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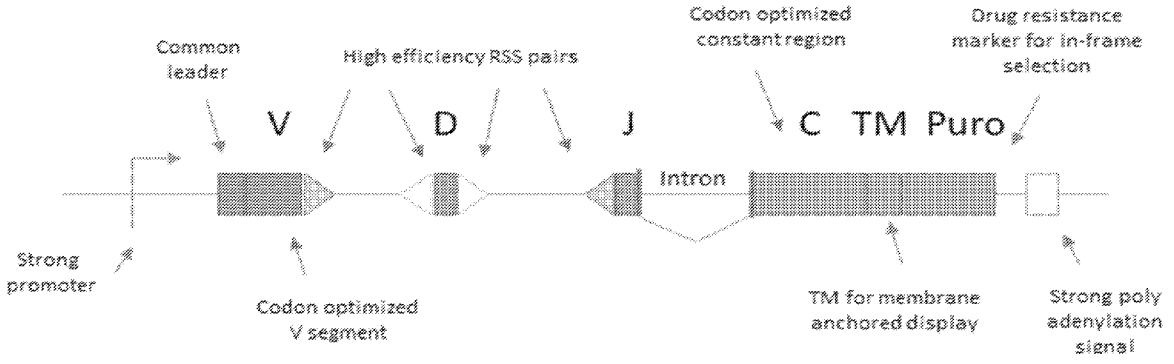
(54) Title: POLYPEPTIDES TARGETING MAGE-A3 PEPTIDE-MHC COMPLEXES AND METHODS OF USE THEREOF

FIG. 1



(57) Abstract: The present disclosure relates to antibodies and antigen-binding fragments thereof that bind to a MAGE-A3 peptide-MHC complex (pMHC) thereof. Also provided are chimeric antigen receptors and host cells comprising them. The compositions of the disclosure are useful in the diagnosis and treatment of cancer, including hematological malignancies, solid tumors, and epithelial cancers. In particular, provided are polypeptides that specifically bind to a pMHC complex displaying a MAGE-A3 peptide over a EPS8L2 pMHC complex displaying the EPS8L2 peptide SAAELVHFL (SEQ ID NO: 333), and methods of use thereof in treating cancer.

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[Continued on next page]

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POLYPEPTIDES TARGETING MAGE-A3 PEPTIDE-MHC COMPLEXES AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application Serial No. 62/981,940 filed on February 26, 2020 and U.S. Provisional Patent Application Serial No. 63/076,123 filed on September 9, 2020, the contents of which are hereby incorporated by reference in their entireties.

INCORPORATION OF THE SEQUENCE LISTING

[0002] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: A2BI_013_01WO_SeqList_ST25.txt, date created on February 24, 2021, file size 206 kb).

BACKGROUND

[0003] Melanoma-associated antigen 3 (MAGE-A3) is a protein that in humans is encoded by the *MAGEA3* gene. MAGE-A3 is a tumor-specific protein that has been identified in many tumors including melanoma, non-small cell lung cancer, and hematologic malignancies, among others. The presence of the antigen in tumor cells has been associated with worse prognosis, including shorter survival duration in subjects with lung adenocarcinoma. However, therapies targeting MAGE-A3 have failed in clinical trials.

[0004] There is an unmet need for selective therapies targeting MAGE-A3 for the treatment of cancer.

BRIEF SUMMARY

[0005] The present disclosure relates to novel antibodies, or antigen-binding fragments thereof, specific for MAGE-A3:peptide-MHC complexes, chimeric antigen receptors comprising the same, and methods of use thereof. The antibody and CAR compositions may be used in the treatment of a variety of cancers.

[0006] In one aspect, the disclosure provides a polypeptide, comprising an antibody or antigen-binding fragment thereof that specifically binds to a peptide comprising the sequence of FLWGPRALV (SEQ ID NO: 1) and has an EC50 of less than 5 nM. In some embodiments, the antibody or antigen-binding fragment thereof binds to the peptide within a peptide-MHC complex. In some embodiments, the antibody or antigen-binding fragment thereof is a human antibody or an antigen-binding fragment thereof. In some embodiments, the antigen-binding fragment is a single-chain variable fragment (scFv) or a single chain Fab (scFab). In some embodiments, the antigen-binding fragment is an scFv and comprises a variable heavy chain (VH)-linker-variable light chain (VL) orientation.

[0007] In some embodiments, the antigen-binding fragment is an scFv and comprises a VL-linker-VH orientation.

[0008] In some embodiments, the antibody or antigen-binding fragment thereof has an EC50 of less than 1 nM.

[0009] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain CDR1 comprising the sequence of SEQ ID NO: 221; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 222; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 223; a light chain CDR1 comprising the sequence of SEQ ID NO: 224; a light chain CDR2 comprising the sequence of SEQ ID NO: 225; and a light chain CDR3 comprising the sequence of SEQ ID NO: 226.

[0010] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 5; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 6; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 7; a light chain CDR1 comprising the sequence of SEQ ID NO: 8; a light chain CDR2 comprising the sequence of SEQ ID NO: 9; and a light chain CDR3 comprising the sequence of SEQ ID NO: 10; b) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 77; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 78; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 79; a light chain CDR1 comprising the sequence of SEQ ID NO: 80; a light chain CDR2 comprising the sequence of SEQ ID NO: 81; and a light chain CDR3 comprising the sequence of SEQ ID NO: 82; c) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 85; a heavy chain CDR2 comprising the

sequence of SEQ ID NO: 86; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 87; a light chain CDR1 comprising the sequence of SEQ ID NO: 88; a light chain CDR2 comprising the sequence of SEQ ID NO: 89; and a light chain CDR3 comprising the sequence of SEQ ID NO: 90; d) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 93; a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 94); a heavy chain CDR3 comprising the sequence of SEQ ID NO: 95; a light chain CDR1 comprising the sequence of SEQ ID NO: 96; a light chain CDR2 comprising the sequence of SEQ ID NO: 97; and a light chain CDR3 comprising the sequence of SEQ ID NO: 98; e) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 101; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 102; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 103; a light chain CDR1 comprising the sequence of SEQ ID NO: 104; a light chain CDR2 comprising the sequence of SEQ ID NO: 105; and a light chain CDR3 comprising the sequence of SEQ ID NO: 106; f) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 109; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 110; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 111; a light chain CDR1 comprising the sequence of SEQ ID NO: 112; a light chain CDR2 comprising the sequence of SEQ ID NO: 113; and a light chain CDR3 comprising the sequence of SEQ ID NO: 114; g) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 117; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 118; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 119; a light chain CDR1 comprising the sequence of SEQ ID NO: 120; a light chain CDR2 comprising the sequence of SEQ ID NO: 121; and a light chain CDR3 comprising the sequence of SEQ ID NO: 122; h) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 125; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 126; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 127; a light chain CDR1 comprising the sequence of SEQ ID NO: 128; a light chain CDR2 comprising the sequence of SEQ ID NO: 129; and a light chain CDR3 comprising the sequence of SEQ ID NO: 130; i) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 133; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 134; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 135; a light chain CDR1 comprising the sequence of SEQ ID NO: 136; a light chain CDR2 comprising the sequence of SEQ ID NO: 137; and a light chain CDR3 comprising the sequence of SEQ ID NO: 138; j) a heavy chain CDR1 comprising the

sequence of SEQ ID NO: 141; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 142; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 143; a light chain CDR1 comprising the sequence of SEQ ID NO: 144; a light chain CDR2 comprising the sequence of SEQ ID NO: 145; and a light chain CDR3 comprising the sequence of SEQ ID NO: 146; or k) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 149; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 150; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 151; a light chain CDR1 comprising the sequence of SEQ ID NO: 152; a light chain CDR2 comprising the sequence of SEQ ID NO: 153; and a light chain CDR3 comprising the sequence of SEQ ID NO: 154.

[0011] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a variable heavy chain comprising the sequence of SEQ ID NO: 11 and a variable light chain comprising the sequence of SEQ ID NO: 12; b) a variable heavy chain comprising the sequence of SEQ ID NO: 83 and a variable light chain comprising the sequence of SEQ ID NO: 84; c) a variable heavy chain comprising the sequence of SEQ ID NO: 91 and a variable light chain comprising the sequence of SEQ ID NO: 92; d) a variable heavy chain comprising the sequence of SEQ ID NO: 99 and a variable light chain comprising the sequence of SEQ ID NO: 100; e) a variable heavy chain comprising the sequence of SEQ ID NO: 107 and a variable light chain comprising the sequence of SEQ ID NO: 108; f) a variable heavy chain comprising the sequence of SEQ ID NO: 115 and a variable light chain comprising the sequence of SEQ ID NO: 116; g) a variable heavy chain comprising the sequence of SEQ ID NO: 123 and a variable light chain comprising the sequence of SEQ ID NO: 124; h) a variable heavy chain comprising the sequence of SEQ ID NO: 131 and a variable light chain comprising the sequence of SEQ ID NO: 132; i) a variable heavy chain comprising the sequence of SEQ ID NO: 139 and a variable light chain comprising the sequence of SEQ ID NO: 140; j) a variable heavy chain comprising the sequence of SEQ ID NO: 147 and a variable light chain comprising the sequence of SEQ ID NO: 148; or k) a variable heavy chain comprising the sequence of SEQ ID NO: 155 and a variable light chain comprising the sequence of SEQ ID NO: 156.

[0012] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) SEQ ID NO: 246; b) SEQ ID NO: 247; c) SEQ ID NO: 248; d) SEQ ID NO: 249; e) SEQ ID

NO: 250; f) SEQ ID NO: 251; g) SEQ ID NO: 252; h) SEQ ID NO: 253; i) SEQ ID NO: 254; j) SEQ ID NO: 255; or k) SEQ ID NO: 256.

[0013] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain CDR1 comprising the sequence of SEQ ID NO: 227; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 228; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 229; a light chain CDR1 comprising the sequence of SEQ ID NO: 230; a light chain CDR2 comprising the sequence of SEQ ID NO: 231; and a light chain CDR3 comprising the sequence of SEQ ID NO: 232.

[0014] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 13; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 14; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 15; a light chain CDR1 comprising the sequence of SEQ ID NO: 16; a light chain CDR2 comprising the sequence of SEQ ID NO: 17; and a light chain CDR3 comprising the sequence of SEQ ID NO: 18; b) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 157; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 158; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 159; a light chain CDR1 comprising the sequence of SEQ ID NO: 160; a light chain CDR2 comprising the sequence of SEQ ID NO: 161; and a light chain CDR3 comprising the sequence of SEQ ID NO: 162; or c) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 165; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 166; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 167; a light chain CDR1 comprising the sequence of SEQ ID NO: 168; a light chain CDR2 comprising the sequence of SEQ ID NO: 169; and a light chain CDR3 comprising the sequence of SEQ ID NO: 170.

[0015] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a variable heavy chain comprising the sequence of SEQ ID NO: 19 and a variable light chain comprising the sequence of SEQ ID NO: 20; b) a variable heavy chain comprising the sequence of SEQ ID NO: 163 and a variable light chain comprising the sequence of SEQ ID NO: 164; or c) a variable heavy chain comprising the sequence of SEQ ID NO: 171 and a variable light chain comprising the sequence of SEQ ID NO: 172.

[0016] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) SEQ ID NO: 257; b) SEQ ID NO: 258; c) SEQ ID NO: 259.

[0017] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain CDR1 comprising the sequence of SEQ ID NO: 233; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 234; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 235); a light chain CDR1 comprising the sequence of SEQ ID NO: 236; a light chain CDR2 comprising the sequence of SEQ ID NO: 237; and a light chain CDR3 comprising the sequence of SEQ ID NO: 238.

[0018] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 29; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 30; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 31; a light chain CDR1 comprising the sequence of SEQ ID NO: 32; a light chain CDR2 comprising the sequence of SEQ ID NO: 33; and a light chain CDR3 comprising the sequence of SEQ ID NO: 34; b) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 173; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 174; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 175; a light chain CDR1 comprising the sequence of SEQ ID NO: 176; a light chain CDR2 comprising the sequence of SEQ ID NO: 177; and a light chain CDR3 comprising the sequence of SEQ ID NO: 178; c) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 181; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 182; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 183; a light chain CDR1 comprising the sequence of SEQ ID NO: 184; a light chain CDR2 comprising the sequence of SEQ ID NO: 185; and a light chain CDR3 comprising the sequence of SEQ ID NO: 186; d) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 189; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 190; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 191; a light chain CDR1 comprising the sequence of SEQ ID NO: 192; a light chain CDR2 comprising the sequence of SEQ ID NO: 193; and a light chain CDR3 comprising the sequence of SEQ ID NO: 194; or e) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 197; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 198; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 199; a light chain CDR1 comprising the sequence of SEQ ID NO: 200; a light chain

CDR2 comprising the sequence of SEQ ID NO: 201; and a light chain CDR3 comprising the sequence of SEQ ID NO: 202.

[0019] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a variable heavy chain comprising the sequence of SEQ ID NO: 35 and a variable light chain comprising the sequence of SEQ ID NO: 36; b) a variable heavy chain comprising the sequence of SEQ ID NO: 179 and a variable light chain comprising the sequence of SEQ ID NO: 180; c) a variable heavy chain comprising the sequence of SEQ ID NO: 187 and a variable light chain comprising the sequence of SEQ ID NO: 188; d) a variable heavy chain comprising the sequence of SEQ ID NO: 195 and a variable light chain comprising the sequence of SEQ ID NO: 196; or e) a variable heavy chain comprising the sequence of SEQ ID NO: 203 and a variable light chain comprising the sequence of SEQ ID NO: 204.

[0020] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) SEQ ID NO: 260; b) SEQ ID NO: 261; c) SEQ ID NO: 262; d) SEQ ID NO: 263; e) SEQ ID NO: 264.

[0021] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain CDR1 comprising the sequence of SEQ ID NO: 239; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 240; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 241; a light chain CDR1 comprising the sequence of SEQ ID NO: 242; a light chain CDR2 comprising the sequence of SEQ ID NO: 243; and a light chain CDR3 comprising the sequence of SEQ ID NO: 244.

[0022] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 45; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 46; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 47; a light chain CDR1 comprising the sequence of SEQ ID NO: 48; a light chain CDR2 comprising the sequence of SEQ ID NO: 49; and a light chain CDR3 comprising the sequence of SEQ ID NO: 50; b) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 205; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 206; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 207; a light chain CDR1 comprising the sequence of SEQ ID NO: 208; a light chain CDR2 comprising the sequence of SEQ ID NO: 209; and a light chain CDR3 comprising the sequence of SEQ ID NO: 210; or c) a heavy

chain CDR1 comprising the sequence of SEQ ID NO: 213; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 214; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 215; a light chain CDR1 comprising the sequence of SEQ ID NO: 216; a light chain CDR2 comprising the sequence of SEQ ID NO: 217; and a light chain CDR3 comprising the sequence of SEQ ID NO: 218.

[0023] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a variable heavy chain comprising the sequence of SEQ ID NO: 51 and a variable light chain comprising the sequence of SEQ ID NO: 52; b) a variable heavy chain comprising the sequence of SEQ ID NO: 211 and a variable light chain comprising the sequence of SEQ ID NO: 212; or c) a variable heavy chain comprising the sequence of SEQ ID NO: 219 and a variable light chain comprising the sequence of SEQ ID NO: 220.

[0024] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) SEQ ID NO: 265; b) SEQ ID NO: 266; c) SEQ ID NO: 267.

[0025] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 21; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 22; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 23; a light chain CDR1 comprising the sequence of SEQ ID NO: 24; a light chain CDR2 comprising the sequence of SEQ ID NO: 25; and a light chain CDR3 comprising the sequence of SEQ ID NO: 26; b) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 37; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 38; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 39; a light chain CDR1 comprising the sequence of SEQ ID NO: 40; a light chain CDR2 comprising the sequence of SEQ ID NO: 41; and a light chain CDR3 comprising the sequence of SEQ ID NO: 42; c) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 53; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 54; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 55; a light chain CDR1 comprising the sequence of SEQ ID NO: 56; a light chain CDR2 comprising the sequence of SEQ ID NO: 57; and a light chain CDR3 comprising the sequence of SEQ ID NO: 58; d) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 61; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 62; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 63; a light chain CDR1 comprising the

sequence of SEQ ID NO: 64; a light chain CDR2 comprising the sequence of SEQ ID NO: 65; and a light chain CDR3 comprising the sequence of SEQ ID NO: 66; or e) a heavy chain CDR1 comprising the sequence of SEQ ID NO: 69; a heavy chain CDR2 comprising the sequence of SEQ ID NO: 70; a heavy chain CDR3 comprising the sequence of SEQ ID NO: 71; a light chain CDR1 comprising the sequence of SEQ ID NO: 72; a light chain CDR2 comprising the sequence of SEQ ID NO: 73; and a light chain CDR3 comprising the sequence of SEQ ID NO: 74.

[0026] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a variable heavy chain comprising the sequence of SEQ ID NO: 27 and a variable light chain comprising the sequence of SEQ ID NO: 28; b) a variable heavy chain comprising the sequence of SEQ ID NO: 43 and a variable light chain comprising the sequence of SEQ ID NO: 44; c) a variable heavy chain comprising the sequence of SEQ ID NO: 59 and a variable light chain comprising the sequence of SEQ ID NO: 60; d) a variable heavy chain comprising the sequence of SEQ ID NO: 67 and a variable light chain comprising the sequence of SEQ ID NO: 68; or e) a variable heavy chain comprising the sequence of SEQ ID NO: 75 and a variable light chain comprising the sequence of SEQ ID NO: 76.

[0027] In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) SEQ ID NO: 268; b) SEQ ID NO: 269; c) SEQ ID NO: 270; d) SEQ ID NO: 271; e) SEQ ID NO: 272.

[0028] In some embodiments, the antibody or antigen-binding fragment thereof is a monoclonal antibody or antigen-binding fragment thereof.

[0029] In some embodiments, the polypeptide is a chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the extracellular antigen-binding domain comprises the antibody or antigen-binding fragment thereof. In some embodiments, the transmembrane domain comprises the transmembrane domain of a protein selected from the group consisting of CD8-alpha, CD4, CD28, CD137, CD80, CD86, CD152, and PD1. In some embodiments, the intracellular signaling domain comprises an intracellular signaling domain of an immune effector cell. In some embodiments, the intracellular signaling domain comprises the intracellular signaling domain from CD3-zeta.

[0030] In some embodiments, the CAR comprises a co-stimulatory domain. In some embodimetns, the CAR comprises a co-stimulatory domain from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, CD40L, CD3, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, and DAP10.

[0031] In some embodiments, the CAR comprises a hinge domain between the extracellular antigen-binding domain and the transmembrane domain. In some embodiments, the CAR comprises a signal peptide. In some embodiments, the CAR comprises a signal peptide comprising the sequence of SEQ ID NO: 245.

[0032] In some embodiments, the CAR exhibits sub-nanomolar potency. In some embodiments the CAR preferentially binds to a peptide-MHC complex containing a peptide having the sequence of SEQ ID NO: 1 over a peptide-MHC complex containing a peptide having a sequence that differs from SEQ ID NO: 1 by one or more amino acids.

[0033] In some embodiments, the hinge, transmembrane, and intracellular domains of the CAR comprise a sequence at least 95%, 99%, or 100% identical to SEQ ID NO: 273.

[0034] In one aspect, the disclosure provides a polypeptide, comprising an antibody or antigen-binding fragment thereof that specifically binds to a peptide:MHC (pMHC) complex displaying a MAGE-A3$_{112-120}$ peptide comprising the sequence of KVAELVHFL (SEQ ID NO: 332). In one embodiment, the antibody or antigen-binding fragment thereof exhibits at least 6-fold lower selectivity for an EPS8L2 pMHC complex displaying the EPS8L2 peptide SAAELVHFL (SEQ ID NO: 333) than for the MAGE-A3$_{112-120}$ pMHC complex; and/or at least 10-fold lower selectivity for a MAGE-A12 pMHC complex displaying the MAGE-A12 peptide KMAELVHFL (SEQ ID NO: 370) than for the MAGE-A3$_{112-120}$ pMHC complex. In some embodiments, the antibody or antigen-binding fragment thereof comprises: a heavy-chain complementarity-determining region 1 (CDR-H1) comprising the sequence of SEQ ID NO: 287, a CDR-H2 comprising the sequence of SEQ ID NO: 288, a CDR-H3 comprising the sequence of SEQ ID NO: 289, a CDR-L1 comprising the sequence of any one of SEQ ID NOs: 290-292, a CDR-L2 comprising the sequence of any one of SEQ ID NOs: 293-300, and a CDR-L3 comprising the sequence of any one of SEQ ID NOs: 301-310.

[0035] In some embodiments, the antibody or antigen-binding fragment thereof comprises a CDR-H1 comprising the sequence of SEQ ID NO: 287, a CDR-H2 comprising the sequence

of SEQ ID NO: 288, a CDR-H3 comprising the sequence of SEQ ID NO: 289, a CDR-L1 comprising the sequence of SEQ ID NO: 290, a CDR-L2 comprising the sequence of SEQ ID NO: 293, and a CDR-L3 comprising the sequence of SEQ ID NO: 306.

[0036] In some embodiments, the polypeptide comprises a heavy-chain sequence according to SEQ ID NOs: 311. In some embodiments, the polypeptide comprises a light-chain sequence according to SEQ ID NOs: 312-328. In some embodiments, polypeptide comprises a light-chain sequence according to SEQ ID NO: 320. In some embodiments, the polypeptide is chimeric antigen receptor. In some embodiments, the polypeptide is a monoclonal antibody. In some embodiments, the polypeptide is a bispecific antibody.

[0037] In some embodiments, the polypeptides provided herein are soluble.

[0038] In some aspects, the disclosure provides a nucleic acid comprising a polynucleotide sequence encoding the polypeptide described herein. In some embodiments, the disclosure provides a vector comprising the nucleic acid described herein. In some embodiments, the disclosure provides a cell, host cell, or immune cell comprising the nucleic acid or vectors described herein.

[0039] In some aspects, disclosure provides a chimeric antigen receptor, comprising the polypeptide described herein. In some embodiments, the disclosure provides a recombinant immune cell expressing the chimeric antigen receptor described herein. In some embodiments, the immune cell is a T cell. In some embodiments, the immune cell is an NK cell.

[0040] In some aspects, the disclosure provides a pharmaceutical composition comprising a polypeptide described herein and a pharmaceutically acceptable carrier, diluent, or excipient. In some embodiments, the pharmaceutical composition comprises a recombinant immune cell described herein and a pharmaceutically acceptable carrier, diluent, or excipient.

[0041] In some aspects, the disclosure provides a method for diagnosing a disease involving MAGE-A3 positive cells, the method comprising detecting or measuring MAGE-A3 positive cells using the polypeptide described herein.

[0042] In some aspects, the disclosure provides a method for treating a cancer that expresses MAGE-A3 in a subject in need thereof, the method comprising administering a polypeptide

described herein or a pharmaceutical composition described herein to the subject. In some embodiments, the method comprises administering a recombinant immune cell described herein or a pharmaceutical composition described herein. In some embodiments, the cancer is a hematologic malignancy. In some embodiments, the cancer is multiple myeloma. In some embodiments, the cancer is an epithelial cancer. In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is melanoma, head and neck cancer, breast cancer, lung cancer, or synovial sarcoma.

