DD SISVYYWS (SEQ ID NO: 75) (Extended)	VYSSGNINYNPSL ES (SEQ ID NO: 76) (Kabat); YSSGN (SEQ ID NO: 77) (Chothia)	GLDAFDI (SEQ ID NO: 78)
64F9	SYAMS (SEQ ID NO: 79) (Kabat); GFTFTSY (SEQ ID NO: 80) (Chothia); GFTFTSYAMS (SEQ ID NO: 81) (Extended)	RVYSSGNINYNPS LES (SEQ ID NO: 82) (Kabat); YSSGN (SEQ ID NO: 83) (Chothia)	GLDAFDI (SEQ ID NO: 84)
72C2	TYAIS (SEQ ID NO: 85) (Kabat); GGTFITY (SEQ ID NO: 86) (Chothia); GGTFITYAIS (SEQ ID NO: 87) (Extended)	GIIPFFGTANYAQ KFQG (SEQ ID NO: 88) (Kabat); IPFFGT (SEQ ID NO: 89) (Chothia)	WELFFFDF (SEQ ID NO: 90)
2F10	YYSMN (SEQ ID NO: 91) (Kabat); GFTFTYY (SEQ ID NO: 92) (Chothia); GFTFTYYSMN (SEQ ID NO: 93) (Extended)	HISIRSSTIYFADS AKG (SEQ ID NO: 94) (Kabat); SIRSST (SEQ ID NO: 95) (Chothia)	GSGWYGDYFDY (SEQ ID NO: 96)
4F11	NARMGVT (SEQ ID NO: 97) (Kabat); GFSLSNARM (SEQ ID NO: 98) (Chothia); GFSLSNARMGVT (SEQ ID NO: 99) (Extended)	HIFSNDEKSYSTS LKS (SEQ ID NO: 100) (Kabat); FSNDE (SEQ ID NO: 101) (Chothia)	IRDYYDISSYYDY (SEQ ID NO: 102)

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
10H10	NHNIH (SEQ ID NO: 103) (Kabat); GFTFSNH (SEQ ID NO: 104) (Chothia); GFTFSNHNIH (SEQ ID NO: 105) (Extended)	YISRSSSTIYYADS VKG (SEQ ID NO: 106) (Kabat); SRSSST (SEQ ID NO: 107) (Chothia)	DHAQWYGMDV (SEQ ID NO: 108)
17G6	SYWMS (SEQ ID NO: 109) (Kabat); GFTFSSY (SEQ ID NO: 110) (Chothia); GFTFSSYWMS (SEQ ID NO: 111) (Extended)	SIKQDGSEKYYV DSVKG (SEQ ID NO: 112) (Kabat); KQDGSE (SEQ ID NO: 113) (Chothia)	EGVNWGWRLYW HFDL (SEQ ID NO: 114)
65E11	SYSMN (SEQ ID NO: 115) (Kabat); GFTFSSY (SEQ ID NO: 116) (Chothia); GFTFSSYSMN (SEQ ID NO: 117) (Extended)	HSSISRGNIYFADS VKG (SEQ ID NO: 118) (Kabat); SISRGN (SEQ ID NO: 119) (Chothia)	GSGWYG DYFDY (SEQ ID NO: 120)
P02B10	NYAMS (SEQ ID NO: 121) (Kabat); GFAFSNY (SEQ ID NO: 122) (Chothia); GFAFSNYAMS (SEQ ID NO: 123) (Extended)	AIRGGGGSTYYA DSVKG (SEQ ID NO: 124) (Kabat); RGGGGS (SEQ ID NO: 125) (Chothia)	DFISGTWYPDY (SEQ ID NO: 126)
P07D03	SYWIG (SEQ ID NO: 127) (Kabat); GYRFTSY (SEQ ID NO: 128) (Chothia); GYRFTSYWIG (SEQ ID NO: 129) (Extended)	SIYPDDSDTRYSP SFQG (SEQ ID NO: 130) (Kabat); YPDDSD (SEQ ID NO: 131) (Chothia)	STVDYPGYSYFD Y (SEQ ID NO: 132)

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
P08A02	NYWIA (SEQ ID NO: 133) (Kabat); GYTFTNY (SEQ ID NO: 134) (Chothia); GYTFTNYWIA (SEQ ID NO: 135) (Extended)	IIYPDGSDTRYSPS FQG (SEQ ID NO: 136) (Kabat); YPDGSD (SEQ ID NO: 137) (Chothia)	DITSWYYGEPAF DI (SEQ ID NO: 138)
P08E02	SSWIG (SEQ ID NO: 139) (Kabat); GYSFTSS (SEQ ID NO: 140) (Chothia); GYSFTSSWIG (SEQ ID NO: 141) (Extended)	IIYPGDS DTRYSPS FQG (SEQ ID NO: 142) (Kabat); YPGDS D (SEQ ID NO: 143) (Chothia)	GLSQAMTGFGFD Y (SEQ ID NO: 144)
P08F08	SYWIG (SEQ ID NO: 145) (Kabat); GYGFTSY (SEQ ID NO: 146) (Chothia); GYGFTSYWIG (SEQ ID NO: 147) (Extended)	IIHPDDSDTKYSPS FQG (SEQ ID NO: 148) (Kabat); HPDDSD (SEQ ID NO: 149) (Chothia)	SYLRGLWGGYFD Y (SEQ ID NO: 150)
P08G02	SSWIG (SEQ ID NO: 151) (Kabat); GYTFPSS (SEQ ID NO: 152) (Chothia); GYTFPSSWIG (SEQ ID NO: 153) (Extended)	IIYPDTS HTRYSPS FQ (SEQ ID NO: 154) (Kabat); YPDTS H (SEQ ID NO: 155) (Chothia)	ASYFDRGTGYSS WWMDV (SEQ ID NO: 156)
P12B09	QYSMS (SEQ ID NO: 157) (Kabat); GFTFSQY (SEQ ID NO: 158) (Chothia); GFTFSQYSMS (SEQ ID NO: 159) (Extended)	AISGGGVSTYYA DSVKG (SEQ ID NO: 160) (Kabat); SGGGVS (SEQ ID NO: 161) (Chothia)	DISDSGGSHWYF DY (SEQ ID NO: 162)

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
P12F02	SYAMS (SEQ ID NO: 163) (Kabat); GFTFSSY (SEQ ID NO: 164) (Chothia); GFTFSSYAMS (SEQ ID NO: 165) (Extended)	TISGTGGTTYAD SVKG (SEQ ID NO: 166) (Kabat); SGTGGT (SEQ ID NO: 167) (Chothia)	VRAGIDPTASDV (SEQ ID NO: 168)
P12G07	NFAMS (SEQ ID NO: 169) (Kabat); GFTFNNF (SEQ ID NO: 170) (Chothia); GFTFNNFAMS (SEQ ID NO: 171) (Extended)	GISGSGDNTYYA DSVKG (SEQ ID NO: 172) (Kabat); SGSGDN (SEQ ID NO: 173) (Chothia)	DRDIGLGWYSYY LDV (SEQ ID NO: 174)
P13F04	SYAIS (SEQ ID NO: 175) (Kabat); GGTFSSY (SEQ ID NO: 176) (Chothia); GGTFSSY AIS (SEQ ID NO: 177) (Extended)	EIIPFGTASYAQK FQG (SEQ ID NO: 178) (Kabat); IIPFGT (SEQ ID NO: 179) (Chothia)	AGWDDSWFDY (SEQ ID NO: 180)
P15D02	SYWIG (SEQ ID NO: 181) (Kabat); GYSFASY (SEQ ID NO: 182) (Chothia); GYSFASYWIG (SEQ ID NO: 183) (Extended)	VIYPGTSETRYSPS FQG (SEQ ID NO: 184) (Kabat); YPGTSE (SEQ ID NO: 185) (Chothia)	GLSASASGYSFQ Y (SEQ ID NO: 186)
P16C05	DYWIG (SEQ ID NO: 187) (Kabat); GYSFTDY (SEQ ID NO: 188) (Chothia); GYSFTDYWIG (SEQ ID NO: 189) (Extended)	MISPGGSTTIYRPS FQG (SEQ ID NO: 190) (Kabat); SPGGST (SEQ ID NO: 191) (Chothia)	MYTGGYGGSWY FDY (SEQ ID NO: 192)

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
10A1	YYYWT (SEQ ID NO: 382) (Kabat); GGISYY (SEQ ID NO: 383) (Chothia); GGISYYYWT (SEQ ID NO: 384) (Extended)	HIYYSGSTNYP LKS (SEQ ID NO: 385) (Kabat); YYSGS (SEQ ID NO: 386) (Chothia)	AEGSIDAFDF (SEQ ID NO: 387)
10E2	SNYMT (SEQ ID NO: 388) (Kabat); GFTVSSN (SEQ ID NO: 389) (Chothia); GFTVSSNYMT (SEQ ID NO: 390) (Extended)	VIYSGGSTYYADS VKG (SEQ ID NO: 391) (Kabat); YSGGS (SEQ ID NO: 392) (Chothia)	NWGDYW (SEQ ID NO: 393)
11A1	YYFWN (SEQ ID NO: 394) (Kabat); GGSIDYY (SEQ ID NO: 395) (Chothia); GGSIDYYFWN (SEQ ID NO: 396) (Extended)	HVYDIGNTKYNP SLKS (SEQ ID NO: 397) (Kabat); YDIGN (SEQ ID NO: 398) (Chothia)	GEGAIDAFDI (SEQ ID NO: 399)
11C1	YYYWT (SEQ ID NO: 400) (Kabat); GGISYY (SEQ ID NO: 401) (Chothia); GGISYYYWT (SEQ ID NO: 402) (Extended)	HVIYSGTTNYP LKS (SEQ ID NO: 403) (Kabat); IYSGT (SEQ ID NO: 404) (Chothia)	AEGSIDAFDL (SEQ ID NO: 405)
11D1	DYGIH (SEQ ID NO: 406) (Kabat); GFTFSDY (SEQ ID NO: 407) (Chothia); GFTFSDYGIH (SEQ ID NO: 408) (Extended)	VIWYDGSiKKYSD SVKG (SEQ ID NO: 409) (Kabat); WYDGSi (SEQ ID NO: 410) (Chothia)	DEVGtGAFDF (SEQ ID NO: 411)

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
11E1	SdgYYWS (SEQ ID NO: 412) (Kabat); GGSISSdgY (SEQ ID NO: 413) (Chothia); GGSISSdgYYWS (SEQ ID NO: 414) (Extended)	YMYYSGSTYYNP SLKS (SEQ ID NO: 415) (Kabat); YYSGS (SEQ ID NO: 416) (Chothia)	DFGWYFDL (SEQ ID NO: 417)
12A2	SdgYYWS (SEQ ID NO: 418) (Kabat); GGSVSSdgY (SEQ ID NO: 419) (Chothia); GGSVSSdgYYWS (SEQ ID NO: 420) (Extended)	YIYYRRITDYNPS LKS (SEQ ID NO: 421) (Kabat); YYRRI (SEQ ID NO: 422) (Chothia)	DFGWYFDL (SEQ ID NO: 423)
12C4	GYYLH (SEQ ID NO: 424) (Kabat); GYTFTGY (SEQ ID NO: 425) (Chothia); GYTFTGYYLH (SEQ ID NO: 426) (Extended)	WINpNSGGTNYA QKFQG (SEQ ID NO: 427) (Kabat); NpNSGG (SEQ ID NO: 428) (Chothia)	DRGVtmivDGMD D (SEQ ID NO: 429)
12C5	DYGMH (SEQ ID NO: 430) (Kabat); GFTFSDY (SEQ ID NO: 431) (Chothia); GFTFSDYGMH (SEQ ID NO: 432) (Extended)	VIWYDGSnKYA DSVKG (SEQ ID NO: 433) (Kabat); WYDGSn (SEQ ID NO: 434) (Chothia)	DEVGfvGAFDI (SEQ ID NO: 435)
12C6	DYGMH (SEQ ID NO: 663) (Kabat); GFTFSDY (SEQ ID NO: 664) (Chothia); GFTFSDYGMH (SEQ ID NO: 665) (Extended)	VIWYDGSnKYA DSVKG (SEQ ID NO: 666) (Kabat); WYDGSn (SEQ ID NO: 667) (Chothia)	DEVGfvGAFDI (SEQ ID NO: 668)

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
12D3	SdgYYWS (SEQ ID NO: 436) (Kabat); GGSISSdgY (SEQ ID NO: 437) (Chothia); GGSISSdgYYWS (SEQ ID NO: 438) (Extended)	YMYYSGITYHNP SLKS (SEQ ID NO: 439) (Kabat); YYSGI (SEQ ID NO: 440) (Chothia)	DFGWYFDL (SEQ ID NO: 441)
12D6	SdaYYWS (SEQ ID NO: 442) (Kabat); GGSISSdaY (SEQ ID NO: 443) (Chothia); GGSISSdaYYWS (SEQ ID NO: 444) (Extended)	YMYYSGITYYNP SLKS (SEQ ID NO: 445) (Kabat); YYSGI (SEQ ID NO: 446) (Chothia)	DFGWYFDL (SEQ ID NO: 447)
12D7	DYGIH (SEQ ID NO: 448) (Kabat); GFTFSDY (SEQ ID NO: 449) (Chothia); GFTFSDYGIH (SEQ ID NO: 450) (Extended)	VIWYDGSiKKYSD SVKG (SEQ ID NO: 451) (Kabat); WYDGSi (SEQ ID NO: 452) (Chothia)	DEVGtfGAFDF (SEQ ID NO: 453)
12F5	NAWMS (SEQ ID NO: 454) (Kabat); GFTFSNA (SEQ ID NO: 455) (Chothia); GFTFSNAWMS (SEQ ID NO: 456) (Extended)	RIKsktGGGTTDYA APVKG (SEQ ID NO: 457) (Kabat); KsktGGGT (SEQ ID NO: 458) (Chothia)	LIVGaiSLFDY (SEQ ID NO: 459)
12H4	YYFWT (SEQ ID NO: 460) (Kabat); GGSISYY (SEQ ID NO: 461) (Chothia); GGSISYYFWT (SEQ ID NO: 462) (Extended)	QIYYSGNTNSNPS LKS (SEQ ID NO: 463) (Kabat); YYSGN (SEQ ID NO: 464) (Chothia)	AEGSIDAFDI (SEQ ID NO: 465)

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
8C8	SYSMN (SEQ ID NO: 466) (Kabat); GFTFSSY (SEQ ID NO: 467) (Chothia); GFTFSSYSMN (SEQ ID NO: 468) (Extended)	SISStSSNYIHYADS LQG (SEQ ID NO: 469) (Kabat); StSSNY (SEQ ID NO: 470) (Chothia)	DKGTtltnWYFDL (SEQ ID NO: 471)
8F7	SYGMH (SEQ ID NO: 472) (Kabat); GFTFSSY (SEQ ID NO: 473) (Chothia); GFTFSSYGMH (SEQ ID NO: 474) (Extended)	VIWYDGSnKYA DSLKG (SEQ ID NO: 475) (Kabat); WYDGSn (SEQ ID NO: 476) (Chothia)	DGYSgssDAFDI (SEQ ID NO: 477)
8F8	YYYWS (SEQ ID NO: 478) (Kabat); GGISISYY (SEQ ID NO: 479) (Chothia); GGISISYYYWS (SEQ ID NO: 480) (Extended)	NINYMGN TIYNPS LKS (SEQ ID NO: 481) (Kabat); NYMGN (SEQ ID NO: 482) (Chothia)	AEGSIDAFDF (SEQ ID NO: 483)
9D8	GYIY (SEQ ID NO: 484) (Kabat); GYIFTGY (SEQ ID NO: 485) (Chothia); GYIFTGYIY (SEQ ID NO: 486) (Extended)	WINpSSGGTNYA QKFQG (SEQ ID NO: 487) (Kabat); NpSSGG (SEQ ID NO: 488) (Chothia)	DRKReyyynFGMD V (SEQ ID NO: 489)
9E10	SHYIY (SEQ ID NO: 490) (Kabat); GYTFTSH (SEQ ID NO: 491) (Chothia); GYTFTSHYIY (SEQ ID NO: 492) (Extended)	WINpNSGGTNYA QKFQD (SEQ ID NO: 493) (Kabat); NpNSGG (SEQ ID NO: 494) (Chothia)	DRKReyyynFGMD V (SEQ ID NO: 495)

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
9E5	SHYIY (SEQ ID NO: 496) (Kabat); GFTFTSH (SEQ ID NO: 497) (Chothia); GFTFTSHYIY (SEQ ID NO: 498) (Extended)	WINpNSGGTKYA QKFQD (SEQ ID NO: 499) (Kabat); NpNSGG (SEQ ID NO: 500) (Chothia)	DRKReyyynFGMD V (SEQ ID NO: 501)
9F4	IYAIH (SEQ ID NO: 502) (Kabat); GFTLSIY (SEQ ID NO: 503) (Chothia); GFTLSIYAIH (SEQ ID NO: 504) (Extended)	SFGgRGSSTYFAD SVKG (SEQ ID NO: 505) (Kabat); GgRGSS (SEQ ID NO: 506) (Chothia)	EKDWgRGFDY (SEQ ID NO: 507)
9F8	NYSMN (SEQ ID NO: 508) (Kabat); GFTFSNY (SEQ ID NO: 509) (Chothia); GFTFSNYSMN (SEQ ID NO: 510) (Extended)	SISsSTIYIYYADS VKG (SEQ ID NO: 511) (Kabat); SsSTIY (SEQ ID NO: 512) (Chothia)	DIGWevftLGFDY (SEQ ID NO: 513)

Table 2B

Light Chain			
mAb	CDRL1	CDRL2	CDRL3
31H1	RSSQSLVHSDGNTYLS (SEQ ID NO: 193);	KISNRFS (SEQ ID NO: 194)	MQATQFPLT (SEQ ID NO: 195)
63B2	RSSQSLVHSDGNTYLS (SEQ ID NO: 196);	KISNRFS (SEQ ID NO: 197)	MQATQFPLT (SEQ ID NO: 198)
40E3	RASQGISNYLA (SEQ ID NO: 199);	AASSLQS (SEQ ID NO: 200)	QQYNSYPLT (SEQ ID NO: 201)

Light Chain			
mAb	CDRL1	CDRL2	CDRL3
42C3	RSSQSLVYSIDENTYLN (SEQ ID NO: 202);	QVSNRDS (SEQ ID NO: 203)	MQGTYWPPT (SEQ ID NO: 204)
45F11	RASQSVSSSLA (SEQ ID NO: 205);	GASTRAT (SEQ ID NO: 206)	QQYINWPH (SEQ ID NO: 207)
64F9	QASQDISNYLN (SEQ ID NO: 208);	GASNLET (SEQ ID NO: 209)	QQYDNFPIT (SEQ ID NO: 210)
72C2	RASQSVSSNLA (SEQ ID NO: 211);	SASTRAS (SEQ ID NO: 212)	QQYDNWPPLT (SEQ ID NO: 213)
2F10	RASQSVSSSYLA (SEQ ID NO: 214);	GASSRAT (SEQ ID NO: 215)	QQYGSSPLT (SEQ ID NO: 216)
4F11	RASQDISNYLA (SEQ ID NO: 217);	AASSLQS (SEQ ID NO: 218)	LQLNSFPFT (SEQ ID NO: 219)
10H10	RASQGISSWLA (SEQ ID NO: 220);	AASSLQS (SEQ ID NO: 221)	QQAFSFPFT (SEQ ID NO: 222)
17G6	KSSQSVLYSYNNKNYVA (SEQ ID NO: 223);	WASTRES (SEQ ID NO: 224)	QQYYSTLT (SEQ ID NO: 225)
65E11	RASQSVSSSYLA (SEQ ID NO: 226);	DASSRAT (SEQ ID NO: 227)	QQYGSSPLT (SEQ ID NO: 228)
P02B10	SGSSSNIGSNYVY (SEQ ID NO: 229);	RNNQRPS (SEQ ID NO: 230)	AAWDDSLSGVV (SEQ ID NO: 231)
P07D03	SGSRNIGSNYVY (SEQ ID NO: 232);	RNNQRPS (SEQ ID NO: 233)	ASWDGSLSAVV (SEQ ID NO: 234)
P08A02	SGSSSNIGSNYVY (SEQ ID NO: 235);	RNNQRPS (SEQ ID NO: 236)	ATWDDSLGSPV (SEQ ID NO: 237)
P08E02	RASQISRYLN (SEQ ID NO: 238);	AASILQT (SEQ ID NO: 239)	QQSYSTMWT (SEQ ID NO: 240)
P08F08	SGSSSNIGSNYVN (SEQ ID NO: 241);	GDYQRPS (SEQ ID NO: 242)	ATRDDSLSGSVV (SEQ ID NO: 243)

Light Chain			
mAb	CDRL1	CDRL2	CDRL3
P08G02	RASQSIYDYLH (SEQ ID NO: 244);	DASNLQS (SEQ ID NO: 245)	QQSYTTPLFT (SEQ ID NO: 246)
P12B09	RASQYIGRYLN (SEQ ID NO: 247);	GATSLAS (SEQ ID NO: 248)	QQSYSTTSPT (SEQ ID NO: 249)
P12F02	SGSTSNIGRNYVY (SEQ ID NO: 250);	RTNQRPS (SEQ ID NO: 251)	AAWDDSLSGRV (SEQ ID NO: 252)
P12G07	SGSSNIGSNYVY (SEQ ID NO: 253);	MNNQRPS (SEQ ID NO: 254)	AAWDDSLSAVV (SEQ ID NO: 255)
P13F04	SGSNSNIGTNYVS (SEQ ID NO: 256);	RSSRRPS (SEQ ID NO: 257)	AAWDGSLSGHW V (SEQ ID NO: 258)
P15D02	RASQSIDTYLN (SEQ ID NO: 259);	SASSLHS (SEQ ID NO: 260)	QQSYSTTAWT (SEQ ID NO: 261)
P16C05	RASQSIGQSLN (SEQ ID NO: 262);	GASSLQS (SEQ ID NO: 263)	QQSYSTPIT (SEQ ID NO: 264)
10A1	RASQSISTWLA (SEQ ID NO: 514);	KASSLES (SEQ ID NO: 515)	QQYKSYSHT (SEQ ID NO: 516)
10E2	RASQSISSWLA (SEQ ID NO: 517);	KASSLES (SEQ ID NO: 518)	QQYKSFSLT (SEQ ID NO: 519)
11A1	RASQSISSWLA (SEQ ID NO: 520);	KASTLES (SEQ ID NO: 521)	QQYNSYSYT (SEQ ID NO: 522)
11C1	RASQSVSSWLA (SEQ ID NO: 523);	KASSLES (SEQ ID NO: 524)	QQYNTYSHT (SEQ ID NO: 525)
11D1	RASQGIRNDLG (SEQ ID NO: 526);	AASSLQS (SEQ ID NO: 527)	LQDYNYPFT (SEQ ID NO: 528)
11E1	RASQDIDNYLA (SEQ ID NO: 529);	AASALQS (SEQ ID NO: 530)	QNYNSGPRT (SEQ ID NO: 531)
12A2	RASQDISNYLT (SEQ ID NO: 532);	AASALQS (SEQ ID NO: 533)	QNYNSAPRT (SEQ ID NO: 534)

Light Chain			
mAb	CDRL1	CDRL2	CDRL3
12C4	RSSQSLLHSNGYNYLD (SEQ ID NO: 535);	LGSNRAS (SEQ ID NO: 536)	MQTLQTPFT (SEQ ID NO: 537)
12C5	RASQGINSHLA (SEQ ID NO: 538);	YASTLPS (SEQ ID NO: 539)	QQLNHYPIT (SEQ ID NO: 540)
12C6	RASQGINSHLA (SEQ ID NO: 669);	YASTLPS (SEQ ID NO: 670)	QQLNHYPIT (SEQ ID NO: 671)
12D3	RASQGISNYLA (SEQ ID NO: 541);	AASTLHS (SEQ ID NO: 542)	QKYNSAPRT (SEQ ID NO: 543)
12D6	RASQDISNYLA (SEQ ID NO: 544);	AASTLHS (SEQ ID NO: 545)	QKYNSAPRT (SEQ ID NO: 546)
12D7	RASQDISSFLA (SEQ ID NO: 547);	VASTLQS (SEQ ID NO: 548)	QQLHVYPIT (SEQ ID NO: 549)
12F5	RSSQSLLDSDDGNtYLD (SEQ ID NO: 550);	TLSYRAS (SEQ ID NO: 551)	MQRIEFPFT (SEQ ID NO: 552)
12H4	RASQTISTWLA (SEQ ID NO: 553);	KASNLES (SEQ ID NO: 554)	QQYQTFST (SEQ ID NO: 555)
8C8	RASQGISNYLA (SEQ ID NO: 556);	AASTLQS (SEQ ID NO: 557)	QKYNSAPLT (SEQ ID NO: 558)
8F7	RSSQTLVHSNGYNYLN (SEQ ID NO: 559);	LGSNRAS (SEQ ID NO: 560)	MQAIQTPYT (SEQ ID NO: 561)
8F8	RASQSISSWLA (SEQ ID NO: 562);	KASNLES (SEQ ID NO: 563)	QQYNSYSCT (SEQ ID NO: 564)
9D8	QASQDINNYLH (SEQ ID NO: 565);	DASDWET (SEQ ID NO: 566)	QQYDHLPIT (SEQ ID NO: 567)
9E10	QASQDISNYLH (SEQ ID NO: 568);	DASDLET (SEQ ID NO: 569)	QQYDHLPIT (SEQ ID NO: 570)
9E5	QASQDISNYLH (SEQ ID NO: 571);	DASDLET (SEQ ID NO: 572)	QQYDHLPIT (SEQ ID NO: 573)

Light Chain			
mAb	CDRL1	CDRL2	CDRL3
9F4	QASQDISNYLN (SEQ ID NO: 574);	DASNLET (SEQ ID NO: 575)	QQYDNLPLYT (SEQ ID NO: 576)
9F8	RSSQSLLYSNGYNYLD (SEQ ID NO: 577);	LNSNRAS (SEQ ID NO: 578)	MQALQTPLT (SEQ ID NO: 579)

[0189] The disclosure encompasses modifications to the CARs and polypeptides comprising the sequences shown in Tables 1 or 2A-2B, including functionally equivalent CARs having modifications which do not significantly affect their properties and variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence may be mutated to obtain an antibody with the desired binding affinity to CD70. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or which mature (enhance) the affinity of the polypeptide for its ligand, or use of chemical analogs.

[0190] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the half-life of the antibody in the blood circulation.

[0191] Substitution variants have at least one amino acid residue in the antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 3 under the heading of “conservative substitutions.” If such substitutions result in a change in biological activity, then more substantial changes, denominated “exemplary substitutions” in Table 3, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 3: Amino Acid Substitutions

Original Residue (naturally occurring amino acid)	Conservative Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn
Asn (N)	Gln	Gln; His; Asp, Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn
Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

[0192] In some embodiments, the disclosure provides a CAR comprising an extracellular ligand-binding domain that binds to CD70 and competes for binding to CD70 with a CAR described herein, including CAR comprising an extracellular domain comprising an ScFv comprising the sequences of 31H1, 63B2, 40E3, 42C3, 45F11, 64F9, 72C2, 2F10, 4F11, 10H10, 17G6, 65E11, P02B10, P07D03, P08A02, P08E02, P08F08, P08G02, P12B09, P12F02,

P12G07, P13F04, P15D02, P16C05, 10A1, 10E2, 11A1, 11C1, 11D1, 11E1, 12A2, 12C4, 12C5, 12D3, 12D6, 12D7, 12F5, 12H4, 8C8, 8F7, 8F8, 9D8, 9E10, 9E5, 9F4 or 9F8.

[0193] In some embodiments, the disclosure provides a CAR, which specifically binds to CD70, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 20; and/or a VL region comprising a sequence shown in SEQ ID NO: 19. In some embodiments, the disclosure provides a CAR, which specifically binds to CD70, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 22; and/or a VL region comprising a sequence shown in SEQ ID NO: 21. In some embodiments, the disclosure provides a CAR, which specifically binds to CD70, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 28; and/or a VL region comprising a sequence shown in SEQ ID NO: 27. In some embodiments, the disclosure provides a CAR, which specifically binds to CD70, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 36; and/or a VL region comprising a sequence shown in SEQ ID NO: 35. In some embodiments, the disclosure provides a CAR, which specifically binds to CD70, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 46; and/or a VL region comprising a sequence shown in SEQ ID NO: 45. In some embodiments, the disclosure provides a CAR, which specifically binds to CD70, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 18; and/or a VL region comprising a sequence shown in SEQ ID NO: 17. In some embodiments, the disclosure provides a CAR, which specifically binds to CD70, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 34; and/or a VL region comprising a sequence shown in SEQ ID NO: 33. In some embodiments, the disclosure also provides CARs comprising CDR portions of antibodies to CD70 antibodies based on CDR contact regions. CDR contact regions are regions of an antibody that imbue specificity to the antibody for an antigen. In general, CDR contact regions include the residue positions in the CDRs and Vernier zones which are constrained in order to maintain proper loop structure for the antibody to bind a specific antigen. See, *e.g.*, Makabe et al., J. Biol. Chem., 283:1156-1166, 2007. Determination of CDR contact regions is well within the skill of the art.

[0194] The binding affinity (KD) of the ligand binding domain of the CD70 specific CAR as described herein to CD70 (such as human CD70) can be for example about 0.1 to about 1000 nM, for example between about 0.5nM to about 500nM, or for example between about 1nM to about 250nM. In some embodiments, the binding affinity is about any of 1000 nm, 750 nm, 500 nm, 400 nm, 300 nm, 250 nm, 200 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 45 nM,

40 nM, 35 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 10 nM, 8 nM, 7.5 nM, 7 nM, 6.5 nM, 6 nM, 5.5 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.5 nM, 0.3 nM or 0.1 nM.

[0195] In some embodiments, the binding affinity (KD) of the scFv of the ligand binding domain of the CD70-specific CAR as described herein to CD70 is about 10nM to about 100 nM, about 10nM to about 90nM, about 10nM to about 80nM, about 20nM to about 70nM, about 25nM to about 75nM, or about 40nM to about 110nM. In some embodiments, the binding affinities of the scFv described in this paragraph are for human CD70.

[0196] In some embodiments, the binding affinity is less than about any of 1000 nM, 900 nM, 800 nM, 250 nM, 200 nM, 100 nM, 50 nM, 30 nM, 20 nM, 10 nM, 7.5 nM, 7 nM, 6.5 nM, 6 nM, 5 nM.

[0197] The intracellular signaling domain of a CAR according to the disclosure is responsible for intracellular signaling following the binding of extracellular ligand-binding domain to the target resulting in the activation of the immune cell and immune response. The intracellular signaling domain has the ability to activate of at least one of the normal effector functions of the immune cell in which the CAR is expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of cytokines.