[0043] In some aspects, the disclosure provides a method for treating a cancer that expresses MAGE-A3 in a subject in need thereof, comprising selecting a therapeutic composition comprising an antibody, a recombinant immune cell expressing a chimeric antigen receptor, or a recombinant immune cell expressing a chimeric antigen receptor TCR, that specifically binds to a peptide:MHC (pMHC) complex displaying a MAGE-A3 peptide and exhibits at least 1,000-fold lower reactivity (1,000-fold higher EC_{50}) to a EPS8L2 pMHC complex displaying the EPS8L2 peptide SAAELVHFL (SEQ ID NO: 333) (and/or to any EPS8L2 peptide) than for a MAGE-A3 pMHC complex; and administering an effective amount of the therapeutic composition to the subject.

[0044] In some aspects, the disclosure provides a method for treating a cancer that expresses MAGE-A3 in a subject in need thereof, comprising administering to the subject an effective amount of the therapeutic composition comprising an antibody, a recombinant immune cell expressing a chimeric antigen receptor, or a recombinant immune cell expressing a chimeric antigen receptor TCR, that specifically binds to a peptide:MHC (pMHC) complex displaying a MAGE-A3 peptide and exhibits at least 1,000-fold lower reactivity (1,000-fold higher EC_{50}) for an EPS8L2 pMHC complex displaying the EPS8L2 peptide SAAELVHFL (SEQ ID NO: 333) than for the MAGE-$A3_{112-120}$ pMHC complex.

[0045] In some aspects, the disclosure provides a method for discovering an antibody useful in treating cancer that expresses MAGE-A3, comprising selecting from an antibody-display library one or more antibodies that specifically bind to a peptide:MHC (pMHC) complex displaying a MAGE-$A3_{112-120}$ peptide and exhibit at least 1,000-fold lower reactivity (1,000-fold higher EC_{50}) for an EPS8L2 pMHC complex displaying the EPS8L2 peptide SAAELVHFL (SEQ ID NO: 333) than for the MAGE-$A3_{112-120}$ pMHC complex.

[0046] In some aspects, the disclosure provides a method of making a pharmaceutical composition for treating a cancer that expresses MAGE-A3, comprising selecting an antibody, or a recombinant immune cell expressing a chimeric antigen receptor, that specifically binds to a peptide:MHC (pMHC) complex displaying a MAGE-A3 peptide and exhibits at least 1,000-fold lower reactivity (1,000-fold higher EC_{50}) for an EPS8L2 pMHC complex displaying the EPS8L2 peptide SAAELVHFL (SEQ ID NO: 333) than for the MAGE-$A3_{112-120}$ pMHC complex.

[0047] In some embodiments, the therapeutic composition comprises the antibody. In some embodiments, the therapeutic composition comprises the recombinant immune cell expressing a chimeric antigen receptor. In some embodiments, the therapeutic composition comprises the recombinant immune cell expressing a chimeric antigen receptor TCR.

[0048] In some embodiments, the MAGE-A3 pMHC complex is a MAGE-$A3_{112-120}$ pMHC complex. In some embodiments, the MAGE-A3 pMHC complex is a MAGE-$A3_{271-279}$ pMHC complex.

[0049] In some embodiments, the cancer is a hematologic malignancy. In some embodiments, the cancer is multiple myeloma. In some embodiments, the cancer is an epithelial cancer. In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is melanoma, head and neck cancer, breast cancer, lung cancer, or synovial sarcoma.

[0050] Other features and advantages of the disclosure will be apparent from and encompassed by the following detailed description and claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0051] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0052] **FIG. 1** shows a schematic of an integrated V(D)J tripartite recombination substrate.

[0053] **FIGS. 2A-2B** show design, generation and analysis of an antibody library generated by V(D)J recombination. **FIG. 2A** shows a representation of a heavy chain locus having V

segments, D segments and J segments linked to an IgG1 constant domain, a transmembrane domain (TM) and a puromycin resistance marker. Also shown are a cassette to facilitate Cre-mediated integration (loxP-FLAG-TM-Hygro), a DNTT gene driven by a strong promoter and a common light chain gene. **FIG. 2B** shows antibody library generation using tetracycline to induce V(D)J recombination and puromycin to select for in-frame heavy chain genes.

[0054] FIGS. 3A-3D show sequence analysis of heavy chain genes generated by V(D)J recombination. **FIG. 3A** shows a comparison of heavy chain V gene usage in a library generated by V(D)J recombination and a previously described library derived from 654 healthy human donors. **FIG. 3B** shows the frequency of V-J combinations observed in a library generated by V(D)J recombination. **FIG. 3C** shows the composition of CDR-H3 sequences fifteen amino acids in length present in a library generated by V(D)J recombination and a human CDR-H3 database. **FIG. 3D** shows the distribution of CDR-H3 amino acid length in a library generated by V(D)J recombination and a human CDR-H3 database.

[0055] FIGS. 4A-4C show FACS analysis of antibody binding to peptide-pMHC complex. **FIG. 4A** shows FACS-based enrichment of a V(D)J repertoire for cells encoding MAGE-A3/HLA-A*02:01-specific antibodies. **FIG. 4B** shows characterization of the enriched library by FACS for membrane-anchored antibody expression (anti-human kappa-PE) and binding to on target (MAGE-A3/HLA-A*02:01) or off target (NY-ESO-1 or HIV-1 gag p17/HLA-A*02:01) pMHC complex. **FIG. 4C** shows characterization of a panel of antibodies (CDR-H3; SEQ ID NOs:274-280) identified by NGS analysis of the enriched library.

[0056] FIGS. 5A-5B shows affinity maturation of a library of MAGE-A3/HLA-A*02:01-specific antibodies diversified at CDR-L1 (SEQ ID NOs: 24 and 192), CDR-L2 (SEQ ID NO: 25) or CDR-L3 (SEQ ID NOs: 26, 281 and 282) by RAG-mediated recombination. **FIG. 5A** shows FACS-based enrichment for high affinity MAGE-A3/HLA-A*02:01-specific antibodies. In **FIG. 5B**, cells expressing membrane-anchored Mab3 or the indicated affinity matured derivate were analyzed by flow cytometry for binding to MAGE-A3/HLA-A*02:01 or the control pMHC complex PSA-1/HLA-A*02:01.

[0057] FIGS. 6A-6B show functional analysis of recombinant CARs using T2/Jurkat-NFAT assay. **FIG. 6A** shows reporter gene expression (luciferase activity) by Jurkat/NFAT-Luc reporter cells expressing CAR3 (upside down triangles) or an affinity matured derivative (CAR3-a, circles; CAR3-b, triangles; CAR3-c, squares) when co-cultured with T2 cells loaded with MAGE-A3 peptide. **FIG. 6B** shows Jurkat/NFAT-Luc reporter cells expressing CAR3-a, CAR3-b or CAR3-c co-cultured with T2 cells loaded with MAGE-A3 (SEQ ID NO: 1; blue circles) or the indicated related peptide (SEQ ID NOs: 283-286; green triangles).

[0058] FIG. 7 shows an analysis of peptide-mediated MHC stabilization on T2 cells loaded with different concentrations of MAGE-A3 peptide or related peptide variants bound with anti-HLA antibody. Sequences shown from left to right are SEQ ID NO: 1, SEQ ID NO: 335, SEQ ID NO: 336, SEQ ID NO: 337, and SEQ ID NO: 338.

[0059] FIG. 8 shows designated names and sequences for peptides and constructs described herein. Sequences shown from top to bottom are SEQ ID NO: 1 and SEQ ID NO: 332.

[0060] FIG. 9 shows the expression of genes of interest in primary tissue and cell lines referenced herein.

[0061] FIG. 10 shows a summary of selectivity for MAGE-A3$_{112\text{-}120}$ CARs and TRCs determined using the Jurkat/T2 assay. The selectivity window is the ratio of EC50s or ECmin of on-target peptide over off-target peptide (e.g. MAGE-A12:A3 or EPS8L2:A3). ECmin is the minimum peptide concentration with statistically significant difference from baseline. N/A: not applicable; N/D: not determined. Sequences shown from top to bottom are SEQ ID NO: 339, SEQ ID NO: 340, SEQ ID NO: 332, SEQ ID NO: 341, SEQ ID NO: 342, SEQ ID NO: 343, SEQ ID NO: 344, SEQ ID NO: 345, SEQ ID NO: 346, SEQ ID NO: 347, SEQ ID NO: 348, SEQ ID NO: 349, SEQ ID NO: 350, SEQ ID NO: 351, SEQ ID NO: 352, SEQ ID NO: 353, SEQ ID NO: 354, SEQ ID NO: 355.

[0062] FIG. 11 shows MAGE-A3$_{271\text{-}279}$ CAR and TCR selectivity in T2/Jurkat cell assays. The selectivity window is the ratio of EC50s of on-target peptide over off-target peptide. N/A: not applicable. Sequences shown from top to bottom are SEQ ID NO: 1, SEQ ID NO: 356, SEQ ID NO: 357, SEQ ID NO: 358, SEQ ID NO: 359, SEQ ID NO: 360, SEQ ID NO: 361, SEQ ID NO: 362, SEQ ID NO: 363, SEQ ID NO: 364, SEQ ID NO: 365, SEQ ID NO: 366, SEQ ID NO: 367, SEQ ID NO: 368, and SEQ ID NO: 369.

[0063] FIG. 12 shows selectivity of MAGE-A3$_{112-120}$ CAR and TCR in primary T cell assays with MCF7or HEK293 cells loaded with peptide. Sequences shown from top to bottom are SEQ ID NO: 332, SEQ ID NO: 333, and SEQ ID NO: 370.

[0064] FIG. 13 shows the specific probes designed to determine expression of MAGE-A12 in the brain and cell lines for RNA fluorescent in situ hybridization. The BaseScope™ probe can potentially cross react with MAGE-A2, A2b, and A10. However, the RNA expression of these genes is extremely low in the brain and significantly lower than A12. Green: identical nucleotide acid; yellow: BaseScope™ probe. Sequences shown on left table from top to bottom are SEQ ID NO: 371, SEQ ID NO: 372, SEQ ID NO: 373, SEQ ID NO: 374, SEQ ID NO: 375, SEQ ID NO: 376, SEQ ID NO: 377, SEQ ID NO: 378, and SEQ ID NO: 379. Sequences shown on right table from top to bottom are SEQ ID NO: 380, SEQ ID NO: 381, SEQ ID NO: 382, and SEQ ID NO: 383.

[0065] FIG. 14 shows detection of MAGE-A12 using cell lines with known MAGE-A12 expression levels. Examples of stain dots are highlighted with red arrows. RPKM values were extracted from TRON database.

[0066] FIG. 15 shows MAGE-A12 expression in the brain. ~3 million brain cells from 8 donors were stained using MAGE-A12-specific probe and scanned by eye and ImageJ software. No expression of MAGE-A12 higher than the negative control was detected. No rare cells with very high expression of MAGE-A12 was observed.

[0067] FIG. 16 shows MAGE-A3 CAR and TCR selectivity against related MAGE-family peptides in T2/Jurkat cell assays.

[0068] FIG. 17 shows MAGE-A3 CAR and TCR selectivity against non-MAGE-A family in T2Jurkat cell assays.

[0069] FIG. 18 shows MAGE-A3 CAR and TCR selectivity against A12 and EPS8L2 in T2/Jurkat cell assays.

[0070] FIG. 19 shows primary T cell cytotoxicity of MAGE CARs and TCRs on peptide-loaded MCF7 and HEK293 cells. MCF7 (no/low expression of MAGE; Positive for EPS8L2; homogeneous peptide loading). HEK293 (no/low expression of MAGE and EPS8L2; QuIK peptide loading). Averages of two measurements are plotted.

[0071] FIG. 20 shows cytotoxicity data for MAGE-A3$_{112-120}$ TCR in A375 and CAPAN2 cell lines. At 27:1 E:T ratio, the TCR showed significantly higher level of killing on CAPAN2 (HLA-A*02+) than CAPAN2 HLA-A*02 KO cells. The level is similar to A375 cells, which has high expression of MAGE-A3 and MAGE-A12, as well as moderate expression of EPS8L2. Expression of MAGE-A3 and MAGE-A12 is low (<1TPM) in CAPAN2, while EPS8L2 is high (~450 TPM), indicating killing is mediated by EPS8L2.

[0072] FIG. 21 shows a summary of mass spectrometry data and cell line gene expression data. EPS8L2 was identified in PANC-1, but not CaSki cells, despite the RNA levels being 2x higher in CaSki, compared to PANC-1.

[0073] FIG. 22 shows a comparison of sensitivity in Jurkat cells co-cultured with peptide loaded T2, MCF7, or HEK293. Sequences shown from top to bottom are SEQ ID NO: 1, SEQ ID NO: 332, SEQ ID NO: 1, SEQ ID NO: 332, SEQ ID NO: 1, and SEQ ID NO: 332.

[0074] FIG. 23 shows EPS8L2 and MAGE-A12 expression in the brain from GTEx database.

DETAILED DESCRIPTION

[0075] The present disclosure provides novel antibodies and antigen-binding fragments thereof that selectively bind to a MAGE-A3 peptide. Also provided are chimeric antigen receptors (CARs) binding to this target. These antibodies and CARs may be used to treat cancer. In the context of the present disclosure, the following definitions are provided.

DEFINITIONS

[0076] As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

[0077] As used herein, the "administration" of an agent, *e.g.*, an anti-MAGE-A3:pMHC antibody or CAR-expressing cell, to a subject or subject includes any route of introducing or delivering to a subject a compound to perform its intended function. Suitable dosage formulations and methods of administering the agents are known in the art. Route of administration can also be determined and method of determining the most effective route of administration are known to those of skill in the art and will vary with the composition used

for treatment, the purpose of the treatment, the health condition or disease stage of the subject being treated and target cell or tissue. Non-limiting examples of route of administration include parenteral, enteral, and topical routes of administration. Administration includes self-administration and the administration by another. It is also to be appreciated that the various modes of treatment or prevention of medical conditions as described are intended to mean "substantial", which includes total but also less than total treatment or prevention, and wherein some biologically or medically relevant result is achieved.

[0078] As used herein, the term "animal" refers to living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term "mammal" includes both human and non-human mammals. Similarly, the term "subject" or "patient" includes both human and veterinary subjects, for example, humans, non-human primates, dogs, cats, sheep, mice, horses, and cows.

[0079] As used herein, the term "antibody" collectively refers to immunoglobulins or immunoglobulin-like molecules including by way of example and without limitation, IgA, IgD, IgE, IgG and IgM, combinations thereof, and similar molecules produced during an immune response in any vertebrate, for example, in mammals such as humans, goats, rabbits and mice, as well as non-mammalian species, such as shark immunoglobulins. The term "antibody" includes intact immunoglobulins and "antibody fragments" or "antigen binding fragments" that specifically bind to a molecule of interest (or a group of highly similar molecules of interest) to the substantial exclusion of binding to other molecules (for example, antibodies and antibody fragments that have a binding constant for the molecule of interest that is at least $10^3\ M^{-1}$ greater, at least $10^4\ M^{-1}$ greater or at least $10^5\ M^{-1}$ greater than a binding constant for other molecules in a biological sample). The term "antibody" also includes genetically engineered forms such as chimeric antibodies (for example, humanized murine antibodies), heteroconjugate antibodies (such as, bispecific antibodies). See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.); Kuby, J., *Immunology* 3rd, Ed., W.H. Freeman & Co., New York, 1997. The term antibody herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies) formed from at least two

intact antibodies, and antibody fragments, so long as they exhibit the desired biological activity.

[0080] "Antibody fragments" or "antigen binding fragments" include proteolytic antibody fragments (such as F(ab′)2 fragments, Fab′ fragments, Fab′-SH fragments and Fab fragments as are known in the art), recombinant antibody fragments (such as sFv fragments, dsFv fragments, bispecific sFv fragments, bispecific dsFv fragments, F(ab)′2 fragments, single chain Fv proteins ("scFv"), disulfide stabilized Fv proteins ("dsFv"), diabodies, and triabodies (as are known in the art), and camelid antibodies (see, for example, U.S. Pat. Nos. 6,015,695; 6,005,079; 5,874,541; 5,840,526; 5,800,988; and 5,759,808). An scFv protein is a fusion protein in which a light chain variable region of an immunoglobulin and a heavy chain variable region of an immunoglobulin are bound by a linker, while in dsFvs, the chains have been mutated to introduce a disulfide bond to stabilize the association of the chains.

[0081] As used herein, the term "antigen" refers to a compound, composition, or substance that may be specifically bound by the products of specific humoral or cellular immunity, such as an antibody molecule or T-cell receptor. Antigens can be any type of molecule including, for example, haptens, simple intermediary metabolites, sugars (*e.g.*, oligosaccharides), lipids, and hormones as well as macromolecules such as complex carbohydrates (*e.g.*, polysaccharides), phospholipids, and proteins. Common categories of antigens include, but are not limited to, viral antigens, bacterial antigens, fungal antigens, protozoa and other parasitic antigens, tumor antigens, antigens involved in autoimmune disease, allergy and graft rejection, toxins, and other miscellaneous antigens.

[0082] As used herein, "binding affinity" refers to the tendency of one molecule to bind (typically non-covalently) with another molecule, such as the tendency of a member of a specific binding pair for another member of a specific binding pair. A binding affinity can be measured as a dissociation constant, which for a specific binding pair (such as an antibody/antigen pair) can be lower than 1×10^{-5} M, lower than 1×10^{-6} M, lower than 1×10^{-7} M, lower than 1×10^{-8} M, lower than 1×10^{-9} M, lower than 1×10^{-10} M, lower than 1×10^{-11} M or lower than 1×10^{-12} M. In one aspect, binding affinity is calculated by a modification of the Scatchard method described by Frankel et al., *Mol. Immunol.,* 16:101-106, 1979. In another aspect, binding affinity is measured by a binding constant. In another aspect, binding affinity

is measured by an antigen/antibody dissociation rate. In yet another aspect, a high binding affinity is measured by a competition radioimmunoassay.

[0083] The terms "cancer," "neoplasm," and "tumor," used interchangeably and in either the singular or plural form, refer to cells that have undergone a malignant transformation that makes them pathological to the host organism. Non-limiting examples of cancers that may be treated according to the methods of the present disclosure include hematological malignancies and solid tumors.

[0084] The term "chimeric antigen receptors" or "CARs" as used herein, may refer to artificial T-cell receptors, chimeric T-cell receptors, or chimeric immunoreceptors, for example, and encompass engineered receptors that graft an artificial specificity onto a particular immune effector cell, such as a helper T cell (CD4+), cytotoxic T cell (CD8+) or NK cell. CARs may be employed to impart the specificity of a monoclonal antibody onto a T cell, thereby allowing a large number of specific T cells to be generated, for example, for use in adoptive cell therapy. In specific embodiments, CARs direct specificity of the cell to a tumor associated antigen, *e.g.*, MAGE-A3. In some embodiments, CARs comprise an intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising a tumor associated antigen binding region. In some embodiments, CARs comprise fusions of single-chain variable fragments (scFvs) or scFabs derived from monoclonal antibodies, fused to a transmembrane domain and intracellular signaling domain. Either heavy-light (H-L) and light-heavy (L-H) scFvs may be used. The specificity of CAR designs may be derived from ligands of receptors (*e.g.*, peptides) or from Dectins. In some embodiments, CARs comprise domains for additional co-stimulatory signaling, such as CD3, FcR, CD27, CD28, CD137, DAP10, and/or 0X40. In some embodiments, molecules can be co-expressed with the CAR, including co-stimulatory molecules, reporter genes for imaging (*e.g.*, for positron emission tomography), gene products that conditionally ablate the T cells upon addition of a pro-drug, homing receptors, cytokines, and cytokine receptors. As used herein, characteristics attributed to a chimeric antigen receptor may be understood to refer to the receptor itself or to a host cell comprising the receptor.

[0085] As used herein, an "epitope" or "antigenic determinant" refers to particular chemical groups or contiguous or non-contiguous peptide sequences on a molecule that are antigenic,

i.e., that elicit a specific immune response. An antibody binds a particular antigenic epitope. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0086] The polypeptides described herein may be "isolated" polypeptides, meaning that the removed from living host. Isolated polypeptide may be expressed in a host cell, such as an immune cell, as a receptor and be displayed on the surface of that cell. The disclosure further provided soluble embodiments, include monoclonal antibodies, single-chain fragments (scFv's), bispecific or multispecific antibodies, and the like. It will be readily understood that the complementarity determining regions (CDRs) of antibodies can be grafted onto various scaffolds including antibody-based scaffolds, designed protein scaffolds, and non-protein scaffolds. The polypeptides of the disclosure may also be expressed *in vivo* (that is, in a subject organism, *e.g.*, a human subject) rather than *in vitro*, such as by administration to the subject of a vector comprising a polynucleotide encoding the polypeptide.

[0087] As used herein, "homology" or "identical", percent "identity" or "similarity", when used in the context of two or more nucleic acids or polypeptide sequences, refers to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, *e.g.*, at least 60% identity, preferably at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region (*e.g.*, nucleotide sequence encoding an antibody described herein or amino acid sequence of an antibody described herein). Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. The alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Current Protocols in Molecular Biology (Ausubel et al., eds. 1987) Supplement 30, section 7.7.18,

Table 7.7.1. Preferably, default parameters are used for alignment. A preferred alignment program is BLAST, using default parameters. In particular, preferred programs are BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+SwissProtein+SPupdate+PIR. Details of these programs can be found at the following Internet address: ncbi.nlm.nih.gov/cgi-bin/BLAST. The terms "homology" or "identical", percent "identity" or "similarity" also refer to, or can be applied to, the complement of a test sequence. The terms also include sequences that have deletions and/or additions, as well as those that have substitutions. As described herein, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is at least 50-100 amino acids or nucleotides in length. An "unrelated" or "non-homologous" sequence shares less than 40% identity, or alternatively less than 25% identity, with one of the sequences of the present disclosure.

[0088] The term " human antibody" as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the disclosure may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis or by somatic mutation *in vivo*). However, the term "human antibody" as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a rabbit, have been grafted onto human framework sequences. Thus, as used herein, the term "human antibody" refers to an antibody in which substantially every part of the protein (*e.g.*, CDR, framework, C_L, C_H domains (*e.g.*, C_{H1}, C_{H2}, C_{H3}), hinge, V_L, V_H) is substantially non-immunogenic in humans, with only minor sequence changes or variations. Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent (mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a

chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (*e.g.*, heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an Fv can comprise a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

[0089] As used herein, "MAGE-A3 antibody" and "anti-MAGE-A3 antibody" are used interchangeably, and refer to an antibody that specifically binds to MAGE-A3 polypeptide and/or MAGE-A3 peptide-MHC complex as antigen. In some embodiments, the antibody specifically binds a MAGE-A3 peptide-MHC complex and is referred to as "anti-MAGE-A3:peptide-MHC complex (pMHC) antibody" or "anti-MAGE-A3:pMHC antibody". Similarly, the term "MAGE-A3", as mentioned in "MAGE-A3 binding", "MAGE-A3 targeting", "MAGE-A3 specific", "MAGE-A3 expression" or other similar terms here, refers to both MAGE-A3 polypeptide and MAGE-A3 peptide-MHC complex.