[0198] In some embodiments, an intracellular signaling domain for use in a 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. Intracellular signaling domains comprise two distinct classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation, and those that act in an antigen- independent manner to provide a secondary or co-stimulatory signal. Primary cytoplasmic signaling sequences can comprise signaling motifs which are known as immunoreceptor tyrosine-based activation motifs of ITAMs. ITAMs are well defined signaling motifs found in the intracytoplasmic tail of a variety of receptors that serve as binding sites for syk/zap70 class tyrosine kinases. Examples of ITAM used in the disclosure can include as non limiting examples those derived from TCR ζ , FcR γ , FcR β , FcR ϵ , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b and CD66d. In some embodiments, the intracellular signaling domain of the CAR can comprise the CD3 ζ signaling domain which has amino acid sequence with at least about 70%, at least 80%, at least 90%, 95%, 97%, or 99% sequence identity with an amino acid

sequence shown in SEQ ID NO: 272 or 683. In some embodiments the intracellular signaling domain of the CAR of the disclosure comprises a domain of a co-stimulatory molecule.

[0199] In some embodiments, the intracellular signaling domain of a CAR of the disclosure comprises a part of co-stimulatory molecule selected from the group consisting of fragment of 41BB (GenBank: AAA53133.) and CD28 (NP_006130.1). In some embodiments, the intracellular signaling domain of the CAR of the disclosure comprises amino acid sequence which comprises at least 70%, at least 80%, at least 90%, 95%, 97%, or 99% sequence identity with an amino acid sequence shown in SEQ ID NO: 271 or 682 and SEQ ID NO: 275. In some embodiments, the intracellular signaling domain of the CAR of the disclosure comprises amino acid sequence which comprises at least 70%, at least 80%, at least 90%, 95%, 97%, or 99% sequence identity with an amino acid sequence shown in SEQ ID NO: 271 or 682 and/or at least 70%, at least 80%, at least 90%, 95%, 97%, or 99% sequence identity with an amino acid sequence shown in SEQ ID NO: 276.

[0200] CARs are expressed on the surface membrane of the cell. Thus, the CAR can comprise a transmembrane domain. Suitable transmembrane domains for a CAR disclosed herein have the ability to (a) be expressed at the surface of a cell, which is in some embodiments an immune cell such as, for example without limitation, a lymphocyte cell, such as a T helper (T_h) cell, cytotoxic T (T_c) cell, T regulatory (T_{reg}) cell, or Natural killer (NK) cells, and/or (b) interact with the ligand-binding domain and intracellular signaling domain for directing cellular response of an immune cell against a predefined target cell. The transmembrane domain can be derived either from a natural or from a synthetic source. The transmembrane domain can be derived from any membrane-bound or transmembrane protein. As non-limiting examples, the transmembrane polypeptide can be a subsequence or subunit of the T cell receptor such as α , β , γ or δ , polypeptide constituting CD3 complex, IL-2 receptor p55 (α chain), p75 (β chain) or γ chain, subunit chain of Fc receptors, in particular Fc γ receptor III or CD proteins. Alternatively, the transmembrane domain can be synthetic and can comprise predominantly hydrophobic residues such as leucine and valine. In some embodiments said transmembrane domain is derived from the human CD8 α chain (*e.g.*, NP_001139345.1). The transmembrane domain can further comprise a stalk domain between the extracellular ligand-binding domain and said transmembrane domain. A stalk domain may comprise up to 300 amino acids—in some embodiments 10 to 100 amino acids or in some embodiments 25 to 50 amino acids. Stalk region may be derived from all or part of naturally occurring molecules, such as from all or part of the

extracellular region of CD8, CD4, CD28, 4-1BB, or IgG (in particular, the hinge region of an IgG), or from all or part of an antibody heavy-chain constant region. Alternatively the stalk domain may be a synthetic sequence that corresponds to a naturally occurring stalk sequence, or may be an entirely synthetic stalk sequence. In some embodiments said stalk domain is a part of human CD8 α chain (*e.g.*, NP_001139345.1). In another particular embodiment, said hinge and transmembrane domains comprise a part of human CD8 α chain, which in some embodiments comprises at least 70%, at least 80%, at least 90%, 95% 97%, or 99% sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 268 and 270. In some embodiments, the stalk domain of CARs described herein comprises a subsequence of CD8 α , an IgG1, or an Fc γ RIII α , in particular the hinge region of any of an CD8 α , an IgG1, or an Fc γ RIII α . In some embodiments, the stalk domain comprises a human CD8 α hinge, a human IgG1 hinge, or a human Fc γ RIII α hinge. In some embodiments, CARs disclosed herein can comprise an extracellular ligand-binding domain that specifically binds CD70. In some embodiments the CARs disclosed herein comprise an scFv, CD8 α human hinge and transmembrane domains, the CD3 ζ signaling domain, and 4-1BB signaling domain.

[0201] Table 4 provides exemplary sequences of domains which can be used in the CARs disclosed herein.

Table 4: Exemplary sequences of CAR Components

Domain	Amino Acid Sequence	SEQ ID NO:
CD8 α signal peptide	MALPVTALLLPLALLHAARP	266
Fc γ RIII α hinge	GLAVSTISSFFPPGYQ	267
CD8 α hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACD	268

Domain	Amino Acid Sequence	SEQ ID NO:
IgG1 hinge	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIART PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSK LTVDKSRWQQGNVFSVMSVMEALHNHYTQKSLSLSPGK	269
CD8 α transmembrane (TM) domain	IYIWAPLAGTCGVLLLSLVITLYC	270
41BB intracellular signaling domain (ISD)	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGG CEL	271
41BB intracellular signaling domain (ISD)	GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL	682
CD3 ζ intracellular signaling domain (ISD)	RVKFSSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	272
CD3 ζ intracellular signaling domain (ISD)	RVKFSSRSADAPAYKQGQNQLYNELNLGRREEYDVLDK RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	683
Fc ϵ RI α -TM-IC (Fc ϵ RI α chain transmembrane and intracellular domain)	FFIPLLVVILFAVDTGLFISTQQQVTFLLKIKRTRKGFRL NPHPKPNPKNN	273

Domain	Amino Acid Sequence	SEQ ID NO:
FcεRIβ-ΔITAM (FcεRI β chain without ITAM)	MDTESNRRANLALPQEPSSVPAFEVLEISPQEVSSGRLLK SASSPPLHTWLTVLKKEQEFLGVTQILTAMICLCFGTVV CSVLDISHIEGDIFSSFKAGYPFWGAIFFSISGMLSIIISERR NATYLVRGSLGANTASSIAGGTGITILIINLKKSLAYIH SCQKFFETKCFMASFSTEIVVMMLFLTILGLGSAVSLTIC GAGEELKGNKVPE	274
CD28-IC (CD28 co-stimulatory domain)	RSKRSRGGHSDYMNMTPRRPGPTRKHYPYAPPRDFAA YRS	276
FcεRIγ-SP (signal peptide)	MIPAVVLLLLLLVEQAAA	277
FcεRI γ-ΔITAM (FcεRI γ chain without ITAM)	LGEPQLCYILDAILFLYGIVLTLLYCRLKIQVRKAAITSYE KS	278
GSG-P2A (GSG-P2A ribosomal skip polypeptide)	GSGATNFSLLKQAGDVEENPGP	279
GSG-T2A (GSG-T2A ribosomal skip polypeptide)	GSGEGRGSLTTCGDVEENPGP	280

[0202] Downregulation or mutation of target antigens is commonly observed in cancer cells, creating antigen-loss escape variants. Thus, to offset tumor escape and render immune cell more specific to target, the CD70-specific CAR can comprise one or more additional extracellular ligand-binding domains, to simultaneously bind different elements in target thereby augmenting immune cell activation and function. In some embodiments, the extracellular ligand-binding domains can be placed in tandem on the same transmembrane polypeptide, and optionally can be separated by a linker. In some embodiments, said different extracellular ligand-binding domains can be placed on different transmembrane polypeptides composing the CAR. In some

embodiments, the disclosure relates to a population of CARs, each CAR comprising a different extracellular ligand-binding domain. In a particular, the disclosure relates to a method of engineering immune cells comprising providing an immune cell and expressing at the surface of the cell a population of CARs, each CAR comprising different extracellular ligand-binding domains. In another particular embodiment, the disclosure relates to a method of engineering an immune cell comprising providing an immune cell and introducing into the cell polynucleotides encoding polypeptides composing a population of CAR each one comprising different extracellular ligand-binding domains. By population of CARs, it is meant at least two, three, four, five, six or more CARs each one comprising different extracellular ligand-binding domains. The different extracellular ligand-binding domains according to the disclosure can, in some embodiments, simultaneously bind different elements in target thereby augmenting immune cell activation and function. The disclosure also relates to an isolated immune cell which comprises a population of CARs each one comprising different extracellular ligand-binding domains.

[0203] In another aspect, the disclosure provides polynucleotides encoding any of the CARs and polypeptides described herein. Polynucleotides can be made and expressed by procedures known in the art.

[0204] In another aspect, the disclosure provides compositions (such as a pharmaceutical compositions) comprising any of the cells of the disclosure. In some embodiments, the composition comprises a cell comprising a polynucleotide encoding any of the CARs described herein. In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 297 and SEQ ID NO:298, SEQ ID NO: 299 and SEQ ID NO:300, SEQ ID NO: 301 and SEQ ID NO:302, SEQ ID NO: 303 and SEQ ID NO:304, SEQ ID NO: 305 and SEQ ID NO:306, SEQ ID NO: 307 and SEQ ID NO:308 or SEQ ID NO: 309 and SEQ ID NO:310, below:

4F11 heavy chain variable region

CAGGTCACCTTGAAGGAGTCTGGTCCTGTGCTGGTGAACCCACAGAGACCCTCAC
GCTGACCTGCACCGTCTCTGGGTCTCACTCAGTAATGCTAGAATGGGTGTGACCTG
GATCCGTCAGCCCCCAGGGAAGGCCCTGGAGTGGCTTGACACATTTTTTCGAATGA
CGAAAAATCCTACAGTACATCTCTGAAGAGCAGGCTCACCATCTCCAAGGACACTT
CCAAAACCCAGGTGGTCCTTACCATGACCAACATGGACCCTGTGGACACAGCCACA

TATTACTGTGCACGGATACGAGATTACTATGACATTAGTAGTTATTATGACTACTGG
GGCCAGGGAACCCTGGTCAGCGTCTCCTCA (SEQ ID NO: 297)

4F11 light chain variable region

GACATCCAGATGACCCAGTCTCCATCTGCCATGTCTGCATCTGTAGGAGACAGAGTC
ACCATCACTTGTCGGGCGAGTCAGGACATTAGCAATTATTTAGCCTGGTTTCAGCAG
AAACCAGGGAAAGTCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGG
GGTCCCATCAAGGTTTCAGCGGCAGTGGATCGGGGACAGAATTCACTCTCACAATCA
GCAGCCTGCTGCCTGAAGATTTTGCAACTTATTACTGTCTACAGCTTAATAGTTTCCC
GTTCACTTTTGGCGGAGGGACCAAGGTGGAGATCAAC (SEQ ID NO: 298)

[0205] In still other embodiments, the composition comprises either or both of the
polynucleotides shown in SEQ ID NO: 299 and SEQ ID NO:300 below:

17G6 heavy chain variable region

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAG
ACTCTCCTGTGTAGCCTCTGGATTCACCTTTAGTAGTTATTGGATGAGCTGGGTCCG
CCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAGCATAAAGCAAGATGGAAGT
GAGAAATACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGC
CAAGAACTCAGTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGGTGTGT
ATTACTGTGCGAGAGAAGGAGTCAACTGGGGATGGAGACTCTACTGGCACTTCGAT
CTCTGGGGCCGTGGAACCCTGGTCACTGTCTCCTCA (SEQ ID NO: 299)

17G6 light chain variable region

GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCC
ACCATCAACTGCAAGTCCAGCCAGAGTGTTTTATACAGCTACAACAATAAGAACTA
CGTAGCTTGGTACCAGCAGAAACCAGGACAACCTCCTAACCTACTCATTTTCTGGGC
ATCTACCCGGGAATCCGGGGTCCCTGACCGATTTCAGTGGCAGCGGGTCTGGGACAG
ATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAGTTTACTACTGTC
AGCAATATTATAGTACGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA
(SEQ ID NO: 300).

[0206] In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 301 and SEQ ID NO:302 below:

10H10 heavy chain variable region

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAG
ACTCTCCTGTGCAGTCTCTGGATTACCTTCAGTAACCATAACATACTGGGTCCG
CCAGGCTCCAGGGAAGGGGCTGGAGTGGATTTTCATACATTAGTCGAAGTAGTAGTA
CCATATATTACGCAGACTCTGTGAAGGGCCGATTCACAATCTCCAGAGACAATGCC
AAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGACGAAGACACGGCTGTGTA
TTACTGTGCGAGAGATCACGCTCAGTGGTACGGTATGGACGTTTGGGGCCAAGGGA
CCACGGTCACCGTCTCCTCA (SEQ ID NO: 301).

10H10 light chain variable region

GACATCCAGATGACCCAGTCTCCATCTTCCGTGTCTGCATCGGTAGGAGACAGAGTC
ACCATCACTTGTCTGGGCGAGTCAGGGTATTAGCAGCTGGTTAGCCTGGTATCAGCA
GAAACCAGGGAAAGCCCCTAAGGTCCTGATCTATGCTGCATCCAGTTTGCAAAGTG
GGGTCCCATCAAGGTTACGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCA
GCAGCCTGCAGCCTGAAGATTTTGCAACTTACTATTGTCAACAGGCTTTCAGTTTCC
CATTCATTTTCGGCCCTGGGACCAAAGTGGATATCAAA (SEQ ID NO: 302).

[0207] In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 303 and SEQ ID NO:304 below:

P07D03 heavy chain variable region

GAAGTGCAGCTTGTCCAGAGCGGAGCCGAAGTGAAGAAGCCTGGCGAGAGCCTGA
AGATCAGCTGCAAGGGCTCCGGATATCGCTTCACAAGTTACTGGATAGGGTGGGTG
CGCCAGATGCCTGGTAAGGGACTGGAATGGATGGGCTCTATATATCCTGATGATTCC
GACACACGTTATAGCCCAAGCTTTCAGGGCCAGGTCACAATCAGCGCTGACAAGAG
CATCAGCACCGCCTACCTTCAGTGGTCGTCTCTGAAGGCCAGCGACACCGCAATGTA
CTACTGCGCCTCTAGCACAGTTGACTACCCGGGATACAGTTACTTCGACTACTGGGG
CCAAGGTACACTGGTTCACCGTCAGCAGC (SEQ ID NO: 303)

P07D03 light chain variable region

GAGCTCCAGAGCGTGCTGACCCAGCCTCCTAGCGCAAGCGGCACCCCTGGACAGCG
 TGTGACAATTAGCTGTAGCGGAAGTCGTAGCAATATCGGATCAAACCTATGTGTATTG
 GTATCAGCAATTGCCCCGGTACAGCACCCAAATTGCTCATATATAGAAATAATCAGA
 GACCTAGCGGAGTGCCTGATCGTTTTAGCGGTAGCAAAAGCGGCACCAGCGCATCA
 CTGGCAATTTTCAGGCCTGCGTAGCGAAGATGAGGCGGATTATTACTGTGCGAGTTG
 GGATGGTTCGCTGAGTGCTGTTGTGTTTCGGCACCGGTACAAAACCTGACCGTTCTG
 (SEQ ID NO: 304)

[0208] In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 305 and SEQ ID NO:306 below:

P08G02 heavy chain variable region

GAAGTGCAGCTTGTCCAGAGCGGAGCCGAAGTGAAGAAGCCTGGCGAGAGCCTGA
 AGATCAGCTGCAAGGGCTCCGGATACACCTTTCCTTCATCATGGATAGGTTGGGTGC
 GCCAGATGCCTGGTAAGGGACTGGAATGGATGGGCATCATATACCCTGATACTAGC
 CATACCCGTTACAGCCCAAGCTTTCAGGGCCAGGTCACAATCAGCGCTGACAAGAG
 CATCAGCACCGCCTACCTTCAGTGGTCGTCTCTGAAGGCCAGCGACACCGCAATGTA
 CTA CTGTGCCCCGTGCGAGCTATTTTCGATCGTGGAACAGGGTATAGTTCTTGGTGGAT
 GGATGTGTGGGGCCAAGGTACACTGGTCACCGTCAGCAGC (SEQ ID NO: 305)

P08G02 light chain variable region

GAGCTCGATATTCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCAAGCGTGGGCGA
 TAGAGTGACCATTACCTGTAGGGCCTCACAATCCATATACGACTATTTGCACTGGTA
 TCAGCAGAAACCCGGGAAAGCACCCAAACTGCTGATTTACGATGCTTCCAACCTAC
 AGAGTGGCGTTCCTTCACGTTTTAGCGGTAGCGGTTTCAGGCACCGATTTACCCCTGA
 CCATTAGCAGCCTTCAGCCCGAAGATTTTCGCTACGTATTATTGCCAGCAATCATACA
 CCACGCCGTTGTTTACATTCGGCCAGGGTACCAAAGTGGAATCAAA (SEQ ID NO: 306)

[0209] In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 307 and SEQ ID NO: 308 below:

P08F08 heavy chain variable region

GAAGTGCAGCTTGTCCAGAGCGGAGCCGAAGTGAAGAAGCCTGGCGAGAGCCTGA
AGATCAGCTGCAAGGGCTCCGGATACGGATTACAAGTTATTGGATAGGTTGGGTG
CGCCAGATGCCTGGTAAGGGACTGGAATGGATGGGTATCATTCATCCCGATGATAG
CGACACCAAATACAGCCCAAGCTTTCAGGGCCAGGTCACAATCAGCGCTGACAAGA
GCATCAGCACCGCCTACCTTCAGTGGTCGTCTCTGAAGGCCAGCGACACCGCAATGT
ACTACTGTGCCTCTAGCTATTTGCGTGGCTTGTGGGGAGGCTATTTTGACTATTGGG
GCCAAGGTACACTGGTCACCGTCAGCAGC (SEQ ID NO: 307)

P08F08 light chain variable region

GAGCTCCAGAGCGTGCTGACCCAGCCTCCTAGCGCAAGCGGCACCCCTGGACAGCG
TGTGACAATTAGCTGTAGCGGATCAAGCTCAAACATTGGCTCAAATTATGTGAATTG
GTATCAGCAATTGCCCCGGTACAGCACCCAACTGCTCATTTATGGAGATTATCAACG
ACCTAGCGGAGTGCCTGATCGTTTTAGCGGTAGCAAAAGCGGCACCAGCGCATCAC
TGGCAATTTAGGCCTGCGTAGCGAAGATGAGGCGGATTATTACTGTGCTACCCGC
GACGATTCGTTATCTGGGTCTGTCTGTTTTGGCACCGGTACAAAAGTACCGGTGCTG
(SEQ ID NO: 308)

[0210] In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 309 and SEQ ID NO:310 below:

P15D02 heavy chain variable region

GAAGTGCAGCTTGTCCAGAGCGGAGCCGAAGTGAAGAAGCCTGGCGAGAGCCTGA
AGATCAGCTGCAAGGGCTCCGGATACAGTTTTGCCTCATACTGGATCGGTTGGGTGC
GCCAGATGCCTGGTAAGGGACTGGAATGGATGGGCGTAATTTACCCCGGAACTAGC
GAGACACGTTACAGCCCAAGCTTTCAGGGCCAGGTCACAATCAGCGCTGACAAGAG
CATCAGCACCGCCTACCTTCAGTGGTCGTCTCTGAAGGCCAGCGACACCGCAATGTA
CTACTGCGCTAAAGGGTTGAGTGCGAGTGCAAGTGGATATTCTTTCCAATATTGGGG
CCAAGGTACACTGGTCACCGTCAGCAGC (SEQ ID NO: 309)

P15D032 light chain variable region

GAGCTCGATATTCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCAAGCGTGGGCGA
TAGAGTGACCATTACCTGTAGGGCCTCACAAAGCATCGACACATATTTAACTGGT
ATCAGCAGAAACCCGGGAAAGCACCCAACTGCTGATTTATTCAGCTAGTAGCCTA

CACAGTGGCGTTCCTTCACGTTTTAGCGGTAGCGGTTCAGGCACCGATTTCACCCTG
ACCATTAGCAGCCTTCAGCCCGAAGATTTCGCTACGTATTATTGCCAACAATCATAC
AGCACAACTGCTTGGACATTCGGCCAGGGTACCAAAGTGGAAATCAAA (SEQ ID
NO: 310)

[0211] Expression vectors, and administration of polynucleotide compositions are further described herein.

[0212] In another aspect, the disclosure provides a method of making any of the polynucleotides described herein.

[0213] Polynucleotides complementary to any such sequences are also encompassed by the disclosure. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the disclosure, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

[0214] Polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes an antibody or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants contain one or more substitutions, additions, deletions and/or insertions such that the immunoreactivity of the encoded polypeptide is not diminished, relative to a native immunoreactive molecule. The effect on the immunoreactivity of the encoded polypeptide may generally be assessed as described herein. Variants embodiments exhibit at least about 70% identity, at least about 80% identity, at least about 90% identity, or at least about 95% identity to a polynucleotide sequence that encodes a native antibody or a portion thereof.

[0215] Two polynucleotide or polypeptide sequences are said to be “identical” if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A “comparison window” as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, or 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

[0216] Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O., 1978, A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., 1990, Unified Approach to Alignment and Phylogenies pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., 1989, CABIOS 5:151-153; Myers, E.W. and Muller W., 1988, CABIOS 4:11-17; Robinson, E.D., 1971, Comb. Theor. 11:105; Santou, N., Nes, M., 1987, Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R., 1973, Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J., 1983, Proc. Natl. Acad. Sci. USA 80:726-730.

[0217] The “percentage of sequence identity” is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.* the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

[0218] Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a native antibody (or a complementary sequence).

[0219] Suitable “moderately stringent conditions” include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1 % SDS.

[0220] As used herein, “highly stringent conditions” or “high stringency conditions” are those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt’s solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

[0221] It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the disclosure. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the disclosure. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

[0222] The polynucleotides of this disclosure can be obtained using chemical synthesis, recombinant methods, or PCR. Methods of chemical polynucleotide synthesis are well known in the art and need not be described in detail herein. One of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to produce a desired DNA sequence.

[0223] For preparing polynucleotides using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification, as further discussed herein. Polynucleotides may be inserted into host cells by any means known in the art. Cells are transformed by introducing an exogenous polynucleotide by direct uptake, endocytosis,

transfection, F-mating or electroporation. Once introduced, the exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide so amplified can be isolated from the host cell by methods well known within the art. See, *e.g.*, Sambrook et al., 1989.

[0224] Alternatively, PCR allows reproduction of DNA sequences. PCR technology is well known in the art and is described in U.S. Patent Nos. 4,683,195, 4,800,159, 4,754,065 and 4,683,202, as well as PCR: The Polymerase Chain Reaction, Mullis et al. eds., Birkhäuser Press, Boston, 1994.

[0225] RNA can be obtained by using the isolated DNA in an appropriate vector and inserting it into a suitable host cell. When the cell replicates and the DNA is transcribed into RNA, the RNA can then be isolated using methods well known to those of skill in the art, as set forth in Sambrook et al., 1989, *supra*, for example.

[0226] Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, *e.g.*, pUC18, pUC19, Bluescript (*e.g.*, pBS SK+) and its derivatives, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Strategene, and Invitrogen.

[0227] Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide according to the disclosure. It is implied that an expression vector must be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO87/04462, or the lentiviral pLVX vector available from Clontech. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (*i.e.*,

translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

[0228] The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (*e.g.*, where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[0229] A polynucleotide encoding a CD70-specific CAR disclosed herein may exist in an expression cassette or expression vector (*e.g.*, a plasmid for introduction into a bacterial host cell, or a viral vector such as a baculovirus vector for transfection of an insect host cell, or a plasmid or viral vector such as a lentivirus for transfection of a mammalian host cell). In some embodiments, a polynucleotide or vector can include a nucleic acid sequence encoding ribosomal skip sequences such as, for example without limitation, a sequence encoding a 2A peptide. 2A peptides, which were identified in the Aphthovirus subgroup of picornaviruses, causes a ribosomal “skip” from one codon to the next without the formation of a peptide bond between the two amino acids encoded by the codons (see (Donnelly and Elliott 2001; Atkins, Wills et al. 2007; Doronina, Wu et al. 2008)). By “codon” is meant three nucleotides on an mRNA (or on the sense strand of a DNA molecule) that are translated by a ribosome into one amino acid residue. Thus, two polypeptides can be synthesized from a single, contiguous open reading frame within an mRNA when the polypeptides are separated by a 2A oligopeptide sequence that is in frame. Such ribosomal skip mechanisms are well known in the art and are known to be used by several vectors for the expression of several proteins encoded by a single messenger RNA.

[0230] To direct transmembrane polypeptides into the secretory pathway of a host cell, in some embodiments, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in a polynucleotide sequence or vector sequence. The secretory signal sequence is operably linked to the transmembrane nucleic acid sequence, *i.e.*, the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the nucleic acid sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the nucleic acid

sequence of interest (see, *e.g.*, Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830). In some embodiments the signal peptide comprises the amino acid sequence shown in SEQ ID NO: 266 or 277. Those skilled in the art will recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. In some embodiments, nucleic acid sequences of the disclosure are codon-optimized for expression in mammalian cells, or in some embodiments for expression in human cells. Codon-optimization refers to the exchange in a sequence of interest of codons that are generally rare in highly expressed genes of a given species by codons that are generally frequent in highly expressed genes of such species, such codons encoding the amino acids as the codons that are being exchanged.

CD70-SPECIFIC ANTIBODIES AND METHODS OF MAKING THEREOF

[0231] Provided herein are CD70 antibodies.

[0232] In some embodiments, a CD70 antibody of the disclosure comprises any one of the partial light chain sequence as listed in Table 1 and/or any one of the partial heavy chain sequence as listed in Table 1. In Table 1, the underlined sequences are CDR sequences according to Kabat and in bold according to Chothia.

[0233] Tables 2A-2B provide examples of CDR sequences of the CD70 antibodies provided herein.

[0234] In some embodiments, the disclosure provides an antibody (*e.g.* including antibody fragments, such as single chain variable fragments (scFvs) which specifically binds to Cluster of Differentiation 70 (CD70), wherein the antibody comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 49, 50, 51, 55, 56, 57, 61, 62, 63, 67, 68, 69, 73, 74, 75, 79, 80, 81, 85, 86, 87, 91, 92, 93, 97, 98, 99, 103, 104, 105, 109, 110, 111, 115, 116, 117, 121, 122, 123, 127, 128, 129, 133, 134, 135, 139, 140, 141, 145, 146, 147, 151, 152, 153, 157, 158, 159, 163, 164, 165, 169, 170, 171, 175, 176, 177, 181, 182, 183, 187, 188, 189, 382, 383, 384, 388, 389, 390, 394, 395, 396, 400, 401, 402, 406, 407, 408, 412, 413, 414, 418, 419, 420, 424, 425, 426, 430, 431, 432, 663, 664, 665, 436, 437, 438, 442, 443, 444, 448, 449, 450, 454, 455, 456, 460, 461, 462, 466, 467, 468, 472, 473, 474, 478, 479, 480, 484, 485, 486, 490, 491, 492, 496, 497, 498, 502, 503, 504, 508, 509, or 510; (ii) a VH CDR2 comprising the sequence shown in SEQ ID NO: 52, 53, 58, 59, 64, 65, 70, 71, 76, 77, 82, 83, 88, 89, 94, 95, 100, 101, 106, 107, 112, 113, 118, 119, 124, 125, 130, 131, 136, 137, 142, 143, 148, 149, 154, 155, 160, 161, 166, 167,

172, 173, 178, 179, 184, 185, 190, 191, 385, 386, 391, 392, 397, 398, 403, 404, 409, 410, 415, 416, 421, 422, 427, 428, 433, 434, 666, 667, 439, 440, 445, 446, 451, 452, 457, 458, 463, 464, 469, 470, 475, 476, 481, 482, 487, 488, 493, 494, 499, 500, 505, 506, 511, or 512; and iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120, 126, 132, 138, 144, 150, 156, 162, 168, 174, 180, 186, 192, 387, 393, 399, 405, 411, 417, 423, 429, 435, 668, 441, 447, 453, 459, 465, 471, 477, 483, 489, 495, 501, 507, or 513; and/or a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 193, 196, 199, 202, 205, 208, 211, 214, 217, 220, 223, 226, 229, 232, 235, 238, 241, 244, 247, 250, 253, 256, 259, 262, 514, 517, 520, 523, 526, 529, 532, 535, 538, 669, 541, 544, 547, 550, 553, 556, 559, 562, 565, 568, 571, 574, or 577; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 194, 197, 200, 203, 206, 209, 212, 215, 218, 221, 224, 227, 230, 233, 236, 239, 242, 245, 248, 251, 254, 257, 260, 263, 515, 518, 521, 524, 527, 530, 533, 536, 539, 670, 542, 545, 548, 551, 554, 557, 560, 563, 566, 569, 572, 575, or 578; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 195, 198, 201, 204, 207, 210, 213, 216, 219, 222, 225, 228, 231, 234, 237, 240, 243, 246, 249, 252, 255, 258, 261, 264, 516, 519, 522, 525, 528, 531, 534, 537, 540, 671, 543, 546, 549, 552, 555, 558, 561, 564, 567, 570, 573, 576, or 579.

[0235] In some embodiments, the disclosure provides an antibody (e.g. a scFv), which specifically binds to Cluster of Differentiation 70 (CD70), wherein the antibody comprises a heavy chain variable (VH) region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 339, 341, 343, 345, 347, 349, 351, 353, 355, 662, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, or 381; and/or a light chain variable (VL) region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 338, 340, 342, 344, 346, 348, 350, 352, 354, 661, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, or 380.

[0236] In some embodiments, the disclosure provides an isolated antibody which specifically binds to CD70 and competes with any of the foregoing antibodies.

[0237] In some embodiments, the present invention provides an antibody that binds to CD70 and competes with an antibody as described herein, including 31H1, 63B2, 40E3, 42C3, 45F11, 64F9, 72C2, 2F10, 4F11, 10H10, 17G6, 65E11, P02B10, P07D03, P08A02, P08E02, P08F08, P08G02, P12B09, P12F02, P12G07, P13F04, P15D02, P16C05, 10A1, 10E2, 11A1, 11C1,

11D1, 11E1, 12A2, 12C4, 12C5, 12D3, 12D6, 12D7, 12F5, 12H4, 8C8, 8F7, 8F8, 9D8, 9E10, 9E5, 9F4 or 9F8.

[0238] In some embodiments, the invention also provides CDR portions of antibodies to CD70 antibodies based on CDR contact regions. CDR contact regions are regions of an antibody that imbue specificity to the antibody for an antigen. In general, CDR contact regions include the residue positions in the CDRs and Vernier zones which are constrained in order to maintain proper loop structure for the antibody to bind a specific antigen. See, *e.g.*, Makabe et al., *J. Biol. Chem.*, 283:1156-1166, 2007. Determination of CDR contact regions is well within the skill of the art.