[0090] As used herein, the term "monoclonal antibody" refers to an antibody produced by a single clone of B-lymphocytes or by a cell into which the light and heavy chain genes of a single antibody have been transfected. Monoclonal antibodies are produced by methods known to those of skill in the art, for instance by making hybrid antibody-forming cells from a fusion of myeloma cells with immune spleen cells. Monoclonal antibodies include humanized monoclonal antibodies.

[0091] The terms "polynucleotide" and "oligonucleotide" are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, RNAi, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes

and primers. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any aspect of this disclosure that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form. A polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. Thus, the term "polynucleotide sequence" is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching.

[0092] The term "protein", "peptide" and "polypeptide" are used interchangeably and in their broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs or peptidomimetics. The subunits may be linked by peptide bonds. In another aspect, the subunit may be linked by other bonds, *e.g.*, ester, ether, etc. A protein or peptide must contain at least two amino acids and no limitation is placed on the maximum number of amino acids which may comprise a protein's or peptide's sequence. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D and L optical isomers, amino acid analogs and peptidomimetics.

[0093] As used herein, "treating" or "treatment" of a disease in a subject refers to (1) preventing the symptoms or disease from occurring in a subject that is predisposed or does not yet display symptoms of the disease; (2) inhibiting the disease or arresting its development; or (3) ameliorating or causing regression of the disease or the symptoms of the disease. As understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. For the purposes of this disclosure, beneficial or desired results can include one or more, but are not limited to, alleviation or amelioration of one or more symptoms, diminishment of extent of a condition (including a disease),

stabilized (*i.e.*, not worsening) state of a condition (including disease), delay or slowing of condition (including disease), progression, amelioration or palliation of the condition (including disease), states and remission (whether partial or total), whether detectable or undetectable. Preferred are compounds that are potent and can be administered locally at very low doses, thus minimizing systemic adverse effects.

ANTI-MAGE-A3:pMHC POLYPEPTIDES

[0094] The present disclosure provides anti-MAGE-A3:peptide-MHC complex (pMHC) antibodies, antigen-binding antibody fragments, chimeric antigen receptors (CARs), CAR-expressing cells, and compositions comprising.

[0095] pMHCs are a rich source of targets for new medicines directed at peptides derived from proteins whose tissue expression pattern and cellular compartment are well known. Although the therapeutic opportunity is apparent, the limited literature on this modality reflects the technical difficulty of this target class. The historical challenges of generating TCRm antibody therapeutics are supported by the fact that only one molecule has advanced to the clinic: Eureka Therapeutics' ET1402L1-ARTEMIS™2 T cells for alpha fetoprotein (AFP) expressing hepatocellular carcinoma is currently in a Phase 1/2 clinical trial for liver cancer.

[0096] However, in the present disclosure, *in vitro* V(D)J recombination was employed to isolate and engineer fully human monoclonal antibodies capable of binding pMHCs with high avidity and selectivity.

ANTI-MAGE-A3:pMHC ANTIBODIES

[0097] The present disclosure provides anti-MAGE-A3:peptide-MHC complex (pMHC) antibodies and antigen-binding fragments thereof.

[0098] The general structure of antibodies is known in the art. Briefly, an immunoglobulin monomer comprises two heavy chains and two light chains connected by disulfide bonds. Each heavy chain is paired with one of the light chains to which it is directly bound via a disulfide bond. Each heavy chain comprises a constant region (which varies depending on the isotype of the antibody) and a variable region. The variable region comprises three hypervariable regions (or complementarity determining regions) which are designated CDRH1, CDRH2 and CDRH3 and which are supported within framework regions. Each light

chain comprises a constant region and a variable region, with the variable region comprising three hypervariable regions (designated CDRL1, CDRL2 and CDRL3) supported by framework regions in an analogous manner to the variable region of the heavy chain.

[0099] The hypervariable regions of each pair of heavy and light chains mutually cooperate to provide an antigen binding site that is capable of binding a target antigen. The binding specificity of a pair of heavy and light chains is defined by the sequence of CDR1, CDR2 and CDR3 of the heavy and light chains. Thus once a set of CDR sequences (*i.e.* the sequence of CDR1, CDR2 and CDR3 for the heavy and light chains) is determined which gives rise to a particular binding specificity, the set of CDR sequences can, in principle, be inserted into the appropriate positions within any other antibody framework regions linked with any antibody constant regions in order to provide a different antibody with the same antigen binding specificity.

[0100] In some embodiments, provided herein is an antibody that binds to MAGE-A3.

[0101] The *MAGE-A3* gene is expressed during embryogenesis and in a wide variety of tumors. It is presented to specific T cells by HLA molecules at the cell surface as a tumor-specific antigen. It is not expressed in normal adult tissues, except testis and placenta. The latter does not compromise the tumor specificity of MAGE-A3, because these cells do not bear HLA molecules and thus do not present MAGE-A3 antigen. Consequently, MAGE-A3 antigen is a genuinely selective target for tumor-specific active immunotherapy.

[0102] In some embodiments, provided herein is an antibody that binds to a MAGE-A3 peptide comprising the amino acid sequence FLWGPRALV (SEQ ID NO: 1). In some embodiments, provided herein is an antibody that binds to a MAGE-A3 peptide comprising the amino acid sequence of SEQ ID NO: 1 within the context of a peptide-MHC complex. In some embodiments, the antibody has a half maximal effective concentration (EC_{50}) of less than 10 nM. In some embodiments, the EC_{50} is less than 100 nM, less than 50 nM, less than 40 nM, less than 30 nM, less than 20 nM, less than 10 nM, less than 5 nM, or less than 1 nM. In some embodiments, the EC_{50} is less than 5 nM.

[0103] In some embodiments, the antibody binds to the peptide within the context of a peptide-MHC complex. The peptide having SEQ ID NO: 1 was identified as an HLA-A*02:01 restricted peptide. In some embodiments, the antibody binds to the peptide within

the context of a peptide-HLA-A*02:01 complex. In some embodiments, the antibody binds to the peptide within the context of a peptide-HLA-A*02 complex.

[0104] In some embodiments, the antibody has a higher EC_{50} (decreased binding) for a peptide that differs from that of SEQ ID NO: 1 by one amino acid, two amino acids, three amino acids, four amino acids, five amino acids, or more. In some embodiments, the antibody does not bind to an NY-ESO-1 peptide. In some embodiments, the antibody shows decreased binding for peptides isolated from MAGE-A1, A2, A4, A6, A8, A9 A10, A11. In some embodiments, the antibody has a two-fold higher, five-fold higher, 10-fold higher, 15-fold higher, 20-fold higher, 50-fold higher, or 100-fold higher EC_{50} for a peptide that differs from SEQ ID NO:1. In some embodiments, the antibody has a ten-fold lower EC_{50} for the peptide of SEQ ID NO: 1 than for the analogous peptide isolated from another MAGE-A protein.

[0105] In some embodiments, the monoclonal antibody that binds MAGE-A3 is a single domain antibody.

[0106] In some embodiments, the monoclonal antibody that binds, such as specifically binds, MAGE-A3 is a Fab fragment, a Fab′ fragment, a $F(ab)'_2$ fragment, a single chain variable fragment (scFv), or a disulfide stabilized variable fragment (dsFv). In other embodiments, the antibody is an immunoglobulin molecule. In particular examples, the antibody is an IgG.

[0107] In some embodiments, the antibody is a fully human antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a chimeric antibody.

[0108] The monoclonal antibodies disclosed herein can be of any isotype. The monoclonal antibody can be, for example, an IgM or an IgG antibody, such as IgG_1 or an IgG_2. The class of an antibody that specifically binds MAGE-A3 can be switched with another (for example, IgG can be switched to IgM), according to well-known procedures. Class switching can also be used to convert one IgG subclass to another, such as from IgG_1 to IgG_2.

[0109] Antibody fragments are also encompassed by the present disclosure, such as single-domain antibodies (*e.g.*, VH domain antibodies), Fab, $F(ab')_2$, and Fv. These antibody fragments retain the ability to selectively bind with the antigen. These fragments include:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

(2) Fab′, the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab′ fragments are obtained per antibody molecule;

(3) $(Fab')_2$, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; $F(ab')_2$ is a dimer of two Fab′ fragments held together by two disulfide bonds;

(4) Fv, a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains;

(5) Single chain antibody (such as scFv), a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule;

(6) A dimer of a single chain antibody ($scFv_2$), defined as a dimer of a scFv (also known as a “miniantibody”);

(7) VH single-domain antibody, an antibody fragment consisting of a heavy chain variable domain; and

(8) A single chain Fab fragment (scFab), which can be formed by the introduction of a polypeptide linker between the Fd and the light chain to result in the formation of a single chain Fab fragment.

[0110] Methods of making these fragments are known in the art (see for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, 1988).

[0111] In some cases, antibody fragments can be prepared by proteolytic hydrolysis of the antibody or by expression in a host cell (such as *E. coli*) of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic

cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab′ monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab′ fragments and an Fc fragment directly (see U.S. Pat. No. 4,036,945 and U.S. Pat. No. 4,331,647).

[0112] Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

[0113] Antibody Purification. The antibodies of the present disclosure can be purified to homogeneity. The separation and purification of the antibodies can be performed by employing conventional protein separation and purification methods.

[0114] By way of example only, the antibody can be separated and purified by appropriately selecting and combining use of chromatography columns, filters, ultrafiltration, salt precipitation, dialysis, preparative polyacrylamide gel electrophoresis, isoelectric focusing electrophoresis, and the like. *Strategies for Protein Purification and Characterization: A Laboratory Course Manual*, Daniel R. Marshak et al. eds., Cold Spring Harbor Laboratory Press (1996); *Antibodies: A Laboratory Manual*. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988).

[0115] Non-limiting examples of chromatography include affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration chromatography, reverse phase chromatography, and adsorption chromatography. In one aspect, chromatography can be performed by employing liquid chromatography such as HPLC or FPLC.

Exemplary sequences

[0116] Illustrative sequences of anti-MAGE-A3 antibodies are provided herein. Tables 1A and 1B provide non-limiting illustrative heavy chain CDR sequences for antibodies according to the disclosure.

Table 1A: Illustrative heavy chain CDR sequences for antibodies that specifically bind MAGE-A3$_{271-279}$ pMHC complex

Construct name	Parent Ab	Ab ID	VH-1	SEQ ID NO	VH-2	SEQ ID NO	VH-3	SEQ ID NO
C-000364	mAb1		SGGYSWS	5	YIYHSGSTYYNPSLKS	6	CARDGDVPYWYFDLW	7
C-000365	mAb2		ELSMH	13	GFDPEDGETIYAQKFQG	14	CATDLYSSSWYCDAFDIW	15
C-000366	mAb3		DYYMH	21	LVDPEDGETIYAEKFQG	22	CALLSGWYIDAFDIW	23
C-000367	mAb4		SSNWWG	29	YIYYSGSTYYNPSLKS	30	CARIPFGDWWYFDLW	31
C-000368	mAb5		SYDMH	37	AIGTAGDTYYPGSVKG	38	CARDLYYDILTGYPDAFDIW	39
C-000369	mAb6		DYAMH	45	GISWNSGSIGYADSVKG	46	CAKDSTSWVNFPYYMDVW	47
C-000370	mAb7		SSSYYWG	53	SIYYSGSTYYNPSLKS	54	CARRDGGNWYFDLW	55
C-000371	mAb8		SSNWWG	61	YIYYSGSTYYNPSLKS	62	CARNTDPQLEWYFDLW	63
C-000372	mAb9		SYSMN	69	YISSSSSTIYYADSVKG	70	CARDDYSSEYFQHW	71
C-000373	mAb1	V10	SGGYSWS	77	YIYHSGSTYYNPSLKS	78	CARDGDVPYWYFDLW	79
C-000374	mAb1	V12	SGGYSWS	85	YIYHSGSTYYNPSLKS	86	CARDGDVPYWYFDLW	87
C-000375	mAb1	V19	SGGYSWS	93	YIYHSGSTYYNPSLKS	94	CARDGDVPYWYFDLW	95
C-000376	mAb1	V20	SGGYSWS	101	YIYHSGSTYYNPSLKS	102	CARDGDVPYWYFDLW	103
C-000377	mAb1	V21	SGGYSWS	109	YIYHSGSTYYNPSLKS	110	CARDGDVPYWYFDLW	111
C-000378	mAb1	V22	SGGYSWS	117	YIYHSGSTYYNPSLKS	118	CARDGDVPYWYFDLW	119
C-000379	mAb1	V23	SGGYSWS	125	YIYHSGSTYYNPSLKS	126	CARDGDVPYWYFDLW	127
C-000380	mAb1	V24	SGGYSWS	133	YIYHSGSTYYNPSLKS	134	CARDGDVPYWYFDLW	135
C-000456	mAb1	V25	SGGYSWS	141	YIYHSGSTYYNPSLKS	142	CARDGDVPYWYFDLW	143
C-000457	mAb1	V26	SGGYSWS	149	YIYHSGSTYYNPSLKS	150	CARDGDVPYWYFDLW	151
C-000458	mAb2	V27	ELSMH	157	GFDPEDGETIYAQKFQG	158	CATDLYSSSWYCDAFDIW	159
C-000459	mAb2	V29	ELSMH	165	GFDPEDGETIYAQKFQG	166	CATDLYSSSWYCDAFDIW	167

Construct name	Parent Ab	Ab ID	VH-1	SEQ ID NO	VH-2	SEQ ID NO	VH-3	SEQ ID NO
C-000381	mAb4	V15	SSNWWG	173	YIYYSGSTYYNPSLKS	174	CARIPFGDWWYFDLW	175
C-000382	mAb4	V16	SSNWWG	181	YIYYSGSTYYNPSLKS	182	CARIPFGDWWYFDLW	183
C-000383	mAb4	V17	SSNWWG	189	YIYYSGSTYYNPSLKS	190	CARIPFGDWWYFDLW	191
C-000460	mAb4	V34	SSNWWG	197	YIYYSGSTYYNPSLKS	198	CARIPFGDWWYFDLW	199
C-000384	mAb6	V18	DYAMH	205	GISWNSGSIGYADSVKG	206	CAKDSTSWVNFPYYMDVW	207
C-000461	mAb6	V36	DYAMH	213	GISWNSGSIGYADSVKG	214	CAKDSTSWVNFPYYMDVW	215

Table 1B: Illustrative heavy chain CDR sequences for antibodies that specifically bind MAGE-A3$_{112-120}$ pMHC complex

Construct name	Parent Ab	Ab ID	VH-1	SEQ ID NO	VH-2	SEQ ID NO	VH-3	SEQ ID NO
C-000479	mAb22	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-000499	mAb22	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001375	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001376	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001505	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001506	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001507	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001508	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001509	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001510	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001511	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289

C-001512	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001539	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001540	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001541	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001542	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001543	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001544	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001635	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001637	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001638	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289
C-001640	C-000479	V706	SYAIS	287	GIIPIFGTANYAQKFQG	288	DMDTFSMVTLFDY	289

[0117] Tables 2A and 2B provide non-limiting illustrative light chain CDR sequences for antibodies according to the disclosure.

Table 2A: Illustrative light chain CDR sequences for antibodies that specifically bind MAGE-A3$_{271-279}$ pMHC complex

Construct name	Parent Ab	Ab ID	VL-1	SEQ ID NO	VL-2	SEQ ID NO	VL-3	SEQ ID NO
C-000364	mAb1		RASQSISSYLN	8	AASSLQS	9	QQSYSTPLT	10
C-000365	mAb2		RASQSISSYLN	16	AASSLQS	17	QQSYSTPLT	18
C-000366	mAb3		RASQSISSYLN	24	AASSLQS	25	QQSYSTPLT	26
C-000367	mAb4		RASQSISSYLN	32	AASSLQS	33	QQSYSTPLT	34
C-000368	mAb5		RASQSISSYLN	40	AASSLQS	41	QQSYSTPLT	42
C-000369	mAb6		RASQSISSYLN	48	AASSLQS	49	QQSYSTPLT	50

Construct name	Parent Ab	Ab ID	VL-1	SEQ ID NO	VL-2	SEQ ID NO	VL-3	SEQ ID NO
C-000370	mAb7		RASQSISSYLN	56	AASSLQS	57	QQSYSTPLT	58
C-000371	mAb8		RASQSISSYLN	64	AASSLQS	65	QQSYSTPLT	66
C-000372	mAb9		RASQSISSYLN	72	AASSLQS	73	QQSYSTPLT	74
C-000373	mAb1	V10	RASQIYVSSYLN	80	AASSLQS	81	QQSYSTPLT	82
C-000374	mAb1	V12	RASQSASFVSSYLN	88	AASSLQS	89	QQSYSTPLT	90
C-000375	mAb1	V19	RASQPYVSSYLN	96	AASSLQS	97	QQSYSTPLT	98
C-000376	mAb1	V20	RASQLYVSSYLN	104	AASSLQS	105	QQSYSTPLT	106
C-000377	mAb1	V21	RASHPYVSSYLN	112	AASSLQS	113	QQSYSTPLT	114
C-000378	mAb1	V22	RASQSTLYVSSYLN	120	AASSLQS	121	QQSYSTPLT	122
C-000379	mAb1	V23	RASQSSDLVSSYLN	128	AASSLQS	129	QQSYSTPLT	130
C-000380	mAb1	V24	RASQSTLLVSSYLN	136	AASSLQS	137	QQSYSTPLT	138
C-000456	mAb1	V25	RASQSYVSGYLN	144	AASSLQS	145	QQSYSTPLT	146
C-000457	mAb1	V26	RASQFGFFVGGYLN	152	AASSLQS	153	QQSYSTPLT	154
C-000458	mAb2	V27	RASQSISSYLN	160	AASSLHYVS	161	QQSYSTPLT	162
C-000459	mAb2	V29	RASQSISSYLN	168	AASSLQS	169	QQSWASTPLT	170
C-000381	mAb4	V15	RASQSISSYLN	176	AASSLQS	177	QQSYSFVLT	178
C-000382	mAb4	V16	RASQSISSYLN	184	AASSLQS	185	QQSYGYVLT	186
C-000383	mAb4	V17	RASQSIGPFLSYLN	192	AASSLQS	193	QQSYSTPLT	194
C-000460	mAb4	V34	RASQSISSYLN	200	AASSLQS	201	QQSWGTPLT	202
C-000384	mAb6	V18	RASQSISSGLSYLN	208	AASSLQS	209	QQSYSTPLT	210
C-000461	mAb6	V36	RASQSISSGVSYLN	216	AASSLQS	217	QQSYSTPLT	218

Table 2B: Illustrative light chain CDR sequences for antibodies that specifically bind MAGE-A3$_{112-120}$ pMHC complex

Construct name	Parent Ab	Ab ID	VH-1	SEQ ID NO	VH-2	SEQ ID NO	VH-3	SEQ ID NO
C-000479	mAb22	V706	RASQSISSYLN	290	AASSLQS	293	QQSYSTPLT	301
C-000499	mAb2	V706	RASQSISSYLN	290	AASSLQS	293	QQSYSTPLT	301
C-001375	C-000479	V706	RASQSISSYLN	290	AASSLVQS	294	QQSYSTPLT	301
C-001376	C-000479	V706	RASQSISSYLN	290	AACSLQS	295	QQSYSTPLT	301
C-001505	C-000479		RASQSISSYLN	290	AACSLQS	295	QQSYSTPLT	301
C-001506	C-000479		RASQSISSYLN	290	AASSLQS	293	QQSYSSPLT	302
C-001507	C-000479		RASQSISSYLN	290	AASSLQS	293	QQSYRTPLT	303
C-001508	C-000479		RASQSISSYLN	290	AASSLQS	293	QQSYLTPLT	304
C-001509	C-000479		RASQSISSYLN	290	AASSLQS	293	QQSYSPPLT	305
C-001510	C-000479		RASVSISSYLN	291	AASSLQS	293	QQSYSTPLT	301
C-001511	C-000479		RASQSISSYLN	290	AASSLQS	293	QQSYSWPLT	306
C-001512	C-000479		RASQSISSYLN	290	AASSLQS	293	QQSYMTPLT	307
C-001539	C-000479		RASQSISSYLN	290	AAYVSSLQS	296	QQSYSTPLT	301
C-001540	C-000479		RASQSISSYLN	290	AASVSSLQS	297	QQSYSTPLT	301
C-001541	C-000479		RASQSISSYLN	290	AAETLQS	298	QQSYSTPLT	301
C-001542	C-000479		RASQSISSYLN	290	AASGIKASSLQS	299	QQSYSTPLT	301
C-001543	C-000479		RASQSISSYLN	290	AASSLQS	293	QQSWDTPLT	308
C-001544	C-000479		RASQSISSYLN	290	AASSLQS	293	QQIVSTPLT	309
C-001635	C-000479		RASQSISSYLN	290	AASSLQS	293	QQSWSTPLT	310
C-001637	C-000479		RASQSISSYLN	290	AAVSSLQS	300	QQSYSTPLT	301

C-001638	C-000479		RASLSISSYLN	292	AASSLQS	293	QQSYSTPLT	301
C-001640	C-000479		RASQSISSYLN	290	AASSLVQS	294	QQSYSTPLT	301

[0118] Tables 3A and 3B provide illustrative heavy and light chain sequences for antibodies according to the disclosure.

Table 3A: Illustrative full-length VH and VL chain sequences for antibodies that specifically bind MAGE-A3$_{271-279}$ pMHC complex

Construct name	Parent Ab	Ab ID	VH full length	SEQ ID NO	VL full length	SEQ ID NO
C-000364	mAb1		QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLEWIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYYCARDGDVPYWYFDLWGRGTLVTVSS	11	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIK	12
C-000365	mAb2		QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDLYSSSWYCDAFDIWGQGTMVTVSS	19	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIK	20
C-000366	mAb3		EVQLVQSGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITADTSTDTAYMEL	27	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGV	28

Construct name	Parent Ab	Ab ID	VH full length	SEQ ID NO	VL full length	SEQ ID NO
			SSLRSEDTAVYYCALLSGWYIDAFDIWG QGTMVTVSS		PSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVEIK	
C-000367	mAb4		QVQLQESGPGLVKPSDTLSLTCAVSGYSI SSSNWWGWIRQPPGKGLEWIGYIYYSGS TYYNPSLKSRVTMSVDTSKNQFSLKLSSV TAVDTAVYYCARIPFGDWWYFDLWGRG TLVTVSS	35	DIQMTQSPSSLSASVGDRVTITCRASQSIS SYLNWYQQKPGKAPKLLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVEIK	36
C-000368	mAb5		EVQLVESGGGLVQPGGSLRLSCAASGFTF SSYDMHWVRQATGKGLEWVSAIGTAGD TYYPGSVKGRFTISRENAKNSLYLQMNSL RAGDTAVYYCARDLYYDILTGYPDAFDI WGQGTMVTVSS	43	DIQMTQSPSSLSASVGDRVTITCRASQSIS SYLNWYQQKPGKAPKLLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVEIK	44
C-000369	mAb6		EVQLVESGGGLVQPGRSLRLSCAASGFTF DDYAMHWVRQAPGKGLEWVSGISWNSG SIGYADSVKGRFTISRDNAKNSLYLQMNS LRAEDTALYYCAKDSTSWVNFPYYMDV WGKGTTVTVSS	51	DIQMTQSPSSLSASVGDRVTITCRASQSIS SYLNWYQQKPGKAPKLLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVEIK	52
C-000370	mAb7		QLQLQESGPGLVKPSETLSLTCTVSGGSIS SSYYWGWIRQPPGKGLEWIGSIYYSGST YYNPSLKSRVTISVDTSKNQFSLKLSSVTA	59	DIQMTQSPSSLSASVGDRVTITCRASQSIS SYLNWYQQKPGKAPKLLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVEIK	60

Construct name	Parent Ab	Ab ID	VH full length	SEQ ID NO	VL full length	SEQ ID NO
			ADTAVYYCARRDGGNWYFDLWGRGTL VTVSS			
C-000371	mAb8		QVQLQESGPGLVKPSDTLSLTCAVSGYSI SSSNWWGWIRQPPGKGLEWIGYIYYSGS TYYNPSLKSRVTMSVDTSKNQFSLKLSSV TAVDTAVYYCARNTDPQLEWYFDLWGR GTLVTVSS	67	DIQMTQSPSSLSASVGDRVTITCRASQSIS SYLNWYQQKPGKAPKLLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVEIK	68
C-000372	mAb9		EVQLVESGGGLVQPGGSLRLSCAASGFTF SSYSMNWVRQAPGKGLEWVSYISSSSSTI YYADSVKGRFTISRDNAKNSLYLQMNSL RAEDTAVYYCARDDYSSEYFQHWGQGT LVTVSS	75	DIQMTQSPSSLSASVGDRVTITCRASQSIS SYLNWYQQKPGKAPKLLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVEIK	76
C-000373	mAb1	V10	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS SGGYSWSWIRQPPGKGLEWIGYIYHSGST YYNPSLKSRVTISVDRSKNQFSLKLSSVT AADTAVYYCARDGDVPYWYFDLWGRG TLVTVSS	83	DIQMTQSPSSLSASVGDRVTITCRASQIYV SSYLNWYQQKPGKAPKLLIYAASSLQSG VPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYSTPLTFGGGTKVEIK	84
C-000374	mAb1	V12	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS SGGYSWSWIRQPPGKGLEWIGYIYHSGST YYNPSLKSRVTISVDRSKNQFSLKLSSVT	91	DIQMTQSPSSLSASVGDRVTITCRASQSAS FVSSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYSTPLTFGGGTKVEIK	92

Construct name	Parent Ab	Ab ID	VH full length	SEQ ID NO	VL full length	SEQ ID NO
			AADTAVYYCARDGDVPYWYFDLWGRG TLVTVSS			
C-000375	mAb1	V19	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS SGGYSWSWIRQPPGKGLEWIGYIYHSGST YYNPSLKSRVTISVDRSKNQFSLKLSSVT AADTAVYYCARDGDVPYWYFDLWGRG TLVTVSS	99	DIQMTQSPSSLSASVGDRVTITCRASQPY VSSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYSTPLTFGGGTKVEIK	100
C-000376	mAb1	V20	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS SGGYSWSWIRQPPGKGLEWIGYIYHSGST YYNPSLKSRVTISVDRSKNQFSLKLSSVT AADTAVYYCARDGDVPYWYFDLWGRG TLVTVSS	107	DIQMTQSPSSLSASVGDRVTITCRASQLY VSSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYSTPLTFGGGTKVEIK	108
C-000377	mAb1	V21	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS SGGYSWSWIRQPPGKGLEWIGYIYHSGST YYNPSLKSRVTISVDRSKNQFSLKLSSVT AADTAVYYCARDGDVPYWYFDLWGRG TLVTVSS	115	DIQMTQSPSSLSASVGDRVTITCRASHPY VSSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYSTPLTFGGGTKVEIK	116
C-000378	mAb1	V22	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS SGGYSWSWIRQPPGKGLEWIGYIYHSGST YYNPSLKSRVTISVDRSKNQFSLKLSSVT	123	DIQMTQSPSSLSASVGDRVTITCRASQSTL YVSSYLNWYQQKPGKAPKLLIYAASSLQ SGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCQQSYSTPLTFGGGTKVEIK	124

Construct name	Parent Ab	Ab ID	VH full length	SEQ ID NO	VL full length	SEQ ID NO
			AADTAVYYCARDGDVPYWYFDLWGRG TLVTVSS			
C-000379	mAb1	V23	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS SGGYSWSWIRQPPGKGLEWIGYIYHSGST YYNPSLKSRVTISVDRSKNQFSLKLSSVT AADTAVYYCARDGDVPYWYFDLWGRG TLVTVSS	131	DIQMTQSPSSLSASVGDRVTITCRASQSSD LVSSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYSTPLTFGGGTKVEIK	132
C-000380	mAb1	V24	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS SGGYSWSWIRQPPGKGLEWIGYIYHSGST YYNPSLKSRVTISVDRSKNQFSLKLSSVT AADTAVYYCARDGDVPYWYFDLWGRG TLVTVSS	139	DIQMTQSPSSLSASVGDRVTITCRASQSTL LVSSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYSTPLTFGGGTKVEIK	140
C-000456	mAb1	V25	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS SGGYSWSWIRQPPGKGLEWIGYIYHSGST YYNPSLKSRVTISVDRSKNQFSLKLSSVT AADTAVYYCARDGDVPYWYFDLWGRG TLVTVSS	147	DIQMTQSPSSLSASVGDRVTITCRASQSY VSGYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYSTPLTFGGGTKVEIK	148
C-000457	mAb1	V26	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS SGGYSWSWIRQPPGKGLEWIGYIYHSGST YYNPSLKSRVTISVDRSKNQFSLKLSSVT	155	DIQMTQSPSSLSASVGDRVTITCRASQFGF FVGGYLNWYQQKPGKAPKLLIYAASSLQ SGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCQQSYSTPLTFGGGTKVEIK	156

Construct name	Parent Ab	Ab ID	VH full length	SEQ ID NO	VL full length	SEQ ID NO
			AADTAVYYCARDGDVPYWYFDLWGRG TLVTVSS			
C-000458	mAb2	V27	QVQLVQSGAEVKKPGASVKVSCKVSGYT LTELSMHWVRQAPGKGLEWMGGFDPED GETIYAQKFQGRVTMTEDTSTDTAYMEL SSLRSEDTAVYYCATDLYSSSWYCDAFDI WGQGTMVTVSS	163	DIQMTQSPSSLSASVGDRVTITCRASQSIS SYLNWYQQKPGKAPKLLIYAASSLHYVS GVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYSTPLTFGGGTKVEIK	164
C-000459	mAb2	V29	QVQLVQSGAEVKKPGASVKVSCKVSGYT LTELSMHWVRQAPGKGLEWMGGFDPED GETIYAQKFQGRVTMTEDTSTDTAYMEL SSLRSEDTAVYYCATDLYSSSWYCDAFDI WGQGTMVTVSS	171	DIQMTQSPSSLSASVGDRVTITCRASQSIS SYLNWYQQKPGKAPKLLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSWASTPLTFGGGTKVEIK	172
C-000381	mAb4	V15	QVQLQESGPGLVKPSDTLSLTCAVSGYSI SSSNWWGWIRQPPGKGLEWIGYIYYSGS TYYNPSLKSRVTMSVDTSKNQFSLKLSSV TAVDTAVYYCARIPFGDWWYFDLWGRG TLVTVSS	179	DIQMTQSPSSLSASVGDRVTITCRASQSIS SYLNWYQQKPGKAPKLLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSFVLTFGGGTKVEIK	180
C-000382	mAb4	V16	QVQLQESGPGLVKPSDTLSLTCAVSGYSI SSSNWWGWIRQPPGKGLEWIGYIYYSGS TYYNPSLKSRVTMSVDTSKNQFSLKLSSV	187	DIQMTQSPSSLSASVGDRVTITCRASQSIS SYLNWYQQKPGKAPKLLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYGYVLTFGGGTKVEIK	188

Construct name	Parent Ab	Ab ID	VH full length	SEQ ID NO	VL full length	SEQ ID NO
			TAVDTAVYYCARIPFGDWWYFDLWGRG TLVTVSS			
C-000383	mAb4	V17	QVQLQESGPGLVKPSDTLSLTCAVSGYSI SSSNWWGWIRQPPGKGLEWIGYIYYSGS TYYNPSLKSRVTMSVDTSKNQFSLKLSSV TAVDTAVYYCARIPFGDWWYFDLWGRG TLVTVSS	195	DIQMTQSPSSLSASVGDRVTITCRASQSIG PFLSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYSTPLTFGGGTKVEIK	196
C-000460	mAb4	V34	QVQLQESGPGLVKPSDTLSLTCAVSGYSI SSSNWWGWIRQPPGKGLEWIGYIYYSGS TYYNPSLKSRVTMSVDTSKNQFSLKLSSV TAVDTAVYYCARIPFGDWWYFDLWGRG TLVTVSS	203	DIQMTQSPSSLSASVGDRVTITCRASQSIS SYLNWYQQKPGKAPKLLIYAASSLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSWGTPLTFGGGTKVEIK	204
C-000384	mAb6	V18	EVQLVESGGGLVQPGRSLRLSCAASGFTF DDYAMHWVRQAPGKGLEWVSGISWNSG SIGYADSVKGRFTISRDNAKNSLYLQMNS LRAEDTALYYCAKDSTSWVNFPYYMDV WGKGTTVTVSS	211	DIQMTQSPSSLSASVGDRVTITCRASQSIS SGLSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYSTPLTFGGGTKVEIK	212
C-000461	mAb6	V36	EVQLVESGGGLVQPGRSLRLSCAASGFTF DDYAMHWVRQAPGKGLEWVSGISWNSG SIGYADSVKGRFTISRDNAKNSLYLQMNS	219	DIQMTQSPSSLSASVGDRVTITCRASQSIS SGVSYLNWYQQKPGKAPKLLIYAASSLQ SGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCQQSYSTPLTFGGGTKVEIK	220

Construct name	Parent Ab	Ab ID	VH full length	SEQ ID NO	VL full length	SEQ ID NO
			LRAEDTALYYCAKDSTSWVNFPYYMDV WGKGTTVTVSS			

Table 3B: Illustrative full-length VH and VL chain sequences for antibodies that specifically bind MAGE-A3$_{112-120}$ pMHC complex

Construct name	Parent Ab	VH full length	SEQ ID NO	VL full length	SEQ ID NO
C-000479	mAb22	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYST PLTFGGGTKVEIK	312
C-000499	mAb22	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYST PLTFGGGTKVEIK	312
C-001375	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLVQSGVPSR	313

Construct name	Parent Ab	VH full length	SEQ ID NO	VL full length	SEQ ID NO
		AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS		FSGSGSGTDFTLTISSLQPEDFATYYCQQSYS TPLTFGGGTKVEIK	
C-001376	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAACSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYST PLTFGGGTKVEIK	314
C-001505	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAACSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYST PLTFGGGTKVEIK	314
C-001506	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYSS PLTFGGGTKVEIK	315
C-001507	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYR TPLTFGGGTKVEIK	316

Construct name	Parent Ab	VH full length	SEQ ID NO	VL full length	SEQ ID NO
		AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS			
C-001508	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYLT PLTFGGGTKVEIK	317
C-001509	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYV TPLTFGGGTKVEIK	318
C-001510	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSLST PLTFGGGTKVEIK	319
C-001511	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSSST PLTFGGGTKVEIK	320

Construct name	Parent Ab	VH full length	SEQ ID NO	VL full length	SEQ ID NO
		AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS			
C-001512	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYLT PLTFGGGTKVEIK	317
C-001539	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYST PLSGTFGGGTKVEIK	321
C-001540	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYSP PLSGTFGGGTKVEIK	322
C-001541	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAAETLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYST PLTFGGGTKVEIK	323

Construct name	Parent Ab	VH full length	SEQ ID NO	VL full length	SEQ ID NO
		AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS			
C-001542	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASVSSLQSGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQS YSTPLTFGGGTKVEIK	324
C-001543	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQIVST PLTFGGGTKVEIK	325
C-001544	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSWD TPLTFGGGTKVEIK	326
C-001635	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT	311	DIQMTQSPSSLSASVGDRVTITCRASLSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYST PLTFGGGTKVEIK	327

Construct name	Parent Ab	VH full length	SEQ ID NO	VL full length	SEQ ID NO
		AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS			
C-001637	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASVSSLQSGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQS YSTPLTFGGGTKVEIK	324
C-001638	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSWS TPLTFGGGTKVEIK	328
C-001640	C-000479	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANY AQKFQGRVTITADESTSTAYMELSSLRSEDT AVYYCARDMDTFSMVTLFDYWGQGTLVT VSS	311	DIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSLST PLTFGGGTKVEIK	319

[0119] In some embodiments, an anti-MAGE-A3 antibody may comprise a sequence matching a motif or consensus sequence of antibodies provided herein. For example, mAb1, mAb2, mAb4, and mAb6 related antibodies share the same heavy chain CDR sequences, but differ in the light chain CDR sequences, but with a light chain CDR consensus as shown in Table 4A; mAb22 related

antibodies share the same heavy chain CDR sequences, but differ in the light chain CDR sequences, but with a light chain CDR consensus as shown in Table 4B. Consensus sequences may also be generated among different antibodies stemming from different parental antibodies. Consensus sequences are not limited to those provided herein. Methods of generating such sequences are known in the art and may be produced using, *e.g.*, multiple sequence alignment tools, such as Clustal-W.

Table 4A: Illustrative consensus light chain CDR sequences for antibodies that specifically bind MAGE-A3$_{271-279}$ pMHC complex

Antibody	VL-1	SEQ ID NO	VL-2	SEQ ID NO	VL-3	SEQ ID NO
mAb1 variants	RAS [Q/H] [S/P/I/L/F] [-/G/A/S/T] [-F/S/D/L] [-/Y/F/L] [I/V] [S/G] [S/G] VLN	224	AASSLQS	225	QQSYSTPLT	226
mAb2 variants	RASQSISSYLN	230	AASSL [Q/H] [-/Y] [-/V] S	231	QQS [Y/W] [-/A] STPLT	232
mAb4 variants	RASQSI [S/G] [-/P] [-/F] [-/L] SYLN	236	AASSLQS	237	QQS [Y/W] [S/G] [F/Y/T] [V/P] LT	238
mAb6 variants	RASQSISS [-/G] [-/L/V] [-/S] YLN	242	AASSLQS	243	QQSYSTPLT	244

Table 4B: Illustrative consensus light chain CDR sequences for antibodies that specifically bind MAGE-A3$_{112-120}$ pMHC complex

Antibody	VL-1	SEQ ID NO	VL-2	SEQ ID NO	VL-3	SEQ ID NO
mAb22 variants	RAS[L/Q/V]S[S/Y]L N	329	AA[-/S/Y][-/G/V][-/KA][C/E/S][S/T][L[-/V]QS	330	QQ[I/S][V/W/Y][S/D/L/M/R][T/P/S/W]PL T	331

Variations

[0120] In some embodiments, one or more amino acid residues in a CDR of the antibodies provided herein are substituted with another amino acid. The substitution may be "conservative" in the sense of being a substitution within the same family of amino acids. The naturally occurring amino acids may be divided into the following four families and conservative substitutions will take place within those families.

1) Amino acids with basic side chains: lysine, arginine, histidine.

2) Amino acids with acidic side chains: aspartic acid, glutamic acid

3) Amino acids with uncharged polar side chains: asparagine, glutamine, serine, threonine, tyrosine.

4) Amino acids with nonpolar side chains: glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, cysteine.

[0121] In another aspect, one or more amino acid residues are added to or deleted from one or more CDRs of an antibody. Such additions or deletions may occur at the N or C termini of the CDR or at a position within the CDR.

[0122] By varying the amino acid sequence of the CDRs of an antibody by addition, deletion or substitution of amino acids, various effects such as increased binding affinity for the target antigen may be obtained.

[0123] It is to be appreciated that antibodies of the disclosure comprising such varied CDR sequences may still bind MAGE-A3 with similar specificity and sensitivity profiles. This may be tested by way of the binding assays disclosed in Examples described herein. The constant regions of antibodies may also be varied. For example, antibodies may be provided with Fe regions of any isotype: IgA (IgA1, IgA2), IgD, IgE, IgG (IgG1, IgG2, IgG3, IgG4) or IgM.

[0124] One of skill will realize that conservative variants of the antibodies can be produced. Such conservative variants employed in antibody fragments, such as dsFv fragments or in scFv fragments, will retain critical amino acid residues necessary for correct folding and stabilizing between the V_H and the V_L regions. In some embodiments, the variants will retain the charge characteristics of the residues, for example.

[0125] Amino acid substitutions (such as at most one, at most two, at most three, at most four, at most five, at most six, at most seven, at most eight, at most nine, at most ten, at most 11, at most 12, at most 13, at most 14, at most 15, at most 16, at most 17, at most 18, at most 19, or at most 20 amino acid substitutions) can be made in the V_H and/or the V_L regions to increase yield. Conservative amino acid substitution tables providing functionally similar amino acids are well known to one of ordinary skill in the art. The following six groups are examples of amino acids that are considered to be conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

[0126] Amino acid substitutions, deletions, and additions, and other such sequence variations, may be performed based on sequence alignment techniques using existing sequence alignment tools.

Modifications

[0127] As used herein, the term "detectable label" refers to a molecule or material that can produce a detectable (such as visually, electronically or otherwise) signal that indicates the presence and/or concentration of the label in a sample. When conjugated to a specific binding molecule, the detectable label can be used to locate and/or quantify the target to which the specific binding molecule is directed. Thereby, the presence and/or concentration of the target in a sample can be detected by detecting the signal produced by the detectable label. A detectable label can be detected directly or indirectly, and several different detectable labels conjugated to different specific-binding molecules can be used in combination to detect one or more targets. For example, a first detectable label conjugated to an antibody specific to a target can be detected indirectly through the use of a second detectable label that is conjugated to a molecule that specifically binds the first detectable label. Multiple detectable labels that can be separately detected can be conjugated to different specific binding

molecules that specifically bind different targets to provide a multiplexed assay that can provide simultaneous detection of the multiple targets in a sample. A detectable signal can be generated by any mechanism including absorption, emission and/or scattering of a photon (including radio frequency, microwave frequency, infrared frequency, visible frequency and ultra-violet frequency photons). Detectable labels include colored, fluorescent, phosphorescent and luminescent molecules and materials, catalysts (such as enzymes) that convert one substance into another substance to provide a detectable difference (such as by converting a colorless substance into a colored substance or vice versa, or by producing a precipitate or increasing sample turbidity), haptens that can be detected through antibody-hapten binding interactions using additional detectably labeled antibody conjugates, and paramagnetic and magnetic molecules or materials. Non-limiting examples of detectable labels include enzymes such as horseradish peroxidase, alkaline phosphatase, acid phosphatase, glucose oxidase, β-galactosidase or β-glucuronidase; fluorphores such as fluoresceins, luminophores, coumarins, BODIPY dyes, resorufins, and rhodamines (many additional examples of fluorescent molecules can be found in *The Handbook—A Guide to Fluorescent Probes and Labeling Technologies*, Molecular Probes, Eugene, Oreg.); nanoparticles such as quantum dots (obtained, for example, from QuantumDot Corp, Invitrogen Nanocrystal Technologies, Hayward, Calif.; see also, U.S. Pat. Nos. 6,815,064, 6,682,596 and 6,649,138, each of which patents is incorporated by reference herein); metal chelates such as DOTA and DPTA chelates of radioactive or paramagnetic metal ions like Gd^{3+}; and liposomes, for example, liposomes containing trapped fluorescent molecules. Where the detectable label includes an enzyme, a detectable substrate such as a chromogen, a fluorogenic compound, or a luminogenic compound can be used in combination with the enzyme to generate a detectable signal (A wide variety of such compounds are commercially available, for example, from Invitrogen Corporation, Eugene Oreg.). Non-limiting examples of chromogenic compounds include diaminobenzidine (DAB), 4-nitrophenylphospate (pNPP), fast red, bromochloroindolyl phosphate (BCIP), nitro blue tetrazolium (NBT), BCIP/NBT, fast red, AP Orange, AP blue, tetramethylbenzidine (TMB), 2,2′-azino-di-[3-ethylbenzothiazoline sulphonate] (ABTS), o-dianisidine, 4-chloronaphthol (4-CN), nitrophenyl-β-D-galactopyranoside (ONPG), o-phenylenediamine (OPD), 5-bromo-4-chloro-3-indolyl-β-galactopyranoside (X-Gal), methylumbelliferyl-β-D-galactopyranoside (MU-

Gal), p-nitrophenyl-α-D-galactopyranoside (PNP), 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc), 3-amino-9-ethyl carbazol (AEC), fuchsin, iodonitrotetrazolium (INT), tetrazolium blue and tetrazolium violet. Alternatively, an enzyme can be used in a metallographic detection scheme. Metallographic detection methods include using an enzyme such as alkaline phosphatase in combination with a water-soluble metal ion and a redox-inactive substrate of the enzyme. The substrate is converted to a redox-active agent by the enzyme, and the redox-active agent reduces the metal ion, causing it to form a detectable precipitate. (See, for example, co-pending U.S. patent application Ser. No. 11/015,646, filed Dec. 20, 2004, PCT Publication No. 2005/003777 and U.S. Patent Application Publication No. 2004/0265922; each of which is incorporated by reference herein). Metallographic detection methods include using an oxido-reductase enzyme (such as horseradish peroxidase) along with a water soluble metal ion, an oxidizing agent and a reducing agent, again to form a detectable precipitate. (See, for example, U.S. Pat. No. 6,670,113, which is incorporated by reference herein). Haptens are small molecules that are specifically bound by antibodies, although by themselves they will not elicit an immune response in an animal and must first be attached to a larger carrier molecule such as a protein to generate an immune response. Examples of haptens include di-nitrophenyl, biotin, digoxigenin, and fluorescein. Additional examples of oxazole, pyrazole, thiazole, nitroaryl, benzofuran, triperpene, urea, thiourea, rotenoid, coumarin and cyclolignan haptens are disclosed in U.S. Provisional Patent Application No. 60/856,133, filed Nov. 1, 2006, which is incorporated by reference herein. In some embodiments, the detectable label comprises a non-endogenous hapten (*e.g.* not biotin), such as, for example, the haptens disclosed in U.S. Pat. Nos. 7,695,929, 8,618,265 and 8,846,320 (incorporated herein by reference), including for example pyrazoles, nitrophenyl compounds, benzofurazans, triterpenes, ureas and thioureas, rotenone and rotenone derivatives, oxazoles and thiazoles, coumarin and coumarin derivatives, and cyclolignans.