[0239] The binding affinity (K_D) of the CD70 antibody as described herein to CD70 (such as human CD70 (*e.g.*, (SEQ ID NO: 335))) can be about 0.001 to about 5000 nM. In some embodiments, the binding affinity is about any of 5000 nM, 4500 nM, 4000 nM, 3500 nM, 3000 nM, 2500 nM, 2000 nM, 1789 nM, 1583 nM, 1540 nM, 1500 nM, 1490 nM, 1064 nM, 1000 nM, 933 nM, 894 nM, 750 nM, 705 nM, 678 nM, 532 nM, 500 nM, 494 nM, 400 nM, 349 nM, 340 nM, 353 nM, 300 nM, 250 nM, 244 nM, 231 nM, 225 nM, 207 nM, 200 nM, 186 nM, 172 nM, 136 nM, 113 nM, 104 nM, 101 nM, 100 nM, 90 nM, 83 nM, 79 nM, 74 nM, 54 nM, 50 nM, 45 nM, 42 nM, 40 nM, 35 nM, 32 nM, 30 nM, 25 nM, 24 nM, 22 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 12 nM, 10 nM, 9 nM, 8 nM, 7.5 nM, 7 nM, 6.5 nM, 6 nM, 5.5 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.5 nM, 0.3 nM, 0.1 nM, 0.01 nM, or 0.001 nM. In some embodiments, the binding affinity is less than about any of 5000 nM, 4000 nM, 3000 nM, 2000 nM, 1000 nM, 900 nM, 800 nM, 250 nM, 200 nM, 100 nM, 50 nM, 30 nM, 20 nM, 10 nM, 7.5 nM, 7 nM, 6.5 nM, 6 nM, 5 nM, 4.5 nM, 4 nM, 3.5 nM, 3 nM, 2.5 nM, 2 nM, 1.5 nM, 1 nM, or 0.5 nM.

[0240] In some embodiments, the disclosure provides a nucleic acid encoding any of the foregoing isolated antibodies. In some embodiments, the disclosure provides a vector comprising such a nucleic acid. In some embodiments, the disclosure provides a host cell comprising such a nucleic acid.

[0241] The disclosure further provides any of the antibodies of the foregoing antibodies for use as a medicament. In some embodiments, the medicament is for use in treatment of a CD70-related cancer selected from the group consisting of Renal Cell Carcinoma, Glioblastoma, glioma such as low grade glioma, Non-Hodgkin's Lymphoma (NHL), Hodgkin's Disease (HD), Waldenstrom's macroglobulinemia, Acute Myeloid Leukemia, Multiple Myeloma, diffuse large-cell lymphoma, follicular lymphoma or Non-Small Cell Lung Cancer.

[0242] In some embodiments, the disclosure provides a method of treating a subject in need thereof, comprising providing any of the foregoing antibodies, and administering said antibody to said subject.

[0243] In some embodiments, the disclosure provides a pharmaceutical composition comprising any of the foregoing antibodies.

[0244] In some embodiments, the disclosure provides a method of treating a condition associated with malignant cells expressing CD70 in a subject comprising administering to a subject in need thereof an effective amount of any one of the foregoing antibodies or a pharmaceutical composition comprising any one of the foregoing antibodies. In some embodiments, the condition is cancer. In some embodiments, the cancer is an CD70 related cancer selected from the group consisting of Renal Cell Carcinoma, Glioblastoma, glioma such as low grade glioma, Non-Hodgkin's Lymphoma (NHL), Hodgkin's Disease (HD), Waldenstrom's macroglobulinemia, Acute Myeloid Leukemia, Multiple Myeloma, diffuse large-cell lymphoma, follicular lymphoma or Non-Small Cell Lung Cancer.

[0245] In some embodiments, the disclosure provides, a method of inhibiting tumor growth or progression in a subject who has malignant cells expressing CD70, comprising administering to the subject in need thereof an effective amount of a pharmaceutical composition of the disclosure to the subject.

[0246] In some embodiments, the disclosure provides, a method of inhibiting metastasis of malignant cells expressing CD70 in a subject, comprising administering to the subject in need thereof an effective amount of a pharmaceutical composition of the disclosure to the subject.

[0247] In some embodiments, the disclosure provides, a method of inducing tumor regression in a subject who has malignant cells expressing CD70, comprising administering to the subject in need thereof an effective amount of a pharmaceutical composition of the disclosure to the subject.

[0248] In some embodiments, the antibody, comprising culturing the host cell of the disclosure under conditions that result in production of the antibody, and isolating the antibody from the host cell or culture.

[0249] The antibodies useful in the present invention can encompass monoclonal antibodies, polyclonal antibodies, antibody fragments (*e.g.*, Fab, Fab', F(ab')₂, Fv, Fc, etc.), chimeric antibodies, bispecific antibodies, heteroconjugate antibodies, single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion (*e.g.*, a domain antibody), humanized

antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. The antibodies may be murine, rat, human, or any other origin (including chimeric or humanized antibodies).

[0250] In some embodiments, the CD70 monospecific antibody as described herein is a monoclonal antibody. For example, the CD70 monospecific antibody is a human monoclonal antibody.

[0251] The disclosure further provides the following illustrative embodiments:

1. An isolated antibody, which specifically binds to Cluster of Differentiation 70 (CD70), wherein the antibody comprises

(a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 49, 50, 51, 55, 56, 57, 61, 62, 63, 67, 68, 69, 73, 74, 75, 79, 80, 81, 85, 86, 87, 91, 92, 93, 97, 98, 99, 103, 104, 105, 109, 110, 111, 115, 116, 117, 121, 122, 123, 127, 128, 129, 133, 134, 135, 139, 140, 141, 145, 146, 147, 151, 152, 153, 157, 158, 159, 163, 164, 165, 169, 170, 171, 175, 176, 177, 181, 182, 183, 187, 188, 189, 382, 383, 384, 388, 389, 390, 394, 395, 396, 400, 401, 402, 406, 407, 408, 412, 413, 414, 418, 419, 420, 424, 425, 426, 430, 431, 432, 663, 664, 665, 436, 437, 438, 442, 443, 444, 448, 449, 450, 454, 455, 456, 460, 461, 462, 466, 467, 468, 472, 473, 474, 478, 479, 480, 484, 485, 486, 490, 491, 492, 496, 497, 498, 502, 503, 504, 508, 509, or 510; (ii) a VH CDR2 comprising the sequence shown in SEQ ID NO: 52, 53, 58, 59, 64, 65, 70, 71, 76, 77, 82, 83, 88, 89, 94, 95, 100, 101, 106, 107, 112, 113, 118, 119, 124, 125, 130, 131, 136, 137, 142, 143, 148, 149, 154, 155, 160, 161, 166, 167, 172, 173, 178, 179, 184, 185, 190, 191, 385, 386, 391, 392, 397, 398, 403, 404, 409, 410, 415, 416, 421, 422, 427, 428, 433, 434, 666, 667, 439, 440, 445, 446, 451, 452, 457, 458, 463, 464, 469, 470, 475, 476, 481, 482, 487, 488, 493, 494, 499, 500, 505, 506, 511, or 512; and (iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120, 126, 132, 138, 144, 150, 156, 162, 168, 174, 180, 186, 192, 387, 393, 399, 405, 411, 417, 423, 429, 435, 668, 441, 447, 453, 459, 465, 471, 477, 483, 489, 495, 501, 507, or 513; and/or

(b) a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 193, 196, 199, 202, 205, 208, 211, 214, 217, 220, 223, 226, 229, 232, 235, 238, 241, 244, 247, 250, 253, 256, 259, 262, 514, 517, 520, 523, 526, 529, 532, 535, 538, 669, 541, 544, 547, 550, 553, 556, 559, 562, 565, 568, 571, 574, or 577; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 194, 197, 200, 203, 206, 209, 212, 215, 218, 221, 224, 227, 230, 233, 236, 239, 242, 245, 248, 251, 254, 257, 260, 263, 515, 518, 521, 524, 527, 530, 533, 536, 539, 670, 542, 545, 548, 551, 554, 557, 560, 563, 566, 569, 572, 575, or 578; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 195, 198, 201, 204, 207, 210, 213, 216, 219, 222, 225, 228, 231, 234, 237, 240, 243, 246, 249, 252, 255, 258, 261, 264, 516, 519, 522, 525, 528, 531, 534, 537, 540, 671, 543, 546, 549, 552, 555, 558, 561, 564, 567, 570, 573, 576, or 579.

2. An isolated antibody which specifically binds to Cluster of Differentiation 70 (CD70), wherein the antibody comprises:

(a) a VH region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 339, 341, 343, 345, 347, 349, 351, 353, 355, 662, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, or 381; and/or

(b) a VL region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 338, 340, 342, 344, 346, 348, 350, 352, 354, 661, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, or 380.

3. An isolated antibody which specifically binds to CD70 and competes with the antibody of embodiment 1.

4. A nucleic acid encoding the antibody of any one of embodiments 1-3.

5. A vector comprising the nucleic acid of embodiment 4.

6. A host cell comprising the nucleic acid of embodiment 4.

7. The antibody of any one of embodiments 1-3 for use as a medicament.

8. The antibody of embodiment 7, wherein the medicament is for use in treatment of an CD70 related cancer selecting from the group consisting of Renal Cell Carcinoma, Glioblastoma, glioma such as low grade glioma, Non-Hodgkin's Lymphoma (NHL), Hodgkin's Disease (HD), Waldenstrom's macroglobulinemia, Acute Myeloid Leukemia, Multiple Myeloma, diffuse large-cell lymphoma, follicular lymphoma or Non-Small Cell Lung Cancer.

9. A method of treating a subject in need thereof comprising:

- a. providing the antibody according to any one of embodiments 1-3; and
- b. administering said antibody to said subject.

10. A pharmaceutical composition comprising the antibody of any one of embodiments 1-3.

11. A method of treating a condition associated with malignant cells expressing CD70 in a subject comprising administering to a subject in need thereof an effective amount of the antibody of any one of embodiments 1-3 or the pharmaceutical composition of embodiment 10.

12. The method of embodiment 11, wherein the condition is a cancer.

13. The method of embodiment 12, wherein the cancer is an CD70 related cancer selected from the group consisting of Renal Cell Carcinoma, Glioblastoma, glioma such as low grade glioma, Non-Hodgkin's Lymphoma (NHL), Hodgkin's Disease (HD), Waldenstrom's macroglobulinemia, Acute Myeloid Leukemia, Multiple Myeloma, diffuse large-cell lymphoma, follicular lymphoma or Non-Small Cell Lung Cancer.

14. A method of inhibiting tumor growth or progression in a subject who has malignant cells expressing CD70, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of embodiment 10 to the subject.

15. A method of inhibiting metastasis of malignant cells expressing CD70 in a subject, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of embodiment 10 to the subject.

16. A method of inducing tumor regression in a subject who has malignant cells expressing CD70, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of embodiment 10 to the subject.

17. A method of producing an antibody, comprising culturing the host cell of embodiment 6 under conditions that result in production of the antibody, and isolating the antibody from the host cell or culture.

METHODS OF ENGINEERING AN IMMUNE CELL

[0252] Methods of preparing immune cells for use in immunotherapy are provided herein. In some embodiments, the methods comprise introducing a CAR according to the disclosure into immune cells, and expanding the cells. In some embodiments, the disclosure relates to a method of engineering an immune cell comprising: providing a cell and expressing at the surface of the cell at least one CAR as described above. Methods for engineering immune cells are described in, for example, PCT Patent Application Publication Nos. WO/2014/039523, WO/2014/184741, WO/2014/191128, WO/2014/184744, and WO/2014/184143, each of which is incorporated herein by reference in its entirety. In some embodiments, the method comprises: transfecting the cell with at least one polynucleotide encoding CAR as described above, and expressing the polynucleotides in the cell.

[0253] In some embodiments, the polynucleotides are present in lentiviral vectors for stable expression in the cells.

[0254] In some embodiments, the method can further comprise a step of genetically modifying a cell by disrupting or inactivating at least one gene expressing, for example without limitation, a component of the TCR, a target for an immunosuppressive agent, an HLA gene, CD70 and/or an immune checkpoint protein such as, for example, PDCD1 or CTLA-4. By disrupting or inactivating a gene it is intended that the gene of interest is not expressed in a functional protein form. In some embodiments, the gene to be disrupted or inactivated is selected from the group consisting of, for example without limitation, TCR α , TCR β , CD52, GR, PD-1, CD70 and CTLA-4. In some embodiments the method comprises disrupting or inactivating one or more genes by introducing into the cells a rare-cutting endonuclease able to selectively inactivate a gene by selective DNA cleavage. In some embodiments the rare-cutting endonuclease can be, for example, a zinc finger nuclease (ZFN), megaTAL nuclease, meganuclease, transcription activator-like effector nuclease (TALE-nuclease), or CRISPR-associated endonuclease.

[0255] In some embodiments, an additional catalytic domain is used with a rare-cutting endonuclease to enhance its capacity to inactivate targeted genes. For example, an additional catalytic domain can be a DNA end-processing enzyme. Non-limiting examples of DNA end-processing enzymes include 5-3' exonucleases, 3-5' exonucleases, 5-3' alkaline exonucleases, 5'

flap endonucleases, helicases, phosphatase, hydrolases and template-independent DNA polymerases. Non-limiting examples of such catalytic domain comprise of a protein domain or catalytically active derivative of the protein domain selected from the group consisting of hExoI (EXO1_HUMAN), Yeast ExoI (EXO1_YEAST), *E. coli* ExoI, Human TREX2, Mouse TREX1, Human TREX1, Bovine TREX1, Rat TREX1, TdT (terminal deoxynucleotidyl transferase) Human DNA2, Yeast DNA2 (DNA2_YEAST). In some embodiments, an additional catalytic domain can have a 3'-5'-exonuclease activity, and In some embodiments, said additional catalytic domain is TREX, such as a TREX2 catalytic domain (WO2012/058458). In some embodiments, said catalytic domain is encoded by a single chain TREX polypeptide. The additional catalytic domain may be fused to a nuclease fusion protein or chimeric protein. In some embodiments, the additional catalytic domain is fused using, for example, a peptide linker.

[0256] In some embodiments, the method further comprises a step of introducing into cells an exogenous nucleic acid comprising at least a sequence homologous to a portion of the target nucleic acid sequence, such that homologous recombination occurs between the target nucleic acid sequence and the exogenous nucleic acid. In some embodiments, said exogenous nucleic acid comprises first and second portions which are homologous to region 5' and 3' of the target nucleic acid sequence, respectively. The exogenous nucleic acid may also comprise a third portion positioned between the first and the second portion which comprises no homology with the regions 5' and 3' of the target nucleic acid sequence. Following cleavage of the target nucleic acid sequence, a homologous recombination event is stimulated between the target nucleic acid sequence and the exogenous nucleic acid. In some embodiments, homologous sequences of at least about 50 bp, greater than about 100 bp, or greater than about 200 bp can be used within the donor matrix. The exogenous nucleic acid can be, for example without limitation, from about 200 bp to about 6000 bp, or from about 1000 bp to about 2000 bp. Shared nucleic acid homologies are located in regions flanking upstream and downstream the site of the break, and the nucleic acid sequence to be introduced is located between the two arms.

[0257] In some embodiments, a nucleic acid successively comprises a first region of homology to sequences upstream of said cleavage; a sequence to inactivate a targeted gene selected from the group consisting of TCR α , TCR β , CD52, CD70, glucocorticoid receptor (GR), deoxycytidine kinase (DCK), and an immune checkpoint protein such as for example programmed death-1 (PD-1); and a second region of homology to sequences downstream of the cleavage. The polynucleotide introduction step can be simultaneous, before or after the

introduction or expression of the rare-cutting endonuclease. Depending on the location of the target nucleic acid sequence wherein break event has occurred, such exogenous nucleic acid can be used to knock-out a gene, *e.g.* when exogenous nucleic acid is located within the open reading frame of the gene, or to introduce new sequences or genes of interest. Sequence insertions by using such exogenous nucleic acid can be used to modify a targeted existing gene, by correction or replacement of the gene (allele swap as a non-limiting example), or to up- or down-regulate the expression of the targeted gene (promoter swap as non-limiting example), the targeted gene correction or replacement. In some embodiments, inactivation of a gene selected from the group consisting of TCR α , TCR β , CD52, CD70, GR, DCK, and immune checkpoint proteins, can be done at a precise genomic location targeted by a specific TALE-nuclease, wherein said specific TALE-nuclease catalyzes a cleavage and wherein the exogenous nucleic acid successively comprising at least a region of homology and a sequence to inactivate one targeted gene selected from the group consisting of TCR α , TCR β , CD52, CD70, GR, DCK, immune checkpoint proteins which is integrated by homologous recombination. In some embodiments, several genes can be, successively or at the same time, disrupted or inactivated by using several TALE-nucleases respectively and specifically targeting one defined gene and several specific polynucleotides for specific gene inactivation.

[0258] In some embodiments, the method comprises inactivation of one or more additional genes selected from the group consisting of TCR α , TCR β , CD52, CD70, GR, DCK, and immune checkpoint proteins. In some embodiments, inactivation of a gene can be accomplished by introducing into the cells at least one rare-cutting endonuclease such that the rare-cutting endonuclease specifically catalyzes cleavage in a targeted sequence of the cell genome; and optionally, introducing into the cells an exogenous nucleic acid successively comprising a first region of homology to sequences upstream of the cleavage, a sequence to be inserted in the genome of the cell, and a second region of homology to sequences downstream of the cleavage; wherein the introduced exogenous nucleic acid inactivates a gene and integrates at least one exogenous polynucleotide sequence encoding at least one recombinant protein of interest. In some embodiments, the exogenous polynucleotide sequence is integrated within a gene encoding a protein selected from the group consisting of TCR α , TCR β , CD52, CD70, GR, DCK, and immune checkpoint protein.

[0259] In another aspect, a step of genetically modifying cells can comprise: modifying T cells by disrupting or inactivating at least one gene expressing a target for an immunosuppressive

agent, and; expanding the cells, optionally in presence of the immunosuppressive agent. An immunosuppressive agent is an agent that suppresses immune function by one of several mechanisms of action. An immunosuppressive agent can diminish the extent and/or voracity of an immune response. Non-limiting examples of immunosuppressive agents include calcineurin inhibitors, targets of rapamycin, interleukin-2 α -chain blockers, inhibitors of inosine monophosphate dehydrogenase, inhibitors of dihydrofolic acid reductase, corticosteroids, and immunosuppressive antimetabolites. Some cytotoxic immunosuppressants act by inhibiting DNA synthesis. Others may act through activation of T cells or by inhibiting the activation of helper cells. The methods according to the disclosure allow conferring immunosuppressive resistance to T cells for immunotherapy by disrupting or inactivating the target of the immunosuppressive agent in T cells. As non-limiting examples, targets for immunosuppressive agent can be a receptor for an immunosuppressive agent such as for example without limitation CD52, glucocorticoid receptor (GR), FKBP family gene members, and cyclophilin family gene members.

[0260] In some embodiments, the genetic modification of the method involves expression, in provided cells to engineer, of one rare-cutting endonuclease such that the rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene, thereby disrupting or inactivating the targeted gene. In some embodiments, a method of engineering cells comprises at least one of the following steps: providing a T cell, such as from a cell culture or from a blood sample; selecting a gene in the T cell expressing a target for an immunosuppressive agent; introducing into the T cell a rare-cutting endonuclease able to selectively inactivate by DNA cleavage (in some embodiments by double-strand break) the gene encoding a target for the immunosuppressive agent, and expanding the cells, optionally in presence of the immunosuppressive agent.

[0261] In some embodiments, the method comprises: providing a T cell, such as from a cell culture or from a blood sample; selecting a gene in the T cell wherein the gene expresses a target for an immunosuppressive agent; transfecting the T cell with nucleic acid encoding a rare-cutting endonuclease able to selectively inactivate by DNA cleavage (in some embodiments by double-strand break) the gene encoding a target for the immunosuppressive agent, and expressing the rare-cutting endonucleases into the T cells; and expanding the cells, optionally in presence of the immunosuppressive agent.

[0262] In some embodiments, the rare-cutting endonuclease specifically targets CD52 or GR. In some embodiments, the gene selected for inactivation encodes CD52, and the immunosuppressive treatment comprises a humanized antibody targeting CD52 antigen. In some embodiments, the gene selected for inactivation encodes GR, and the immunosuppressive treatment comprises a corticosteroid such as dexamethasone. In some embodiments, the gene selected for inactivation is a FKBP family gene member or a variant thereof and the immunosuppressive treatment comprises FK506, also known as Tacrolimus or fujimycin. In some embodiments, the FKBP family gene member is FKBP12 or a variant thereof. In some embodiments, gene selected for inactivation is a cyclophilin family gene member or a variant thereof and the immunosuppressive treatment comprises cyclosporine.

[0263] In some embodiments, the rare-cutting endonuclease can be, for example, zinc finger nuclease (ZFN), megaTAL nuclease, meganuclease, transcription activator-like effector nuclease (TALE-nuclease), or CRISPR-associated endonuclease. In some embodiments, the rare-cutting endonuclease is a TALE-nuclease. In some embodiments, the rare-cutting nuclease is a CRISPR nuclease, with a guide RNA at least partially complementary or fully complementary to a target site.

[0264] Generally, a CRISPR-associated nuclease is supplied with a guide RNA (gRNA) or the functional equivalent. The gRNA is comprised of two parts; a crispr-RNA (crRNA) that is specific for a target genomic DNA sequence, and a trans-activating RNA (tracrRNA) that facilitates Cas binding to the DNA. In some embodiments, the crRNA and tracrRNA may be present in the same RNA oligonucleotide, referred to as a single guide-RNA (sgRNA). In some embodiments, the crRNA and tracrRNA may be present as separate RNA oligonucleotides. As used herein, the term “guide RNA” or “gRNA” refers to the combination of a tracrRNA and a crRNA, present as either an sgRNA or a crRNA:tracrRNA duplex. In some embodiments, the CRISPR-associated nuclease is a Cas9 nuclease. In some embodiments, the Cas9 protein can be derived from *Streptococcus pyogenes* (SpCas9). In some embodiments, the Cas9 protein can be derived from other bacteria strains including *Staphylococcus aureus* (SaCas9). In some embodiments, the Cas endonuclease is selected from the group comprising SpCas9, SpCas9-HF1, SpCas9-HF2, SpCas9-HF3, SpCas9-HF4, SaCas9, FnCpf, FnCas9, eSpCas9, C2C1, C2C3, Cpf1, Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Cse3, Csa5, Csn2, Csm2, Csm3, Csm4,

Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, or Csf4.

[0265] Studies suggest that adoptive transfer of T cells derived from less-differentiated (*i.e.*, T_{SCM} or T_{CM}) subset leads to prolonged persistence *in vivo* (see, *e.g.*, Berger, C. et al., The Journal of Clinical Investigation, 118(1): 294-305 (2008)). Thus, genetic knockdown of CD70 in the CAR T product is an important consideration to prevent T cell differentiation.

[0266] In some embodiments, the genetic modification of the method involves expression, in provided cells to engineer, of one rare-cutting endonuclease such that the rare-cutting endonuclease specifically catalyzes cleavage in the CD70 gene, thereby disrupting or inactivating the CD70 gene. In some embodiments, a method of engineering cells comprises at least one of the following steps: providing a T cell, such as from a cell culture or from a blood sample; introducing into the T cell a rare-cutting endonuclease able to selectively inactivate by DNA cleavage (in some embodiments by double-strand break) the gene encoding CD70, and expanding the cells.

[0267] In some embodiments, the method comprises: providing a T cell, such as from a cell culture or from a blood sample; transfecting the T cell with nucleic acid encoding a rare-cutting endonuclease able to selectively inactivate by DNA cleavage (in some embodiments by double-strand break) the gene encoding CD70, and expressing the rare-cutting endonucleases into the T cells; and expanding the cells.

[0268] In some embodiments, the rare-cutting endonuclease can be, for example, a meganuclease, a zinc finger nuclease, or a TALE-nuclease (TALEN). In some embodiments, the rare-cutting endonuclease is a TALE-nuclease. In some embodiments, the rare-cutting nuclease is a CRISPR-associated nuclease, with a guide RNA at least partially complementary or fully complementary to a target site.

[0269] Also provided herein are methods of engineering T cells, suitable for immunotherapy, wherein the methods comprise: genetically modifying T cells by disrupting or inactivating at least immune checkpoint protein. In some embodiments the immune checkpoint protein is, for example, PD-1 and/or CTLA-4. In some embodiments, methods of genetically modifying a cell comprise: modifying T cells by disrupting or inactivating at least one immune checkpoint protein; and expanding the cells. Immune checkpoint proteins include, but are not limited to Programmed Death 1 (PD-1, also known as PDCD1 or CD279, accession number: NM—005018), Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4, also known as CD152, GenBank

accession number AF414120.1), LAG3 (also known as CD223, accession number: NM—002286.5), Tim3 (also known as HAVCR2, GenBank accession number: JX049979.1), BTLA (also known as CD272, accession number: NM—181780.3), BY55 (also known as CD160, GenBank accession number: CR541888.1), TIGIT (also known as VSTM3, accession number: NM—173799), B7H5 (also known as C10orf54, homolog of mouse vista gene, accession number: NM—022153.1), LAIR1 (also known as CD305, GenBank accession number: CR542051.1), SIGLEC10 (GeneBank accession number: AY358337.1), 2B4 (also known as CD244, accession number: NM—001166664.1), which directly inhibit immune cells. For example, CTLA-4 is a cell-surface protein expressed on certain CD4 and CD8 T cells; when engaged by its ligands (B7-1 and B7-2) on antigen presenting cells, T cell activation and effector function are inhibited.

[0270] In some embodiments, said method to engineer cells comprises at least one of the following steps: providing a T cell, such as from a cell culture or from a blood sample; introducing into the T cell a rare-cutting endonuclease able to selectively inactivate by DNA cleavage (in some embodiments by double-strand break) one gene encoding a immune checkpoint protein; and expanding the cells. In some embodiments, the method comprises: providing a T cell, such as from a cell culture or from a blood sample; transfecting said T cell with nucleic acid encoding a rare-cutting endonuclease able to selectively inactivate by DNA cleavage (in some embodiments by double-strand break) a gene encoding a immune checkpoint protein; expressing the rare-cutting endonucleases into the T cells; expanding the cells. In some embodiments, the rare-cutting endonuclease specifically targets a gene selected from the group consisting of: PD-1, CTLA-4, LAG3, Tim3, BTLA, BY55, TIGIT, B7H5, LAIR1, SIGLEC10, 2B4, TCR α , and TCR β . In some embodiments, the rare-cutting endonuclease can be a meganuclease, a zinc finger nuclease or a TALE-nuclease. In some embodiments, the rare-cutting endonuclease is a TALE-nuclease. In some embodiments, the rare-cutting nuclease is a Cas9 nuclease, with a guide RNA at least partially complementary or fully complementary to a target site.

[0271] In some embodiments, the present disclosure can be particularly suitable for allogeneic immunotherapy. In such embodiments, cells may be modified by a method comprising: disrupting or inactivating at least one gene encoding a component of the T cell receptor (TCR) in T cells; and expanding the T cells. In some embodiments, the genetic modification of the method relies on the expression, in provided cells to engineer, of one rare-cutting endonuclease

such that the rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene thereby disrupting or inactivating the targeted gene. In some embodiments, said method to engineer cells comprises at least one of the following steps: providing a T cell, such as from a cell culture or from a blood sample; introducing into the T cell a rare-cutting endonuclease able to selectively inactivate by DNA cleavage (in some embodiments by double-strand break) at least one gene encoding a component of the T cell receptor (TCR), and expanding the cells.

[0272] In some embodiments, the method comprises: providing a T cell, such as from a cell culture or from a blood sample; transfecting said T cell with nucleic acid encoding a rare-cutting endonuclease able to selectively inactivate by DNA cleavage (in some embodiments by double-strand break) at least one gene encoding a component of the T cell receptor (TCR); expressing the rare-cutting endonucleases into the T cells; sorting the transformed T cells, which do not express TCR on their cell surface; and expanding the cells.

[0273] In some embodiments, the rare-cutting endonuclease can be a meganuclease, a zinc finger nuclease or a TALE-nuclease. In some embodiments, the rare-cutting endonuclease is a TALE-nuclease. In some embodiments the TALE-nucleases recognize and cleave a sequence encoding TCR α or TCR β . In some embodiments a TALE-nuclease comprises a polypeptide sequence selected from the amino acid sequence shown in SEQ ID NO: 281, 282, 283, 284, 285, 286, 287, 288, 289 or 290.

TALE-nuclease polypeptide sequences:

Repeat TRAC T01-L

LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQR
LLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDG
GKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPE
QVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVL
CQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQAL
ETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAH
GLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQ
RLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNG
GGRPALE (SEO ID NO: 281).

Repeat TRAC T01-R

LTPEQVVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQR
LLPVLCQAHGLTPEQVVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVVAIASNIGG
KQALETVQALLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ
VVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQRLLPVL
CQAHGLTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQA
LETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVA
IASNIGGKQALETVQALLPVLCQAHGLTPEQVVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPEQVVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVVAIASHDGGKQALETVQR
LLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNGG
GRPALE (SEQ ID NO: 282).

Repeat TRBC T01-L

LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQR
 LLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGG
 GKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQ
 QVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPV
 LCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQA
 LETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAI
 ASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAH
 GLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQ
 RLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNGG
 GRPALE (SEQ ID NO: 283).

Repeat TRBC T01-R

NPQRSTUVWXYZTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNNGG
KQALETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQ
VVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVVAISHDGGKQALETVQRLLPVL
CQAHGLTPEQVVVAISHDGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQAL
ETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQALETV

QRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN
GGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLT
PQQVVAIASNNGGRPAALE (SEQ ID NO: 284).

Repeat TRBC_T02-L

LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQR
LLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGG
KQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQ
VVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLC
QAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQAL
ETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETV
QRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN
GGGRPAALE (SEQ ID NO: 285).

Repeat TRBC_T02-R

LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQR
LLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGG
KQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ
VVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLC
QAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQAL
ETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETV
QRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN
GGGRPAALE (SEQ ID NO: 286).

Repeat CD52_T02-L

LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQR
LLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNG
GKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPE

QVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQA
LETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVA
IASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAH
GLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQ
RLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNGG
GRPALE (SEQ ID NO: 287).

Repeat CD52 T02-R

LTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQR
LLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGG
GKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQ
QVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQA
LETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVA
IASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETV
QALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN
GGGRPALE (SEQ ID NO: 288).

Repeat CD70-L

LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQR
LLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDG
GKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQ
QVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPV
LCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQA
LETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVA
IASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQ
ALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNG
GGRPALE (SEQ ID NO: 289)

Repeat CD70-R

LTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQR
LLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNGG
GKQALETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPE
QVVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQRLLPV
LCQAHGLTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVVAIASHDGGKQA
LETVQRLLPVLCQAHGLTPEQVVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPEQVVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPQQVVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNNGGKQALETVQ
RLLPVLCQAHGLTPEQVVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVVAIASNG
GGRPALE (SEQ ID NO: 290)

[0274] In another aspect, one another step of genetically modifying cell can be a method of expanding TCR α deficient T cells comprising introducing into the T cell pT α (also known as preTCR α) or a functional variant thereof and expanding the cells, optionally through stimulation of the CD3 complex. In some embodiments, the method comprises: a) transfecting the cells with nucleic acid encoding at least a fragment of pT α to support CD3 surface expression; b) expressing said pT α into the cells; and c) expanding the cells, optionally through stimulation of the CD3 complex.