[0128] The antibodies of the present disclosure may be multimerized to increase the affinity for an antigen. The antibody to be multimerized may be one type of antibody or a plurality of antibodies which recognize a plurality of epitopes of the same antigen. As a method of multimerization of the antibody, binding of the IgG CH3 domain to two scF_v molecules, binding to streptavidin, introduction of a helix-turn-helix motif and the like can be exemplified.

[0129] The antibody compositions of the present disclosure may be in the form of a conjugate formed between any of these antibodies and another agent (immunoconjugate). In one aspect, the antibodies of the present disclosure are conjugated to radioactive material. In another aspect, the antibodies of the present disclosure can be bound to various types of molecules such as polyethylene glycol (PEG).

[0130] The disclosed monoclonal antibodies specific for MAGE-A3 can be conjugated to a therapeutic agent or effector molecule including, but are not limited to, molecules in which there is a covalent linkage of a therapeutic agent to an antibody. A therapeutic agent is an agent with a particular biological activity directed against a particular target molecule or a cell bearing a target molecule. One of skill in the art will appreciate that therapeutic agents can include various drugs such as vinblastine, daunomycin and the like, cytotoxins such as native or modified *Pseudomonas* exotoxin or Diphtheria toxin, encapsulating agents (such as liposomes) which themselves contain pharmacological compositions, radioactive agents such as ^{125}I, ^{32}P, ^{14}C, ^{3}H and ^{35}S and other labels, target moieties and ligands.

[0131] The choice of a particular therapeutic agent depends on the particular target molecule or cell, and the desired biological effect. Thus, for example, the therapeutic agent can be a cytotoxin that is used to bring about the death of a particular target cell (such as a tumor cell). Conversely, where it is desired to invoke a non-lethal biological response, the therapeutic agent can be conjugated to a non-lethal pharmacological agent or a liposome containing a non-lethal pharmacological agent.

[0132] With the therapeutic agents and antibodies described herein, one of skill can readily construct a variety of clones containing functionally equivalent nucleic acids, such as nucleic acids which differ in sequence but which encode the same effector moiety or antibody sequence. Thus, the present disclosure provides nucleic acids encoding antibodies and conjugates and fusion proteins thereof.

[0133] Effector molecules can be linked to an antibody of interest using any number of means known to those of skill in the art. Both covalent and noncovalent attachment means may be used. The procedure for attaching an effector molecule to an antibody varies according to the chemical structure of the effector. Polypeptides typically contain a variety of functional groups; such as carboxylic acid (COOH), free amine (—NH_2) or sulfhydryl (—

SH) groups, which are available for reaction with a suitable functional group on an antibody to result in the binding of the effector molecule. Alternatively, the antibody is derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of known linker molecules. The linker can be any molecule used to join the antibody to the effector molecule. The linker is capable of forming covalent bonds to both the antibody and to the effector molecule. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the antibody and the effector molecule are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (such as through a disulfide linkage to cysteine) or to the alpha carbon amino and carboxyl groups of the terminal amino acids.

[0134] In some circumstances, it is desirable to free the effector molecule from the antibody when the immunoconjugate has reached its target site. Therefore, in these circumstances, immunoconjugates will comprise linkages that are cleavable in the vicinity of the target site. Cleavage of the linker to release the effector molecule from the antibody may be prompted by enzymatic activity or conditions to which the immunoconjugate is subjected either inside the target cell or in the vicinity of the target site.

[0135] In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, label (such as enzymes or fluorescent molecules) drugs, toxins, and other agents to antibodies, one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or other polypeptide.

[0136] The antibodies or antibody fragments disclosed herein can be derivatized or linked to another molecule (such as another peptide or protein). In some cases, the antibody or antibody fragment (such as a VH domain) is fused to a heterologous protein, for example an Fc protein. In some embodiments, the antibody or antibody fragment is fused to a part of a chimeric antigen receptor (CAR) protein. In some embodiments, the antibody or antibody fragment is fused to a CD8 hinge, a CD28 transmembrane domain and a CD28-4-1BB-CD3ζ signaling domain.

[0137] In general, the antibodies or portion thereof is derivatized such that the binding to the target antigen is not affected adversely by the derivatization or labeling. For example, the antibody can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (for example, a bispecific antibody or a diabody), a detection agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

[0138] One type of derivatized antibody is produced by cross-linking two or more antibodies (of the same type or of different types, such as to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (such as m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (such as disuccinimidyl suberate). Such linkers are commercially available.

[0139] An antibody that binds (for example specifically binds) MAGE-A3 or a fragment thereof can be labeled with a detectable moiety. Non-limiting examples of detection agents include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like. Bioluminescent markers are also of use, such as luciferase, Green fluorescent protein (GFP), Yellow fluorescent protein (YFP). An antibody can also be labeled with enzymes that are useful for detection, such as horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When an antibody is labeled with a detectable enzyme, it can be detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is visually detectable. An antibody may also be labeled with biotin, and detected through indirect measurement of avidin or streptavidin binding. It should be noted that the avidin itself can be labeled with an enzyme or a fluorescent label.

[0140] An antibody may be labeled with a magnetic agent, such as gadolinium. Antibodies can also be labeled with lanthanides (such as europium and dysprosium), and manganese. Paramagnetic particles such as superparamagnetic iron oxide are also of use as labels. An

antibody may also be labeled with a predetermined polypeptide epitopes recognized by a secondary reporter (such as leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0141] An antibody can also be labeled with a radiolabeled amino acid. The radiolabel may be used for both diagnostic and therapeutic purposes. For instance, the radiolabel may be used to detect MAGE-A3 by x-ray, emission spectra, or other diagnostic techniques. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionucleotides: ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I.

[0142] An antibody can also be derivatized with a chemical group such as polyethylene glycol (PEG), a methyl or ethyl group, or a carbohydrate group. These groups may be useful to improve the biological characteristics of the antibody, such as to increase serum half-life or to increase tissue binding.

[0143] Toxins can be employed with the monoclonal antibodies described herein to produce immunotoxins. Exemplary toxins include ricin, abrin, diphtheria toxin and subunits thereof, as well as botulinum toxins A through F. These toxins are readily available from commercial sources (for example, Sigma Chemical Company, St. Louis, Mo.). Contemplated toxins also include variants of the toxins described herein (see, for example, see, U.S. Pat. Nos. 5,079,163 and 4,689,401). In some embodiments, the toxin is *Pseudomonas* exotoxin (PE) (U.S. Pat. No. 5,602,095). As used herein "*Pseudomonas* exotoxin" refers to a full-length native (naturally occurring) PE or a PE that has been modified. Such modifications can include, but are not limited to, elimination of domain Ia, various amino acid deletions in domains Ib, II and III, single amino acid substitutions and the addition of one or more sequences at the carboxyl terminus (for example, see Siegall et al., *J. Biol. Chem.* 264:14256-14261, 1989).

[0144] PE employed with the monoclonal antibodies described herein can include the native sequence, cytotoxic fragments of the native sequence, and conservatively modified variants of native PE and its cytotoxic fragments. Cytotoxic fragments of PE include those which are cytotoxic with or without subsequent proteolytic or other processing in the target cell. Cytotoxic fragments of PE include PE40, PE38, and PE35. For additional description of PE

and variants thereof, see for example, U.S. Pat. Nos. 4,892,827; 5,512,658; 5,602,095; 5,608,039; 5,821,238; and 5,854,044; PCT Publication No. WO 99/51643; Pai et al., *Proc. Natl. Acad. Sci. USA* 88:3358-3362, 1991; Kondo et al., *J. Biol. Chem.* 263:9470-9475, 1988; Pastan et al., *Biochim. Biophys. Acta* 1333:C1-C6, 1997.

[0145] The antibodies described herein can also be used to target any number of different diagnostic or therapeutic compounds to cells expressing MAGE-A3 or MAGE-A3 peptide-MHC complex. Thus, an antibody of the present disclosure can be attached directly or via a linker to a drug that is to be delivered directly to cells expressing cell-surface MAGE-A3 peptide-MHC complex. This can be done for therapeutic, diagnostic or research purposes. Therapeutic agents include such compounds as nucleic acids, proteins, peptides, amino acids or derivatives, glycoproteins, radioisotopes, lipids, carbohydrates, or recombinant viruses. Nucleic acid therapeutic and diagnostic moieties include antisense nucleic acids, derivatized oligonucleotides for covalent cross-linking with single or duplex DNA, and triplex forming oligonucleotides.

[0146] Alternatively, the molecule linked to an anti-MAGE-A3 antibody can be an encapsulation system, such as a liposome or micelle that contains a therapeutic composition such as a drug, a nucleic acid (for example, an antisense nucleic acid), or another therapeutic moiety that is preferably shielded from direct exposure to the circulatory system. Means of preparing liposomes attached to antibodies are well known to those of skill in the art (see, for example, U.S. Pat. No. 4,957,735; Connor et al., *Pharm. Ther.* 28:341-365, 1985).

[0147] Antibodies described herein can also be covalently or non-covalently linked to a detectable label. Detectable labels suitable for such use include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels include magnetic beads, fluorescent dyes (for example, fluorescein isothiocyanate, Texas red, rhodamine, green fluorescent protein, and the like), radiolabels (for example, ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (such as horseradish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (such as polystyrene, polypropylene, latex, and the like) beads.

[0148] Means of detecting such labels are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted illumination. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

CHIMERIC ANTIGEN RECEPTORS

[0149] The present disclosure provides chimeric antigen receptors (CARs) that bind to MAGE-A3. In some embodiments, the CAR binds to a MAGE-A3 peptide, the MAGE-A3 peptide comprising the sequence of FLWGPRALV (SEQ ID NO: 1). In some embodiments, the CAR binds to a peptide of SEQ ID NO: 1 within the context of a peptide-MHC complex. The CAR may comprise different domains, including an extracellular, antigen-binding domain; a hinge domain; a transmembrane domain; an intracellular signaling domain; and a co-stimulatory domain. It may comprise one or more of each of these domains.

[0150] In some embodiments, the CAR, or a host cell expressing such a CAR, binds to a MAGE-A3:pMHC with an EC_{50} of less than 10 nM. In some embodiments, the CAR, or a host cell expressing such a CAR, binds to a MAGE-A3:pMHC with an EC_{50} of less than 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM. In some embodiments the CAR, or a host cell comprising, binds to a MAGE-A3:pMHC with an EC_{50} of less than 5 nM. In some embodiments, the CAR shows a 1000-5000 fold improvement in EC_{50} compared to the parent antibody from which the antigen-binding domain is derived. In some embodiments, the CAR shows a 1000 fold improvement in EC_{50} compared to the parent antibody from which the antigen-binding domain is derived.

Antigen-binding domain

[0151] The antigen-binding domain of the CAR of the present disclosure may be derived from an anti-MAGE-A3 antibody or antigen-binding fragment thereof as disclosed herein. In some embodiments, the antibody binds to a MAGE-A3 peptide having the sequence of SEQ ID NO: 1. In some embodiments, the antibody binds to a MAGE-A3 peptide having the sequence of SEQ ID NO :1 within the context of a peptide-MHC complex. In some embodiments, the antigen-binding domain comprises an scFv fragment of an antibody

disclosed herein. In some embodiments, the antigen-binding domain comprises an scFab fragment of an antibody disclosed herein. In some embodiments, the antigen-binding domain comprises a single chain fragment antigen-binding fragment of an antibody according to the present disclosure. Examples of antibody fragments that may be comprised by the antigen-binding domain include, but are not limited to, fragment antigen-binding (Fab) fragments, single chain Fab (scFab) fragments, F(ab′)2 fragments, Fab′ fragments, Fv fragments, recombinant IgG (rIgG) fragments, single chain antibody fragments, single chain variable fragments (scFv), single domain antibodies (*e.g.*, sdAb, sdFv, nanobody) fragments, diabodies, and multi-specific antibodies formed from antibody fragments. In particular embodiments, the antibodies are single-chain antibody fragments comprising a variable heavy chain region and/or a variable light chain region, such as scFvs.

[0152] In some embodiments, the antigen-binding domain may comprise an scFv having a VH-linker-VL orientation. In some embodiments, the antigen-binding domain may comprise an scFv having a VL-linker-VH orientation.

[0153] In some embodiments, the antigen-binding domain further comprises a leader sequence or signal peptide. In embodiments where the antigen-binding domain comprises an scFv, the signal peptide may be positioned at the amino terminus of the scFv. In some embodiments, when the heavy chain variable region is N-terminal, the signal peptide may be positioned at the amino terminus of the heavy chain variable region. In some embodiments, when the light chain variable region is N-terminal, the signal peptide may be positioned at the amino terminus of the light chain variable region. The signal peptide may comprise any suitable signal peptide. In some embodiments, the signal peptide comprises the sequence of MDMRVPAQLLGLLLLWLRGARC (SEQ ID NO: 245).

Hinge domain

[0154] In some embodiments, the CAR comprises a linker, spacer, or hinge sequence between the antigen-binding domain and the transmembrane domain. One of ordinary skill in the art will appreciate that a hinge sequence is a short sequence of amino acids that, in at least some instances, facilitates flexibility (see, *e.g.*, Woof et al., Nat. Rev. Immunol., 4(2): 89-99 (2004)). The hinge sequence can be any suitable sequence derived or obtained from any suitable molecule. In some embodiments, the length of the hinge sequence may be optimized

based on the distance between the CAR and the MAGE-A3 or MAGE-A3 peptide-MHC complex (pMHC) binding epitope, *e.g.*, longer hinges may be optimal for membrane proximal MAGE-A3 or MAGE-A3 peptide-MHC complex (pMHC) epitopes.

[0155] In some embodiments, the CAR, such as the antigen-binding portion thereof, further includes a hinge, linker or spacer. The hinge may be derived from or include at least a portion of an immunoglobulin Fc region, for example, an IgG1 Fc region, an IgG2 Fc region, an IgG3 Fc region, an IgG4 Fc region, an IgE Fc region, an IgM Fc region, or an IgA Fc region. In certain embodiments, the spacer domain includes at least a portion of an IgG1, an IgG2, an IgG3, an IgG4, an IgE, an IgM, or an IgA immunoglobulin Fc region that falls within its CH2 and CH3 domains. In some embodiments, the spacer domain may also include at least a portion of a corresponding immunoglobulin hinge region. In some embodiments, the hinge is derived from or includes at least a portion of a modified immunoglobulin Fc region, for example, a modified IgG1 Fc region, a modified IgG2 Fc region, a modified IgG3 Fc region, a modified IgG4 Fc region, a modified IgE Fc region, a modified IgM Fc region, or a modified IgA Fc region. The modified immunoglobulin Fc region may have one or more mutations (*e.g.*, point mutations, insertions, deletions, duplications) resulting in one or more amino acid substitutions, modifications, or deletions that cause impaired binding of the spacer domain to an Fc receptor (FcR). In some aspects, the modified immunoglobulin Fc region may be designed with one or more mutations which result in one or more amino acid substitutions, modifications, or deletions that cause impaired binding of the spacer domain to one or more FcR including, but not limited to, FcγRI, FcγR2A, FcγR2B1, FcγR2B2, FcγR3A, FcγR3B, FcεRI, FcεR2, FcαRI, Fcα/μR, or FcRn.

[0156] In some aspects, a portion of the immunoglobulin constant region serves as a spacer region between the antigen-binding domain, *e.g.*, scFv, and transmembrane domain. The spacer can be of a length that provides for increased responsiveness of the cell following antigen-binding, as compared to in the absence of the spacer. Exemplary spacers include those having at least about 10 to 229 amino acids, about 10 to 200 amino acids, about 10 to 175 amino acids, about 10 to 150 amino acids, about 10 to 125 amino acids, about 10 to 100 amino acids, about 10 to 75 amino acids, about 10 to 50 amino acids, about 10 to 40 amino acids, about 10 to 30 amino acids, about 10 to 20 amino acids, or about 10 to 15 amino acids,

and including any integer between the endpoints of any of the listed ranges. In some embodiments, a spacer region has about 12 amino acids or less, about 119 amino acids or less, or about 229 amino acids or less. In some embodiments, the spacer is at or about 12 amino acids in length. Exemplary spacers include a CD28 hinge, IgG4 hinge alone, IgG4 hinge linked to CH2 and CH3 domains, or IgG4 hinge linked to the CH3 domain. Exemplary spacers include, but are not limited to, those described in Hudecek et al. (2013) Clin. Cancer Res., 19:3153, international patent application publication number WO2014031687, U.S. Pat. No. 8,822,647 or published app. No. US2014/0271635.

[0157] In some embodiments, the hinge sequence is derived from the human CD8-alpha molecule or a CD28 molecule. In some embodiments, the hinge sequence is derived from CD8-alpha.

[0158] Optionally, a short oligo- or polypeptide linker may form the linkage between the transmembrane domain and the intracellular signaling domain(s) of the CAR. A glycine-serine doublet may provide a suitable linker. Alternatively, or in addition, poly-glycine and poly-serine sequences may provide suitable linkers. In some embodiments, the polypeptide linker is between 2 and 10 amino acids in length.

Transmembrane domain

[0159] With respect to the transmembrane domain, the CAR can be designed to comprise a transmembrane domain that is fused to the antigen-binding domain of the CAR. In some embodiments, the transmembrane domain that naturally is associated with one of the domains in the CAR is used. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0160] The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Typically, the transmembrane domain denotes a single transmembrane alpha helix of a transmembrane protein, also known as an integral protein. Transmembrane regions of particular use in this invention may be derived from (*i.e.* comprise at least the transmembrane region(s) of) CD28, CD3 epsilon, CD4, CD5, CD8, CD9, CD16,

CD22, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD154, TCR alpha, TCR beta, or CD3 zeta and/or transmembrane regions containing functional variants thereof such as those retaining a substantial portion of the structural, *e.g.*, transmembrane, properties thereof.

[0161] Alternatively, the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. In some embodiments, a triplet of phenylalanine, tryptophan and valine can be used at one or both ends of a synthetic transmembrane domain. A transmembrane domain of the invention can be thermodynamically stable in a membrane. It may be a single alpha helix, a transmembrane beta barrel, a beta-helix of gramicidin A, or any other structure. In some embodiments, transmembrane helices are about 20 amino acids in length.

[0162] In some embodiments, the transmembrane domain in the CAR of the invention is the CD28 transmembrane domain.

Intracellular domain

[0163] The intracellular signaling domain or otherwise the cytoplasmic domain of the CAR of the invention triggers or elicits activation of at least one of the normal effector functions of the immune cell in which the CAR has been placed. The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus, the term "intracellular signaling domain" refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While the entire intracellular signaling domain may be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

[0164] Preferred examples of intracellular signaling domains for use in the CAR of the invention include the cytoplasmic sequences of the T cell receptor (TCR) 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.

[0165] Signals generated through one intracellular signaling domain alone may be insufficient for full activation of an immune cell, and a secondary or co-stimulatory signal may also be required. For example, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). Primary cytoplasmic signaling sequences regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

[0166] Examples of ITAM-containing primary cytoplasmic signaling sequences that are useful as intracellular signaling domains according to the present disclosure include those derived from an intracellular signaling domain of a lymphocyte receptor chain, a TCR/CD3 complex protein, an Fc receptor subunit, an IL-2 receptor subunit, CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, CD66d, CD278(ICOS), FcεRI, DAP10, and DAP12. In some embodiments, the intracellular signaling domain in the CAR of the invention comprises a cytoplasmic signaling sequence derived from CD3.

Co-stimulatory domains

[0167] In some embodiments, the CAR comprises a costimulatory domain. The costimulatory domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient response of lymphocytes to an antigen.

[0168] Various co-stimulatory domains have been reported to confer differing properties. For example, the 4-1BB co-stimulatory domain showed enhanced persistence in *in vivo* xenograph models (Milone et al. Mol Ther 2009; 17:1453-1464; Song et al. Cancer Res 2011; 71:4617-4627). Additionally, these different co-stimulatory domains produce different

cytokine profiles which, in turn, may produce effects on target cell-mediated cytotoxicity and the tumor microenvironment. DAP10 signaling in NK cells has been associated with an increase in Th1 and inhibition of Th2 type cytokine production in CD8+ T cells (Barber et al. Blood 2011; 117:6571-6581).

[0169] Non-limiting examples of co-stimulatory molecules include an MHC class I molecule, TNF receptor proteins, immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, a Toll ligand receptor, B7-H3, BAFFR, BTLA, BLAME (SLAMF8), CD2, CD4, CD5, CD7, CD8alpha, CD8beta, CD11a, LFA-1 (CD11a/CD18), CD11b, CD11c, CD11d, CD18, CD19, CD19a, CD27, CD28, CD29, CD30, CD40, CD49a, CD49D, CD49f, CD69, CD84, CD96 (Tactile), CD100 (SEMA4D), CD103, CRTAM, OX40 (CD134), 4-1BB (CD137), SLAM (SLAMF1, CD150, IPO-3), CD160 (BY55), SELPLG (CD162), DNAM1 (CD226), Ly9 (CD229), SLAMF4 (CD244, 2B4), ICOS (CD278), CEACAM1, CDS, CRTAM, DAP10, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB1, ITGB2, ITGB7, KIRDS2, LAT, LFA-1, LIGHT, LTBR, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), PAG/Cbp, PD-1, PSGL1, SLAMF6 (NTB-A, Ly108), SLAMF7, SLP-76, TNFR2, TRANCE/RANKL, VLA1, VLA-6, a ligand that specifically binds with CD83, and the like. Thus, while the invention is exemplified primarily with regions of CD28, and/or 4-1BB as the co-stimulatory signaling elements, other costimulatory elements are within the scope of the invention.

[0170] The cytoplasmic signaling sequences within the intracellular signaling domain of the CAR of the invention may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, may form the linkage. In some embodiments, the linker comprises glycine-serine doublet. In some embodiments, the linker is between 2 and 10 amino acids in length.

[0171] In some embodiments, the intracellular domain comprises the intracellular signaling domain of CD3-ζ and a costimulatory domain derived from CD28. In some embodiments, the intracellular domain comprises the intracellular signaling domain of CD3-ζ and a costimulatory domain derived from 4-1BB. In some embodiments, the intracellular domain

comprises the intracellular signaling domain of CD3-ζ and costimulatory domains derived from both CD28 and 4-1BB.

Further Modifications

[0172] The CARs of the present invention, nucleotide sequences encoding the same, vectors encoding the same, and cells comprising nucleotide sequences encoding said CARs may be further modified, engineered, optimized, or appended in order to provide or select for various features. These features may include, but are not limited to, efficacy, persistence, target specificity, reduced immunogenicity, multi-targeting, enhanced immune response, expansion, growth, reduced off-tumor effect, reduced subject toxicity, improved target cytotoxicity, improved tumor infiltration, detection, selection, targeting, and the like. For example, the cells may be engineered to express another CAR, a suicide mechanism, and may be modified to remove or modify expression of an endogenous receptor or molecule such as a TCR and/or MHC molecule.