[0275] Also provided are methods of preparing T cells for immunotherapy comprising steps of the method for expansion for T cell. In some embodiments, the pTα polynucleotide sequence can be introduced randomly or by homologous recombination. In some embodiments, the insertion can be associated with the inactivation of the TCRα gene.

[0276] Different functional variants of pTα can be used. A “functional variant” of the peptide refers to a molecule substantially similar to either the entire peptide or a fragment thereof. A “fragment” of the pTα or functional variant thereof refers to any subset of the molecule, that is, a shorter peptide than the full-length pTα. In some embodiments, pTα or functional variants can be, for example, full-length pTα or a C-terminal truncated pTα version. C-terminal truncated pTα lacks in C-terminal end one or more residues. As non limiting examples, C-terminal truncated pTα version lacks 18, 48, 62, 78, 92, 110 or 114 residues from the C-terminus of the protein. Amino acid sequence variants of the peptide can be prepared by mutations in the DNA which encodes the peptide. Such functional variants include, for example, deletions from, or

insertions or substitutions of, residues within the amino acid sequence. Any combination of deletion, insertion, and substitution may also be made to arrive at the final construct, provided that the final construct possesses the desired activity, in particular the restoration of a functional CD3 complex. In some embodiments, at least one mutation is introduced in the different pT α versions as described above to affect dimerization. As non limiting example, mutated residue can be at least W46R, D22A, K24A, R102A or R117A of the human pT α protein or aligned positions using CLUSTALW method on pT α family or homologue member. In some embodiments, pT α or variant thereof as described above comprise the mutated residue W46R or the mutated residues D22A, K24A, R102A and R117A. In some embodiments, said pT α or variants are also fused to a signal-transducing domain such as CD28, OX40, ICOS, CD27, CD137 (4-1BB) and CD8 as non limiting examples. The extracellular domain of pT α or variants as described above can be fused to a fragment of the TCR α protein, particularly the transmembrane and intracellular domain of TCR α . pT α variants can also be fused to the intracellular domain of TCR α .

[0277] In some embodiments, pT α versions can be fused to an extracellular ligand-binding domain. In some embodiments, pT α or functional variant thereof is fused to a single chain antibody fragment (scFv) comprising the light and the heavy variable fragment of a target antigen specific monoclonal antibody joined by a flexible linker.

[0278] The term “TCR α deficient T cell” refers to an isolated T cell that lacks expression of a functional TCR α chain. This may be accomplished by different means, as non limiting examples, by engineering a T cell such that it does not express any functional TCR α on its cell surface or by engineering a T cell such that it produces very little functional TCR α chain on its surface or by engineering a T cell to express mutated or truncated form of TCR α chain. TCR α deficient cells can no longer be expanded through CD3 complex. Thus, to overcome this problem and to allow proliferation of TCR α deficient cells, pT α or functional variant thereof is introduced into the cells, thus restoring a functional CD3 complex. In some embodiments, the method further comprises introducing into said T cells rare-cutting endonucleases able to selectively inactivate by DNA cleavage one gene encoding one component of the T cell receptor (TCR). In some embodiments, the rare-cutting endonuclease is a TALE-nuclease.

[0279] In another aspect, engineered T cells obtained by the methods described herein can be contacted with bispecific antibodies. For example, the T cells can be contacted with bispecific antibodies ex vivo prior to administration to a patient, or in vivo following administration to a

patient. Bispecific antibodies comprise two variable regions with distinct antigen properties that facilitate bringing the engineered cells into proximity to a target antigen. As a non-limiting example, a bispecific antibody can be directed against a tumor marker and lymphocyte antigen, such as for example without limitation CD3, and has the potential to redirect and activate any circulating T cells against tumors.

[0280] In some embodiments, polynucleotides encoding polypeptides according to the present disclosure can be mRNA which is introduced directly into the cells, for example by electroporation. In some embodiments, cytoPulse technology can be used to transiently permeabilize living cells for delivery of material into the cells. Parameters can be modified in order to determine conditions for high transfection efficiency with minimal mortality.

[0281] Also provided herein are methods of transfecting T cell. In some embodiments, the method comprises: contacting a T cell with RNA and applying to T cell an agile pulse sequence consisting of: (a) an electrical pulse with a voltage range from about 2250 to 3000 V per centimeter; (b) a pulse width of 0.1 ms; (c) a pulse interval of about 0.2 to 10 ms between the electrical pulses of step (a) and (b); (d) an electrical pulse with a voltage range from about 2250 to 3000 V with a pulse width of about 100 ms and a pulse interval of about 100 ms between the electrical pulse of step (b) and the first electrical pulse of step (c); and (e) four electrical pulses with a voltage of about 325 V with a pulse width of about 0.2 ms and a pulse interval of 2 ms between each of 4 electrical pulses. In some embodiments, a method of transfecting T cell comprising contacting said T cell with RNA and applying to T cell an agile pulse sequence comprising: (a) an electrical pulse with a voltage of about 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2400, 2450, 2500, 2600, 2700, 2800, 2900 or 3000V per centimeter; (b) a pulse width of 0.1 ms; (c) and a pulse interval of about 0.2, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 ms between the electrical pulses of step (a) and (b); (d) one electrical pulse with a voltage range from about 2250, of 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2400, 2450, 2500, 2600, 2700, 2800, 2900 or 3000V with a pulse width of 100 ms and a pulse interval of 100 ms between the electrical pulse of step (b) and the first electrical pulse of step (c); and (e) 4 electrical pulses with a voltage of about 325 V with a pulse width of about 0.2 ms and a pulse interval of about 2 ms between each of 4 electrical pulses. Any values included in the value range described above are disclosed in the present application. Electroporation medium can be any suitable medium known in the art, such as BTXpress Cytoporation[®] Media T4, available from BTX. In some embodiments, the

electroporation medium has conductivity in a range spanning about 0.01 to about 1.0 milliSiemens.

[0282] In some embodiments, as non limiting examples, an RNA encodes a rare-cutting endonuclease, one monomer of the rare-cutting endonuclease such as half-TALE-nuclease, a CAR, at least one component of the multi-chain chimeric antigen receptor, a pTα or functional variant thereof, an exogenous nucleic acid, and/or one additional catalytic domain.

ENGINEERED IMMUNE CELLS

[0283] The disclosure also provides engineered immune cells comprising any of the CAR polynucleotides described herein. In some embodiments, a CAR can be introduced into an immune cell as a transgene via a plasmid vector. In some embodiments, the plasmid vector can also contain, for example, a selection marker which provides for identification and/or selection of cells which received the vector.

[0284] CAR polypeptides may be synthesized in situ in the cell after introduction of polynucleotides encoding the CAR polypeptides into the cell. Alternatively, CAR polypeptides may be produced outside of cells, and then introduced into cells. Methods for introducing a polynucleotide construct into cells are known in the art. In some embodiments, stable transformation methods can be used to integrate the polynucleotide construct into the genome of the cell. In other embodiments, transient transformation methods can be used to transiently express the polynucleotide construct, and the polynucleotide construct not integrated into the genome of the cell. In other embodiments, virus-mediated methods can be used. The polynucleotides may be introduced into a cell by any suitable means such as for example, recombinant viral vectors (*e.g.* retroviruses, adenoviruses), liposomes, and the like. Transient transformation methods include, for example without limitation, microinjection, electroporation or particle bombardment. Polynucleotides may be included in vectors, such as for example plasmid vectors or viral vectors.

[0285] Also provided herein are isolated cells and cell lines obtained by the above-described methods of engineering cells provided herein. In some embodiments, an isolated cell comprises at least one CAR as described above. In some embodiments, an isolated cell comprises a population of CARs, each CAR comprising different extracellular ligand-binding domains.

[0286] Also provided herein are isolated immune cells obtained according to any one of the methods described above. Any immune cell capable of expressing heterologous DNAs can be used for the purpose of expressing the CAR of interest. In some embodiments, the immune cell

is a T cell. In some embodiments, an immune cell can be derived from, for example without limitation, a stem cell. The stem cells can be adult stem cells, non-human embryonic stem cells, more particularly non-human stem cells, cord blood stem cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells. Representative human cells are CD34+ cells. The isolated cell can also be a dendritic cell, killer dendritic cell, a mast cell, a NK-cell, a B-cell or a T cell selected from the group consisting of inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes. In some embodiments, the cell can be derived from the group consisting of CD4+ T-lymphocytes and CD8+ T-lymphocytes.

[0287] In some embodiments, the engineered immune cells expressing at their cell surface membrane a CD70-specific CAR of the disclosure comprise a percentage of stem cell memory and central memory cells greater than 10%, 20%, 30%, 40%, 50%, or 60%. In some embodiments, the engineered immune cells expressing at their cell surface membrane a CD70-specific CAR of the disclosure comprise a percentage of stem cell memory and central memory cells of about 10% to about 60%, about 10% to about 50%, about 10% to about 40%, about 15% to about 50%, about 15% to about 40%, about 20% to about 60%, or about 20% to about 70%.

[0288] In some embodiments, the immune cell is an inflammatory T-lymphocyte that expresses any one of the CARs described herein. In some embodiments, the immune cell is a cytotoxic T-lymphocyte that expresses any one of the CARs described herein. In some embodiments, the immune cell is a regulatory T-lymphocyte that expresses any one of the CARs described herein. In some embodiments, the immune cell is a helper T-lymphocyte that expresses any one of the CARs described herein.

[0289] Prior to expansion and genetic modification, a source of cells can be obtained from a subject through a variety of non-limiting methods. Cells can be obtained from a number of non-limiting sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In some embodiments, any number of T cell lines available and known to those skilled in the art, may be used. In some embodiments, cells can be derived from a healthy donor, from a patient diagnosed with cancer or from a patient diagnosed with an infection. In some embodiments, cells can be part of a mixed population of cells which present different phenotypic characteristics.

[0290] Also provided herein are cell lines obtained from a transformed T cell according to any of the above-described methods. Also provided herein are modified cells resistant to an immunosuppressive treatment. In some embodiments, an isolated cell according to the disclosure comprises a polynucleotide encoding a CAR.

[0291] The immune cells of the disclosure can be activated and expanded, either prior to or after genetic modification of the T cells, using methods as generally described, for example without limitation, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005. T cells can be expanded in vitro or in vivo. Generally, the T cells of the disclosure can be expanded, for example, by contact with an agent that stimulates a CD3 TCR complex and a co-stimulatory molecule on the surface of the T cells to create an activation signal for the T cell. For example, chemicals such as calcium ionophore A23187, phorbol 12-myristate 13-acetate (PMA), or mitogenic lectins like phytohemagglutinin (PHA) can be used to create an activation signal for the T cell.

[0292] In some embodiments, T cell populations may be stimulated in vitro by contact with, for example, an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (*e.g.*, bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. The anti-CD3 antibody and an anti-CD28 antibody can be disposed on a bead or plate or other substrate. Conditions appropriate for T cell culture include an appropriate media (*e.g.*, Minimal Essential Media or RPMI Media 1640 or, X-vivo 5, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (*e.g.*, fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN- γ , IL-4, IL-7, GM-CSF, IL-10, IL-2, IL-15, TGF β , and TNF, or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl- cysteine and 2-mercaptoethanol. Media can include RPMI 1640, A1M-V, DMEM, MEM, α - MEM, F-12, X-Vivo 1, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum

(or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells (*e.g.*, IL-7 and/or IL-15). Antibiotics, *e.g.*, penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (*e.g.*, 37° C) and atmosphere (*e.g.*, air plus 5% CO₂). T cells that have been exposed to varied stimulation times may exhibit different characteristics

[0293] In some embodiments, the cells of the disclosure can be expanded by co-culturing with tissue or cells. The cells can also be expanded *in vivo*, for example in the subject's blood after administering the cell into the subject.

[0294] In some embodiments, an isolated cell according to the present disclosure comprises one disrupted or inactivated gene selected from the group consisting of CD52, CD70, GR, PD-1, CTLA-4, LAG3, Tim3, BTLA, BY55, TIGIT, B7H5, LAIR1, SIGLEC10, 2B4, HLA, TCR α and TCR β and/or expresses a CAR, a multi-chain CAR and/or a pT α transgene. In some embodiments, an isolated cell comprises polynucleotides encoding polypeptides comprising a multi-chain CAR. In some embodiments, the isolated cell according to the present disclosure comprises two disrupted or inactivated genes selected from the group consisting of: CD52 and GR, CD52 and TCR α , CDR52 and TCR β , CD70 and CD52, CD70 and TCR α , CD70 and TCR β , GR and TCR α , GR and TCR β , TCR α and TCR β , PD-1 and TCR α , PD-1 and TCR β , CTLA-4 and TCR α , CTLA-4 and TCR β , LAG3 and TCR α , LAG3 and TCR β , Tim3 and TCR α , Tim3 and TCR β , BTLA and TCR α , BTLA and TCR β , BY55 and TCR α , BY55 and TCR β , TIGIT and TCR α , TIGIT and TCR β , B7H5 and TCR α , B7H5 and TCR β , LAIR1 and TCR α , LAIR1 and TCR β , SIGLEC10 and TCR α , SIGLEC10 and TCR β , 2B4 and TCR α , 2B4 and TCR β and/or expresses a CAR, a multi-chain CAR and a pT α transgene.

[0295] In some embodiments, the isolated cell according to the present disclosure comprises three disrupted or inactivated genes selected from CD52, CD70 and TCR α or CD52, CD70 and TCR β and/or expresses a CAR, a multi-chain CAR and a pT α transgene.

[0296] In some embodiments, TCR is rendered not functional in the cells according to the disclosure by disrupting or inactivating TCR α gene and/or TCR β gene(s). In some embodiments, a method to obtain modified cells derived from an individual is provided, wherein the cells can proliferate independently of the major histocompatibility complex (MHC) signaling pathway. Modified cells, which can proliferate independently of the MHC signaling pathway, susceptible

to be obtained by this method are encompassed in the scope of the present disclosure. Modified cells disclosed herein can be used in for treating patients in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD); therefore in the scope of the present disclosure is a method of treating patients in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD) comprising treating said patient by administering to said patient an effective amount of modified cells comprising disrupted or inactivated TCR α and/or TCR β genes.

[0297] In some embodiments, the immune cells are engineered to be resistant to one or more chemotherapy drugs. The chemotherapy drug can be, for example, a purine nucleotide analogue (PNA), thus making the immune cell suitable for cancer treatment combining adoptive immunotherapy and chemotherapy. Exemplary PNAs include, for example, clofarabine, fludarabine, cyclophosphamide, and cytarabine, alone or in combination. PNAs are metabolized by deoxycytidine kinase (dCK) into mono-, di-, and tri-phosphate PNA. Their tri-phosphate forms compete with ATP for DNA synthesis, act as pro-apoptotic agents, and are potent inhibitors of ribonucleotide reductase (RNR), which is involved in trinucleotide production. Provided herein are CD70-specific CAR-T cells comprising an disrupted or inactivated dCK gene. In some embodiments, the dCK knockout cells are made by transfection of T cells using polynucleotides encoding specific TAL-nulcease directed against dCK genes by, for example, electroporation of mRNA. The dCK knockout CD70-specific CAR-T cells can be resistant to PNAs, including for example clorofarabine and/or fludarabine, and maintain T cell cytotoxic activity toward CD70-expressing cells.

[0298] In some embodiments, isolated cells or cell lines of the disclosure can comprise a pT α or a functional variant thereof. In some embodiments, an isolated cell or cell line can be further genetically modified by disrupting or inactivating the TCR α gene.

MONOCLONAL ANTIBODY-SPECIFIC EPITOPES

[0299] In some embodiments, the extracellular domain of any one of the CD70-specific CARs disclosed herein may comprise one or more epitopes specific for (*i.e.*, specifically recognized by) a monoclonal antibody. These epitopes are also referred to herein as mAb-specific epitopes. Exemplary mAb-specific epitopes are disclosed in International Patent Publication No. WO 2016/120126, which is incorporated herein in its entirety. In these embodiments, the extracellular domain comprises the VH and VL polypeptides that specifically bind to CD70 and

one or more epitopes that bind to one or more monoclonal antibodies (mAbs). CARs comprising the mAb-specific epitopes can be single-chain or multi-chain.

[0300] The inclusion of eptiopes specific for monoclonal antibodies in the extracellular domain of the CARs described herein allows sorting and depletion of engineered immune cells expressing the CARs. In some embodiments, this feature also promotes recovery of endogenous CD70-expressing cells that were depleted by administration of engineered immune cells expressing the CARs.

[0301] Accordingly, in some embodiments, the present disclosure relates to a method for sorting and/or depleting the engineered immune cells endowed with the CARs comprising mAb-specific epitopes and a method for promoting recovery of endogenous CD70-expressing cells, such as bone marrow progeniotr cells.

[0302] Several epitope-monoclonal antibody couples can be used to generate CARs comprising monoclonal antibody specific epitopes; in particular, those already approved for medical use, such as CD20 epitope/rituximab as a non-limiting example.

[0303] The disclosure also encompasses methods for sorting the engineered immune cells endowed with the CD70-specific CARs expressing the mAb-specific epitope(s) and therapeutic methods where the activation of the engineered immune cells endowed with these CARs is modulated by depleting the cells using an antibody that targets the external ligand binding domain of said CARs.

<u>Rituximab</u>		
Mimotope	SEQ ID NO: 293	CPYSNPSLC
<u>Palivizumab</u>		
Epitope	SEQ ID NO: 660	NSELLSLINDMPITNDQKKLMSNN
<u>Cetuximab</u>		
Mimotope 1	SEQ ID NO: 603	CQFDLSTRRLKC
Mimotope 2	SEQ ID NO: 604	CQYNLSSRALKC
Mimotope 3	SEQ ID NO: 605	CVWQRWQKSYVC
Mimotope 4	SEQ ID NO: 606	CMWDRFSRWYKC
<u>Nivolumab</u>		
Epitope 1	SEQ ID NO: 607	SFVLNWYRMSPSNQTDKLAAPEDR
Epitope 2	SEQ ID NO: 608	SGTYLCGAISLAPKAQIKE
<u>QBEND-10</u>		
Epitope 1	SEQ ID NO: 609	ELPTQGTFSTNVSTNVS

Epitope 2	SEQ ID NO: 295	ELPTQGTFSTNVSTNVSPAKPTTTA
<u>Alemtuzumab</u>		
Epitope	SEQ ID NO: 610	GQNDTSQTSSPS

[0304] In some embodiments, the CAR-T cell comprises a polynucleotide encoding a suicide polypeptide, such as for example RQR8. See, *e.g.*, WO2013153391A, which is hereby incorporated by reference in its entirety. In CAR-T cells comprising the polynucleotide, the suicide polypeptide is expressed at the surface of a CAR-T cell. In some embodiments, the suicide polypeptide comprises the amino acid sequence shown in SEQ ID NO: 291.

CPYSNPSLCSGGGGSELPTQGTFSTNVSTNVSPAKPTTTACPYNSPSLCSGGGGSP
APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
LVITLYCNHRNRRRVCKCPRPVV (SEQ ID NO: 291).

[0305] The suicide polypeptide may also comprise a signal peptide at the amino terminus—for example, MGTSLLCWMALCLLGADHADA (SEQ ID NO: 611). In some embodiments, the suicide polypeptide comprises the amino acid sequence shown in SEQ ID NO: 292, which includes the signal sequence of SEQ ID NO: 611.

MGTSLLCWMALCLLGADHADACPYNSPSLCSGGGGSELPTQGTFSTNVSTNVSPAKPTT
TACPYNSPSLCSGGGGSPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIY
IWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVV (SEQ ID NO: 292).

[0306] In some embodiments, the suicide polypeptide comprises the amino acid sequence of SEQ ID NO: 611

[0307] When the suicide polypeptide is expressed at the surface of a CAR-T cell, binding of rituximab to the R epitopes of the polypeptide causes lysis of the cell. More than one molecule of rituximab may bind per polypeptide expressed at the cell surface. Each R epitope of the polypeptide may bind a separate molecule of rituximab. Deletion of CD70-specific CAR-T cells may occur in vivo, for example by administering rituximab to a patient. The decision to delete the transferred cells may arise from undesirable effects being detected in the patient which are attributable to the transferred cells, such as for example, when unacceptable levels of toxicity are detected.

[0308] In some embodiments, upon administration to a patient, engineered immune cells expressing at their cell surface any one of the CD70-specific CARs described herein may reduce, kill or lyse endogenous CD70-expressing cells of the patient. In one embodiment, a percentage

reduction or lysis of CD70-expressing endogenous cells or cells of a cell line expressing CD70 by engineered immune cells expressing any one of the CD70-specific CARs described herein is at least about or greater than 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%. In one embodiment, a percentage reduction or lysis of CD70-expressing endogenous cells or cells of a cell line expressing CD70 by engineered immune cells expressing any one of the CD70-specific CARs described herein is about 5% to about 95%, about 10% to about 95%, about 10% to about 90%, about 10% to about 80%, about 10% to about 70%, about 10% to about 60%, about 10% to about 50%, about 10% to about 40%, about 20% to about 90%, about 20% to about 80%, about 20% to about 70%, about 20% to about 60%, about 20% to about 50%, about 25% to about 75%, or about 25% to about 60%. In one embodiment, the endogenous CD70-expressing cells are endogenous CD70-expressing bone marrow cells.

[0309] In one embodiment, the percent reduction or lysis of target cells, *e.g.*, a cell line expressing CD70, by engineered immune cells expressing at their cell surface membrane a CD70-specific CAR of the disclosure can be measured using the assay disclosed herein.

Method for sorting CAR-positive immune cells

[0310] In one aspect, provided are methods for in vitro sorting of a population of immune cells, wherein a subset of the population of immune cells comprises engineered immune cells expressing any one of the CD70-specific CARs comprising epitopes specific for monoclonal antibodies described herein. The method comprises contacting the population of immune cells with a monoclonal antibody specific for the epitopes and selecting the immune cells that bind to the monoclonal antibody to obtain a population of cells enriched in engineered immune cells expressing CD70-specific CAR.

[0311] In some embodiments, said monoclonal antibody specific for said epitope is optionally conjugated to a fluorophore. In this embodiment, the step of selecting the cells that bind to the monoclonal antibody can be done by Fluorescence Activated Cell Sorting (FACS). In some embodiments, said monoclonal antibody specific for said epitope is optionally conjugated to a magnetic particle. In this embodiment, the step of selecting the cells that bind to the monoclonal antibody can be done by Magnetic Activated Cell Sorting (MACS).

[0312] In some embodiments, the extracellular binding domain of the CAR comprises a mAb-specific epitope of one or more of SEQ ID NO: 294 or 601-610. In some embodiments, the

extracellular binding domain of the CAR comprises a mAb-specific epitope of SEQ ID NO: 609. In some embodiments, the extracellular binding domain of the CAR comprises a mAb-specific epitope of SEQ ID NO: 295. In some embodiments, the extracellular binding domain of the CAR comprises a mAb-specific epitope of SEQ ID NO: 609 and the antibody used to contact the population of immune cells is QBEND-10. In some embodiments, the extracellular binding domain of the CAR comprises a mAb-specific epitope of SEQ ID NO: 295 and the antibody used to contact the population of immune cells is QBEND-10.

[0313] In some embodiments, the extracellular binding domain of the CAR comprises a mAb-specific epitope of SEQ ID NO: 294. In some embodiments, the extracellular binding domain of the CAR comprises a mAb-specific epitope of SEQ ID NO: 294 and the antibody used to contact the population of immune cells is Rituximab.

[0314] In some embodiments, the population of CAR-expressing immune cells obtained when using the method for in vitro sorting of immune cells described above, comprises at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of CAR-expressing immune cells. In some embodiments, the population of CD70 CAR-expressing immune cells obtained when using the method for in vitro sorting of CAR-expressing immune cells described above, comprises at least 85% of CAR-expressing immune cells.

[0315] According to the disclosure, cells to be administered to the recipient may be enriched in vitro from the source population. Methods of expanding source populations are well known in the art, and may include selecting cells that express an antigen such as CD34 antigen, using combinations of density centrifugation, immuno-magnetic bead purification, affinity chromatography, and fluorescent activated cell sorting, known to those skilled in the art.

[0316] Flow cytometry is widely used in the art and is a method well known to one of ordinary skill to sort and quantify specific cell types within a population of cells. In general, flow cytometry is a method for quantitating components or structural features of cells primarily by optical means. Since different cell types can be distinguished by quantitating structural features, flow cytometry and cell sorting can be used to count and sort cells of different phenotypes in a mixture.

[0317] A flow cytometric analysis involves two basic steps: 1) labeling selected cell types with one or more labeled markers, and 2) determining the number of labeled cells relative to the total number of cells in the population.

[0318] The primary method of labeling cell types is by binding labeled antibodies to markers expressed by the specific cell type. The antibodies are either directly labeled with a fluorescent compound or indirectly labeled using, for example, a fluorescent- labeled second antibody which recognizes the first antibody.

[0319] In some embodiments, the method used for sorting immune cells expressing a CAR is the Magnetic- Activated Cell Sorting (MACS). Magnetic-activated cell sorting (MACS) is a method for separation of various cell populations depending on their surface antigens (CD molecules) by using superparamagnetic nanoparticles and columns. It takes a few simple steps to get pure cell populations. Cells in a single-cell suspension are magnetically labeled with microbeads. The sample is applied to a column composed of ferromagnetic spheres, which are covered with a cell-friendly coating allowing fast and gentle separation of cells. The unlabeled cells pass through while the magnetically labeled cells are retained within the column. The flow-through can be collected as the unlabeled cell fraction. After a short washing step, the column is removed from the separator, and the magnetically labeled cells are eluted from the column.

[0320] In some embodiments, the mAb used in the method for sorting immune cells expressing the CAR is chosen from alemtuzumab, ibritumomab tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10 and/or ustekinumab. In some embodiments, said mAb is rituximab. In another embodiment, said mAb is QBEND-10.

[0321] In some embodiments, the CAR-T cell comprises a selected epitope within the scFv having a specificity to be recognized by a specific antibody. See, *e.g.*, WO2016/120216, which is hereby incorporated by reference in its entirety. Such an epitope facilitates sorting and/or depleting the CAR-T cells. The epitope can be selected from any number of epitopes known in the art. In some embodiments, the epitope can be a target of a monoclonal antibody approved for medical use, such as, for example without limitation, the CD20 epitope recognized by rituximab. In some embodiments, the epitope comprises the amino acid sequence CPYSNPSLC (SEQ ID NO: 293)

[0322] In some embodiments, the epitope is located within the CAR. For example without limitation, the epitope can be located between the scFv and the hinge of a CAR. In some

embodiments, two instances of the same epitope, separated by linkers, may be used in the CAR. For example, the polypeptide comprising the amino acid sequence shown in SEQ ID NO: 294 or in SEQ ID NO: 609 or SEQ ID NO: 295 can be used within a CAR, located between the light chain variable region and the hinge.

GSGGGGSCPYSNPSLCSGGGGSCPYSNPSLCSGGGGGS (SEQ ID NO: 294)

ELPTQGTFSTNVSTNV (SEQ ID NO: 609)

ELPTQGTFSTNVSTNVSPAKPTTTA (SEQ ID NO: 295)

[0323] In some embodiments, the extracellular binding domain of the CAR comprises the following sequence

$V_1-L_1-V_2-(L)_x$ -Epitope1-(L) $_x$;

$V_1-L_1-V_2-(L)_x$ -Epitope1-(L) $_x$ -Epitope2-(L) $_x$;

$V_1-L_1-V_2-(L)_x$ -Epitope1-(L) $_x$ -Epitope2-(L) $_x$ -Epitope3-(L) $_x$;

(L) $_x$ -Epitope1-(L) $_x$ - $V_1-L_1-V_2$;

(L) $_x$ -Epitope1-(L) $_x$ -Epitope2-(L) $_x$ - $V_1-L_1-V_2$;

Epitope1-(L) $_x$ -Epitope2-(L) $_x$ -Epitope3-(L) $_x$ - $V_1-L_1-V_2$;

(L) $_x$ -Epitope1-(L) $_x$ - $V_1-L_1-V_2$ -(L) $_x$ -Epitope2-(L) $_x$;

(L) $_x$ -Epitope1-(L) $_x$ - $V_1-L_1-V_2$ -(L) $_x$ -Epitope2-(L) $_x$ -Epitope3-(L) $_x$;

(L) $_x$ -Epitope1-(L) $_x$ - $V_1-L_1-V_2$ -(L) $_x$ -Epitope2-(L) $_x$ -Epitope3-(L) $_x$ -Epitope4-(L) $_x$;

(L) $_x$ -Epitope1-(L) $_x$ -Epitope2-(L) $_x$ - $V_1-L_1-V_2$ -(L) $_x$ -Epitope3-(L) $_x$;

(L) $_x$ -Epitope1-(L) $_x$ -Epitope2-(L) $_x$ - $V_1-L_1-V_2$ -(L) $_x$ -Epitope3-(L) $_x$ -Epitope4-(L) $_x$;

$V_1-(L)_x$ -Epitope1-(L) $_x$ - V_2 ;

$V_1-(L)_x$ -Epitope1-(L) $_x$ - V_2 -(L) $_x$ -Epitope2-(L) $_x$;

$V_1-(L)_x$ -Epitope1-(L) $_x$ - V_2 -(L) $_x$ -Epitope2-(L) $_x$ -Epitope3-(L) $_x$;

$V_1-(L)_x$ -Epitope1-(L) $_x$ - V_2 -(L) $_x$ -Epitope2-(L) $_x$ -Epitope3-(L) $_x$ -Epitope4-(L) $_x$;

(L) $_x$ -Epitope1-(L) $_x$ - $V_1-(L)_x$ -Epitope2-(L) $_x$ - V_2 ; or,

(L) $_x$ -Epitope1-(L) $_x$ - $V_1-(L)_x$ -Epitope2-(L) $_x$ - V_2 -(L) $_x$ -Epitope3-(L) $_x$;

wherein,

V_1 is V_L and V_2 is V_H or V_1 is V_H and V_2 is V_L ;

L_1 is a linker suitable to link the V_H chain to the V_L chain;

L is a linker comprising glycine and serine residues, and each occurrence of L in the extracellular binding domain can be identical or different to other occurrence of L in the same

extracellular binding domain, which in embodiments comprises or is SGGGG (SEQ ID NO: 614), GGGGS (SEQ ID NO: 615) or SGGGS (SEQ ID NO: 616), and, x is 0 or 1 or 2 and each occurrence of x is selected independently from the others; and, Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are mAb-specific epitopes and can be identical or different and wherein V_H is a heavy chain variable fragment and V_L is a light chain variable fragment. In some embodiments, Epitope 1, Epitope 2 and Epitope 4 are a mAb-specific epitope having an amino acid sequence of SEQ ID NO: 293 and Epitope 3 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO: 295.