[0173] In some embodiments, the vector or nucleic acid sequence encoding the CAR further encodes other genes. The vector or nucleic acid sequence may be constructed to allow for the co-expression of multiple genes using a multitude of techniques including co-transfection of two or more plasmids, the use of multiple or bidirectional promoters, or the creation of bicistronic or multicistronic vectors. The construction of multicistronic vectors may include the encoding of IRES elements.

[0174] The CAR expressing cell may further comprise a disruption to one or more endogenous genes. In some embodiments, the endogenous gene encodes TCRα, TCRβ, CD52, glucocorticoid receptor (GR), deoxycytidine kinase (dCK), or an immune checkpoint protein such as, for example, programmed death-1 (PD-1).

[0175] The CARs of the present invention and cells expressing these CARs may be further modified to improve efficacy against solid tumors. This increased efficacy may be measured by an increase in tumor cytotoxicity, tumor infiltration, and evasion of or resistance to tumor immunosuppressive mediators. In some embodiments, enhanced anti-tumor efficacy may be characterized by increased TCR signaling, increased cytokine release, enhanced killing of tumor cells, increased T cell infiltration of established tumors, improved tumor trafficking, attenuated tumor-induced hypofunction, and improved migration and chemotaxis.

[0176] In one aspect, the CAR expressing cells are further modified to evade or neutralize the activity of immunosuppressive mediators, including, but not limited to prostaglandin E2 (PGE2) and adenosine. In some embodiments, this evasion or neutralization is direct. In other embodiments, this evasion or neutralization is mediated via the inhibition of protein kinase A (PKA) with one or more binding partners, for example ezrin. In a specific embodiment, the CAR-expressing cells further express the peptide "regulatory subunit I anchoring disruptor" (RIAD). RIAD is thought to inhibit the association of protein kinase A (PKA) with ezrin, which thus prevents PKA-mediated inhibition of TCR activation (Newick et al. Cancer Res 2016 August; 76(15 Suppl): Abstract nr B27).

[0177] In some embodiments, the CAR expressing cells of the invention may induce a broad antitumor immune response consistent with epitope spreading.

[0178] In some embodiments, the CAR expressing cells of the invention further comprise a homing mechanism. For example, the cell may transgenically express one or more stimulatory chemokines or cytokines or receptors thereof. In particular embodiments, the cells are genetically modified to express one or more stimulatory cytokines. In certain embodiments, one or more homing mechanisms are used to render the inventive cells resistant to an inhibitory tumor microenvironment. In some embodiments, the CAR expressing cells are further modified to release inducible cytokines upon CAR activation, *e.g.*, to attract or activate innate immune cells to a targeted tumor (so-called fourth generation CARs or TRUCKS). In some embodiments, CARs may co-express homing molecules, *e.g.*, CCR4 or CCR2b, to increase tumor trafficking.

Controlling CAR Expression

[0179] In some instances, it may be advantageous to regulate the activity of the CAR or CAR expressing cells CAR. For example, inducing apoptosis using, *e.g.*, a caspase fused to a dimerization domain (see, *e.g.*, Di et al., N Engl. J. Med. 2011 Nov. 3; 365(18):1673-1683), can be used as a safety switch in the CAR therapy of the instant invention. In another example, CAR-expressing cells can also express an inducible Caspase-9 (iCaspase-9) molecule that, upon administration of a dimerizer drug (*e.g.*, rimiducid (also called AP1903 (Bellicum Pharmaceuticals) or AP20187 (Ariad)) leads to activation of the Caspase-9 and apoptosis of the cells. The iCaspase-9 molecule contains a chemical inducer of dimerization

(CID) binding domain that mediates dimerization in the presence of a CID. This results in inducible and selective depletion of CAR-expressing cells. In some cases, the iCaspase-9 molecule is encoded by a nucleic acid molecule separate from the CAR-encoding vector(s). In some cases, the iCaspase-9 molecule is encoded by the same nucleic acid molecule as the CAR-encoding vector. The iCaspase-9 can provide a safety switch to avoid any toxicity of CAR-expressing cells. See, *e.g.*, Song et al. Cancer Gene Ther. 2008; 15(10):667-75; Clinical Trial Id. No. NCT02107963; and Di Stasi et al. N. Engl. J. Med. 2011; 365:1673-83.

[0180] Alternative strategies for regulating the CAR therapy of the instant invention include utilizing small molecules or antibodies that deactivate or turn off CAR activity, *e.g.*, by deleting CAR-expressing cells, *e.g.*, by inducing antibody dependent cell-mediated cytotoxicity (ADCC). For example, CAR-expressing cells described herein may also express an antigen that is recognized by molecules capable of inducing cell death, *e.g.*, ADCC or compliment-induced cell death. For example, CAR expressing cells described herein may also express a receptor capable of being targeted by an antibody or antibody fragment. Examples of such receptors include EpCAM, VEGFR, integrins (*e.g.*, integrins αvβ3, α4, αI3/4β3, α4β7, α5β1, αvβ3, αv), members of the TNF receptor superfamily (*e.g.*, TRAIL-R1, TRAIL-R2), PDGF Receptor, interferon receptor, folate receptor, GPNMB, ICAM-1, HLA-DR, CEA, CA-125, MUC1, TAG-72, IL-6 receptor, 5T4, GD2, GD3, CD2, CD3, CD4, CD5, CD11, CD11a/LFA-1, CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/lgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41, CD44, CD51, CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5, CD319/SLAMF7, and EGFR, and truncated versions thereof (*e.g.*, versions preserving one or more extracellular epitopes but lacking one or more regions within the cytoplasmic domain). For example, CAR-expressing cells described herein may also express a truncated epidermal growth factor receptor (EGFR) which lacks signaling capacity but retains the epitope that is recognized by molecules capable of inducing ADCC, *e.g.*, cetuximab (ERBITUX.RTM.), such that administration of cetuximab induces ADCC and subsequent depletion of the CAR-expressing cells (see, *e.g.*, WO2011/056894, and Jonnalagadda et al., Gene Ther. 2013; 20(8)853-860).

[0181] In some embodiments, the CAR 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 cells comprising the polynucleotide, the suicide polypeptide may be expressed at the surface of a CAR cell. The suicide polypeptide may also comprise a signal peptide at the amino terminus. Another strategy includes expressing a highly compact marker/suicide gene that combines target epitopes from both CD32 and CD20 antigens in the CAR-expressing cells described herein, which binds rituximab, resulting in selective depletion of the CAR-expressing cells, *e.g.*, by ADCC (see, *e.g.*, Philip et al., Blood. 2014; 124(8)1277-1287). Other methods for depleting CAR-expressing cells described herein include administration of CAMPATH, a monoclonal anti-CD52 antibody that selectively binds and targets mature lymphocytes, *e.g.*, CAR-expressing cells, for destruction, *e.g.*, by inducing ADCC. In other embodiments, the CAR-expressing cell can be selectively targeted using a CAR ligand, *e.g.*, an anti-idiotypic antibody. In some embodiments, the anti-idiotypic antibody can cause effector cell activity, e.g, ADCC or ADC activities, thereby reducing the number of CAR-expressing cells. In other embodiments, the CAR ligand, *e.g.*, the anti-idiotypic antibody, can be coupled to an agent that induces cell killing, *e.g.*, a toxin, thereby reducing the number of CAR-expressing cells. Alternatively, the CAR molecules themselves can be configured such that the activity can be regulated, *e.g.*, turned on and off as described below.

[0182] In some embodiments, a regulatable CAR (RCAR) where the CAR activity can be controlled is desirable to optimize the safety and efficacy of a CAR therapy. In some embodiments, a RCAR comprises a set of polypeptides, typically two in the simplest embodiments, in which the components of a standard CAR described herein, *e.g.*, an antigen-binding domain and an intracellular signaling domain, are partitioned on separate polypeptides or members. In some embodiments, the set of polypeptides include a dimerization switch that, upon the presence of a dimerization molecule, can couple the polypeptides to one another, *e.g.*, can couple an antigen-binding domain to an intracellular signaling domain. Additional description and exemplary configurations of such regulatable CARs are provided herein and in International Publication No. WO 2015/090229, hereby incorporated by reference in its entirety.

[0183] In an aspect, an RCAR comprises two polypeptides or members: I) an intracellular signaling member comprising an intracellular signaling domain, *e.g.*, a primary intracellular signaling domain described herein, and a first switch domain; 2) an antigen-binding member comprising an antigen-binding domain, *e.g.*, that specifically binds a tumor antigen described herein, as described herein and a second switch domain. Optionally, the RCAR comprises a transmembrane domain described herein. In an embodiment, a transmembrane domain can be disposed on the intracellular signaling member, on the antigen-binding member, or on both. Unless otherwise indicated, when members or elements of an RCAR are described herein, the order can be as provided, but other orders are included as well. In other words, in an embodiment, the order is as set out in the text, but in other embodiments, the order can be different. *E.g.*, the order of elements on one side of a transmembrane region can be different from the example, *e.g.*, the placement of a switch domain relative to an intracellular signaling domain can be different, *e.g.*, reversed.

[0184] In some embodiments, the CAR expressing immune cell may only transiently express a CAR. For example, the cells of the invention may be transduced with mRNA comprising a nucleic acid sequence encoding an inventive CAR. In this vein, the present invention also includes an RNA construct that can be directly transfected into a cell. A method for generating mRNA for use in transfection involves in vitro transcription (IVT) of a template with specially designed primers, followed by polyA addition, to produce a construct containing 3' and 5' untranslated sequences ("UTRs"), a 5' cap and/or Internal Ribosome Entry Site (IRES), the nucleic acid to be expressed, and a polyA tail, typically 50-2000 bases in length. RNA so produced can efficiently transfect different kinds of cells. In some embodiments, the template includes sequences for the CAR. In an embodiment, an RNA CAR vector is transduced into a cell by electroporation.

[0185] The CAR expressing cells of the present invention may further comprise one or more additional CARs. These additional CARs may or may not be specific for MAGE-A3 or MAGE-A3 peptide-MHC complex. In some embodiments, the one or more additional CARs may act as inhibitory or activating CARs. In some aspects, the MAGE-A3-targeting or MAGE-A3 peptide-MHC complex-targeting CAR is the stimulatory or activating CAR; in other aspects, it is the costimulatory CAR. In some embodiments, the cells further include

inhibitory CARs (iCARs, see Fedorov et al., Sci. Transl. Medicine, 2013 December; 5(215): 215ra172), such as a CAR recognizing an antigen other than MAGE-A3 or MAGE-A3 peptide-MHC complex, whereby an activating signal delivered through the MAGE-A3-targeting or MAGE-A3 peptide-MHC complex-targeting CAR is diminished or inhibited by binding of the inhibitory CAR to its ligand, *e.g.*, to reduce off-target effects.

[0186] In some embodiments, the CAR expressing cells of the present invention may further comprise one or more additional CARs that may target one or more antigens selected from the group of: BCMA; BCR-Ab1; BST2; CAIX; CD19; CD20; CD22; CD123; CD171; CD30; CD33; CD38; CD44v6; CD44v7/8; CEA; CLL-1; EGFRvIII; EGP-2; EGP-40; ERBB2 (Her2/neu); EPCAM; fetal acetylcholine receptor, FBP; FLT3; Folate receptor alpha; GD2; GD3; Her3 (ErbB3); Her4 (ErbB4); k-light chain; KDR; MAD-CT-1; MAD-CT-2; MAGE-A1; MARTI; ML-IAP; MYCN; Oncofetal antigen (h5T4); NKG2D ligands PDK1; PDL1; PSCA; PSMA; PRSS21; ROR1; SLAMF7; TAG-72; Tn Ag; TSLPR; B7H3 (CD276); KIT (CD17); IL-13Ra2; Mesothelin; IL-11Ra; VEGFR2; LeY; CD24; PDGFR-beta; SSEA-4; CD20; MUC1; EGFR; NCAM; Prostase; PAP; ELF2M; Ephrin B2; FAP; IGF-1 receptor; CAFX; LMP2; gp100; tyrosinase; EphA2; Fucosyl GM1; sLe; ganglioside GM3; TGS5; HMWMAA; OAcGD2; OR51E2; Folate receptor beta; TEM1/CD248; TEM7R; CLDN6; TSHR; GloboH; GPR20; GPRC5D; CXORF61; CD97; CD179a; ADRB3; ALK; Polysialic acid; PANX3; PLAC1; NY-BR-1; NY-ESO-1; UPK2; TIM-1; HAVCR1; LY6K; TARP; WT1; LAGE-1a; ETV6-AML; SPA17; XAGE1; Tie 2; Fos-related antigen 1; p53; p53 mutant; prostein; surviving; telomerase; PCTA-1; Rat sarcoma Ras mutant; hTERT; sarcoma translocation breakpoints; ERG; NA17; PAX3; Androgen receptor; Cyclin B1; RhoC; TRP-2; CYP1B1; BORIS, SART3; PAX5; OY-TES1; LCK; AKAP-4; SSX2; RAGE-1; RU1; RU2; legumain; HPV E6; HPV E7; intestinal carboxyl esterase; mut hsp70-2; CD79a; CD79b; CD72; LAIR1; CD89; LILRA2; CD300LF; CLEC12A; EMR2; FCRL5; GPC3; IGLL1; and LY75.

[0187] In some embodiments, the antigen-binding domain of the inventive CAR is affinity tuned. In particular, the affinity of the anti-MAGE-A3 or anti-MAGE-A3:peptide-MHC complex CAR antigen-binding domain is adjusted to discriminate cells overexpressing MAGE-A3, *e.g.* tumor cells, from normal tissues which express low or minimal physiological

levels of MAGE-A3. This may be accomplished, *e.g.*, through the use of a CAR-expressing T cell with target antigen affinities varying over three orders of magnitude (Liu et al. Cancer Res 2015 September; 75(17):3596-607). Additionally, *in vivo* xenograft models may be used to evaluate the toxicity of affinity tuned anti-MAGE-A3 or anti-MAGE-A3:peptide-MHC complex CARs on normal human tissue (Johnson et al. Sci Transl Med 2015 February; 7(275):275ra22).

[0188] In some embodiments, the antigen-binding domain of the CAR is or is part of an immunoconjugate, in which the antigen-binding domain is conjugated to one or more heterologous molecule(s), such as, but not limited to, a cytotoxic agent, an imaging agent, a detectable moiety a multimerization domain or other heterologous molecule. Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.*, At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu); chemotherapeutic agents (*e.g.*, methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins. In some embodiments, the antigen-binding domain is conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (*e.g.*, protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

[0189] In some embodiments, the CAR expressing cells of the invention may be further genetically modified to express the dominant negative form of the transforming growth factor (TGF) beta receptor (DNR).

[0190] In another embodiment, the CAR expressing cell may be specific for another antigen, including a tumor antigen in some cases. In some embodiments, the transformed host cells may be selected for specificity for one or more strong viral antigens or may be transformed to exhibit specificity for these antigens. In specific embodiments, the cells are pp65CMV-specific T cells, CMV-specific T cells, EBV-specific T cells, Varicella Virus-specific T cells, Influenza Virus-specific T cells and/or Adenovirus-specific T cells.

[0191] To increase persistence, the cells of the invention may be further modified to overexpress pro-survival signals, reverse anti-survival signals, overexpress Bcl-xL, overexpress hTERT, lack Fas, or express a TGFβ dominant negative receptor. Persistence may also be facilitated by the administration of cytokines, *e.g.*, IL-2, IL-7, and IL-15.

Variations

[0192] Included in the scope of the invention are functional portions of the inventive CARs described herein. The term "functional portion" when used in reference to a CAR refers to any part or fragment of the CAR of the invention, which part or fragment retains the biological activity of the CAR of which it is a part (the parent CAR). Functional portions encompass, for example, those parts of a CAR that retain the ability to recognize target cells, or detect, treat, or prevent a disease, to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent CAR.

[0193] The functional portion can comprise additional amino acids at the amino or carboxyl terminus of the portion, or at both termini, which additional amino acids are not found in the amino acid sequence of the parent CAR. In some embodiments, the additional amino acids do not interfere with the biological function of the functional portion, *e.g.*, recognize target cells, detect cancer, treat or prevent cancer, etc. In some embodiments, the additional amino acids enhance the biological activity, as compared to the biological activity of the parent CAR.

[0194] Included in the scope of the invention are functional variants of the inventive CARs described herein. The term "functional variant" as used herein refers to a CAR, polypeptide, or protein having substantial or significant sequence identity or similarity to a parent CAR, which functional variant retains the biological activity of the CAR of which it is a variant. Functional variants encompass, for example, those variants of the CAR described herein (the parent CAR) that retain the ability to recognize target cells to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional variant can, for instance, be at least about 30%, 50%, 75%, 80%, 90%, 98% or more identical in amino acid sequence to the parent CAR.

[0195] A functional variant can, for example, comprise the amino acid sequence of the parent CAR with at least one conservative amino acid substitution. Alternatively or

additionally, the functional variants can comprise the amino acid sequence of the parent CAR with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent CAR.

[0196] Amino acid substitutions of the inventive CARs are preferably conservative amino acid substitutions. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same or similar chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic/negatively charged polar amino acid substituted for another acidic/negatively charged polar amino acid (*e.g.*, Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (*e.g.*, Ala, Gly, Val, Ile, Leu, Met, Phe, Pro, Trp, Cys, Val, etc.), a basic/positively charged polar amino acid substituted for another basic/positively charged polar amino acid (*e.g.* Lys, His, Arg, etc.), an uncharged amino acid with a polar side chain substituted for another uncharged amino acid with a polar side chain (*e.g.*, Asn, Gin, Ser, Thr, Tyr, etc.), an amino acid with a beta-branched side-chain substituted for another amino acid with a beta-branched side-chain (*e.g.*, Ile, Thr, and Val), an amino acid with an aromatic side-chain substituted for another amino acid with an aromatic side chain (*e.g.*, His, Phe, Trp, and Tyr), etc.

[0197] Also, amino acids may be added or removed from the sequence based on vector design.

[0198] The CAR can consist essentially of the specified amino acid sequence or sequences described herein, such that other components, *e.g.*, other amino acids, do not materially change the biological activity of the functional variant.

[0199] The CARs of embodiments of the invention (including functional portions and functional variants) can be of any length, *i.e.*, can comprise any number of amino acids, provided that the CARs (or functional portions or functional variants thereof) retain their biological activity, *e.g.*, the ability to specifically bind to antigen, detect diseased cells in a

mammal, or treat or prevent disease in a mammal, etc. For example, the CAR can be about 50 to about 5000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more amino acids in length.

[0200] The CARs of embodiments of the invention (including functional portions and functional variants of the invention) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

[0201] The CARs of embodiments of the invention (including functional portions and functional variants) can be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, *e.g.*, a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated, or undergo additional post-translational modifications.

[0202] The CARs of embodiments of the invention (including functional portions and functional variants thereof) can be obtained by methods known in the art. The CARs may be made by any suitable method of making polypeptides or proteins. Suitable methods of de novo synthesizing polypeptides and proteins are described in references, such as Chan et al., Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2000; Peptide and Protein Drug Analysis, ed. Reid, R., Marcel Dekker, Inc., 2000; Epitope Mapping, ed. Westwood et al., Oxford University Press, Oxford, United Kingdom, 2001; and U.S. Pat. No. 5,449,752. Also, polypeptides and proteins can be recombinantly produced using the nucleic acids described herein using standard recombinant methods. See, for

instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, N Y, 1994. Further, some of the CARs of the invention (including functional portions and functional variants thereof) can be isolated and/or purified from a source, such as a plant, a bacterium, an insect, a mammal, *e.g.*, a rat, a human, etc. Methods of isolation and purification are well-known in the art. Alternatively, the CARs described herein (including functional portions and functional variants thereof) can be commercially synthesized by companies. In this respect, the inventive CARs can be synthetic, recombinant, isolated, and/or purified.

NUCLEIC ACIDS AND VECTORS

[0203] The present disclosure also provides nucleic acids encoding an antibody, antigen-binding antibody fragment, or chimeric antigen receptor as disclosed herein. The present invention also provides vectors in which a nucleic acid of the present invention is inserted. Vectors derived from retroviruses are suitable tools to achieve long-term gene transfer since they allow for genetic stability and high expression, in addition to having a flexible genome. Furthermore, clinical experience with retroviral vectors provides guidance for optimizing efficacy and safety in their use.

[0204] In brief summary, the expression of natural or synthetic nucleic acids encoding CARs is typically achieved by operably linking a nucleic acid encoding the CAR polypeptide or portions thereof to a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration in eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

[0205] The expression constructs of the present invention may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, *e.g.*, U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties. In another embodiment, the invention provides a gene therapy vector.

[0206] The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a

phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[0207] Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, gammaretroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (*e.g.*, WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[0208] A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In some embodiments, adeno-associated virus vectors are used. In some embodiments, retrovirus vectors are used.

[0209] Additional promoter elements, *e.g.*, enhancers, regulate the frequency of transcriptional initiation. In some embodiments, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

[0210] Various promoter sequences may be used, including, but not limited to the immediate early cytomegalovirus (CMV) promoter, Elongation Growth Factor-1α (EF-1α), simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human

immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

[0211] In order to assess the expression of a CAR polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

[0212] Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, *e.g.*, enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5′ flanking region showing the highest level of expression of reporter gene is identified as the promoter.

Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

[0213] Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, *e.g.*, mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

[0214] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York). A preferred method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection.

[0215] Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, *e.g.*, human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[0216] Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle in vitro and in vive is a liposome (*e.g.*, an artificial membrane vesicle).

[0217] In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (in vitro, ex vivo or in vivo). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the

liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

[0218] Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma, St. Louis, Mo.; dicetyl phosphate ("DCP") can be obtained from K & K Laboratories (Plainview, N.Y.); cholesterol ("Choi") can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about −20 degrees Celsius. "Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., 1991 Glycobiology 5: 505-10). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

[0219] Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; "biochemical" assays, such as detecting the presence or absence of a particular peptide, *e.g.*, by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

HOST CELLS

[0220] Also provided are cells, cell populations, and compositions containing the cells, *e.g.*, cells comprising a nucleic acid sequence encoding an anti-MAGE-A3 or anti-MAGE-A3:peptide-MHC complex chimeric antigen receptor. Among the compositions are pharmaceutical compositions and formulations for administration, such as for adoptive cell therapy. Also provided are therapeutic methods for administering the cells and compositions to subjects, *e.g.*, patients.

[0221] Thus also provided are cells expressing the anti-MAGE-A3 or anti-MAGE-A3:peptide-MHC complex CARs. The cells generally are eukaryotic cells, such as mammalian cells, and typically are human cells, more typically primary human cells, *e.g.*, allogeneic or autologous donor cells. The cells for introduction of the CAR may be isolated from a sample, such as a biological sample, *e.g.*, one obtained from or derived from a subject. In some embodiments, the subject from which the cell is isolated is one having the disease or condition or in need of a cell therapy or to which cell therapy will be administered. The subject in some embodiments is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered. In some embodiments, the cells are derived from the blood, bone marrow, lymph, or lymphoid organs, are cells of the immune system, such as cells of the innate or adaptive immune systems, *e.g.*, myeloid or lymphoid cells, including lymphocytes, typically T cells and/or NK cells. Other exemplary cells include stem cells, such as multipotent and pluripotent stem cells, including induced pluripotent stem cells (iPSCs). The cells typically are primary cells, such as those isolated directly from a subject and/or isolated from a subject

and frozen. In some embodiments, the cells include one or more subsets of T cells or other cell types, such as whole T cell populations, $CD4^+$ cells, $CD8^+$ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for differentiation, expansion, recirculation, localization, and/or persistence capacities, antigen-specificity, type of antigen receptor, presence in a particular organ or compartment, marker or cytokine secretion profile, and/or degree of differentiation.