[0324] In some embodiments, the extracellular binding domain of the CAR comprises the following sequence

V₁-L₁-V₂-(L)_x-Epitope1-(L)_x;
 V₁-L₁-V₂-(L)_x-Epitope1-(L)_x-Epitope2-(L)_x;
 V₁-L₁-V₂-(L)_x-Epitope1-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x;
 (L)_x-Epitope1-(L)_x-V₁-L₁-V₂;
 (L)_x-Epitope1-(L)_x-Epitope2-(L)_x-V₁-L₁-V₂;
 Epitope1-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-V₁-L₁-V₂;
 (L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x;
 (L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x;
 (L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;
 (L)_x-Epitope1-(L)_x-Epitope2-(L)_x-V₁-L₁-V₂-(L)_x-Epitope3-(L)_x;
 (L)_x-Epitope1-(L)_x-Epitope2-(L)_x-V₁-L₁-V₂-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;
 V₁-(L)_x-Epitope1-(L)_x-V₂;
 V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x;
 V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x;
 V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;
 (L)_x-Epitope1-(L)_x-V₁-(L)_x-Epitope2-(L)_x-V₂; or,
 (L)_x-Epitope1-(L)_x-V₁-(L)_x-Epitope2-(L)_x-V₂-(L)_x-Epitope3-(L)_x;

wherein,

V₁ is V_L and V₂ is V_H or V₁ is V_H and V₂ is V_L;

L₁ is a linker suitable to link the V_H chain to the V_L chain;

L is a linker comprising glycine and serine residues, and each occurrence of L in the extracellular binding domain can be identical or different to other occurrence of L in the same

extracellular binding domain, which in embodiments comprises or is SGGGG (SEQ ID NO: 614), GGGGS (SEQ ID NO: 615) or SGGGS (SEQ ID NO: 616), and, x is 0 or 1 or 2 and each occurrence of x is selected independently from the others; and, Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are mAb-specific epitopes and can be identical or different and wherein V_H is an heavy chain variable fragment and V_L is a light chain variable fragment. In some embodiments, Epitope 1, Epitope 2 and Epitope 4 are a mAb-specific epitope having an amino acid sequence of SEQ ID NO: 293 and Epitope 3 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO: 609.

[0325] In some embodiments, the extracellular binding domain of the CAR comprises the following sequence

V₁-L₁-V₂-(L)_x-Epitope1-(L)_x-Epitope2-(L)_x; or, (L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x- wherein V₁, V₂, L₁, L, x and Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are as defined above.

[0326] In some embodiments, the extracellular binding domain of the CAR comprises the following sequence

(L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x- wherein V₁, V₂, L₁, L, x are as defined above and wherein (L)_x-Epitope1-(L)_x is

GGGGSCPYSNPSLCSGGGSGGGGS (SEQ ID NO: 617),

(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4 is

GSGGGGSCPYSNPSLCSGGGSELPTQGTFSTNVSTNVSPAKPTTTACPYSNPSLC (SEQ ID NO: 618) and

and V₁, V₂, L₁, L, x are as defined above.

[0327] In some embodiments, the epitope-specific antibody may be conjugated with a cytotoxic drug. It is also possible to promote CDC cytotoxicity by using engineered antibodies on which are grafted component(s) of the complement system. In some embodiments, activation of the CAR-T cells can be modulated by depleting the cells using an antibody which recognizes the epitope.

THERAPEUTIC APPLICATIONS

[0328] Isolated cells obtained by the methods described above, or cell lines derived from such isolated cells, can be used as a medicament. In some embodiments, such a medicament can be used for treating cancer. In some embodiments, the cancer is Renal Cell Carcinoma, Glioblastoma, glioma such as low grade glioma, Non-Hodgkin's Lymphoma (NHL), Hodgkin's

Disease (HD), Waldenstrom's macroglobulinemia, Acute Myeloid Leukemia, Multiple Myeloma, diffuse large-cell lymphoma, follicular lymphoma or Non-Small Cell Lung Cancer.

[0329] In some embodiments, the cancer is of hematopoietic origin, such as a lymphoma or leukemia. In some embodiments, the cancer is selected from the group consisting of multiple myeloma, malignant plasma cell neoplasm, Hodgkin's lymphoma, nodular lymphocyte predominant Hodgkin's lymphoma, Kahler's disease and Myelomatosis, plasma cell leukemia, plasmacytoma, B-cell prolymphocytic leukemia, hairy cell leukemia, B-cell non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), follicular lymphoma, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's macroglobulinemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B-cell lymphoma, lymphoplasmacytic lymphoma, Waldenström macroglobulinemia, nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, primary central nervous system lymphoma, primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, diffuse large B-cell lymphoma associated with inflammation, intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, plasmablastic lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, and other hematopoietic cells related cancer, e.g. ALL or AML.

[0330] In some embodiments, an isolated cell according to the disclosure, or cell line derived from the isolated cells, can be used in the manufacture of a medicament for treatment of a cancer in a patient in need thereof.

[0331] Also provided herein are methods for treating patients. In some embodiments the method comprises providing an immune cell of the disclosure to a patient in need thereof. In some embodiments, the method comprises a step of administering transformed immune cells of the disclosure to a patient in need thereof.

[0332] In some embodiments, T cells of the disclosure can undergo robust in vivo T cell expansion and can persist for an extended amount of time.

[0333] Methods of treatment of the disclosure can be ameliorating, curative or prophylactic. The method of the disclosure may be either part of an autologous immunotherapy or part of an allogeneic immunotherapy treatment. The disclosure is particularly suitable for allogeneic immunotherapy. T cells from donors can be transformed into non-alloreactive cells using standard protocols and reproduced as needed, thereby producing CAR-T cells which may be administered to one or several patients. Such CAR-T cell therapy can be made available as an “off the shelf” therapeutic product.

[0334] Cells that can be used with the disclosed methods are described in the previous section. Treatment can be used to treat patients diagnosed with, for example, cancer. Cancers that may be treated include, for example without limitation, cancers that involve B lymphocytes, including any of the above-listed cancers. Types of cancers to be treated with the CARs and CAR-T cells of the disclosure include, but are not limited to certain leukemia or lymphoid malignancies. Adult tumors/cancers and pediatric tumors/cancers are also included. In some embodiments, the treatment can be in combination with one or more therapies against cancer selected from the group of antibodies therapy, chemotherapy, cytokines therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy and radiation therapy.

[0335] In some embodiments, treatment can be administered into patients undergoing an immunosuppressive treatment. Indeed, the methods of the disclosure, in some embodiments, rely on cells or population of cells, which have been made resistant to at least one immunosuppressive agent due to the inactivation of a gene encoding a receptor for such immunosuppressive agent. In this aspect, the immunosuppressive treatment should help the selection and expansion of the T cells according to the disclosure within the patient. The administration of the cells or population of cells according to the disclosure may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, infusion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection or infusion, or intraperitoneally. In some embodiments, the cell compositions of the disclosure are administered by intravenous injection or infusion.

[0336] In some embodiments the administration of the cells or population of cells can comprise administration of, for example, about 10^4 to about 10^9 cells per kg body weight including all integer values of cell numbers within those ranges. In some embodiments the administration of the cells or population of cells can comprise administration of about 10^5 to about 10^6 cells per kg body weight including all integer values of cell numbers within those ranges. The cells or population of cells can be administered in one or more doses. In some embodiments, said effective amount of cells can be administered as a single dose. In some embodiments, said effective amount of cells can be administered as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. The cells or population of cells may be obtained from any source, such as the patient, a blood bank, or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administered will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. In some embodiments, an effective amount of cells or composition comprising those cells are administered parenterally. In some embodiments, administration can be an intravenous administration. In some embodiments, administration can be directly done by injection within a tumor.

[0337] In some embodiments, the methods involve (a) administering to a subject having a disease a first dose of allogeneic CAR-T cells. In some embodiments, the first dose contains about 1×10^4 cells, about 5×10^4 cells, about 1×10^5 cells, about 5×10^5 cells, about 1×10^6 cells, about 5×10^6 cells, about 6×10^6 cells, about 1×10^7 cells, about 6×10^7 cells, about 1×10^8 , about 1.8×10^8 cells, or about 4.8×10^8 cells. In some embodiments, the methods further involve (b) administering to the subject a subsequent dose of CAR-T cells at a time point that is at least or more than about 5 weeks after and less than about 24 weeks after initiation of the administration in (a).

[0338] In some embodiments, a subject with relapsed/refractory disease (*e.g.*, relapsed/refractory RCC) is administered a first and subsequent dose of allogeneic CAR-T cells each contain about 6×10^6 cells, and the subsequent dose of CAR-T cells in (b) is administered about 99 days after initiation of the administration in (a).

[0339] In some embodiments, the methods further involve the administration of additional subsequent or subsequent doses, such that a first and multiple subsequent doses are administered, e.g., in accordance with the dosing amounts and timing schedules as specified for the first and subsequent doses. In some embodiments, the first of one or more subsequent doses is administered at a time that is at least or greater than 5 weeks after the initiation of the administration of the subsequent dose. In some embodiments, the administration of the first, subsequent, and subsequent doses includes administering at least three of the doses within at or about 5 weeks. In some embodiments, the subsequent dose is administered at about 16 weeks following the initiation of administration of the first dose, and an additional subsequent or subsequent dose is administered at week 17 following the initiation of administration of the first dose. In some embodiments, additional subsequent doses are administered at week 17 and/or week 34 following the initiation of administration of the first dose.

[0340] In some aspects, the time of administering the subsequent dose(s) is further one at which the subject does not exhibit an immune response, e.g., does not exhibit a detectable adaptive host immune response specific for the CAR-T said first (or prior) dose.

[0341] In some embodiments, the time between the administration of the first dose (initial dose), e.g., the initiation of the administration of the first or prior dose, and the initiation of the administration of the subsequent dose (e.g., the initiation of the administration of the subsequent dose) is greater than about 4 weeks, e.g., greater than about 5, 6, 7, 8, or 9 days, e.g., greater than about 20 weeks, e.g., between about 9 and about 35 weeks, between about 14 and about 28 weeks, between 15 and 27 weeks, or between 16 weeks and about 18 weeks; and/or at or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27 weeks. In some embodiments, administration of the subsequent dose (e.g., initiation thereof) is more than about 5 weeks after and less than about 24 weeks after administration of the first or prior dose (e.g., initiation thereof). In some embodiments, the administration of the subsequent dose is initiated 17 weeks following the initiation of the first dose. In some embodiments, the time between administration of the first and the subsequent dose (e.g., initiation thereof) or prior and next subsequent dose is greater than about 5 weeks and less than about 24 weeks, such as between 10 and 24 weeks, such as about 17 weeks. In some embodiments, the time between administration of the first and the subsequent dose (e.g., initiation thereof) is about 17 weeks.

[0342] In some embodiments of the disclosure, cells are administered to a patient in conjunction with (e.g., before, simultaneously or following) any number of relevant treatment

modalities, including but not limited to treatment with agents such as monoclonal antibody therapy, CCR2 antagonist (*e.g.*, INC-8761), antiviral therapy, cidofovir and interleukin-2, cytarabine (also known as ARA-C) or natalizimab treatment for MS patients or efalizumab treatment for psoriasis patients or other treatments for PML patients. In some embodiments, CD70-specific CAR-T cells are administered to a patient in conjunction with one or more of the following: an anti-PD-1 antibody (*e.g.*, nivolumab, pembrolizumab, or PF-06801591), an anti-PD-L1 antibody (*e.g.*, avelumab, atezolizumab, or durvalumab), an anti-OX40 antibody (*e.g.*, PF-04518600), an anti-4-1BB antibody (*e.g.*, PF-05082566), an anti-MCSF antibody (*e.g.*, PD-0360324), an anti-GITR antibody, and/or an anti-TIGIT antibody. In some embodiments, a CD70-specific CAR comprising the amino acid sequence shown in SEQ ID NO: 319 or 327 is administered to a patient in conjunction with anti-PD-L1 antibody avelumab. In further embodiments, the T cells of the disclosure may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and/or irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Henderson, Naya et al. 1991; Liu, Albers et al. 1992; Bierer, Hollander et al. 1993). In further embodiments, the T cells of the disclosure may be used in combination with Receptor Tyrosine Kinase inhibitors such as Midostaurin, Sunitinib and axitinib, mTOR inhibitors such as Rapamycin and Everolimus, epigenetic modulators such as Vorinostat, proteasome inhibitors such as Bortezomib, immunomodulatory agents such as lenalidomide, Hedgehog inhibitors such as Erismodegib and PF-04449913 or Isocitrate Dehydrogenase (IDH) inhibitors such as AG-120 and AG-221. In a further embodiment, the cell compositions of the disclosure are administered to a patient in conjunction with (*e.g.*, before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In some embodiments, the cell compositions of the disclosure are administered following B-cell ablative therapy such as agents that react with CD20, *e.g.*, Rituxan. For example, in some embodiments, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments,

following the transplant, subjects receive an infusion of the expanded immune cells of the disclosure. In some embodiments, expanded cells are administered before or following surgery.

[0343] In some embodiments, provided are methods for depleting CD70-specific CAR-expressing engineered immune cells from a subject administered with said cells. Depletion can be by inhibition or elimination.

[0344] In one aspect, a method for depleting engineered immune cells expressing a CD70-specific CAR comprising an epitope specific for a monoclonal antibody comprises contacting said engineered immune cell with a monoclonal antibody specific for the epitope.

[0345] In some embodiments, a method for depleting from a subject administered with engineered immune cells expressing a CD70-specific CAR comprising an epitope specific for a monoclonal antibody comprises administering to the subject a monoclonal antibody specific for the epitope. In these embodiments, administration of the monoclonal antibody specific for the epitope present in the extracellular domain of the CAR to the subject eliminates or inhibits the activity of engineered CAR-expressing immune cells from the subject. In one aspect, depletion of engineered CAR expressing immune cells allows for recovery of an endogenous population of CD70-expressing cells.

[0346] In one aspect, the disclosure relates to a method for promoting recovery of endogenous CD70-expressing cells in a subject administered with engineered immune cells expressing at cell surface a CD70-specific CAR comprising an epitope specific for a monoclonal antibody, the method comprising administering a monoclonal antibody specific for the epitope to the subject. In some embodiments, endogenous CD70-expressing cells are endogenous CD70-expressing bone marrow cells. In one aspect, the term “recovery” refers to increasing the number of endogenous CD70-expressing cells. The number of endogenous CD70-expressing cells may increase due to increase in proliferation of endogenous CD70-expressing cells and/or due to reduction in elimination of endogenous CD70-expressing cells by CAR expressing engineered immune cells. In some embodiments, administration of the monoclonal antibody to the subject depletes the CAR expressing engineered immune cells and increases the number of endogenous CD70-expressing cells, *e.g.*, endogenous CD70-expressing bone marrow progenitor cells, in the subject. In one embodiment, administration of the monoclonal antibody to the subject increases the number of endogenous CD70-expressing cells by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, compared to the

number of endogenous CD70-expressing cells prior to administration of the monoclonal antibody.

[0347] In one aspect, provided is a method for treating a CD70-mediated condition in a subject, the method comprising: (a) administering to the subject engineered immune cells expressing at cell surface CD70-specific CARs comprising one or more epitopes specific for one or more monoclonal antibodies; and (b) subsequently depleting the engineered immune cells from the subject by administering one or more monoclonal antibodies specific for the epitope to the subject.

[0348] In some embodiments, the mAbs used in the method for depleting CAR-expressing engineered immune cells are selected from alemtuzumab, ibritumomab tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10, ustekinumab, and combinations thereof.

[0349] In some embodiments, said epitope specific for a monoclonal antibody (mAb-specific epitope) is a CD20 epitope or mimotope, *e.g.* SEQ ID NO: 609, SEQ ID NO: 294, or SEQ ID NO: 295, and the mAb specific for the epitope is rituximab.

[0350] In some embodiments, the step of administering a monoclonal antibody to the subject comprises infusing the subject with the monoclonal antibody. In some embodiments, the amount of epitope-specific mAb administered to the subject is sufficient to eliminate at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the CAR-expressing immune cell in the subject.

[0351] In some embodiments, the step of administering a monoclonal antibody to the subject comprises infusing the subject with 375mg/m² of rituximab, once or several times weekly.

[0352] In some embodiments, when immune cells expressing a CAR comprising an mAb-specific epitope (CAR-expressing immune cells) are depleted in a CDC assay using epitope-specific mAb, the amount of viable CAR-expressing immune cells decreases, *e.g.* by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90%.

[0353] In some embodiments, a cytotoxic drug is coupled to the epitope-specific mAbs which are used to deplete CAR-expressing immune cells. By combining targeting capabilities of monoclonal antibodies with the cell-killing ability of cytotoxic drugs, antibody-drug conjugate (ADC) allows a sensitive discrimination between healthy and diseased tissue when compared to

the use of the drug alone. Market approvals were received for several ADCs; the technology for making them -particularly on linkers- is abundantly presented in the following prior art (Payne, G. (2003) *Cancer Cell* 3:207-212; Trail et al (2003) *Cancer Immunol. Immunother.* 52:328-337; Syrigos and Epenetos (1999) *Anticancer Research* 19:605-614; Niculescu-Duvaz and Springer (1997) *Adv. Drug Del. Rev.* 26:151-172; U.S. Pat. No. 4,975,278).

[0354] In some embodiments, the epitope-specific mAb to be infused is conjugated beforehand with a molecule able to promote complement dependent cytotoxicity (CDC). Therefore, the complement system helps or complements the ability of antibodies to clear pathogens from the organism. When stimulated by one of several, is triggered an activation cascade as a massive amplification of the response and activation of the cell-killing membrane attack complex. Different molecule may be used to conjugate the mAb, such as glycans [Courtois, A, Gac-Breton, S., Berthou, C, Guezennec, J., Bordron, A. and Boisset, C. (2012), Complement dependent cytotoxicity activity of therapeutic antibody fragments is acquired by immunogenic glycan coupling, *Electronic Journal of Biotechnology* ISSN: 0717-3458; <http://www.ejbiotechnology.info> DOI: 10.2225/voll5-issue5).

KITS

[0355] The disclosure also provides kits for use in the instant methods. Kits of the disclosure include one or more containers comprising a polynucleotide encoding a CD70-specific CAR, or an engineered immune cell comprising a polynucleotide encoding a CD70-specific CAR as described herein, and instructions for use in accordance with any of the methods of the disclosure described herein. Generally, these instructions comprise a description of administration of the engineered immune cell for the above described therapeutic treatments. The kit may include one or more agents for lymphodepletion (*e.g.* alemtuzumab, cytoxan, fludarabine, cyclophosphamide, or temozolomide).

[0356] The instructions relating to the use of the engineered immune cells as described herein generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the disclosure are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk) are also acceptable. In some embodiments, the containers are identifiable (*e.g.*, by label, barcode, or

radio-frequency identification (RFID)), trackable, or imprinted with a machine-readable container identifier.

[0357] The kits of this disclosure are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. In some embodiments, the container (*e.g.*, plastic bag) is suitable for intravenous infusion. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (*e.g.*, an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a CD70-specific CAR. The container may further comprise a second pharmaceutically active agent.

[0358] Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container.

[0359] The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the disclosure in any way. Indeed, various modifications of the disclosure in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

[0360] The deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Pfizer, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. Section 122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. Section 1.14 with particular reference to 886 OG 638).

[0361] The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the disclosure in contravention of the rights granted under the authority of any government in accordance with its patent laws.

EXAMPLES

Example 1. Generation of CD70-specific CAR-T cells

[0362] The following codon-optimized CD70 CAR sequences listed in Table 5 below were, synthesized and subcloned into the following lentiviral vectors pLVX-EF1a-TurboGFP-P2A-CD70 CAR (Clontech) or pCLS-EF1a-BFP-P2A-CD70 CAR (Collectis) using the XmaI (5') and MluI (3') restriction sites (thus cloning the CAR following the P2A site).

Table 5: Exemplary CD70-specific CARs

CAR	CAR Amino Acid Sequence	Components
31H1	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKK PGSSVKVSCKASGGTFSSYGF SWVRQAPGQGLEW MGGIIPIFGSANYAQKFQGRVTITADKSTSTVYME LISLRSEDTAVYYCARGGSSSPFAYWGQGT LVTVS SGGGGSGGGGSGGGGSGGGGSDIVMTQNPLSSPVT LGQPASISC RSSQSLVHSDGNTYLS WLQQRPGQSP RLLIYKISNRFSGVPDRFSGSGAGTDFTLKISRVEA EDVGVYYCMQATQFPLTIGGGGSKVEIKTTTPAPRP PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIF KQPFMRPVQTTQEEDGCSRFP EEEEGGCEL RVKF SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR (SEQ ID NO: 311)	CD8 α signal peptide; 31H1 VH; GS linker; 31H1 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD

CAR	CAR Amino Acid Sequence	Components
63B2	<p>MALPVTALLLPLALLLHAARPQVQLVQSGAEVKK PGSSVKVSCKASGGTFSSYGFSWVRQAPGQGLEW MGGIPIFGTANYAQKFQGRVTITADKSTSTVFME LISLRSEYTAVYYCARGGSSSPFAYWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSDIVMTQTPLSSPVT LGQPASISCRSSQSLVHSDGNTYLSWLQQRPGQSP RLLIYKISNRFSGVPDRFSGSGAGTDFTLKISRVEA EDVGVYYCMQATQFPLTIGGGSKVEIK TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACDIYWAPLAGTCGVLLLSL VITLYCKRG RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG CELRVKFSRSADAPAYQQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR (SEQ ID NO: 312)</p>	<p>CD8α signal peptide; 63B2 VH; GS linker; 63B2 VL; GS linker; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD</p>
40E3	<p>MALPVTALLLPLALLLHAARPQVQLQESGPGLVKP SETLSLTCTVSGGSISSYYWNWIRQPPGKGLEWIG YIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLRSV TAADTAVYYCARTDIRTWGQGTLVTVSSGGGGSG GGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTI TCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQS GVPSKFSGSGSGTDFTLTISLQPEDFATYYCQQYN SYPLTFGGGTKVEIKTTTPAPRPPTPAPTIASQPLSL RPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCG VLLLSL VITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG HDGLYQGLSTATKDTYDALHMQALPPR</p>	<p>CD8α signal peptide; 40E3 VH; GS linker; 40E3 VL; GS linker; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD</p>

CAR	CAR Amino Acid Sequence	Components
	(SEQ ID NO: 313)	
42C3	<p>MALPVTALLLPLALLLHAARPEVQLVESGGGLVQP GGSLRLSCAASGFTFRNSWMSWVRQAPGKGLEW VANIKRDGSEKYYYVDSVKGRFTISRDNAKNSLYL QMNSLRAEDTAVYYCARDQTGSFDYWGQGT</p> <p>LVTVSSGGGGSGGGGSGGGGSGGGGSDVVM</p> <p>TSPLSLPVT</p> <p>LGQPASISCRSSQSLVSDENTYLNWFQQRPG QSLRRLIYQVSNRDSGVDPDRFSGSGSGTDFTLKISR VEAEDVGVYFCMQGTYPPTFGGGTKVEIKTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACDIYWAPLAGTCGVLLLSLVITLYCKRGRKK LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR</p> <p>(SEQ ID NO: 314)</p>	<p>CD8α signal peptide;</p> <p>42C3 VH;</p> <p>GS linker;</p> <p>42C3 VL;</p> <p>GS linker;</p> <p>CD8α hinge;</p> <p>CD8α TM domain;</p> <p>41BB ISD;</p> <p>CD3ζ ISD</p>
45F11	<p>MALPVTALLLPLALLLHAARPQVQLRSGSPGLVKP SETLSLTCTVSDDDISVYYWSWIRQPAGKGLEWIG RVYSSGNINYNPSLESRVMTMSVDTSKSRFSLNLSSV TAADTAVYYCARGLDAFDIWGQGTMTVTVSSGGG GSGGGGSGGGGSGGGGSEIVMTQSPATLSMSLGER ATLSCRASQSVSSSLAWYQQKPGQAPRLLIYGAST RATGIPARFGGSGSGTEFTLTISSLQSEDFAVYYCQ QYINWPHFGGGTKVEIKTTTPAPRPPTPAPTIASQP LSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAG TCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQ QQGQNQLYNELNLGRREEYDVL</p> <p>DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR</p>	<p>CD8α signal peptide;</p> <p>45F11 VH;</p> <p>GS linker;</p> <p>45F11 VL;</p> <p>GS linker;</p> <p>CD8α hinge;</p> <p>CD8α TM domain;</p> <p>41BB ISD;</p> <p>CD3ζ ISD</p>

CAR	CAR Amino Acid Sequence	Components
	RGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 315)	
64F9	MALPVTALLLPLALLLHAARPEVQLLES GGGLVQP GESLRLSCEVSGFTFTSYAMSWVRQVPKGLEWV SIISGVAFTTYADSVKGRFTISR D HSKNTLYLQMN GLRAEDTAVYYCVKVDGEVYWGQGLVTVSSGG GGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGD RVTITCQASQDISNYLNWYQQKPGKAPKILY GAS NLETGVPSRFSGSGSGTDFTFAISSLQPEDVATYYC QQYDNFPITFGQGRLEIKTTTPAPRPPTPAPTIA SQ PLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLA GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPA YQQGQNQLYNELNLGRREEYDVL DKRRGRDP EM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 316)	CD8 α signal peptide; 64F9 VH; GS linker; 64F9 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
72C2	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKK PGSSVKVSCEASGGTFITYAISWVRQAPGQGLEW MGGIIPFFGTANYAQKFQGRVTITADKSTSTASME LRSLRSED TAMY YCAQWELFFDFWGQGPVTVS SGGGGSGGGGSGGGGSGGGGSEIVMTQSPDTLSVS PGERAILSCRASQSVSSNLAWYQQKPGQAPRLLIY SASTRASGIPARFSGSGSGTEFTLSISLQSEDFAVY YCQQYDNWPPLTFGGG TKVEIKTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYW APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVL DKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM	CD8 α signal peptide; 72C2 VH; GS linker; 72C2 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD

CAR	CAR Amino Acid Sequence	Components
	KGERRRGKGHDGLYQGLSTATKDTYDALHMQAL PPR (SEQ ID NO: 317)	
2F10	MALPVTALLPLALLHAARPAVQLVESGGGLVQP GGSLRLSCAASGFTFTYYSMNWVRQAPGKGLEW VSHISIRSSSTIYFADSAKGRFTISRDNKNSLYLQM NSLRDEDTAVYYCAR <u>GSGWYGDYFDYWGGTL</u> VTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGT LSLSPGERATLSC <u>RASQSVSSSYLA</u> WYQQQPGQAP RLLIY <u>GASSRAT</u> GIPDRFSGSGSGTDFTLTISRLEPE DFAIYYC <u>QQYGSSPLT</u> FGGGTKVEIK TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACDIYWAPLAGTCGVLLSLVITLYCKRG RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG CELRVKFSRSADAPAYQQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR (SEQ ID NO: 318)	CD8 α signal peptide; 2F10 VH; GS linker; 2F10 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
4F11	MALPVTALLPLALLHAARPQVTLKESGPVLVKP TETLTCTVSGFSLSNARMGVTWIRQPPGKALE WLAHIFSNDEKSYSTSLKSRLTISKDTSKTQVVLTM TNMDPVDATYYCAR <u>IRDYYDISSYYDY</u> WGQGT VSVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPS AMSASVGDRVTITC <u>RASQDISNYLA</u> WFQQKPGKV PKRLIY <u>AASSLQSG</u> VPSRFSGSGSGTEFTLTISLLPE DFATYYC <u>LQLNSFPFT</u> FGGGTKVEIN TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACDIYWAPLAGTCGVLLSLVITLYCKRG RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG	CD8 α signal peptide; 4F11 VH; GS linker; 4F11 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD;

CAR	CAR Amino Acid Sequence	Components
	CELRVKFSRSADAPAYQQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR (SEQ ID NO: 319)	CD3ζ ISD
10H10	MALPVTALLPLALLHAARPEVQLVESGGGLVQP GGSRLSCAVSGFTFS <u>NHNIHWVRQAPGKGLEWIS</u> <u>YISRSSSTIYYADSVKGRFTISRDNAKNSLYLQMNS</u> LRDEDTAVYYCAR <u>DHAQWYGMDV</u> WGQGTTVTV SSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSVSA SVGDRVTITC <u>RASQGISSWLA</u> WYQQKPGKAPKVL IY <u>AASSLOS</u> GVPSRFSGSGSGTDFTLTISSLQPEDFA TYYC <u>QQAESFPFT</u> FGPGTKVDIKTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYW APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM RPVQTTQEEDGCSCRFEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM KGERRRGKGHDGLYQGLSTATKDTYDALHMQAL PPR (SEQ ID NO: 320)	CD8α signal peptide; 10H10 VH; GS linker; 10H10 VL; GS linker; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
17G6	MALPVTALLPLALLHAARPEVQLVESGGGLVQP GGSRLSCVASGFTFS <u>SYWMSWVRQAPGKGLEW</u> <u>VASIKQDGSEKYYVDSVKGRFTISRDNAKNSVYL</u> <u>QMNSLRAEDTGVIYCAR</u> <u>EGVNWGWRLYW</u> <u>HFD</u> <u>LWGRGTLVTVSSGGGGSGGGGSGGGGSGGGGSDI</u> <u>VMTQSPDSLAVSLGERATINC</u> <u>KSSQSVLYSYNNKN</u> <u>YVAWYQQKPGQPNNLLIF</u> <u>WASTRES</u> GVPRDFSGS GSGTDFTLTISSLQAEDVAVYYC <u>QYYSTLT</u> TFGGG TKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAA	CD8α signal peptide; 17G6 VH; GS linker; 17G6 VL; GS linker; CD8α hinge; CD8α TM domain;

CAR	CAR Amino Acid Sequence	Components
	GGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIT LYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF PEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG LYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR (SEQ ID NO: 321)	41BB ISD; CD3 ζ ISD
65E11	MALPVTALLLPLALLLHAARPEVQVVESGGGLVQP GGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWV SHSSISRGNIYFADSVKGRFTISRDNKNSLYLQMN SLRDEDTAVYYCARGSGWYGDYFDYWGQGTLVT VSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTL LSPGERVTLSCRASQSVSSSYLAWYQQKPGQAPRL LIYDASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQYGSSPLTFGGGTKVEIKTTTPAPRPPTP APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIY IWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQ PFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRS ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI GMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR (SEQ ID NO: 322)	CD8 α signal peptide; 65E11 VH; GS linker; 65E11 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P02B10	MALPVTALLLPLALLLHAARPEVQLLES GGGLVQP GGSLRLSCAASGFAFSNYAMSWVRQAPGKGLEW VSAIRGGGGSTYYADSVKGRFTISRDNKNTLYLQ MNSLRAEDTAVYYCARDFISGTWYPDYWGQGTL VTVSSGGGGSGGGGSGGGGSGGGGSELQSVLTQPP SASGTPGQRVTISCSGSSSNIGSNYVYWYQQLPGT APKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGL	CD8 α signal peptide; P02B10 VH; GS linker; P02B10 VL; GS linker;