[0222] With reference to the subject to be treated, the cells may be allogeneic and/or autologous. Among the methods included are off-the-shelf methods. In some aspects, such as for off-the-shelf technologies, the cells are pluripotent and/or multipotent, such as stem cells, induced pluripotent stem cells (iPSCs), or T cells that either lack or are engineered to be deficient in T cell receptor function. In some embodiments, the methods include isolating cells from the subject, preparing, processing, culturing, and/or engineering them, as described herein, and re-introducing them into the same patient, before or after cryopreservation.

[0223] Among the sub-types and subpopulations of T cells and/or of $CD4^+$ and/or of $CD8^+$ T cells are naïve T (TN) cells, effector T cells (TEFF), memory T cells and sub-types thereof, such as stem cell memory T (TSCM), central memory T (TCM), effector memory T (TEM), or terminally differentiated effector memory T cells, tumor-infiltrating lymphocytes (TIL), immature T cells, mature T cells, helper T cells, cytotoxic T cells, mucosa-associated invariant T (MAIT) cells, naturally occurring and adaptive regulatory T (Treg) cells, helper T cells, such as TH1 cells, TH2 cells, TH3 cells, TH17 cells, TH9 cells, TH22 cells, follicular helper T cells, alpha/beta T cells, and delta/gamma T cells.

[0224] In some embodiments, the cells are natural killer (NK) cells, Natural Killer T (NKT) cells, cytokine-induced killer (CIK) cells, tumor-infiltrating lymphocytes (TIL), lymphokine-activated killer (LAK) cells, or the like. In some embodiments, the cells are monocytes or granulocytes, *e.g.*, myeloid cells, macrophages, neutrophils, dendritic cells, mast cells, eosinophils, and/or basophils.

[0225] In some embodiments, the cells are derived from cell lines, *e.g.*, T cell lines. The cells in some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, non-human primate, and pig.

METHODS OF USING ANTI-MAGE-A3:pMHC ANTIBODIES AND CARS

[0226] The methods of the present disclosure relate, in part, to the discovery by the present applicant that cross-reactivity to antigens other than MAGE-A12 peptide-MHC complexes may underly the failure of prior MAGE-A3 clinical trials. Thus, an advantage of some embodiments of the methods of the present disclosure is that they select for (or use known) polypeptides that specifically bind to a MAGE-A3 antigen or pMHC complex thereof over other peptide antigens beside MAGE-A12, in particular the EPSL8 peptide SAAELVHFL (SEQ ID NO: 333). The present applicant's recognition that the leading antibodies and receptors targeting MAGE-A3, which generally are specific to the peptide KVAELVHFL (SEQ ID NO: 332), have failed in clinical trials due to cross-reactivity with the EPSL8 peptide **SA**AELVHFL (SEQ ID NO: 333) rather than (as is the prevailing theory) due to cross-reactivity with the MAGE-A12 peptide K**M**AELVHFL (SEQ ID NO: 370) suggests the need to identify novel binding agents. This need is satisfied by the present disclosure. Significantly, this newly recognized mechanism cannot be identified by sequence comparison alone as the MAGE-A12 peptide has only one amino acid change (underlined) rather than two changes in the EPSL8 peptide.

[0227] Beyond using binding agents selected for specific binding to MAGE-A3$_{112-120}$ peptide KVAELVHFL (SEQ ID NO: 332) over EPSL8 peptide **SA**AELVHFL (SEQ ID NO: 333), the method of the present disclosure further comprise selection of a MAGE-A3 peptide other than MAGE-A3$_{112-120}$ as the target for therapy. Given the field's prior focus on the MAGE-A3$_{112-120}$ peptide, selection of other peptide targets, including but not limited to MAGE-A3$_{271-279}$ has surprising and unexpected benefits for therapy, such as reduced adverse events and toxicity of therapy. In some cases, experimental verification is necessary for selection of binding agents having the desired specificity for MAGE-A3 over EPSL8. The methods of the present disclosure permit selection of prior MAGE-A3 binding agents for use in treatment as well as the discovery of additional binding agent, as exemplified in Tables 1A through 4B.

[0228] In an aspect, the present disclosure provides methods of using the disclosed anti-MAGE-A3:pMHC antibodies and antigen binding fragments thereof for the treatment of cancer. In an aspect, the present disclosure provides methods of using the disclosed anti-MAGE-A3:pMHC chimeric antigen receptors (CARs) and cells comprising same for the

treatment of cancer. In some embodiments, the compositions of the present disclosure may be used in diagnostic methods to detect and/or quantify the presence of MAGE-A3 expressing cells.

Disease indications

[0229] Cancers that may be treated include tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. The cancers may comprise non-solid tumors (such as hematological tumors, for example, leukemias and lymphomas) or may comprise solid tumors. Types of cancers to be treated with the CARs of the invention include, but are not limited to, solid tumors, epithelial cancers, and hematological malignancies. In some embodiments, the cancer is a hematologic malignancy, multiple myeloma, an epithelial cancer, a solid tumor, melanoma, head and neck cancer, breast cancer, lung cancer, or synovial sarcoma. In some embodiments the cancer may be selected from a carcinoma, blastoma, and sarcoma, and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies *e.g.*, sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

[0230] Hematologic cancers are cancers of the blood or bone marrow. Examples of hematological (or hematogenous) cancers include leukemias, including acute leukemias (such as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma (indolent and high grade forms), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia.

[0231] Solid tumors are abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumors can be benign or malignant. Different types of solid tumors are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). Examples of solid tumors, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leciomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid

malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, seminoma, bladder carcinoma, melanoma, and CNS tumors (such as a glioma (such as brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme) astrocytoma, CNS lymphoma, germinoma, medulloblastoma, Schwannoma craniopharyogioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, neuroblastoma, retinoblastoma and brain metastases).

[0232] In some embodiments, the CAR expressing cells of the invention are used to treat a cancer the tumorous cells are positive for MAGE-A3 expression. In some embodiments, the cells of the invention may be used to treat MAGE-A3 positive breast cancer, skin cancer, and lung cancer. In general, MAGE-A3 positive tumor cells may be identified via known methods. For example, MAGE-A3 expression on tumor cells may be identified via immunofluorescence or flow cytometry using anti-MAGE-A3 antibodies. Alternatively, MAGE-A3 expression may be measured functionally through CAR cytotoxicity against target cells.

[0233] Methods of testing a CAR for the ability to recognize target cells and for antigen specificity are known in the art. For instance, Clay et al., J. Immunol., 163: 507-513 (1999), teaches methods of measuring the release of cytokines (*e.g.*, interferon-γ, granulocyte/monocyte colony stimulating factor (GM-CSF), tumor necrosis factor a (TNF-α) or interleukin 2 (IL-2)). In addition, CAR function can be evaluated by measurement of cellular cytotoxicity, as described in Zhao et al., J. Immunol., 174: 4415-4423 (2005).

[0234] A biopsy is the removal of tissue and/or cells from an individual. Such removal may be to collect tissue and/or cells from the individual in order to perform experimentation on the removed tissue and/or cells. This experimentation may include experiments to determine if the individual has and/or is suffering from a certain condition or disease-state. The condition or disease may be, *e.g.*, cancer. With respect to detecting the presence of MAGE-

A3 expressing tumor cells in a host, the sample comprising cells of the host can be a sample comprising whole cells, lysates thereof, or a fraction of the whole cell lysates, *e.g.*, a nuclear or cytoplasmic fraction, a whole protein fraction, or a nucleic acid fraction. If the sample comprises whole cells, the cells can be any cells of the host, *e.g.*, the cells of any organ or tissue, including blood cells or endothelial cells.

[0235] Melanoma-associated antigen (MAGE)-A3 and MAGE-C2 are antigens encoded by cancer-germline genes, and have been recognized as potential prognostic biomarkers and attractive targets for immunotherapy in multiple types of cancer. In non small cell lung cancer, MAGE-A3/C2 mRNA expression was identified in 73% (151/206) and 53% (109/206) of patients with NSCLC, respectively. MAGE-A3/C2 protein expression was identified in 58% (44/76) and 53% (40/76) of NSCLC cases, respectively. MAGE-A3 mRNA expression was observed to be associated with smoking history, disease stage and lymph node metastasis. MAGE-A3/C2-positive patients had a poorer survival rate compared with MAGE-A3/C2-negative patients. Multivariate analysis identified that MAGE-A3 expression may serve as an independent marker of poor prognosis in patients with NSCLC. Downregulation of MAGE-A3 mRNA expression in A549 cells resulted in lower migration and colony formation rates, and a higher amount of epithelial marker and lower amount of mesenchymal marker expression compared with the control group. These results indicate that MAGE-A3 serves a role in NSCLC cell metastasis through the induction of epithelial-mesenchymal transition. In conclusion, MAGE-A3 may serve as a diagnostic and prognostic biomarker for patients with NSCLC, due to its association with tumor progression and poor clinical outcome.

Subjects

[0236] The subject referred to herein may be any living subject. In some embodiments, the subject is a mammal. The mammal referred to herein can be any mammal. As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. The mammals may be from the order Carnivora, including Felines (cats) and Canines (dogs). The mammals may be from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). The mammals may

be of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes)

[0237] In some embodiments, the subject, to whom the cells, cell populations, or compositions are administered is a primate, such as a human. In some embodiments, the primate is a monkey or an ape. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some examples, the patient or subject is a validated animal model for disease, adoptive cell therapy, and/or for assessing toxic outcomes such as cytokine release syndrome (CRS).

[0238] In some embodiments, the subject has persistent or relapsed disease, *e.g.*, following treatment with another immunotherapy and/or other therapy, including chemotherapy, radiation, and/or hematopoietic stem cell transplantation (HSCT), *e.g.*, allogenic HSCT. In some embodiments, the administration effectively treats the subject despite the subject having become resistant to another therapy. In some embodiments, the subject has not relapsed but is determined to be at risk for relapse, such as at a high risk of relapse, and thus the compound or composition is administered prophylactically, *e.g.*, to reduce the likelihood of or prevent relapse.

[0239] In some embodiments, the methods include administration of CAR expressing cells or a composition containing the cells to a subject, tissue, or cell, such as one having, at risk for, or suspected of having a disease, condition or disorder associated with the expression of MAGE-A3. In some embodiments, the cells, populations, and compositions are administered to a subject having the particular disease or condition to be treated, *e.g.*, via adoptive cell therapy, such as adoptive T cell therapy. In some embodiments, the cells or compositions are administered to the subject, such as a subject having or at risk for the disease or condition. In some aspects, the methods thereby treat, *e.g.*, ameliorate one or more symptom of the disease or condition, such as by lessening tumor burden in a MAGE-A3 expressing cancer.

Dose, dosing, and administration

[0240] The compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired.

[0241] In general, administration may be topical, parenteral, or enteral.

[0242] In the case of adoptive cell therapy, methods for administration of cells for adoptive cell therapy are known and may be used in connection with the provided methods and compositions. For example, adoptive T cell therapy methods are described, *e.g.*, in US Patent Application Publication No. 2003/0170238 to Gruenberg et al; U.S. Pat. No. 4,690,915 to Rosenberg; Rosenberg (2011) Nat Rev Clin Oncol. 8(10):577-85). See, *e.g.*, Themeli et al. (2013) Nat Biotechnol. 31(10): 928-933; Tsukahara et al. (2013) Biochem Biophys Res Commun 438(1): 84-9; Davila et al. (2013) PLoS ONE 8(4): e61338.

[0243] The compositions of the invention are suitable for parenteral administration. As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue, thus generally resulting in the direct administration into the blood stream, into muscle, or into an internal organ. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal, intravenous, intraarterial, intrathecal, intraventricular, intraurethral, intracranial, intratumoral, intrasynovial injection or infusions; and kidney dialytic infusion techniques. In some embodiments, parenteral administration of the compositions of the present invention comprises intravenous or intraarterial administration.

[0244] The disclosure provides pharmaceutical compositions comprising polypeptides comprising the disclosed anti-MAGE-A3:pMHC antigen binding domains, anti-MAGE-A3:pMHC chimeric antigen receptors (CARs) and cells comprising same, and a pharmaceutically acceptable carrier, diluent or excipient.

[0245] Formulations of a pharmaceutical composition suitable for parenteral administration typically generally comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage

form, such as in ampoules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and the like. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In some embodiments of a formulation for parenteral administration, the active ingredient is provided in dry (*i.e.* powder or granular) form for reconstitution with a suitable vehicle (*e.g.* sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition. Parenteral formulations also include aqueous solutions which may contain excipients such as salts, carbohydrates and buffering agents, but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. Exemplary parenteral administration forms include solutions or suspensions in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, or in a liposomal preparation. Formulations for parenteral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

[0246] The terms "oral", "enteral", "enterally", "orally", "non-parenteral", "non-parenterally", and the like, refer to administration of a compound or composition to an individual by a route or mode along the alimentary canal. Examples of "oral" routes of administration of a composition include, without limitation, swallowing liquid or solid forms of a composition from the mouth, administration of a composition through a nasojejunal or gastrostomy tube, intraduodenal administration of a composition, and rectal administration, *e.g.*, using suppositories for the lower intestinal tract of the alimentary canal.

[0247] In some embodiments, the formulated composition comprising isolated anti-MAGE-A3 or anti-MAGE-A3:pMHC CAR-expressing cells is suitable for administration via injection.

[0248] Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids,

semi-solids, monophasic compositions, multiphasic compositions (*e.g.*, oil-in-water, water-in-oil), foams, microsponges, liposomes, nanoemulsions, aerosol foams, polymers, fullerenes, and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

[0249] Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

[0250] Compositions and formulations for parenteral, intrathecal, or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carder compounds and other pharmaceutically acceptable carriers or excipients.

[0251] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

[0252] The pharmaceutical compositions of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0253] The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, aerosols, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0254] In some embodiments of the present invention the pharmaceutical compositions may be formulated and used as foams. Pharmaceutical foams include formulations such as, but not limited to, emulsions, microemulsions, creams, jellies and liposomes. While basically similar in nature these formulations vary in the components and the consistency of the final product. Agents that enhance uptake of oligonucleotides at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (WO 97/30731), also enhance the cellular uptake of oligonucleotides.

[0255] The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

[0256] Formulations comprising anti-MAGE-A3 antibodies or anti-MAGE-A3:pMHC CAR expressing cells may include pharmaceutically acceptable excipient(s). Excipients included in the formulations will have different purposes depending, for example, on the CAR construct, the subpopulation of cells used, and the mode of administration. Examples of generally used excipients include, without limitation: saline, buffered saline, dextrose, water-for-infection, glycerol, ethanol, and combinations thereof, stabilizing agents, solubilizing agents and surfactants, buffers and preservatives, tonicity agents, bulking agents, and lubricating agents. The formulations comprising anti-MAGE-A3 antibodies or anti-MAGE-A3:pMHC CAR

expressing cells will typically have been prepared and cultured in the absence of any non-human components, such as animal serum (*e.g.*, bovine serum albumin).

[0257] The formulation or composition may also contain more than one active ingredient useful for the particular indication, disease, or condition being treated with the binding molecules or cells, preferably those with activities complementary to the binding molecule or cell, where the respective activities do not adversely affect one another. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. Thus, in some embodiments, the pharmaceutical composition further includes other pharmaceutically active agents or drugs, such as chemotherapeutic agents, *e.g.*, asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine, etc. In some embodiments, the pharmaceutically active agents or drugs may comprise immune checkpoint inhibitors, *e.g.*, drugs that target PD-1, MAGE-A3, PD-L2, LAG3, CTLA4, KIR, CD244, B7-H3, B7-H4, BTLA, HVEM, GAL9, TIM3, and/or A2aR. Examples of these inhibitors include, but are not limited to, pidilizumab, nivolumab, pembrolizumab, atezolizumab, MDX-1105, BMS-936559, MED14736, MPDL3280A, MSB0010718C, tremelimumab, and ipilimumab, which may be administered alone or in combination with other agents, *e.g.*, GM-CSF.

[0258] The pharmaceutical composition in some aspects can employ time-released, delayed release, and sustained release delivery systems such that the delivery of the composition occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. Many types of release delivery systems are available and known. Such systems can avoid repeated administrations of the composition, thereby increasing convenience to the subject and the physician.

[0259] Administration can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician.

[0260] The pharmaceutical composition in some embodiments contains the anti-MAGE-A3 antibodies or anti-MAGE-A3 CAR cells in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is monitored by periodic assessment of treated subjects. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and can be determined. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0261] In the context of antibody dosing, although the dose or the frequency of administration varies depending on the objective therapeutic effect, administration method, treating period, age, body weight and the like, in some embodiments, the dose may be between 10 μg/kg to 10 mg/kg per day.

[0262] In certain embodiments, in the context of genetically engineered cells expressing the CARs, a subject is administered the range of about one million to about 100 billion cells, such as, *e.g.*, 1 million to about 50 billion cells (*e.g.*, about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion cells, about 40 billion cells, or a range defined by any two of the foregoing values), such as about 10 million to about 100 billion cells (*e.g.*, about 20 million cells, about 30 million cells, about 40 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases about 100 million cells to about 50 billion cells (*e.g.*, about 120 million cells, about 250 million cells, about 350 million cells, about 450 million cells, about 650 million cells, about 800 million cells, about 900 million cells, about 3 billion cells, about 30 billion cells, about 45 billion cells) or any value in between these ranges, and/or such a number of cells per kilogram of body weight of the subject. For example, in some embodiments the administration of the cells or population of cells can comprise

administration of about 10.sup.3 to about 10.sup.9 cells per kg body weight including all integer values of cell numbers within those ranges.

[0263] The cells or population of cells can be administrated in one or more doses. In some embodiments, said effective amount of cells can be administrated as a single dose. In some embodiments, said effective amount of cells can be administrated 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 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 administrated 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 administrated parenterally. In some embodiments, administration can be an intravenous administration. In some embodiments, administration can be directly done by injection within a tumor.

[0264] For purposes of the invention, the amount or dose of the inventive CAR material administered should be sufficient to effect a therapeutic or prophylactic response in the subject or animal over a reasonable time frame. For example, the dose of the inventive CAR material should be sufficient to bind to antigen, or detect, treat or prevent disease in a period of from about 2 hours or longer, *e.g.*, about 12 to about 24 or more hours, from the time of administration. In certain embodiments, the time period could be even longer. The dose will be determined by the efficacy of the particular inventive CAR material and the condition of the animal (*e.g.*, human), as well as the body weight of the animal (*e.g.*, human) to be treated.

[0265] For purposes of the invention, an assay, which comprises, for example, comparing the extent to which target cells are lysed or IFN-γ is secreted by T cells expressing the inventive CAR, polypeptide, or protein upon administration of a given dose of such T cells to a mammal, among a set of mammals of which is each given a different dose of the T cells, could be used to determine a starting dose to be administered to a mammal. The extent to

which target cells are lysed or IFN-γ is secreted upon administration of a certain dose can be assayed by methods known in the art.

[0266] In some embodiments, the cells are administered as part of a combination treatment, such as simultaneously with or sequentially with, in any order, another therapeutic intervention, such as an antibody or engineered cell or receptor or agent, such as a cytotoxic or therapeutic agent. The cells or antibodies in some embodiments are co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. In some contexts, the cells are co-administered with another therapy sufficiently close in time such that the cell populations enhance the effect of one or more additional therapeutic agents, or vice versa. In some embodiments, the cells or antibodies are administered prior to the one or more additional therapeutic agents. In some embodiments, the cells or antibodies are administered after to the one or more additional therapeutic agents.

[0267] In embodiments, a lymphodepleting chemotherapy is administered to the subject prior to, concurrently with, or after administration (*e.g.*, infusion) of CAR cells. In an example, the lymphodepleting chemotherapy is administered to the subject prior to administration of the cells. For example, the lymphodepleting chemotherapy ends 1-4 days (*e.g.*, 1, 2, 3, or 4 days) prior to CAR cell infusion. In embodiments, multiple doses of CAR cells are administered, *e.g.*, as described herein. In embodiments, a lymphodepleting chemotherapy is administered to the subject prior to, concurrently with, or after administration (*e.g.*, infusion) of a CAR-expressing cell described herein. Examples of lymphodepletion include, but may not be limited to, nonmyeloablative lymphodepleting chemotherapy, myeloablative lymphodepleting chemotherapy, total body irradiation, etc. Examples of lymphodepleting agents include, but are not limited to, antithymocyte globulin, anti-CD3 antibodies, anti-CD4 antibodies, anti-CD8 antibodies, anti-CD52 antibodies, anti-CD2 antibodies, TCRαβ blockers, anti-CD20 antibodies, anti-CD19 antibodies, Bortezomib, rituximab, anti-CD 154 antibodies, rapamycin, CD3 immunotoxin, fludarabine, cyclophosphamide, busulfan, melphalan, Mabthera, Tacrolimus, alefacept, alemtuzumab, OKT3, OKT4, OKT8, OKT11, fingolimod, anti-CD40 antibodies, anti-BR3 antibodies, Campath-1H, anti-CD25 antibodies,

calcineurin inhibitors, mycophenolate, and steroids, which may be used alone or in combination.

Diagnostic indications

[0268] The antibodies of the disclosure are useful in methods known in the art relating to the localization and/or quantitation of a MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex (*e.g.*, for use in measuring levels of the MAGE-A3 polypeptide within appropriate physiological samples, for use in diagnostic methods, for use in imaging the polypeptide, and the like). The antibodies of the disclosure are useful in isolating a MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex by standard techniques, such as affinity chromatography or immunoprecipitation. A MAGE-A3 antibody of the disclosure can facilitate the purification of natural MAGE-A3 peptide-MHC complex from biological samples, *e.g.*, mammalian sera or cells as well as recombinantly-produced MAGE-A3 peptide-MHC complex expressed in a host system. Moreover, MAGE-A3 antibody can be used to detect a MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex (*e.g.*, in plasma, a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the polypeptide. The MAGE-A3 antibodies of the disclosure can be used diagnostically to monitor MAGE-A3 levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen. The detection can be facilitated by coupling (*i.e.*, physically linking) the MAGE-A3 antibody of this disclosure to a detectable substance.

[0269] The antibodies of the disclosure are useful for detection of MAGE-A3 Polypeptide or MAGE-A3 peptide-MHC complex. An exemplary method for detecting the level of MAGE-A3 polypeptides or MAGE-A3 peptide-MHC complex in a biological sample involves obtaining a biological sample from a subject and contacting the biological sample with a MAGE-A3 antibody of the present disclosure which is capable of detecting the MAGE-A3 polypeptides or MAGE-A3 peptide-MHC complex.