CAR	CAR Amino Acid Sequence	Components
	RSEDEADYYC <u>AAWDDSLSGVV</u> FGGGTKLTVLTTT PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSL VITLYCKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCE LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR (SEQ ID NO: 323)	CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P07D03	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKP GESLKISCKGSGYRFTSYWIGWVRQMPGKGLEW MGSIY <u>PDDSD</u> TRYSPSFQGVTVISADKSISTAYLQW SSLKASDTAMYCYCASSTVDYPGYSYFDYWGGQTL VTVSSGGGGSGGGGSGGGGSGGGGSELQSVLTQPP SASGTPGQRTVISC <u>SGSRNIGSNYVY</u> WYQQLPGT APKLLIY <u>RNNQRPS</u> GVDPDRFSGSKSGTSASLAISGL RSEDEADYYC <u>ASWDGSLSAVV</u> FGTGTKLTVLTTT PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSL VITLYCKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCE LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR (SEQ ID NO: 324)	CD8 α signal peptide; P07D03 VH; GS linker; P07D03 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P08A02	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKP GESLKISCKGSGYTFTNYWIAWVRQMPGKGLEW MGIIY <u>PDGSD</u> TRYSPSFQGVTVISADKSISTAYLQW SSLKASDTAMYCYCAR <u>DITSWYYGEPAFDI</u> WGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSELQSVLTQ	CD8 α signal peptide; P08A02 VH; GS linker; P08A02 VL;

CAR	CAR Amino Acid Sequence	Components
	PPSASGTPGQRTVISC <u>SGSSSNIGSNYVY</u> WYQQLP TAPKLLIY <u>RNNQRPS</u> GVPDRFSGSKSGTSASLAISG LRSEDEADYYC <u>ATWDDSLGSPV</u> FGTGTKLTVLTT TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDIYWAPLAGTCGVLLLSLVITLYCKRGR KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR (SEQ ID NO: 325)	GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P08E02	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKP GESLKISCKGSGY <u>SFTSSWIG</u> WVRQMPGKGLEWM G <u>IIPGDS</u> DTRYSPSFQGQVTISADKSISTAYLQWSS LKASDTAMYYCAK <u>GLSQAMTGFGFDY</u> WGQGT LTVSSGGGGSGGGGSGGGGSGGGGSELDIQMTQS PSSLSASVGDRVTITC <u>RASQISIRYLN</u> WYQQKPGK APKLLIY <u>AASILOT</u> GVPSRFSGSGSGTDFTLTISLQ PEDFATYYC <u>QQSYSTMWT</u> FGQGTKVEIKTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDIYWAPLAGTCGVLLLSLVITLYCKRGRKKLL YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA LHMQALPPR (SEQ ID NO: 326)	CD8 α signal peptide; P08E02 VH; GS linker; P08E02 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P08F08	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKP GESLKISCKGSGY <u>GFTSYWIG</u> WVRQMPGKGLEW MG <u>IHPDSD</u> TKYSPSFQGQVTISADKSISTAYLQW	CD8 α signal peptide; P08F08 VH;

CAR	CAR Amino Acid Sequence	Components
	SSLKASDTAMYCYCASS <u>SYLRGLWGGYFDY</u> WGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSELQSVLTQ PPSASGTPGQRVTISC <u>SGSSSNIGSNYVNWY</u> QQLPG TAPKLLIY <u>G DYQRPS</u> GVPDRFSGSKSGTSASLAISG LRSEDEADYYC <u>ATRDDSLSGSVV</u> FGTGTKLTVLTT TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGR KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR (SEQ ID NO: 327)	GS linker; P08F08 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P08G02	MALPVTALLLPLALLHAARPEVQLVQSGAEVKKP GESLKISCKGSGYTFP <u>SSWIG</u> WVRQMPGKGLEWM <u>GIYPDTSH</u> TRYSPSFQGVVTISADKSISTAYLQWSS LKASDTAMYCYCAR <u>ASYFDRGTGYSSWWMDV</u> WG QGTLVTVSSGGGGSGGGGSGGGGSGGGGSELDIQ MTQSPSSLSASVGDRVITITC <u>RASQSIYDYLH</u> WYQQ KPGKAPKLLIY <u>DASN</u> <u>LQS</u> GVPSRFSGSGSGTDFTLT ISSLPEDFATYYC <u>QQSYTTPLFT</u> FGQGTKVEIKTT TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGR KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR (SEQ ID NO: 328)	CD8 α signal peptide; P08G02 VH; GS linker; P08G02 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD

CAR	CAR Amino Acid Sequence	Components
P12B09	<p>MALPVTALLLPLALLLHAARPEVQLLES GGGLVQP GGSLRLSCAASGFTFS<u>QYMS</u>WVRQAPGKGLEWV SAISGGGVSTYYADSVKGRFTISRDN SKNTLYLQM NSLRAEDTAVYYCAS<u>DISDSGGSHWYFDY</u>WGQG TLVTVSSGGGGSGGGGSGGGGSGGGGSELDIQMT QSPSSLSASVGDRVITITC<u>RASQYIGRYLN</u>WYQQKR GKAPKLLIH<u>GATSLAS</u>GVPSRFSGSGSGTDFLTISS LQPEDFATYYC<u>QOSYSTTSPT</u>FGQGTKVEIKTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR (SEQ ID NO: 329)</p>	<p>CD8α signal peptide; P12B09 VH; GS linker; P12B09 VL; GS linker; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD</p>
P12F02	<p>MALPVTALLLPLALLLHAARPEVQLLES GGGLVQP GGSLRLSCAASGFTFS<u>SYAMS</u>WVRQAPGKGLEWV STISGTGGTTYADSVKGRFTISRDN SKNTLYLQM NSLRAEDTAVYYCAK<u>VRAGIDPTASDV</u>WGQGT VTVSSGGGGSGGGGSGGGGSGGGGSELQSVLTQPP SASGTPGQRTVITSC<u>SGSTSNIGRNYVY</u>WYQQLP GTAPKLLIY<u>RTNQRPS</u>GVPDFRFSKSGTSASLAISGL RSEDEADYYC<u>AAWDDSLSGRV</u>FGTGTKLTVLTTT PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCE LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT</p>	<p>CD8α signal peptide; P12F02 VH; GS linker; P12F02 VL; GS linker; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD</p>

CAR	CAR Amino Acid Sequence	Components
	YDALHMQALPPR (SEQ ID NO: 330)	
P12G07	MALPVTALLLPLALLLHAARPEVQLLES GGGLVQP GGSLRLSCAASGFTFNNFAMSWVRQAPGKGLEW VSGISGSGDNTYYADSVKGRFTISRDN SKNTLYLQ MNSLRAEDTAVYYCAKDRDIGLGWYSYLDVW GQGTLTVTVSSGGGGSGGGGSGGGGSGGGGSELQS VLTQPPSASGTPGQRVTISC SGSSSNIGSNYVYWYQ QLPGTAPKPLIYMNNQRPSGVPDRFSGSKSGTSAS LAISGLRSEDEADYYCAAWDDSLSAVVFGTGTKL TVLTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGA VHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCK RGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEE GGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR (SEQ ID NO: 331)	CD8 α signal peptide; P12G07 VH; GS linker; P12G07 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P13F04	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKK PGSSVKV SCKASGGTFSSYAISWVRQAPGQGLEW MGEIIPIFGTASYAQKFQGRVTITADESTSTAYMEL SSLRSED TAVYYCARAGWDDSWFDYWGQGT LVT VSSGGGGSGGGGSGGGGSGGGGSELQSVLTQPPSA SGTPGQRVTISC SGSNSNIGTNYVSWYQQLPGTAP KLLIYRSSRRPSGVPDRFSGSKSGTSASLAISGLRSE DEADYYCAAWDGSLSGHWVFGTGTGKLTVLTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACDIYWAPLAGTCGVLLLSLVITLYCKRGRKK LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV	CD8 α signal peptide; P13F04 VH; GS linker; P13F04 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD

CAR	CAR Amino Acid Sequence	Components
	LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR (SEQ ID NO: 332)	
P15D02	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKP GESLKISCKGSGYSF <u>ASYWIGWVRQMPGKGLEWM</u> <u>GVIYPGTSETRYSPSFQ</u> QVTISADKSISTAYLQWS SLKASDTAMYYCAK <u>GLSASASGYSFQYWGQ</u> GTL VTVSSGGGGSGGGGSGGGGSGGGGSELDIQMTQS PSSLSASVGDRVTITC <u>RASQSIDTYLNWYQQKPGK</u> APKLLIY <u>SASSLHSGVPSRFSGSGSGTDFTLT</u> ISSLQ PEDFATYYC <u>QOSYSTTAWTF</u> GQGQTKVEIKTTTPAP RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA LHMQALPPR (SEQ ID NO: 333)	CD8 α signal peptide; P15D02 VH; GS linker; P15D02 VL; GS linker; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P16C05	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKP GESLKISCKGSGYSF <u>TDYWIGWVRQMPGKGLEW</u> <u>MGMISPGGSTTIYRPSFQ</u> QVTISADKSISTAYLQW SSLKASDTAMYYCARE <u>EMYTGGYGGSWYFDYWG</u> QGTLVTVSSGGGGSGGGGSGGGGSGGGGSELDIQ MTQSPSSLSASVGDRVTITC <u>RASQSIGQSLNWYQQ</u> KPGKAPKLLIY <u>GASSLOS</u> GVPSRFSGSGSGTDFTLT ISSLQPEDFATYYC <u>QOSYSTPIT</u> FGQGQTKVEIKTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK	CD8 α signal peptide; P16C05 VH; GS linker; P16C05 VL; GS linker; CD8 α hinge; CD8 α TM domain;

CAR	CAR Amino Acid Sequence	Components
	LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR (SEQ ID NO: 334)	41BB ISD; CD3 ζ ISD

[0363] CARs comprising ScFv based on 10A1, 10E2, 11A1, 11C1, 11D1, 11E1, 12A2, 12C4, 12C5, 12D3, 12D6, 12D7, 12F5, 12H4, 8C8, 8F7, 8F8, 9D8, 9E10, 9E5, 9F4 or 9F8 sequences are also prepared and comprise sequences shown in SEQ ID NO: 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600 and 601.

Example 2: Jurkat screen for in vitro characterization of anti-CD70 CARs

[0364] Jurkat cells are an immortalized human T cell line and much like primary T cells, express CD70 upon activation or transduction. Hence, these cells were chosen for transduction with CD70 CARs to study their activation profile. Jurkat cells modified using CRISPR/Cas9 to knockout CD70 (“KO Jurkat”) or parental Jurkat (“WT Jurkat”) were transduced with CD70 CARs. Their auto-activation profile was determined by comparing the percentage of CD69 expression on WT Jurkat (auto-activation and target-dependent activation since WT Jurkat express target *i.e.*, CD70, upon transduction) versus that on KO Jurkat (auto-activation alone, in the absence of target, *i.e.*, CD70) by flow cytometry. Clones that showed target-specific activation and minimal auto-activation were then selected based on the “Activation ratio” (please see below for definition of the term). Auto-activation is a term used to describe the target-independent clustering and activation of CARs. CAR auto-activation can range from minimal/none to high and is thought to be an inherent characteristic or tendency of the scFv to aggregate and cluster. Auto-activation leads to chronic signaling and can lead to T cell differentiation, exhaustion, and decreased cytolytic potential. Screening and elimination of highly auto-activating CARs is an essential step in optimal CAR identification. Ideal CARs have minimal auto-activation.

[0365] Binding of CARs to recombinant antigen is one way to characterize CARs and bin them into unique groups. Strong binding can be an indication that a CAR is highly expressed on

the cell surface and has a moderate to high affinity for its target antigen. Low or minimal binding can indicate low expression and/or weaker affinity. CARs with high and low binding should be further characterized, as optimal affinity and cell-surface expression are unknown.

Materials and methods:

CRISPR/Cas9 knockout of CD70 in Jurkat cells

[0366] Jurkat cells were transfected with CD70-targeting guide RNA vectors containing Cas9 and GFP obtained from DNA 2.0 using Lipofectamine 3000 (Invitrogen). Forty-eight hours after transfection, fluorescence activated single cell sorting was performed to select GFP+ CD70- cells. Individual clones were then expanded and genomic DNA was obtained for PCR by crude cell lysis. PCR products were sequenced to identify clones with indels or frameshifts indicating CD70 knockout.

Jurkat cell transduction with CD70 CARs

[0367] HEK293T cells were plated at 0.5 million cells per mL in 2mL of DMEM (Gibco) supplemented with 10% FBS (Hyclone or JR Scientific) per well of a 6-well plate on Day 0. On Day 1, the lentivirus was prepared by mixing together lentiviral packaging vectors 1.5ug psPAX2, 0.5ug pMD2G, and 2ug of the appropriate transfer CAR vector containing GFP tag in 250uL Opti-MEM (Gibco) per well of the 6-well plate ("DNA mix"). 10uL Lipofectamine 2000 (Invitrogen) in 250uL Opti-MEM was incubated at room temperature for 5 minutes and then added to the DNA mix. The virus was incubated at room temperature for 20 minutes and the total volume of 500uL was slowly added to the sides of the wells containing HEK293T. On Day 2, the media from each well of the 6-well plate was replaced with 2mL per well of Jurkat cell media, *i.e.*, RPMI (Gibco) supplemented with 10% FBS. On Day 3, Jurkat cells (KO or WT) were resuspended at 0.5 million cells per mL in 2mL of RPMI supplemented with 10% FBS per well of a 6-well plate. The lentiviral supernatants from HEK293T cells were harvested and passed through a 0.45 micron filter (EMD Millipore) to remove cell debris, and then added to the Jurkat cells. On Day 6, transduction efficiency was determined by detecting GFP signal via flow cytometry. Cells were expanded into larger flasks as needed using RPMI supplemented with 10% FBS.

Activation profile and ability to bind CD70 protein

[0368] On Day 5, transduced cells were stained with human anti-CD69 antibody conjugated to PE-Cy7 and acquired on a flow cytometer to obtain percentage of CD69+ population for each CAR. Cells were also incubated with recombinant biotinylated human CD70 protein, stained with streptavidin conjugated to PE, and acquired on a flow cytometer to determine protein binding. Activation ratios (WT activation/KO activation) and protein binding were used to rank CARs.

Table 6

CAR	CD69+ in WT (%)	CD69+ in KO (%)	Activation ratio (WT/KO)	Type of activation	Protein binding (%)
31H1	37.2	16.1	2.31	Auto	1.75
63B2	11.1	1.85	6	Target	0.02
40E3	59.2	28.3	2.09	Auto	0.65
42C3	30.8	5.86	5.25	Target	1.48
45F11	16.4	2.46	6.67	Target	1.29
64F9	25.2	6.48	3.89	Target	6.24
72C2	7.85	1.27	6.18	Target	0.09
2F10	22.2	2.86	7.76	Target	0.00
4F11	27.6	2.32	11.90	Target	47.10
10H10	32.8	4.48	7.32	Target	17.90
17G6	78.7	11.5	6.84	Target	37.60
65E11	11.4	1.36	8.38	Target	0.05

[0369] Activation ratio and protein binding of hybridoma CARs were determined in the Jurkat screen and used for *in vitro* characterization of CARs. Activation ratio was determined by calculating the ratio of the percentage of CD69 expression on WT Jurkat cells transduced with CD70 CAR (WT) to that on CD70 knockout Jurkat cells transduced with CD70 CAR (KO). A higher activation ratio indicates target-dependent activation. Protein binding was determined by binding to recombinant biotinylated hCD70 protein and detected using streptavidin conjugated

to phycoerythrin (PE) dye via flow cytometry. Protein binding values indicate the percentage of CD3+ CAR T cells that bind to human CD70 protein.

Table 7

CAR	%CD69+ in WT (%)	CD69+ in KO (%)	Activation ratio (WT/KO)	Type of activation	Protein binding (%)
P02B10	22.50	8.01	2.81	Target	77.90
P07D03	14.40	4.73	3.04	Target	0.68
P08A02	67.60	4.69	14.41	Target	20.70
P08E02	43.70	13.30	3.29	Target	57.10
P08F08	34.90	10.20	3.42	Target	35.90
P08G02	54.70	25.90	2.11	Auto	77.80
P12B09	52.10	12.00	4.34	Target	5.74
P12F02	34.80	2.91	11.96	Target	2.39
P12G07	64.90	5.60	11.59	Target	15.50
P13F04	43.20	10.00	4.32	Target	37.50
P15D02	33.60	12.90	2.60	Target	62.00
P16C05	59.80	26.50	2.26	Auto	70.80

[0370] **Table 7:** Activation ratio and protein binding of phage CARs were determined in the Jurkat screen. Activation ratio was used for *in vitro* characterization of CARs. Activation ratio was determined by calculating the ratio of the percentage of CD69 expression on WT Jurkat cells transduced with CD70 CAR (WT) to that on CD70 knockout Jurkat cells transduced with CD70 CAR (KO). A higher activation ratio indicates target-dependent activation. Protein binding was determined by binding to recombinant biotinylated hCD70 protein and detected using streptavidin conjugated to phycoerythrin (PE) dye via flow cytometry. Protein binding values indicate the percentage of CD3+ CAR T cells that bind to human CD70 protein.

[0371] CARs that showed minimal auto-activation, *i.e.*, minimal expression of CD69 when transduced in CD70 KO Jurkat, were considered more desirable compared to those that are highly activated even in the absence of target since this could lead to a more exhausted CAR

phenotype. Similarly, CARs with higher human CD70 protein binding were considered to be more desirable as this indicated proper expression and folding of the CARs on the surface.

Example 3: Primary T cell screen for in vitro characterization of phage and hybridoma CARs

[0372] Primary human T cells were transduced with CD70 CARs to determine transduction efficiencies, CD70-expression on T cells in culture, and T cell subsets. Transduced CAR T cells were then frozen for use in functional assays.

[0373] Transduction efficiency of CAR constructs can vary greatly between different clones. Greater transduction efficiency leads to increased CAR T cells numbers and a more efficient production process, while CARs with low transduction efficiency may not be suitable for large scale production. High transduction efficiency is, in some embodiments, advantageous.

[0374] T cells have a range of phenotypes or subsets, indicative of differentiation state and antigen exposure. Cell surface marks help identify T cell subsets and the marker, CD62L is generally found on naïve, stem-cell memory, and central memory T cells. These cells are less differentiated relative to other subsets such as effector memory and effector T cells, and thus CAR T cells with higher percentages of CD62L positive cells are, in some embodiments, advantageous.

[0375] CD70 surface expression also varies between different transduced CARs with some CAR T cells expressing 20-40% CD70 due to activation and transduction of T cells and others expressing none.

Materials and methods:

Primary T cell isolation

[0376] T cells were purified from buffy coat samples obtained from Stanford University using Ficoll gradient density medium (Ficoll Paque PLUS / GE Healthcare Life Sciences). The PBMC layer was recovered and T cells were purified using a commercially available T cell isolation kit (Miltenyi Biotec).

T cell transduction with CD70 CARs

[0377] HEK293T cells were plated at 0.5 million cells per mL in 2mL of DMEM (Gibco) supplemented with 10% FBS (Hyclone or JR Scientific) per well of a 6-well plate on Day 0. On Day 1, the lentivirus was prepared by mixing together lentiviral packaging vectors 1.5ug psPAX2, 0.5ug pMD2G, and 2ug of the appropriate transfer CAR vector containing GFP tag in

250uL Opti-MEM (Gibco) per well of the 6-well plate ("DNA mix"). 10uL Lipofectamine 2000 (Invitrogen) in 250uL Opti-MEM was incubated at room temperature for 5 minutes and then added to the DNA mix. The virus was incubated at room temperature for 20 minutes and the total volume of 500uL was slowly added to the sides of the wells containing HEK293T. Purified T cells were activated in X-Vivo-15 medium (Lonza) supplemented with 100IU/mL human IL-2 (Miltenyi Biotec), 10% FBS (Hyclone or JR Scientific), and human T activation CD2/CD3/CD28 beads at a bead:cell ratio 1:2 (Miltenyi Biotec). On Day 2, the media from each well of the 6-well plate was replaced with 2mL per well of T cell transduction media, *i.e.*, X-Vivo-15 supplemented with 10% FBS. On Day 3, T cells were resuspended at 0.5 million cells per mL in 2mL of T cell transduction media per well of a 6-well plate. The lentiviral supernatants from HEK293T cells were harvested and passed through a 0.45 micron filter (EMD Millipore) to remove cell debris, and then added to the T cells along with 100IU/mL human IL-2. On Day 6, transduction efficiency was determined by detecting GFP signal via flow cytometry. Cells were expanded into larger flasks or G-Rex vessels (Wilson Wolf) as needed using T cell expansion media, *i.e.*, X-Vivo-15 supplemented with 5% human AB serum (Gemini Bio).

CD70 expression and T cell subsets of CAR T cells

[0378] On Day 13 post-activation, transduced CAR T cells were stained with anti-human CD70 antibody conjugated to PE and anti-human CD62L antibody conjugated to BV605, and acquired on a flow cytometer to obtain percentage of CD70+ and CD62L+ populations for each CAR. Expanded CAR T cells were then frozen in FBS containing 10% DMSO (Sigma Aldrich) for future use in functional assays.

Table 8

CAR	CAR+ (%)	CD70+ (%)	CD62L+ (%)
31H1	58.7	16.0	53.4
63B2	4.1	30.0	76.3
40E3	60.0	31.3	54.5
42C3	51.5	35.9	58.9
45F11	26.8	33.6	78.8

64F9	57.3	27.3	79.1
72C2	32.2	32.2	83.2
2F10	1.1	27.9	32.3
4F11	89.3	0.1	78.0
10H10	37.4	1.7	49.0
17G6	71.7	0.2	69.0
65E11	56.9	5.0	76.1
P02B10	74.2	43.9	73.5
P07D03	81.0	17.7	76.1
P08A02	22.3	0.81	27.6
P08E02	48.1	1.25	35.6
P08F08	89.2	17.0	78.6
P08G02	66.9	1.22	58.8
P12B09	49.7	3.19	25.7
P12F02	59.8	12.8	27.1
P12G07	26.2	3.90	23.1
P13F04	6.47	6.78	32.9
P15D02	78.8	0.37	26.0
P16C05	60.3	11.8	34.4

[0379] **Table 8:** CAR T cell phenotype at day 13 post-activation was used for *in vitro* characterization of phage and hybridoma CARs. CAR⁺ population was determined by gating on CD3⁺ cells; CD70-expressing population was determined by gating on live CD3⁺ cells; CD62L-expressing population was determined by gating on live CD3⁺ CAR⁺ CD8⁺ cells.

[0380] CAR constructs tested showed varying levels of transduction efficiency. Clones that resulted in low transduction efficiencies were considered less desirable since they would be less suitable for large scale production. The CAR T cell products also showed different phenotype (as measured by CD62L expression) and varying levels of CD70 expression on the surface. In general, CAR T cells that expressed higher levels of CD62L were considered more desirable since these are likely less differentiated.

Example 4: Stress test with phage CARs

[0381] CAR T cells were generated and frozen from 12 different scFvs from the phage library as described in the previous example. These CAR T cells were then thawed and mixed with Raji target cells that are known to express CD70 at an effector:target (E:T) ratio of 1:1 in RPMI supplemented with 10% FBS. Raji cells were added to the CAR T cells every 2 days thereafter in order to maintain an E:T ratio of 1:1. Percentage lysis of target cells and fold expansion of effector CAR T cells were determined at each time-point.

[0382] Stress test is a screening assay that involves repeated exposure of CAR T cells to their target causing the CARs to undergo proliferation and in certain cases, differentiation and exhaustion. The stress test was used to select optimal clones with high target cell lysis and proliferative abilities after several rounds of exposure to target cells.

Materials and methods:

[0383] On Day 0, CAR T cells derived from 12 different scFvs from the phage library were thawed and mixed with Raji cells at an E:T ratio of 1:1. On Day 2, 200uL of cells from the assay were mixed with 50uL of cell counting beads (CountBright, Invitrogen) and acquired on the flow cytometer. The counting beads were used to determine the total number of GFP+ CAR T cells (gated on CD3+) and Raji cells (gated on CD3-) for each CAR treatment. Based on the total CAR T cell count, the number of Raji cells required to maintain E:T ratio of 1:1 was calculated and added to the respective well of the assay. This process was repeated on Day 5 and Day 7. Percentage lysis of target cells was calculated at each time-point by calculating the percentage of live Raji cells for each CAR treatment and then normalizing to non-transduced control. Fold expansion of CAR T cells over the number of CAR T cells plated on Day 0 was calculated at each time-point.

[0384] Optimal clones were those with highest target cell lysis and best fold expansion at the end of the assay on Day 7, for example P08F08.

Table 9

CAR	Serial Killing Assay					
	Target cell lysis (%)			CAR T cell fold expansion		
	Day 2	Day 5	Day 7	Day 2	Day 5	Day 7

P02B10	61.6	-42.9	-12.0	1.56	0.97	0.49
P07D03	80.2	48.9	26.9	1.63	6.11	10.33
P08A02	91.8	12.5	6.9	0.69	1.69	2.65
P08E02	98.8	37.6	-5.7	0.50	0.45	1.06
P08F08	75.9	30.2	57.8	1.52	11.47	24.35
P08G02	98.9	49.9	27.4	0.75	0.56	3.05
P12B09	85.4	11.3	5.4	0.76	2.53	3.34
P12F02	82.8	-53.7	-46.3	2.18	0.96	0.26
P12G07	94.8	36.4	9.1	0.87	1.13	1.58
P13F04	89.3	44.8	41.3	2.15	3.61	5.67
P15D02	98.7	54.6	28.6	1.12	1.29	3.78
P16C05	92.7	18.9	-10.7	1.31	3.13	3.72

[0385] **Table 9:** Target cell lysis and CAR T cell fold expansion in a stress test were used for *in vitro* characterization of phage CARs. CAR T cell phenotype was determined at day 13 post-activation and the cells were then frozen on day 14. Stress test was performed using thawed CAR T cells with target Raji cells at an E:T ratio of 1:1. Target cells lysis was determined via flow cytometry by gating on CD3- target cells 2, 5, and 7 days after co-culturing with CAR T cells. CAR T cell fold expansion was determined using cell counting beads and calculating the number of cells on days 2, 5, and 7 relative to the number of cells added at the beginning of the assay. CARs highlighted in **bold** show high target cells lysis and fold expansion.

Example 5: Repeat of stress test with optimal CARs generated from a second donor

[0386] CAR T cells were generated and frozen from 4 scFvs (P07D03, P08F08, P08G02, P15D02) from the phage library and 2 scFvs from the hybridoma library (4F11, 17G6) as described in Example 3. These CAR T cells were then thawed and mixed with Raji target cells that are known to express CD70 at an effector:target (E:T) ratio of 1:1 in RPMI supplemented with 10% FBS. Raji cells were added to the CAR T cells every 2 days thereafter in order to maintain an E:T ratio of 1:1. Percentage lysis of target cells and fold expansion of effector CAR T cells were determined at each time-point.

[0387] All clones performed well in terms of target cell lysis. Standout performers based on fold expansion were P08F08 and 4F11.

Table 10

CAR	CAR+ (%)	CD70+ (%)	CD62L+ (%)	Serial Killing Assay					
				Target cell lysis (%)			CAR T cell fold expansion		
				Day 2	Day 5	Day 7	Day 2	Day 5	Day 7
P07D03	48.8	3.2	63.4	95.1	98.1	98.7	1.2	8.1	11.8
P08F08	66.9	2.8	58.3	96.2	97.8	98.0	1.4	12.3	15.8
P08G02	37.9	0.1	35.0	98.6	97.2	99.5	0.9	2.4	5.7
P15D02	88.4	0.2	28.4	98.8	97.7	98.8	0.8	4.1	12.2
4F11	88.3	0.3	54.4	98.3	96.9	97.3	0.9	6.0	15.5
17G6	34.8	0.2	28.3	98.7	94.3	90.0	0.5	0.8	2.4

[0388] **Table 10:** Target cell lysis and CAR T cell fold expansion in a stress test were used for *in vitro* characterization of optimal CARs. CAR T cell phenotype was determined at day 14 post-activation and the cells were then frozen on the same day. Stress test was performed using thawed CAR T cells with target Raji cells at an E:T ratio of 1:1. Target cells lysis was determined via flow cytometry by gating on CD3- target cells 2, 5, and 7 days after co-culturing with CAR T cells. CAR T cell fold expansion was determined using cell counting beads and calculating the number of cells on days 2, 5, and 7 relative to the number of cells added at the beginning of the assay. CARs highlighted in **bold** show high target cells lysis and fold expansion.

Example 6: Dose-dependent CAR T *in vivo* efficacy in an RCC S.C. tumor model

[0389] CAR T cells were generated from 4F11 scFv as described in Example 3. NOD scid gamma (NSG) mice were implanted with 786-O tumors subcutaneously and once the tumors attained a volume of 200mm³, the mice were treated with 4F11 CAR T cells at different doses intravenously via tail vein injection to determine the optimal CAR T dose. 786-O cells are available from ATCC® as CRL-1932™. The 4F11 CAR T was highly efficacious *in vivo* and caused complete tumor regression at the 5x10⁶ CAR T dose.

Materials and methods:

[0390] Fifty NOD scid gamma (NSG) mice were shaved and prepared for subcutaneous tumor implant on the right flank. 786-O tumor cells that are known to express CD70 were expanded in

RPMI supplemented with 10% FBS. On Day 0, 786-O cells were resuspended in serum-free RPMI at the required concentration to inject 5 million cells per animal. Tumor cells were injected in 100uL of serum-free RPMI combined with 100uL Matrigel (Corning) per animal subcutaneously. Day 0 baseline body weights were recorded for all animals immediately after tumor implant. Tumors were measured twice a week starting on Day 9 using Digimatic Calipers (Mitutoyo) and body weights recorded. On Day 14, when the tumors attained 200mm³ (standard error 8.39) 40 tumor-bearing mice were randomized to 4 groups of 10 mice each. 4F11 CAR T cells were thawed in RPMI supplemented with 10% FBS and resuspended in serum-free RPMI at the required concentration to inject 1, 3, or 5 million CAR⁺ T cells per animal (calculated based on 4F11 transduction efficiency 57.2%). Number of non-transduced T cells (NTD) required to maintain equal number of total T cells in each group were calculated and added to respective samples. CAR T cells or non-transduced T cell control were injected in 200uL of serum-free RPMI per animal intravenously via tail vein. Tumors were measured and body weights recorded twice a week till Day 43 when the NTD group reached the study end-point (1500mm³ tumor volume).

[0391] Tumor volumes (mean and error SEM) were plotted on GraphPad Prism and statistics were calculated using one-way ANOVA with repeated measures (see FIGs. 1 and 2). While tumors were completely eliminated with the 5 million CAR⁺ dose, the 1 million CAR⁺ dose showed no efficacy as compared with the NTD group. Thus, the 3 million CAR⁺ dose was chosen as the optimal dose for future *in vivo* studies.

Example 7: *In vivo* comparison of CD70 CARs with or without CD70 TALEN knockout in 786-O cells

[0392] 4F11 and P08F08 CAR T cells were generated as described in Example 1 with or without CD70 TALEN DNA electroporation on Day 6 post-activation. NSG mice were implanted with 786-O tumors subcutaneously and once the tumors attained a volume of 200mm³, the mice were treated with CAR T cells intravenously via tail vein injection to determine the CAR and condition with most optimal efficacy. P08F08 CAR T cells were highly efficacious *in vivo* and caused complete tumor regression at the 3x10⁶ CAR T dose regardless of CD70 knockout (KO). 4F11 CAR T cells showed good efficacy when CD70 was knocked out but merely controlled tumor growth in the absence of CD70 knockout. These results suggest that CD70 knock out can improve the activity CD70 CARs.

Materials and methods:

[0393] Seventy-five NSG mice were shaved and prepared for subcutaneous tumor implant on the right flank. 786-O tumor cells that are known to express CD70 were expanded in RPMI supplemented with 10% FBS. On Day 0, 786-O cells were resuspended in serum-free RPMI at the required concentration to inject 5 million cells per animal. Tumor cells were injected in 100uL of serum-free RPMI combined with 100uL Matrigel (Corning) per animal subcutaneously. Day 0 baseline body weights were recorded for all animals immediately after tumor implant. Tumors were measured twice a week starting on Day 7 using Digimatic Calipers (Mitutoyo) and body weights recorded. On Day 20, when the tumors attained 200mm³ (standard error 9.69), 60 tumor-bearing mice were randomized to 6 groups of 10 mice each. On Day 21, CAR T cells were thawed in RPMI supplemented with 10% FBS and resuspended in serum-free RPMI at 3 million CAR+ T cells per animal (calculated based on individual transduction efficiencies). Number of NTD cells required to maintain equal percentage of CAR+ T cells as well as equal number of total T cells in each group were calculated and added to respective samples. CAR T cells or NTD control were injected in 200uL of serum-free RPMI per animal intravenously via tail vein. Tumors were measured and body weights recorded twice a week till Day 56 when the NTD group reached the study end-point (1500mm³ tumor volume) (See FIGs. 3 and 4).

[0394] Tumor volumes (mean and error SEM) were plotted on GraphPad Prism and statistics were calculated using one-way ANOVA with repeated measures. P08F08 CAR T groups, both with or without CD70 knockout, caused complete tumor regression at the 3 million CAR+ dose. 4F11 CAR T group with CD70 knockout also caused complete tumor regression at the 3 million CAR+ dose. However, 4F11 CAR T group without CD70 knockout did not cause complete regression.

[0395] In FIGs. 3 and 4, statistical significance over corresponding NTD control are indicated to the right of the legend (for example, P08F08 with CD70 KO over NTD with CD70 KO). Statistical significance of each CAR group with CD70 KO over corresponding CAR group without CD70 KO are indicated to the left of the legend. Statistics represent RM one-way ANOVA with Dunnett's post-hoc test (ns p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Example 8: *In vivo* comparison of CD70 CARs with or without CD70 TALEN knockout in ACHN metastasis model

[0396] 4F11 and P08F08 CAR T cells from Example 7 were further tested in ACHN cells, a renal cell cancer (RCC)-derived cell line. ACHN cells are described in Simmons et al. Animal Models of Bone Metastasis. *Veterinary Pathology* 52:827-841, 834 (2015). NSG mice were each implanted with 1 million ACHN tumor cells intravenously and 15 days post-tumor cell injection, the mice were treated with CAR T cells intravenously via tail vein injection to determine the CAR and condition with most optimal efficacy. 4F11 CAR T cells dosed at 3 million CAR+ cells per mouse were efficacious across all 3 donors tested regardless of CD70 knockout (KO). P08F08 CAR T cells showed little to no efficacy and were only tested in the absence of CD70 knockout.

Materials and methods:

[0397] Forty-five NSG mice were prepared for intravenous tumor injection via tail vein. ACHN tumor cells that are known to express CD70 were expanded in MEM supplemented with 10% FBS. On Day 0, ACHN tumor cells were resuspended in serum-free MEM at the required concentration to inject 1 million cells per animal. ACHN tumor cells were injected in 200uL of serum-free MEM intravenously. Day 4 baseline body weights were recorded for all animals. Tumor flux was measured twice a week starting on Day 7 using bioluminescence (IVIS Spectrum Imager™ from PerkinElmer™; auto-exposure with a maximum exposure time of 120 seconds), and body weights were recorded. On Day 20, when the tumors attained 200mm³ (standard error 9.69), 20 tumor-bearing mice were randomized to 4 groups of 5 mice each. On Day 15, CAR T cells were thawed in MEM supplemented with 10% FBS and resuspended in serum-free MEM at 3 million CAR+ T cells per animal (calculated based on individual transduction efficiencies). Number of NTD cells required to maintain equal percentage of CAR+ T cells as well as equal number of total T cells in each group were calculated and added to respective samples. CAR T cells or NTD control were injected in 200uL of serum-free MEM per animal intravenously via tail vein. Tumor flux was measured and body weights recorded twice a week till Day 35-49 when the NTD group reached the study end-point ($\geq$ 20% body weight loss) (See FIGs. 5a, 5b and 5c).

[0398] Bioluminescence (mean and error SEM) were plotted on GraphPad Prism and statistics were calculated using one-way ANOVA with repeated measures. 4F11 CAR T groups, both with

or without CD70 knockout, showed anti-tumor efficacy at the 3 million CAR+ dose. P08F08 CAR T group without CD70 knockout showed little to no efficacy at the 3 million CAR+ dose.

Example 9: Activity of CD70-specific CAR T cells expressing CD20 epitopes

Part A: *In vitro* activity

[0399] CD70-specific CAR T cells expressing CD20 epitopes are effective against 786-0 target cells in cell-killing assay

[0400] Six CD70-specific CAR formats were designed (FIG. 6A-6F). Sequences of the constructed CARs are as shown in Tables 11A-11F:

Table 11A

Format	Clone Name	Full Amino Sequence	Seq ID NO:
No Epitope (FIG. 6A)	4F11	MALPVTALLLPLALLLHAARPQVTLKESGPVLVKPTETLTLTCTVSGFSLSNARMGVTWIRQPPGKALEWLAHIFSNDEKSYSTSLKSRLTISKDTSKTQVVLTMTNMDPVDATYYCARIRDYYDISSYYDYWGQGTLSVSSGGGGSGGGSGGGGSDIQMTQSPSAMSASVGDRVTITCRASQDISNYLAWFQQKPGKVPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISLLPEDFATYYCLQLNSFPFTFGGGTKVEINTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR	619
	4F11-2	MALPVTALLLPLALLLHAARPQVTLKESGPVLVKPTETLTLTCTVSGFSLSNARMGVTWIRQPPGKALEWLAHIFSNDEKSYSTSLKSRLTISKDTSKTQVVLTMTNMDPVDATYYCARIRDYYDISSYYDYWGQGTLSVSSGGGGSGGGSGGGGSGGGGSDIQMTQSPSAMSASVGDRVTITCRASQDISNYLAWFQQKPGKVPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISLLPEDFATYYCLQLNSFPFTFGGGTKVEINTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR	672

P08F08	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGESLKISCK GSGYGFTSYWIGWVRQMPGKGLEWMGIIHPDDSDTKYSPSFQG QVTISADKSISTAYLQWSSLKASDTAMYYCASSYLRGLWGGYF DYWGQGTTLVTVSSGGGGSGGGGSGGGGSGGGGSELQSVLTQP PSASGTPGQRTVISCSSSNIGSNYVNWYQQLPGTAPKLLIYGD YQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCATRDDSL GSVVFGTGTKLTVLTTTPAPRPPTPAPTIASQPLSLRPEACRPA GGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL STATKDTYDALHMQALPPR	327
10A1	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKLPSETLSLTCT VSGGSISYYYWTWIRQPPGKGLEWIGHIYYSGSTNYNPSLKS TISIDTSKNLFSKLSSVTAADTAVYYCARAEGSIDAFDFWGGGT MVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSTLSASVGD RVTITCRASQSISTWLAWYQQKPGKAPKVLIIYKASSLESGVPSR FSGSGSGTEFILTINSLQDDFASYCQQYKSYSHTFGQGTKLEI KTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR	580
11C1	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKLPSETLSLNCT VSGGSISYYYWTWIRQPPGKGLEWIGHVIYSGTTNYNPSLKS TISVDTSKNQFSLKLNSVTAADTAVYYCVRAEGSIDAFDLWGQ GTMVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSILSASV GDRVITICRASQSVSSWLAWYQQKPGKAPKVLIIYKASSLESGV PSRFSGTGSGTEFTLTISLQSDDFATYYCQQYNTYSHTFGQGTK LEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRP VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNE LQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA LHMQALPPR	583
11E1	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPLQTLSTCT VSGGSISSdgYYWSWIRQNPNGKLEWIGYMYSGSTYYNPSLKS RVTISVDTSKNQFSLKLRSVTAADTAVYYCTRDFGWYFDLWGR GTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASV GDSITITCRASQDIDNYLAWYQQKTGKVPKVLIIAASALQSGVP SRFSGSGSGTDFTLTISLQPEDVATYYCQNYNSGPRTFGQGTK VEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQN QLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYN ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR	585

12A2	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSQSLSLTCS VSGGSVSSdgYYWSWIRQHPGKGLEWIGYIYYRRITDYNPSLKS RVNISLDTSKNQFSLKLSSVTAADTAVYYCARDGFWYFDLWGR GTLVAVSSGGGGSGGGGSGGGGSGGGGSDIQTQSPSSLASV GDRVITICRASQDISNYLTWYQQKPGRVPEVLIYAASALQSGVP SRFSGSGSGTDFLTITSLQPEDVATYYCQNYNSAPRTFGQGTK VEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQN QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR	586
12C5	MALPVTALLLPLALLLHAARPEVELVESGGGMVQPGRSLRLSC AASGFTFSDYGMHWVRQAPGMGLEWVTVIWYDGSnKYYADS VKGRFTISRDN SKNTVFLQMNSLRAEDTAVYYCARDEVGfvGAF DIWGQGTMTVTVSSGGGGSGGGGSGGGGSGGGGSDIQLTQSPSF LSASVGDRVIITCRASQGINSHLAWYQQKPGKAPKLLIYYASTLP SGVPSRFSGSGSGTEFTLTITSLQPEDFATYYCQQLNHYPITFGQ GTRLDINTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQ GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR	588
12C6	MALPVTALLLPLALLLHAARPEVELVESGGGMVQPGRSLRLSC AASGFTFSDYGMHWVRQAPGMGLEWVTVIWYDGSnKYYADS VKGRFTISRDN SKNTVFLQMNSLRAEDTAVYYCARDEVGfvGAF DIWGQGTMTVTVSSGGGGSGGGGSGGGGSGGGGSDIQLTQSPSF LSASVGDRVIITCRASQGINSHLAWYQQKPGKAPKLLIYYASTLP SGVPSRFSGSGSGTEFTLTITSLQPEDFATYYCQQLNHYPITFGQ GTRLEIKTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQ GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR	673
8F8	MALPVTALLLPLALLLHAARPQVQLQESGPGLVQPSETLSLTCT VSGGSISYYYWSWIRQPPGKGLEWIGNINYMGN TIYNPSLKS RV TISVDTSKDQFSLKLTSVSAADTAVYYCVRAEGSIDAFDFWGQG TLVAVSLGGGGSGGGGSGGGGSGGGGSDIQTQSPSTLSASVG DRVITICRASQSISSWLAWYQQKPGKAPKVLIIYKASNLESGVPS RFSGSGSGTEFTLTITSLQPD DFATYYCQQYNSYSCTFGQGTKLE IKTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA CDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR	596

Table 11B

Format	Clone Name	Full Amino Sequence	Seq ID NO:
SR2 (FIG. 6B)	4F11-SR2	MALPVTALLLPLALLLHAARPQVTLKESGPVLVKPTETLTLTCT VSGFSLSNARMGVTWIRQPPGKALEWLAHIFSNDEKSYSTSLKS RLTISKDTSKTQVVLTMTNMDPVDATYYCARIRDYYDISSYY DYWGQGTLSVSVSSGGGGSGGGGSGGGGSDIQMTQSPSAMSAS VGDRVTITCRASQDISNYLAWFQQKPGKVPKRLIYAASSLQSGV PSRFSGSGSGTEFTLTISSLLPEDFATYYCLQLNSFPFTFGGGTKV EINGSGGGGSCPYSNP SLCSGGGGSCPYSNP SLCSGGGGSTTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNL GRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL PPR	620
	4F11-2-SR2	MALPVTALLLPLALLLHAARPQVTLKESGPVLVKPTETLTLTCT VSGFSLSNARMGVTWIRQPPGKALEWLAHIFSNDEKSYSTSLKS RLTISKDTSKTQVVLTMTNMDPVDATYYCARIRDYYDISSYY DYWGQGTLSVSVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPS AMSASVGDRVTITCRASQDISNYLAWFQQKPGKVPKRLIYAASS LQSGVPSRFSGSGSGTEFTLTISSLLPEDFATYYCLQLNSFPFTFG GGTKVEINGSGGGGSCPYSNP SLCSGGGGSCPYSNP SLCSGGGG STTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGDCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR	674
	P08F08-SR2	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGESLKISCK GSGYGFTSYWIGWVRQMPGKGLEWMGIIHPDDSDTKYSPSFQG QVTISADKSISTAYLQWSSLKASDTAMYYCASSYLRLWGGYF DYWGQGTLSVTVSSGGGGSGGGGSGGGGSGGGGSELQSVLTQP PSASGTPGQRTVISCSSNIGSNYVNWYQQLPGTAPKLLIYGD YQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCATRDDSL SGSVVFGTGTKLTVLGSGGGGSCPYSNP SLCSGGGGSCPYSNP LCSGGGGSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFK QPFMRPVQTTQEEDGDCSCRFPEEEEGGCEL RVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQ GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR	621
	10A1-SR2	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCT VSGGSISYYYWTWIRQPPGKLEWIGHIYYSGSTNYNP SLKSRV TISIDTSKNLFSKLSSVTAADTAVYYCARAEGSIDAFDFWQQG TMVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSTLSASVG DRVITITCRASQSISTWLAWYQQKPGKAPKVLIIYKASSLESGVPS RFSGSGSGTEFILTINSLQPDFAFYCQQYKSYSHFTGQGGTKLE IKSGGGGSCPYSNP SLCSGGGGSCPYSNP SLCSGGGGSTTTTPA RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED	622

Format	Clone Name	Full Amino Sequence	Seq ID NO:
		GCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGDLGYQGLSTATKDTYDALHMQALPPR	
	11C1-SR2	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSSETLSLNCTVSGGSISYYYWTWIRQPPGKGLEWIGHVIYSGTTNPNPSLKSRTISVDTSKNQFSLKLNSVTAADTAVYYCVRAEGSIDAFDLWGQGTMTVTSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSILSASVGDRVTITCRASQSVSSWLAWYQQKPGKAPKVLIIYKASSLESVPSRFSGTGSGTEFTLTISLQSDDFATYYCQQYNTYSHTFGQGTKLEIKSGGGGGSCPYSNPSPSLCSGGGGSCPYSNPSPSLCSGGGGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAFLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGDLGYQGLSTATKDTYDALHMQALPPR	623
	11E1-SR2	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPLQTLSTCTVSGGSISSdgYYWSWIRQNPGKGLEWIGYMYSGSTYYNPSLKSRTISVDTSKNQFSLKLRSVTAADTAVYYCTRDFGWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDSITITCRASQDIDNYLAWYQQKTGKVPKVLIIY AASALQSGVPSRFSGSGSGTDFTLTISLQPEDVATYYCQNYNSGPRTFGQGTKVEIKSGGGGGSCPYSNPSPSLCSGGGGSCPYSNPSPSLCSGGGGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAFLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGDLGYQGLSTATKDTYDALHMQALPPR	624
	12A2-SR2	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSQSLSLTCSVSGGSVSSdgYYWSWIRQHPGKGLEWIGYIYYRRITDYNPSLKS RVNISLDTSKNQFSLKLSSVTAADTAVYYCARDFGWYFDLWGRGTLVAVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDISNYLTWYQQKPGRVPEVLIY AASALQSGVPSRFSGSGSGTDFTLTISLQPEDVATYYCQNYNSAPRTFGQGTKVEIKSGGGGGSCPYSNPSPSLCSGGGGSCPYSNPSPSLCSGGGGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAFLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGDLGYQGLSTATKDTYDALHMQALPPR	625

Format	Clone Name	Full Amino Sequence	Seq ID NO:
	12C5-SR2	MALPVTALLLPLALLLHAARPEVELVESGGGMVQPGRSLRLSC AASGFTFSDYGMHWVRQAPGMGLEWVTVIWYDGSnKYYADS VKGRFTISRDN SKNTVFLQMNSLRAEDTAVYYCARDEVGfvGA FDIWGQGTMTVTVSSGGGGSGGGGSGGGGSGGGGSDIQLTQSPS FLSASVGDRVIITCRASQGINSHLAWYQQKPGKAPKLLIYYAST LPSGVPSRFSGSGSGTEFTLTVTSLQPEDFATYYCQQLNHYPITF GQGTRLDINGSGGGGSCPYSNPSLCSGGGGSCPYSNPSLCSGGG GSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA CDIYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRP VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNE LQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDA LHMQUALPPR	626
	12C6-SR2	MALPVTALLLPLALLLHAARPEVELVESGGGMVQPGRSLRLSC AASGFTFSDYGMHWVRQAPGMGLEWVTVIWYDGSnKYYADS VKGRFTISRDN SKNTVFLQMNSLRAEDTAVYYCARDEVGfvGA FDIWGQGTMTVTVSSGGGGSGGGGSGGGGSGGGGSDIQLTQSPS FLSASVGDRVIITCRASQGINSHLAWYQQKPGKAPKLLIYYAST LPSGVPSRFSGSGSGTEFTLTVTSLQPEDFATYYCQQLNHYPITF GQGTRLEIKSGGGGSCPYSNPSLCSGGGGSCPYSNPSLCSGGG GSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA CDIYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRP VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNE LQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDA LHMQUALPPR	675
	8F8-SR2	MALPVTALLLPLALLLHAARPQVQLQESGPGLVQPSETLSLTCT VSGGSISYYYWSWIRQPPGKGLEWIGNINYMGN TIYNPSLKS RV TISVDTSKDQFSLKLT SVSAADTAVYYCVRAEGSIDAFDFWGQG TLVAVSLGGGGSGGGGSGGGGSGGGGSDIQMTQSPSTLSASVG DRVITITCRASQSISSWLAWYQQKPGKAPKVL IYKASNLESGVPS RFSGSGSGTEFTLTISSLQPD DFATYYCQQYNSYSCTFGQGTKLE IKSGGGGSCPYSNPSLCSGGGGSCPYSNPSLCSGGGGSTTTPAP RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAP LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQUALPP R	627

Table 11C

Format	Clone Name	Full Amino Sequence	Seq ID NO:
RSRQR (FIG. 6C)	4F11- RSRQR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCSGGGGSGG GGSQVTLKESGPVLVKPTETLTCTVSGFSLSNARMGVTWIRQ PPGKALEWLAHIFSNDEKSYSTSLKSRLTISKDTSKTQVVL TMT NMDPVD TATYYCARIRDYYDISSYYDYWGQGT LVS VSSGGGGG GGGGSGGGGSDIQMTQSPSAMSASVGDRVTITCRASQDISNYLA	628

Format	Clone Name	Full Amino Sequence	Seq ID NO:
		WFQQKPGKVPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISLLP EDFATYYCLQLNSFPFTFGGGTKVEINGSGGGGSCPYSNPSLCSG GGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLCTTTAPRP PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLA GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR	
	4F11-2- RSRQR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCSGGGGSGG GGSQVTLKESGPVLVKPTETLTCTVSGFSLSNARMGVTWIRQ PPGKALEWLAHIFSNDEKSYSTSLKSRLTISKDTSKTQVVLMT NMDPVDATATYYCARIRDYDISSYYDYWGQGLVSVSSGGGGG GGGGSGGGSGGGGSDIQMTQSPSAMSASVGDRVTTICRASQD ISNYLAWFQQKPGKVPKRLIYAASSLQSGVPSRFSGSGSGTEFTL TISLLPEDFATYYCLQLNSFPFTFGGGTKVEINGSGGGGSCPY SNPSLCSGGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLCT TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFEEEEEGGCELRVKFSRSADAPAYQQGQNQLYN ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALH MQALPPR	676
	P08F08- RSRQR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCSGGGGSGG GGSEVQLVQSGAEVKKPGESLKISCKGSGYGFTSYWIGWVRQM PGKGLEWMGIIHPDDSDTKYSPSFQGGVTISADKSISTAYLQWSS LKASDTAMYYCASSYLRGLWGGYFDYWGQGLVTVSSGGGGG GGGGSGGGSGGGGSELQSVLTQPPSASGTPGQRTVISCSSSS NIGSNYVNWYQQLPGTAPKLLIYGDYQRPSGVPDRFSGSKSGTS ASLAISGLRSEDEADYYCATRDDSLSGSVVFGTGTCLTVLGS GGSCPYSNPSLCSGGGGSELPTQGTFSNVSTNVSPAKPTTTACPY SNPSLCTTTAPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFEEEEEGGCELRVKFSRSADAPAYQQG QNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGL YNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDT YDALHMQALPPR	629
	10A1- RSRQR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCSGGGGSGG GGSQVQLQESGPGLVKPSETLSLTCTVSGGSISYYYWTWIRQPP GKGLEWIGHIYYSGSTNYPNPSLKSRTISIDTSKNLFSKLSSVTA ADTAVYYCARAEGSIDAFDFWGQGTMTVTVSSGGGGSGGGGSG GGGSGGGGSDIQMTQSPSTLSASVGDRVTTICRASQSISTWLAW YQQKPGKAPKVLIIYKASSLESGVPSRFSGSGSGTEFILTINSLQPD DFASYCQYKSYSHTFGQGTKEIKSGGGGGSCPYSNPSLCSG GGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLCTTTAPRP PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLA GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR	630
	11C1-	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCSGGGGSGG	631

Format	Clone Name	Full Amino Sequence	Seq ID NO:
	RSRQR	GGSQVQLQESGPGLVKPSETLSLNCTVSGGSISYYYWTWIRQPP GKGLEWIGHVIYSGTTNYPNPSLKSRVTISVDTSKNQFSLKLNST AADTAVYYCVRAEGSIDAFDLWGQGTMTVTVSSGGGGSGGGGS GGGGSGGGGSDIQMTQSPSILSASVGDRVITITCRASQSVSSWLA WYQQKPGKAPKVLIIYKASSLESGVPSRFSGTGSGTEFTLTISSLQ SDDFATYYCQYNTYSHTFGQGTKLEIKGSGGGGSCPYSNPSLC SGGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLCCTTTPAP RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP R	
	11E1- RSRQR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCSGGGGSGG GGSQVQLQESGPGLVKPLQTLSTCTVSGGSISdgyYWSWIRQ NPGKLEWIGYMYSGSTYNNPSLKSRVTISVDTSKNQFSLKLR SVTAADTAVYYCTRDFGWYFDLWGRGTLVTVSSGGGGSGGGG SGGGGSGGGGSDIQMTQSPSSLSASVGDSITITCRASQDIDNYLA WYQQKTGKVPKVLIIAASALQSGVPSRFSGSGSGTDFTLTISSL QPEDVATYYCQNYNSGPRTFGQGTKVEIKGSGGGGSCPYSNPSL CSGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLCCTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP PR	632
	12A2- RSRQR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCSGGGGSGG GGSQVQLQESGPGLVKPSQSLSTCSVSGGSVSSdgyYWSWIRQ HPGKLEWIGYIYYRRITDYNPSLKSRVNISLDTSKNQFSLKLSS VTAADTAVYYCARDGWYFDLWGRGTLVAVSSGGGGSGGGG SGGGGSGGGGSDIQMTQSPSSLSASVGDRVITITCRASQDISNYLT WYQQKPGRPVEVLIYAASALQSGVPSRFSGSGSGTDFTLTISSLQ PEDVATYYCQNYNSAPRTFGQGTKVEIKGSGGGGSCPYSNPSLC SGGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLCCTTTPAP RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP R	633
	12C5- RSRQR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCSGGGGSGG GGSEVELVESGGGMVQPGRSLRLSCAASGFTFSDYGMHWVRQ APGMGLEWVTVIWYDGSnKYYADSVKGRFTISRDNSTNTVFLQ MNSLRAEDTAVYYCARDEVGFvGAFDIWGQGTMTVTVSSGGGG SGGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVITITCRASQGI NSHLAWYQQKPGKAPKLLIYYASTLPSGVPSRFSGSGSGTEFTL TVTSLQPEDFATYYCQQLNHYPITFGQGTRLDINGSGGGGSCPY SNPSLCSGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLC TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	634

Format	Clone Name	Full Amino Sequence	Seq ID NO:
		IYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYN ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRRGKGHGGLYQGLSTATKDTYDALH MQALPPR	
	12C6- RSRQR	MALPVTALLLPLALLLHAARPGGGGGSCPYSNPSLCSGGGGSGG GGSEVELVESGGGMVQPGRSLRLSCAASGFTFSDYGMHWVRQ APGMGLEWVTVIWYDGS _n KYYADSVKGRFTISRDNKNTVFLQ MNSLRAEDTAVYYCARDEVGF _v GAFDIWGQGTMTVTVSSGGGG SGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVIITCRASQGI NSHLAWYQQKPGKAPKLLIYYASTLPSGVPSRFSGSGSGTEFTL TVTSLQPEDFATYYCQQLNHYPITFGQGTRLEIKSGGGGSCPY SNPSLCSGGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLC TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD IYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYN ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRRGKGHGGLYQGLSTATKDTYDALH MQALPPR	677
	8F8- RSRQR	MALPVTALLLPLALLLHAARPGGGGGSCPYSNPSLCSGGGGSGG GGSQVQLQESGPGVLVQPSETLSLTCTVSGGSISYYYWSWIRQPP GKGLEWIGNINYMNTIYNPSLKSRTISVDTSKDQFSLKLTSSVS AADTAVYYCVRAEGSIDAFDFWGQGLVAVSLGGGGSGGGGS GGGGSGGGGSDIQMTQSPSTLSASVGDRVITICRASQSISSWLA WYQQKPGKAPKVLIIYKASNLESGVPSRFSGSGSGTEFTLTISLQ PDDFATYYCQQYNSYSCTFGQGTKLEIKSGGGGSCPYSNPSLC SGGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLCCTTTPAP RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRRGKGHGGLYQGLSTATKDTYDALHMQALPP R	635

Table 11D

Format	Clone Name	Full Amino Sequence	Seq ID NO:
RSR (FIG. 6D)	4F11-RSR	MALPVTALLLPLALLLHAARPGGGGGSCPYSNPSLCGGGGSQVT LKESGPVLVKPTETLTCTVSGFSLSNARMGVTVIRQPPGKAL EWLAHIFSNDEKSYSTSLKSRLTISKDTSKTQVVLMTNMDPVD TATYYCARIRDYYDISSYYDYWGQGLVSVSSGGGGSGGGGSG GGGSDIQMTQSPSAMSASVGDRVITICRASQDISNYLAWFQQKP GKVPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISLLPEDFATY YCLQLNSFPFTFGGGTKVEINGGGGSCPYSNPSLCGGGGSTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELN LGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRRGKGHGGLYQGLSTATKDTYDALHMQAL	636

Format	Clone Name	Full Amino Sequence	Seq ID NO:
		PPR	
	4F11-2-RSR	MALPVTALLLPLALLLHAARPGGGGGSCPYSNPSLCGGGGGSQVT LKESGPVLVKPTETLTLTCTVSGFSLSNARMGVTVIRQPPGKAL EWLAHIFSNDEKSYSTSLKSRLTISKDTSKTQVVLTMNMDPVD TATYYCARIRDYYDISSYYDYWGQGTLSVSSGGGGSGGGGSG GGGSGGGGSDIQMTQSPSAMSASVGDRTITCRASQDISNYLA WFQQKPGKVPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISLLP EDFATYYCLQLNSFPFTFGGGTKVEINGGGGSCPYSNPSLCGGG GSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA CDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDAL HMQALPPR	678
	P08F08-RSR	MALPVTALLLPLALLLHAARPGGGGGSCPYSNPSLCGGGGSEVQ LVQSGAEVKKPGESLKISCKGSGYGFTSYWIGWVRQMPGKGLE WMGIIHPDDSDTKYSPSFQGGQVTISADKSISTAYLQWSSLKASDT AMYYCASSYLRGLWGGYFDYWGQGTLSVSSGGGGSGGGGS GGGGSGGGGSELQSVLTQPPSASGTPGQRTISCSGSSSNIGSNY VNWYQQLPGTAPKLLIYG DYQRPSGVPDRFSGSKSGTSASLAIS GLRSEDEADYYCATRDDSLSGSVVFGTGKLTVLGGGGGSCPY SNPSLCGGGGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIF KQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTAT KDTYDALHMQALPPR	637
	10A1-RSR	MALPVTALLLPLALLLHAARPGGGGGSCPYSNPSLCGGGGSQVQ LQESGPGLVKPSETLSLTCTVSGGSISYYYWTWIRQPPGKGLEWI GHIYYSSTNYNPSLKSRVTISIDTSKNLFSKLSSVTAADTAVY YCARAEGSIDAFDFWGQGTMTVTVSSGGGGSGGGGSGGGGSGG GGSDIQMTQSPSTLSASVGDRTITCRASQSISTWLAWYQQKPG KAPKVLIIYKASSLESGVPSRFSGSGSGTEFILTINSLQPDFAFY CQQYKSYSHTFGQGTKLEIKGGGGSCPYSNPSLCGGGGSTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALP PR	638
	11C1-RSR	MALPVTALLLPLALLLHAARPGGGGGSCPYSNPSLCGGGGSQVQ LQESGPGLVKPSETLSLNCTVSGGSISYYYWTWIRQPPGKGLEW IGHVIYSGTTNYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAV YYCVRAEGSIDAFDLWGQGTMTVTVSSGGGGSGGGGSGGGGSG GGGSDIQMTQSPSILSASVGDRTITCRASQSVSSWLAWYQQKP GKAPKVLIIYKASSLESGVPSRFSGTGSGTEFTLTISLQSDDFATY YCQQYNTYSHTFGQGTKLEIKGGGGSCPYSNPSLCGGGGSTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELN	639

Format	Clone Name	Full Amino Sequence	Seq ID NO:
		LGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL PPR	
	11E1-RSR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGGSQVQ LQESGPGLVKPLQTLSTCTVSGGSISSdgYYWSWIRQNPBGKLE WIGYMYYSGSTYYNPSLKSRTISVDTSKNQFSLKLSVTAADT AVYYCTRDFGWYFDLWGRGTLTVSSGGGGSGGGGSGGGGSG GGGSDIQMTQSPSSLSASVGDSITITCRASQDIDNYLAWYQQKT GKVPKVLIIAASALQSGVPSRFSGSGSGTDFTLTISLQPEDVAT YYCQNYNSGPRTFGQGTKVEIKGGGGSCPYSNPSLCGGGGSTTT PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQ EEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR	640
	12A2-RSR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGGSQVQ LQESGPGLVKPSQSLSTCSVSGGSVSSdgYYWSWIRQHPGKGL EWIGYIYYRRITDYNPSLKSRTNISLDTSKNQFSLKLSVTAADT AVYYCARDFGWYFDLWGRGTLVAVSSGGGGSGGGGSGGGGSG GGGSDIQMTQSPSSLSASVGDRVTITCRASQDISNYLTWYQQK PGRVPEVLIYAASALQSGVPSRFSGSGSGTDFTLTISLQPEDVAT YYCQNYNSAPRTFGQGTKVEIKGGGGSCPYSNPSLCGGGGSTTT PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQ EEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR	641
	12C5-RSR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGSEVEL VESGGGMVQPGRSLRLSCAASGFTFSDYGMHWVRQAPGMGLE WVTVIWYDGSNKYYADSVKGRFTISRDN SKNTVFLQMNSLRAE DTAVYYCARDEVGFVGAFDIWGQGTMTVTVSSGGGGSGGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVIITCRASQGINSHLAWY QQKPGKAPKLLIYYASTLPSGVPSRFSGSGSGTEFTLTVTSLQPE DFATYYCQQLNHYPITFGQGTSLDINGGGGSCPYSNPSLCGGGG STTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR	642
	12C6-RSR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGSEVEL VESGGGMVQPGRSLRLSCAASGFTFSDYGMHWVRQAPGMGLE WVTVIWYDGSNKYYADSVKGRFTISRDN SKNTVFLQMNSLRAE DTAVYYCARDEVGFVGAFDIWGQGTMTVTVSSGGGGSGGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVIITCRASQGINSHLAWY QQKPGKAPKLLIYYASTLPSGVPSRFSGSGSGTEFTLTVTSLQPE DFATYYCQQLNHYPITFGQGTSLLEIKGGGGSCPYSNPSLCGGGG STTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC	679

Format	Clone Name	Full Amino Sequence	Seq ID NO:
		DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR	
	8F8-RSR	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGGSQVQLQESGPGLVQPSETLSLTCTVSGGSISYYYWSWIRQPPGKGLEWIGNINYMGN TIYNPSLKSRTVISVDTSKDQFSLKLTSVSAADTAVYYCVRAEGSIDAFDFWGQGLTAVSLGGGGSGGGGSGGGGSGGGSDIQMTQSPSTLSASVGDRVITITCRASQSISSWLAWYQQKPGKAPKVLIIYKASNLESGVPSRFSGSGSGTEFTLTISLQPDFFATYYCQQYNSYSCTFGQGTKLEIKGGGGSCPYSNPSLCGGGGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR	643

Table 11E

Format	Clone Name	Full Amino Sequence	Seq ID NO:
RSR-short (FIG. 6E)	4F11-RSR-short	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGGSQVTLKESGPVLVKPTETLTCTVSGFSLSNARMGV TWIRQPPGKALEWLAHIFSNDEKSYSTSLKSRLTISKDTSKTQVVLTMNMDPVD TATYYCARIRDYDISSYYDYWGQGLT VSVSSGGGGSGGGGSGGGSDIQMTQSPSAMSASVGDRVITITCRASQDISNYLAWFQQKPGKVPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISLLPEDFATY YCLQLNSFPFTFGGGTKVEINGGGGSCPYSNPSLC TTTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR	644
	4F11-2-RSR-short	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGGSQVTLKESGPVLVKPTETLTCTVSGFSLSNARMGV TWIRQPPGKALEWLAHIFSNDEKSYSTSLKSRLTISKDTSKTQVVLTMNMDPVD TATYYCARIRDYDISSYYDYWGQGLT VSVSSGGGGSGGGGSGGGSGGGSDIQMTQSPSAMSASVGDRVITITCRASQDISNYLAWFQQKPGKVPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISLLPEDFATY YCLQLNSFPFTFGGGTKVEINGGGGSCPYSNPSLC TTTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR	680

P08F08- RSR-short	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGSEVQ LVQSGAEVKKPGESLKISCKSGYGFTSYWIGWVRQMPGKGLE WMGIIHPDDSDTKYSPSFQGGQVTISADKSISTAYLQWSSLKASDT AMYYCASSYLRLWGGYFDYWGGQTLTVSSGGGGSGGGGS GGGGSGGGGSELQSVLTQPPSASGTPGQRTVISCSSSSNIGSNY VNWYQQLPGTAPKLLIYG DYQRPSGVPDRFSGSKSGTSASLAIS GLRSEDEADYYCATRDDSLSGSVVFGTGTKLTVLGGGGSCPY NPSLCTTTTPAPRPPTAPTASQPLSLRPEACRPAAGGAVHTRGL DFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQG QNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG LYNELQKDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATKDT YDALHMQALPPR	645
10A1- RSR-short	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGSQVQ LQESGPGLVKPSETLSLTCTVSGGSISYYYWTWIRQPPGKGLEWI GHIYSGSTNYNPSLKSRVTISIDTSKNLFSKLSSVTAADTAVY YCARAEGSIDAFDFWGGQTMVTVSSGGGGSGGGGGSGGGSGG GGSDIQMTQSPSTLSASVGDRVTITCRASQSISTWLAWYQQKPG KAPKVLIIYKASSLESGVPSRFSGSGSGTEFILTINSLQPDFA SYCQQYKSYSHTFGQGTKLEIKGGGGSCPYSNPSLCTTTTPAPRP PTAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG TCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR	646
11C1- RSR-short	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGSQVQ LQESGPGLVKPSETLSLNCTVSGGSISYYYWTWIRQPPGKGLEW IGHVIYSGTTNYNPSLKSRVTISVDTSKNQFSLKLNSVTAADTAV YYCVRAEGSIDAFDLWGQGTMTVTVSSGGGGSGGGGGSGGGSG GGSDIQMTQSPSILSASVGDRVTITCRASQSVSSWLAWYQQKP GKAPKVLIIYKASSLESGVPSRFSGTGSGTEFTLTISLQSDDFATY YCQQYNTYSHTFGQGTKLEIKGGGGSCPYSNPSLCTTTTPAPRP PTAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG TCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR	647
11E1- RSR-short	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGSQVQ LQESGPGLVKPLQTLTLCTVSGGSISSDGYYSWIRQNP GKGL EWIGYMYSGSTYNYNPSLKSRVTISVDTSKNQFSLKLRSVTAAD TAVYYCTRDFGWYFDLWGRGTLTVTVSSGGGGSGGGGGSGGGGS GGGGSDIQMTQSPSSLSASVGDSITITCRASQDIDNYLAWYQQK TGKVPKVLIIY AASALQSGVPSRFSGSGSGTDFTLTISLQPEDVA TYYCQNYNSGPRTFGQGTKVEIKGGGGSCPYSNPSLCTTTTPAPR PPTAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDG CSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA YSEIGMKGERRRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR	648

12A2- RSR-short	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGSQVQ LQESGPGLVKPSQSLSLTCSVSGGSVSSDGYYSWIRQHPGKGL EWIGYIYYRRITDYNPSLKS RVNISLDTSKNQFSLKLSSVTAADT AVYYCARDFGWYFDLWGRGTLVAVSSGGGGSGGGGSGGGGS GGGSDIQMTQSPSSLSASVGDRVTITCRASQDISNYLTWYQQK PGRVPEVLIYAASALQSGVPSRFSGSGSGTDFTLTISSLQPEDVAT YYCQNYNSAPRTFGQGTKVEIKGGGGSCPYSNPSLCCTTPAPRP PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLA GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	649
12C5- RSR-short	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGSEVEL VESGGGMVQPGRSLRLSCAASGFTFSDYGMHWVRQAPGMGLE WVTVIWYDGSNKYYADSVKGRFTISRDN SKNTVFLQMNSLRAE DTAVYYCARDEVGFVGAFDIWGQGTMTVTVSSGGGGSGGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVIITCRASQGINSHLAWY QQKPGKAPKLLIYYASTLPSGVPSRFSGSGSGTEFTLTVTSLQPE DFATYYCQQLNHYPITFGQGTRLDINGGGGSCPYSNPSLCCTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYW APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELN LGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL PPR	650
12C6- RSR-short	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGSEVEL VESGGGMVQPGRSLRLSCAASGFTFSDYGMHWVRQAPGMGLE WVTVIWYDGSNKYYADSVKGRFTISRDN SKNTVFLQMNSLRAE DTAVYYCARDEVGFVGAFDIWGQGTMTVTVSSGGGGSGGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVIITCRASQGINSHLAWY QQKPGKAPKLLIYYASTLPSGVPSRFSGSGSGTEFTLTVTSLQPE DFATYYCQQLNHYPITFGQGTRLEIKGGGGSCPYSNPSLCCTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELN GRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP PR	681
8F8-RSR- short	MALPVTALLLPLALLLHAARPGGGGSCPYSNPSLCGGGGSQVQ LQESGPGLVQPSETLSLTCTVSGGSISYYYWSWIRQPPGKGLEWI GNINYMGN TIYNPSLKS RVTISVDTSKDQFSLKLTSVSAADTAV YYCVRAEGSIDAFDFWGQGTLVAVSLGGGGSGGGGSGGGGSG GGGSDIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKP GKAPKVLIIYKASNLES GVPSRFSGSGSGTEFTLTISSLQPD FAT YYCQQYNSYSCTFGQGTKLEIKGGGGSCPYSNPSLCCTTPAPRP PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLA GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	651

[0401] Testing was performed on: NTD control; the RSRQR format (shown above and schematically in FIG. 6C) of a 4F11 CAR; the SR2 format (shown above and schematically in FIG. 6B) of a P08F08 CAR; the R2S format (shown above and schematically in FIG. 6F) of a P08F08 CAR; and the RSR-short format (shown above and schematically in FIG. 6E) of a P08F08 CAR.

[0402] CAR T cells were generated that express each of the CAR formats. These CAR T cells were then thawed and mixed with 786-0 target cells that are known to express CD70 at an effector:target (E:T) ratio of 3:1, 1:1, 1:3, or 1:9 (or 0 control) in RPMI supplemented with 10% FBS. Percentage lysis of target cells and fold expansion of effector CAR T cells were determined (FIG. 7).

[0403] Percentage lysis of target cells was calculated at each time-point by calculating the percentage of live 786-0 cells for each CAR treatment and then normalizing to no treatment control. Fold expansion of CAR T cells over the number of CAR T cells plated on Day 0 was calculated at each time-point.

[0404] These results demonstrate that CD70-specific CAR-T cells expressing CD20 epitopes can kill target cells effectively at 3:1, 1:1, 1:3, and 1:9 E:T ratios, and ratios therebetween, as well as potentially at ratios outside 3:1 to 1:9 range not test in this experiment.

Part B: Sensitivity to Rituximab *In Vivo*

[0405] Depletion of CD70-specific CAR T cells following rituximab administration allows recovery of CD70-expressing lymphocytes in NSG mice

[0406] The ability of the anti-CD20 antibody rituximab to mediate depletion of CD70-specific CAR T cells expressing CD20 epitopes and facilitate lymphocytes recovery is tested in mice. In this experiment, mice are treated with T cells expressing either a CD70-specific CAR that can bind to mouse CD70 protein on the surface of lymphocytes (α -mouse CD70 CAR Ts) and comprising a CD20 epitope recognized by rituximab or a control CAR, with negligible binding to mouse FLT3 protein. Flow cytometry analysis of lymphocytes is used to demonstrate CAR T cell cytotoxic activity against CD70-expressing lymphocytes, seen as a reduction of lymphocytes compared to mice that received control CAR T cells or to untreated mice. After confirmation of CAR T cell killing activity, mice are given rituximab for four consecutive days and circulating residual CAR T cells are enumerated by flow cytometry at day 13. The CD70-specific CAR T cells in the blood of these mice are depleted compared to the control group, which do not receive

rituximab. Finally, flow cytometry analysis of lymphocytes demonstrates that only the mice that received rituximab (day 20) shows lymphocytes recovery.

[0407] This experiment demonstrates that rituximab-dependent depletion of CD70-specific CAR T cells that express CD20 epitopes mitigates damage to CD70-expressing tissues, allowing for rapid lymphocyte recovery.

Example 10: Activity of CD70-specific CAR T cells

[0408] Target cell killing was assessed using the same assay and similar experimental parameters to those described in Example 9, Part A. The tested cells lines were 786-0, ACHN, and REH (a human acute lymphocytic leukemia cell line). REH cell line shows the best differentiation of CD70-specific CAR T cells and hence will be used to rank scFvs in all future experiments. FIGs. 8A-8D show cell killing of 786-0 (FIG. 8A), ACHN (FIG. 8B), or REH (FIG. 8C) cells using CD70-specific CAR T cells where the CAR extracellular domain comprises the scFvs indicated in the legend (FIG. 8D). The naked CAR format was used for all experiments.

[0409] Table 12 provides the underlying data for FIGs. 8A-8D and additionally the measured percentage positive cells for fluorescently labeled anti-CD70 antibody, CD25 and 4-1BB (activation markers); the stem memory T cell (TSCM) percentage, the BFP percentage (CAR+) before going into the cytotoxicity assay.

Table 12

Clone	%Lysis REH	%Lysis ACHN	%Lysis 786-O	%CD70 D9 (gated on CAR)	%CD25 4-1BB D9 (gated on CAR)	%Tscm D14 (gated on CAR)	%BFP CAR D14
12D6	98.5	75.4	84.6	0.7	15.1	17.2	77.4
8F8	98.1	84.7	94.8	0.0	26.1	28.9	70.5
12H4	97.7	65.8	80.5	0.1	16.9	31.5	83.8
12A2	97.5	76.1	89.3	3.9	6.2	46.5	73.2
12C5	97.5	81.7	91.6	0.0	31.1	24.7	88.2
10A1	97.2	77.0	88.3	0.1	21.5	31.4	73.5
11E1	96.8	84.7	94.5	0.2	34.8	20.4	54.8
12D3	96.0	63.8	75.4	7.5	14.2	35.0	71.0
4F11	96.0	73.4	85.6	4.3	44.0	15.7	36.3
12D7	95.7	84.9	92.7	1.2	48.5	5.4	43.1
11C1	95.6	76.9	90.0	0.5	31.8	30.3	57.9
11A1	73.5	46.0	64.6	2.4	7.0	41.6	82.5
9E10	71.7	77.1	90.0	68.2	21.7	25.2	63.9
8C8	70.7	80.7	90.2	76.6	12.9	26.3	57.6
10E2	59.2	41.5	36.9	52.0	6.4	28.1	34.8

P08F08	45.8	78.0	94.5	30.6	7.8	48.4	43.1
8F7	38.6	39.3	55.4	46.4	3.0	32.3	63.5
12F5	21.7	14.5	16.2	50.9	7.1	29.9	44.7
9E5	13.9	7.0	11.9	13.1	1.3	77.0	63.9
11D1	12.7	-4.4	7.3	32.3	2.0	50.4	58.5
9F8	12.5	8.9	32.6	20.5	1.3	59.4	67.5
9F4	10.4	1.1	8.4	20.8	1.6	63.8	35.7
9D8	5.2	24.6	76.2	29.3	4.6	46.8	70.8
NTD	3.3	7.6	12.0				

[0410] FIG. 9A shows the efficacy of CD70-specific CARs upon repeated exposure to luciferase-labeled 786-O target cells (CAR T cells were transferred to a 96-well plate containing fresh targets every 2-3 days). The E:T ratio was 3:1. The CARs were expressed in cells from donor D503. Similar to the results described in Example 4, target cell lysis in the stress test were used for *in vitro* characterization of CAR scFvs.

[0411] FIG. 9B shows the efficacy of CD70-specific CARs upon repeated exposure to luciferase-labeled ACHN target cells (CAR T cells were transferred to a 96-well plate containing fresh targets every 2-3 days). The E:T ratio was 10:1. The CARs were expressed in cells from donor D503. Similar to the results described in Example 4, target cell lysis in the stress test were used for *in vitro* characterization of CAR scFvs.

[0412] FIG. 9C shows the efficacy of CD70-specific CARs upon repeated exposure to luciferase-labeled REH target cells (2×10^6 cells added at indicated time-points). The E:T ratio was 1:5. The CARs were expressed in cells from donor D503. Similar to the results described in Example 4, target cell lysis in the stress test were used for *in vitro* characterization of CAR scFvs.

[0413] FIG. 10 shows the efficacy of CD70-specific CARs in various formats (as mentioned in example 9 Part A) upon repeated exposure to luciferase-labeled REH target cells (2×10^6 cells added at indicated time-points). The E:T ratio was 1:5. Similar to the results described in Example 4, target cell lysis in the stress test were used for *in vitro* characterization of CAR scFvs.

Example 11: *In vivo* comparison of CD70 CARs in ACHN metastasis model

[0414] CAR T cells containing different CD70 scFvs were generated and tested in ACHN cells, a renal cell cancer (RCC)-derived cell line. ACHN cells are described in Simmons et al. Animal Models of Bone Metastasis. *Veterinary Pathology* 52:827-841, 834 (2015). NSG mice

were each implanted with 1 million ACHN tumor cells intravenously and 15 days post-tumor cell injection, the mice were treated with CAR T cells intravenously via tail vein injection to determine the CAR and condition with most optimal efficacy. 12C5 CAR T cells dosed at 3 million CAR+ cells per mouse showed the best efficacy.

Materials and methods:

[0415] Forty-five NSG mice were prepared for intravenous tumor injection via tail vein. ACHN tumor cells that are known to express CD70 were expanded in MEM supplemented with 10% FBS. On Day 0, ACHN tumor cells were resuspended in serum-free MEM at the required concentration to inject 1 million cells per animal. ACHN tumor cells were injected in 200uL of serum-free MEM intravenously. Day 4 baseline body weights were recorded for all animals. Tumor flux was measured twice a week starting on Day 7 using bioluminescence (IVIS Spectrum Imager™ from PerkinElmer™; auto-exposure with a maximum exposure time of 120 seconds), and body weights were recorded. On Day 20, when the tumors attained 200mm³ (standard error 9.69), 35 tumor-bearing mice were randomized to 7 groups of 5 mice each. On Day 15, CAR T cells were thawed in MEM supplemented with 10% FBS and resuspended in serum-free MEM at 3 million CAR+ T cells per animal (calculated based on individual transduction efficiencies). Number of NTD cells required to maintain equal percentage of CAR+ T cells as well as equal number of total T cells in each group were calculated and added to respective samples. CAR T cells or NTD control were injected in 200uL of serum-free MEM per animal intravenously via tail vein. Tumor flux was measured and body weights recorded twice a week till Day 32 when the NTD group reached the study end-point ($\geq$ 20% body weight loss) (See FIG. 11).

[0416] Bioluminescence (mean and error SEM) were plotted on GraphPad Prism and statistics were calculated using one-way ANOVA with repeated measures. 12C5 CAR T group showed anti-tumor efficacy at the 3 million CAR+ dose.

Example 12: Expression levels of CD70 on patient-derived RCC samples and lysis of patient-derived RCC samples by CD70-specific CAR T cells

[0417] Primary RCC patient samples were obtained from Conversant Bio (frozen dissociated tumor cells) or from CHTN Western/NDRI (fresh tumor fragments which were then dissociated in house using Miltenyi MACS human tumor dissociation kit and GentleMACS). Primary tumor cells were maintained in RPMI supplemented with 20% FBS. To determine cell surface expression of CD70 protein in these cells along with relevant RCC cell lines, we performed flow

cytometry and receptor quantification using anti-CD70 antibody conjugated to Phycoerythrin at a 1:1 ratio. Cell surface receptors were calculated using Quantibrite beads from BD Biosciences and antibody binding capacity (ABC) values were calculated as per manufacturer's recommendation. CD70-specific CAR T cells were generated as previously described and their cytotoxicity against primary RCC cells and ACHN RCC cell line was assessed using the same assay and similar experimental parameters to those described in Example 9, Part A.

Results

[0418] It was confirmed that primary RCC cells express CD70 across ABC values ranging from ~2,000 CD70 receptors per cell (receptors/cell) to ~25,000 receptors/cell (FIG. 12B) with expression on RCC cell lines ranging from ~25,000 receptors/cell to ~400,000 receptors/cell (FIG. 12A). We also confirmed that cells expressing 7,000 CD70 receptors per cell (receptors/cell) (FIG. 13B), 24,000 receptors/cell (FIG. 13A), or 40,000 receptors/cell (FIG. 13C) are effectively killed by CD70-specific CAR T cells, where the CAR is generated from either the 4F11 or P08F08 scFv.

Example 13: Expression levels of CD70 on various hematological tumor cell lines and lysis of these cells by CD70-specific CAR T cells

[0419] The potential to target CD70 across a range of heme tumors including lymphomas, leukemias, and myeloma was characterized. Characterization included expression analysis of both CD70 RNA and cell surface protein in multiple malignancies, followed by efficacy of CAR T cells against cell lines.

Results

[0420] Analysis of RNA expression across acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), non-Hodkins lymphoma (NHL), and multiple myeloma (MM cell) lines using The Cancer Genome Atlas (TCGA) shows that CD70 expression can be observed across all 4 cancer types indicating the potential utility of targeting these cancers with CD70 CAR T cells. To determine cell surface expression of CD70 protein in these cancers, we performed flow cytometry and receptor quantification on a panel of cell lines originating from the selected tumor types. Cell surface protein expression patterns were similar to RNA analyses confirming that CD70 expression was broadly observed in cell lines from all tumor types (FIG. 14A). Next we generated CD70-specific CAR T cells to test their efficacy in an *in vitro*

cytotoxicity assay against the same cell lines. CD70 CAR T cells exhibited robust specific activity against target cells expressing the CD70 antigen (FIG. 14B). Various cell lines can be killed by a CD70-specific CAR (FIG. 14B), indicating that CD70-specific CARs can kill even cells that expression CD70 at a low level. This demonstrates that the activity of the CD70-specific CARs is not restricted to particular cell types. Finally, we showed that these CARs are effective against the MM1S cell line in the *in vivo* assay performed as in Example 7 (FIGs. 15A and 15B). MM1S is a multiple-myeloma cell line expressing a moderate number of CD70 receptors per cell (FIG. 14A). In conclusion, it was observed that CD70 has a broad expression profile across a range of hematological malignancies. Using CD70 CAR T cells either alone or in combination with the other heme targets offers opportunity to target or prevent tumor antigen escape in a wide range of hematologic malignancies.

Example 14: Determination of kinetics and affinity of human CD70/CD70 antibodies interactions at 37°C

[0421] This example determines the binding kinetics and/or affinity of various anti-CD70 antibodies toward human CD70. ScFvs were generated by cloning the variable regions of the anti-CD70 antibodies flanking a (GGGGS)₄ linker (SEQ ID NO: 602) then part of the hinge and Fc from a modified human IgG2 sequence resulting in a scFv-Fc fusion which was expressed using Expi293 then purified by Protein A affinity chromatography. Recombinant human CD70 was generated by fusing an AviTag™, a polyhistidine tag and a trimerization domain from chicken tenascin to the N-terminus of the human CD70 extracellular domain (ECD) which was expressed using Expi293 then purified by immobilized metal affinity chromatography (IMAC) followed by size exclusion chromatography (SEC) as needed.

[0422] The antibody binding kinetics were determined by surface plasmon resonance (Biacore 8K, GE Healthcare Bio-Sciences, Pittsburg PA) at 37°C in HBS-T+ (0.01 M HEPES pH 7.4, 0.15 M NaCl, 0.05% v/v Tween20, 1 mg/mL BSA). Recombinant human CD70 diluted in HBS-T+ was captured on a C1 chip immobilized with an anti-AviTag™ antibody. Purified anti-CD70 scFv-Fc fusions were serially diluted into HBS-T+, injected at 30 uL/min for 2-4 min, dissociation monitored for 10 min then the surface regenerated with 75 mM phosphoric acid between injections. Buffer cycles were collected for each anti-CD70 scFv-Fc fusion for double-referencing purposes (double-referencing as described in Myszk, D.G. Improving biosensor analysis. *J. Mol. Recognit.* **12**, 279-284 (1999)). Kinetic association rates (k_{on}) and dissociation

rates (k_{off}) were obtained simultaneously by fitting the double-referenced sensorgrams globally to a 1:1 Langmuir with mass transport model using Biacore 8K Evaluation Software (GE Healthcare Bio-Sciences, Pittsburg PA) then used to calculate an equilibrium dissociation constant (K_D) from the kinetic rate constants ($K_D = k_{\text{off}}/k_{\text{on}}$). The data was fit to a 1:1 steady state affinity model using Biacore 8K Evaluation Software to determine a steady state equilibrium dissociation constant (SS K_D) as needed.

[0423] The binding kinetics and affinity parameters for the tested anti-CD70 antibodies are shown in Table 13. The antibodies shown in Table 13 share the same scFv sequence as the CARs shown in Tables 5 and 11 having the same name.

Table 13

	scFv format				
	k_a (1/Ms)	k_d (1/s)	$t_{1/2}$ (min)	K_D (nM)	SS K_D (nM)
10A1	5.77E+05	7.73E-04	14.9	1.3	
10H10	4.62E+04	3.20E-03	3.6	69.3	
11C1	2.24E+05	1.86E-04	62.2	0.8	
11E1	5.78E+05	1.00E-03	11.6	1.7	
12A2	-	-	-	Ambiguous	
12C4	1.56E+06	4.55E-04	25.3	0.3	
12D6	7.12E+04	1.94E-03	5.9	27.3	
12D7	4.41E+05	7.55E-04	15.3	1.7	
17G6	-	-	-	Weak binding	
4F11	8.25E+05	1.60E-03	7.2	1.9	
8C8	1.03E+05	1.59E-03	7.3	15.4	
8F8	3.48E+05	7.93E-04	14.6	2.3	
9E10	8.25E+05	2.12E-03	5.4	2.6	
P02B10	-	-	-		355
P07D03	-	-	-		1070
P08A02	-	-	-		1200
P08E02	8.23E+05	4.38E-03	2.6	5.3	
P08F08	1.44E+06	1.53E-02	0.8	10.7	
P08G02	2.39E+04	7.88E-03	1.5	330	
P12B09	3.30E+05	1.35E-03	8.6	4.1	
P12F02	2.75E+05	1.55E-03	7.4	5.7	

P12G07	-	-	-	Weak binding	
P13F04	9.77E+04	2.00E-02	0.6	205	
P15D02	2.86E+05	<8.05E-	>136	<0.3	
P16C05	-	-	-	Weak binding	

[0424] Although the disclosed teachings have been described with reference to various applications, methods, kits, and compositions, it will be appreciated that various changes and modifications can be made without departing from the teachings herein and the claimed invention below. The foregoing examples are provided to better illustrate the disclosed teachings and are not intended to limit the scope of the teachings presented herein. While the present teachings have been described in terms of these exemplary embodiments, the skilled artisan will readily understand that numerous variations and modifications of these exemplary embodiments are possible without undue experimentation. All such variations and modifications are within the scope of the current teachings.

[0425] All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

[0426] The foregoing description and Examples detail certain specific embodiments of the disclosure and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

CLAIMS

1. An isolated antibody, which specifically binds to Cluster of Differentiation 70 (CD70), wherein the antibody comprises:

- (a) a heavy chain variable (VH) region comprising three CDRs from the VH region shown in SEQ ID NO: 18; and a light chain variable (VL) region comprising three CDRs from the VL region shown in SEQ ID NO: 17; wherein 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; or
- (b) a VH region comprising three CDRs from the VH region shown in SEQ ID NO: 371; and a VL region comprising three CDRs from the VL region shown in SEQ ID NO: 370; wherein 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; or
- (c) a VH region comprising three CDRs from the VH region shown in SEQ ID NO: 339; and a VL region comprising three CDRs from the VL region shown in SEQ ID NO: 338; wherein 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; or
- (d) a VH region comprising three CDRs from the VH region shown in SEQ ID NO: 345; and a VL region comprising three CDRs from the VL region shown in SEQ ID NO: 344; wherein 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; or
- (e) a VH region comprising three CDRs from the VH region shown in SEQ ID NO: 349; and a VL region comprising three CDRs from the VL region shown in SEQ ID NO: 348; wherein 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; or
- (f) a VH region comprising three CDRs from the VH region shown in SEQ ID NO: 359; and a VL region comprising three CDRs from the VL region shown in SEQ ID NO: 358;

wherein 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; or

(g) a VH region comprising three CDRs from the VH region shown in SEQ ID NO: 361; and a VL region comprising three CDRs from the VL region shown in SEQ ID NO: 360; wherein 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.

2. The isolated antibody of claim 1, wherein the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 97, 98, or 99; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 100 or 101; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 102; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 217; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 218; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 219.

3. The isolated antibody of claim 2, wherein the VH region comprises the amino acid sequence shown in SEQ ID NO: 18 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 17.

4. The isolated antibody of claim 1, wherein the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 478, 479 or 480; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 481 or 482; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 483; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 562; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 563; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 564.

5. The isolated antibody of claim 4, wherein the VH region comprises the amino acid sequence shown in SEQ ID NO: 371 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 370.

6. An isolated antibody, which specifically binds to Cluster of Differentiation 70 (CD70), wherein the antibody comprises a VH region comprising three CDRs CDRH1, CDRH2, CDRH3, and a VL region comprising three CDRs CDRL1, CDRL2, CDRL3, wherein:

a) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 97, 98 or 99, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 100 or 101, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 102, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 217, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 218, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 219; or

b) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 478, 479 or 480, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 481 or 482, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 483, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 562, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 563, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 564; or

c) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 382, 383 or 384, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 385 or 386, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 387, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 514, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 515, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 516; or

d) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 400, 401 or 402, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 403 or 404, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 405, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 523, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 524, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 525; or

e) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 412, 413 or 414, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 415 or 416, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 417, the CDRL1 comprises the amino acid

sequence of SEQ ID NO: 529, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 530, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 531; or

f) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 442, 443 or 444, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 445 or 446, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 447, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 544, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 545, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 546; or

g) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 448, 449 or 450, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 451 or 452, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 453, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 547, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 548, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 549.

7. The isolated antibody of claim 6, wherein the amino acid sequences for the VL and VH regions, respectively, are selected from one of SEQ IDs:

- a) 17 and 18;
- b) 370 and 371;
- c) 338 and 339
- d) 344 and 345;
- e) 348 and 349;
- f) 358 and 359; and
- g) 360 and 361.

8. The isolated antibody of any one of claims 1-7, which is a bispecific antibody, or a single chain Fv fragment.

9. The isolated antibody of any one of claims 1-8, which is a bispecific antibody.

10. A polynucleotide comprising a nucleic acid sequence encoding the isolated antibody of any one of claims 1-9.

11. An expression vector comprising the polynucleotide of claim 10.
12. The isolated antibody of any one of claims 1-9 for use as a medicament.
13. Use of the isolated antibody of any one of claims 1-9, in the manufacture of a medicament for the treatment of a cancer.
14. The use of claim 13, wherein, the cancer is selected from the group consisting of Renal Cell Carcinoma, Clear Cell Renal Cell Carcinoma, Glioblastoma, glioma, Non-Hodgkin's Lymphoma (NHL), Hodgkin's Disease (HD), Waldenstrom's macroglobulinemia, Acute Myeloid Leukemia, Multiple Myeloma, diffuse large-cell lymphoma, follicular lymphoma and Non-Small Cell Lung Cancer.
15. A pharmaceutical composition comprising the isolated antibody of any one of claims 1-9.
16. A host cell comprising the polynucleotide of claim 10 or the vector of claim 11.
17. A method of producing an anti-CD70 antibody, comprising culturing the host cell of claim 22 under conditions that result in production of the antibody, and isolating the antibody from the host cell or culture.