[0270] In one aspect, the MAGE-A3 antibodies or fragments thereof are detectably labeled. The term "labeled", with regard to the antibody is intended to encompass direct labeling of the antibody by coupling (*i.e.*, physically linking) a detectable substance to the antibody, as well as indirect labeling of the antibody by reactivity with another compound that is directly labeled. Non-limiting examples of indirect labeling include detection of a primary antibody

using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin.

[0271] The detection method of this disclosure can be used to detect expression levels of MAGE-A3 polypeptides or MAGE-A3 peptide-MHC complex in a biological sample in vitro as well as in vivo. In vitro techniques for detection of MAGE-A3 polypeptides or MAGE-A3 peptide-MHC complex include enzyme linked immunosorbent assays (ELISAs), Western blots, flow cytometry, immunoprecipitations, radioimmunoassay, and immunofluorescence (*e.g.*, IHC). Furthermore, in vivo techniques for detection of MAGE-A3 polypeptides or MAGE-A3 peptide-MHC complex include introducing into a subject labeled anti-MAGE-A3 antibody. By way of example only, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. In one aspect, the biological sample contains polypeptide molecules from the test subject.

[0272] Immunoassay and Imaging. A MAGE-A3 antibody of the present disclosure can be used to assay MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex levels in a biological sample (*e.g.*, a cell or tissue sample) using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistochemical (IHC) staining methods. Jalkanen, M. et al., *J. Cell. Biol.* 101: 976-985 (1985); Jalkanen, M. et al., *J. Cell. Biol.* 105: 3087-3096 (1987). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes or other radioactive agents, such as iodine (^{125}I, ^{121}I, ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99}mTc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0273] In addition to assaying MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex levels in a biological sample, MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex levels can also be detected in vivo by imaging. Labels that can be incorporated with anti-MAGE-A3 antibodies for in vivo imaging of MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex levels include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit

detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which can be incorporated into the MAGE-A3 antibody by labeling of nutrients for the relevant scF_v clone.

[0274] A MAGE-A3 antibody which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (*e.g.*, ^{131}I, ^{112}In, ^{99}mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (*e.g.*, parenterally, subcutaneously, or intraperitoneally) into the subject. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99}mTc. The labeled MAGE-A3 antibody will then preferentially accumulate at the location of cells which contain the specific target polypeptide or MAGE-A3 peptide-MHC complex. For example, in vivo tumor imaging is described in S. W. Burchiel et al., *Tumor Imaging: The Radiochemical Detection of Cancer* 13 (1982).

[0275] In some aspects, MAGE-A3 antibodies containing structural modifications that facilitate rapid binding and cell uptake and/or slow release are useful in in vivo imaging detection methods. In some aspects, the MAGE-A3 antibody contains a deletion in the CH2 constant heavy chain region of the antibody to facilitate rapid binding and cell uptake and/or slow release. In some aspects, a Fab fragment is used to facilitate rapid binding and cell uptake and/or slow release. In some aspects, a F(ab)′2 fragment is used to facilitate rapid binding and cell uptake and/or slow release.

[0276] The MAGE-A3 antibody compositions of the disclosure are useful in diagnostic and prognostic methods. As such, the present disclosure provides methods for using the antibodies of the disclosure useful in the diagnosis of MAGE-A3-related medical conditions in a subject. Antibodies of the disclosure may be selected such that they have a high level of epitope binding specificity and high binding affinity to the MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex. In general, the higher the binding affinity of an antibody, the more stringent wash conditions can be performed in an immunoassay to remove nonspecifically bound material without removing the target polypeptide. Accordingly, MAGE-A3 antibodies of the disclosure useful in diagnostic assays usually have binding

affinities of at least 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, or 10^{-12} M. In certain aspects, MAGE-A3 antibodies used as diagnostic reagents have a sufficient kinetic on-rate to reach equilibrium under standard conditions in at least 12 hours, at least 5 hours, at least 1 hour, or at least 30 minutes.

[0277] Some methods of the disclosure employ polyclonal preparations of anti-MAGE-A3 antibodies and anti-MAGE-A3 antibody compositions of the disclosure as diagnostic reagents, and other methods employ monoclonal isolates. In methods employing polyclonal human anti-MAGE-A3 antibodies prepared in accordance with the methods described above, the preparation typically contains an assortment of MAGE-A3 antibodies, *e.g.*, antibodies, with different epitope specificities to the target polypeptide. The monoclonal anti-MAGE-A3 antibodies of the present disclosure are useful for detecting a single antigen in the presence or potential presence of closely related antigens.

[0278] The MAGE-A3 antibodies of the present disclosure can be used as diagnostic reagents for any kind of biological sample. In one aspect, the MAGE-A3 antibodies disclosed herein are useful as diagnostic reagents for human biological samples. MAGE-A3 antibodies can be used to detect MAGE-A3 polypeptides or MAGE-A3 peptide-MHC complex in a variety of standard assay formats. Such formats include immunoprecipitation, Western blotting, ELISA, radioimmunoassay, flow cytometry, IHC and immunometric assays. See Harlow & Lane, *Antibodies, A Laboratory Manual* (Cold Spring Harbor Publications, New York, 1988); U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,879,262; 4,034,074, 3,791,932; 3,817,837; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876. Biological samples can be obtained from any tissue (including biopsies), cell or body fluid of a subject.

[0279] Prognostic Uses of MAGE-A3 Antibodies. The disclosure also provides for prognostic (or predictive) assays for determining whether a subject is at risk of developing a medical disease or condition associated with increased MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex expression or activity (*e.g.*, detection of a precancerous cell). Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a medical disease or condition characterized by or associated with MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex expression.

[0280] Another aspect of the disclosure provides methods for determining MAGE-A3 expression in a subject to thereby select appropriate therapeutic or prophylactic compounds for that subject.

[0281] Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing bladder transitional cell carcinoma, lung adenocarcinoma, breast ductal carcinoma, Hodgkin's lymphoma, pancreas adenocarcinoma, prostate adenocarcinoma, cervical squamous cell carcinoma, skin squamous cell carcinoma, and non-small cell lung cancer. Thus, the disclosure provides a method for identifying a disease or condition associated with increased MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex expression levels in which a test sample is obtained from a subject and the MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex detected, wherein the presence of increased levels of MAGE-A3 polypeptides or MAGE-A3 peptide-MHC complex compared to a control sample is predictive for a subject having or at risk of developing a disease or condition associated with increased MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex expression levels. In some aspects, the disease or condition associated with increased MAGE-A3 polypeptide expression or MAGE-A3 peptide-MHC complex levels is selected from the group consisting of bladder transitional cell carcinoma, lung adenocarcinoma, breast ductal carcinoma, Hodgkin's lymphoma, pancreas adenocarcinoma, prostate adenocarcinoma, cervical squamous cell carcinoma, skin squamous cell carcinoma, and non-small cell lung cancer.

[0282] In, another aspect, the disclosure provides methods for determining whether a subject can be effectively treated with a compound for a disorder or condition associated with increased MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex expression wherein a biological sample is obtained from the subject and the MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex is detected using the MAGE-A3 antibody. The expression level of the MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex in the biological sample obtained from the subject is determined and compared with the MAGE-A3 or MAGE-A3 peptide-MHC complex expression levels found in a biological sample obtained from a subject who is free of the disease. Elevated levels of the MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex in the sample obtained from the subject suspected of having the

disease or condition compared with the sample obtained from the healthy subject is indicative of the MAGE-A3-associated disease or condition in the subject being tested.

[0283] There are a number of disease states in which the elevated expression level of MAGE-A3 polypeptides or MAGE-A3 peptide-MHC complex is known to be indicative of whether a subject with the disease is likely to respond to a particular type of therapy or treatment. Thus, the method of detecting a MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex in a biological sample can be used as a method of prognosis, *e.g.*, to evaluate the likelihood that the subject will respond to the therapy or treatment. The level of the MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex in a suitable tissue or body fluid sample from the subject is determined and compared with a suitable control, *e.g.*, the level in subjects with the same disease but who have responded favorably to the treatment.

[0284] In one aspect, the present disclosure provides for methods of monitoring the influence of agents (*e.g.*, drugs, compounds, or small molecules) on the expression of MAGE-A3 polypeptides or MAGE-A3 peptide-MHC complex. Such assays can be applied in basic drug screening and in clinical trials. For example, the effectiveness of an agent to decrease MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex levels can be monitored in clinical trials of subjects exhibiting elevated expression of MAGE-A3, *e.g.*, patients diagnosed with cancer. An agent that affects the expression of MAGE-A3 polypeptides or MAGE-A3 peptide-MHC complex can be identified by administering the agent and observing a response. In this way, the expression pattern of the MAGE-A3 polypeptide or MAGE-A3 peptide-MHC complex can serve as a marker, indicative of the physiological response of the subject to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the subject with the agent.

[0285] In one aspect, the present disclosure provides for methods of monitoring or predicting the efficacy of therapeutic agents that target MAGE-A3 polypeptides or MAGE-A3 peptide-MHC complex.

[0286] Automated Embodiments. A person of ordinary skill in the art will appreciate that aspects of the methods for using the MAGE-A3 antibodies disclosed herein can be automated. Ventana Medical Systems, Inc. is the assignee of a number of United States patents disclosing systems and methods for performing automated analyses, including U.S.

Pat. Nos. 5,650,327, 5,654,200, 6,296,809, 6,352,861, 6,827,901 and 6,943,029, and U.S. published application Nos. 20030211630 and 20040052685, each of which is incorporated herein by reference. Particular aspects of MAGE-A3 staining procedures can be conducted using various automated processes.

Kits and Articles of Manufacture

[0287] The disclosure provides kits comprising polypeptides comprising the disclosed anti-MAGE-A3:pMHC antigen binding domains or CDRS, or polynucleotides or vectors encoding same.

[0288] The disclosure provides kits comprising the disclosed anti-MAGE-A3:pMHC antibodies or polynucleotides or vectors encoding same.

[0289] The disclosure provides kits comprising the disclosed anti-MAGE-A3:pMHC chimeric antigen receptors (CARs) or polynucleotides or vectors comprising same, or cells comprising same.

[0290] In some embodiments of the kits of the disclosure, the kits further comprise positive controls (e.g. MAGE-A3 antigen), negative controls, appropriate buffers and instructions for use.

EXAMPLES

EXAMPLE 1: Design of a synthetic IGH locus.

[0291] The human IGH locus is large and complex, spanning approximately 1.3 Mb and including V, D, J and constant domain sequences. The functional gene segments can be joined in more than 5000 different V-D-J combinations by recombination; however, the calculated theoretical complexity of the human antibody repertoire is greater than 10^{13}. This diversity is largely due to the action of terminal deoxynucleotidyl transferase (TdT), which introduces sequence diversity at V-D and D-J junctions.

[0292] *In vivo*, regulated control of recombination at the heavy chain locus allows for the productive use of multiple V, D and J segments. Regulation involves both the tight control of expression of the trans factors (RAG1, RAG2 and TdT) that mediate DNA recombination as well as the chromatin structures that mediate access to recombination signal sequences

(RSSs). This allows for sequential recombination to occur such that D-J precedes V-D recombination and the heavy chain precedes light chain recombination. The assembly of a functional B-cell receptor terminates the reaction and prevents further recombination from occurring at that locus. Without this regulation, recombination would proceed until all RSS pairs are consumed, causing the resulting repertoires to over utilize V and J segments distal to the D segments. Here, a heavy chain locus that can produce unbiased repertoires using a simple control system was designed to carry out V(D)J recombination in cultured, recombinant cells, which bypasses the complex signaling required to both detect a productive rearrangement and halt recombination *in vivo*.

[0293] FIG. 1 shows the organization of a representative engineered heavy chain locus. Features of the synthetic locus include the use of a strong promoter, a leader sequence derived from a commonly used V gene, a consensus Kozak sequence, codon-optimized V and constant region sequences and high efficiency RSSs. Together, these features contribute to robust rates of recombination and high levels of antibody expression (FIG. 2B). This synthetic locus is integrated into an engineered HEK293 cell line at a pre-selected chromosomal site using Cre-*lox* recombination.

[0294] While a 1-1-1 recombination substrate massively reduces the size of the *in vitro* locus, it comes at the cost of reduced segmental complexity. This problem was addressed by using a plasmid pool strategy where individual plasmids encode a locus with a single combination of V, D and J sequences but together the plasmid pool encodes all the combinations of functional gene segments found in the *in vivo* repertoire (FIG. 1B). Segmental complexity was further enhanced by using an expanded set of synthetic D sequences which are modified from germline by the randomization of five to twelve bases (A, T, G or C) in the middle of the sequence. When a plasmid pool is introduced into a cell population by Cre-mediated integration, individual cells receive a single plasmid from the pool encoding a randomly selected V, D and J segment; thus, each cell has the potential to express only one heavy chain gene. The cell population represents the entire segmental complexity present in the chosen V-D-J complement, whether it be that corresponding to a natural heavy chain locus or some other preferred combination or ratio of gene segments. With this approach, the size of the heavy chain locus is reduced from 1.3 Mb to approximately 5 kb per cell making it amenable

to standard molecular cloning techniques. The repertoires described in this report were generated using a single human light chain derived from IGKV1-39/J4.

EXAMPLE 2: Engineering a cell line capable of V(D)J recombination.

[0295] In the engineered host cells, *RAG1* is expressed by a cytomegalovirus (CMV) promoter under the control of a tetracycline-inducible regulatory system (Yao, F. et al. Human Gene Therapy 9, 1939-1950 (1998)) and *RAG2* is expressed by a constitutive CMV promoter. Tetracycline upregulates *RAG1* expression allowing for assembly of the RAG1/RAG2 complex. Regulation of RAG levels is important for ensuring that recombination occurs only when a culture has been scaled to the desired cell number, typically greater than ten billion cells, since premature recombination would otherwise limit the complexity of a nascent library and introduce clonal bias. Selection for cells encoding in-frame antibody genes without internal stop codons is made possible by the in-frame linkage of the heavy chain gene to a puromycin resistance marker via a transmembrane domain (TM) (FIG. 1). With this approach, antibody repertoires of more than a billion independently recombined functional antibody genes can be generated using standard culturing methods.

[0296] Junctional diversity is a major contributor to the sequence variation observed in natural antibody repertoires. This is due in part to the activity of TdT, which introduces non-templated nucleotides (N-nucleotides) at gene segment junctions during recombination. With the *in vitro* system, levels of TdT activity were achieved by randomly integrating a CMV promoter-driven *DNTT* gene into the host cell's chromosomal DNA. Additional TdT activity was provided by adding the *DNTT* expression cassette to the *in vitro* antibody locus.

EXAMPLE 3: Design, construction and analysis of a fully human antibody repertoire generated using V(D)J recombination in the engineered cell line.

[0297] FIG. 2A shows a representation of human germline V segments, D segments and human germline J segments. All segments were designed to be present at an even ratio. The *in vitro* antibody loci were introduced into a modified host HEK293 cell line using Cre-*lox* recombination. The resulting cells stably maintained the V, D and J segments in an unrecombined state as evidenced by the lack of antibody present on the cell surface (FIG. 2B). Following expansion of the culture to greater than twelve billion cells, tetracycline-mediated induction of V(D)J recombination for six days resulted in approximately 10% of

cells displaying cell-surface antibody. Subsequent selection with puromycin enriched the population for IgG positive cells resulting in a library of an estimated 1.2 billion independently recombined functional heavy chain sequences. Analysis by next generation sequencing (NGS) revealed that all 282 V-J combinations were present in the library. Most V segments were present near the intended frequency of 2.1% (FIG. 3A). Similarly, J segment usage was also near the intended frequency of 17% (FIG. 3B). CDR-H3 length and amino acid composition were also analyzed and were comparable to that observed in a human CDR-H3 database (FIG. 3C and FIG. 3D). Taken together, these results demonstrate that a diverse human antibody repertoire can be generated *in vitro* using V(D)J recombination.

EXAMPLE 4: Generation of fully human antibodies specific for MAGE-A3$_{279-279}$/HLA-A*02:01 complexes.

[0298] The HuTarg system was used to generate novel, fully human antibodies specific for HLA-A*02:01 restricted MAGE-A3 peptide-MHC complex, wherein the peptide had the sequence of FLWGPRALV (SEQ ID NO: 1). A cell line library of approximately one billion independently recombined human antibodies was sorted by flow cytometry for binding to MAGE-A3/HLA-A*02:01 (FIG. 4A). At each round, a different control pMHC complex was used to gate against off-target binding (PSA-1 or NY-ESO-1 complexed with HLA-A*02:01). In addition, a different fluorescent dye was used to detect on-target binding. This served as another measure to prevent off target cells from being enriched. After three rounds, enriched cells were sorted for next generation sequencing (NGS) analysis to determine the sequence of antibodies that bound specifically to MAGE-A3/HLA-A*02:01. FIG. 4B shows the characterization of the enriched library by FACS for membrane-anchored antibody expression (anti-human kappa-PE) and binding to on target (MAGE-A3/HLA-A*02:01) or off target (NY-ESO-1 or HIV-1 gag p17/HLA-A*02:01) pMHC complex, and the gates and percentage of cells sorted for subsequent NGS analysis. A diverse set of more than 100 novel antibody sequences was identified with most V segments and all J segments in the library represented. CDR-H3 sequences varied in length between seven and twenty-one amino acids (median of thirteen). FIG. 4C shows the binding profiles of antibodies that recognize MAGE-A3/HLA-A*02:01 and not PSA-1/ HLA-A*02:01. These antibodies were also shown not to bind to CMV pp65/HLA-A*02:01, a second control pMHC complex. Cells transfected with membrane-anchored antibody constructs were analyzed by flow cytometry for expression

(anti-human kappa-PE) and binding to biotinylated MAGE-A3/HLA-A*02:01 (blue) or PSA-1/HLA-A*02:01 (red) complexed with SA-Alexa Fluor® 647.

EXAMPLE 5: Affinity maturation of MAGE-A3/HLA-A*02:01-specific antibodies using *in vitro* V(D)J recombination.

[0299] By targeting mutations to the light chain CDR sequences using RAG-mediated recombination, the antibodies were diversified by affinity maturation. DNA constructs encoding a heavy chain and the common light chain were modified to include an RSS cassette within CDR-L1, CDR-L2 or CDR-L3. The constructs were pooled and then stably integrated into modified HEK293 cells using Cre-mediated recombination. Since each cell integrates a single antibody construct, each cell generates antibody diversified at a single light chain CDR location following recombination. Expansion of the culture to approximately six billion cells followed by RAG-mediated recombination and puromycin-mediated selection for in-frame antibody genes generated a library of an estimated 800 million independently recombined antibodies. After three rounds of enrichment by flow cytometry, the enriched pool was characterized for on target (MAGE-A3/HLA-A*02:01) and off target (NY-ESO-1/HLA-A*02:01) binding, and a higher affinity MAGE-A3/HLA-A*02:01 binding population was observed (FIG. 5A). Mab3 variants isolated from the library were confirmed to bind to MAGE-A3/HLA-A*02:01 with higher affinity than their parent (FIG. 5B). In addition, binding to NY-ESO-1/HLA-A*02:01 was not observed, confirming that the antibodies maintained their specificity.

EXAMPLE 6: Characterization of CAR-T cells derived from MAGE-A3$_{271-279}$/HLA-A*02:01-specific antibodies

[0300] Mab3 and three of its affinity matured derivatives were subsequently made as recombinant CARs and subjected to a T2/Jurkat-NFAT assay to assess function. CAR constructs contained a single-chain antigen binding fragment (scFab) domain, a CD8 hinge, a CD28 transmembrane domain and a CD28-4-1BB-CD3ζ signaling domain. All four CAR-Ts were activated by T2 cells loaded with MAGE-A3 peptide. In addition, T cells expressing CARs derived from affinity matured antibodies showed a greater than 1500-fold increase in potency compared to their parent (FIG. 6A).

[0301] In order to evaluate the specificity of the high potency CARs for MAGE-A3/HLA-A*02:01, closely related peptides differing by one amino acid (MAGE-A1/A4/A8 and MAGE-A2/A6), two amino acids (MAGE-A10/A11) or four amino acids (MAGE-A9) were used in the T2/Jurkat-NFAT functional assay. Each CAR-T variant showed preferential activation by T2 cells loaded with MAGE-A3 derived peptide, even when compared to cells loaded with peptides that differed by a single amino acid (FIG. 6B). All peptides were confirmed to load onto T2 cells to a similar extent, ruling out differences in peptide loading as a possible experimental artifact (FIG. 7). Briefly, peptides at 0 μM, 0.015 μM, 0.137 μM, 1.2 μM, 11 μM or 100 μM were loaded on T2 cells. Next, cells were incubated with 1 μg/ml Alexa Fluor® 647 anti-human HLA-A,B,C antibody (Biolegend 311414, clone W6/32) in RPMI1640 medium with 1% (w/v) BSA for one hour at 37°C. Fluorescence signal was measured by FACS after two washes in PBS with 1% (w/v) BSA. Similar levels of MHC stabilization were observed with all peptides, confirming similar loading across peptide variants.

EXAMPLE 7: Generation of fully human antibodies specific for MAGE-A3$_{112-120}$/HLA-A*02:01 complexes.

[0302] The HuTarg system was used to generate novel, fully human antibodies specific for HLA-A*02:01 restricted MAGE-A3 peptide-MHC complex, wherein the peptide had the sequence of KVAELVHFL (SEQ ID NO: 332). A cell line library of approximately one billion independently recombined human antibodies was sorted by flow cytometry for binding to MAGE-A3$_{112-120}$/HLA-A*02:01. At each round, a different control pMHC complex was used to gate against off-target binding (CMV or NY-ESO-1 complexed with HLA-A*02:01). In addition, a different fluorescent dye was used to detect on-target binding. This served as another measure to prevent off target cells from being enriched. After three rounds, enriched cells were sorted for next generation sequencing (NGS) analysis to determine the sequence of antibodies that bound specifically to MAGE-A3/HLA-A*02:01. A diverse set of 142 novel antibody sequences was identified with most V segments and all J segments in the library represented.

[0303] Selected members of this set of 142 antibodies were tested against various peptide MHC antigens. Table 5 shows EC$_{50}$ values for EPS8L2 peptide SAAELVHFL (SEQ ID NO:

333) in complex with HLA-A*02:01 compared to EC_{50} values for MAGE-$A3_{112-120}$/HLA-A*02:01 EC_{50} is reported in micromolar, with lower numbers indicating stronger binding. Only C-001511 showed high specificity for MAGE-$A3_{112-120}$/HLA-A*02:01 over EPS8L2/HLA-A*02:01.

[0304] Table 5

Antibody ID	EPS8L2	MAGE-$A3_{120-120}$
C-000472	0.025	0.91
C-000475	100	9.6
C-000477	0.46	0.37
C-001501	0.16	0.017
C-001511	**4000**	**0.046**
C-001545	0.9	0.028
C-001546	1.6	0.013
C-001636	2.2	0.03
C-001642	100	0.026
C-001643	2	0.0077
C-001644	0.046	0.0043
C-001649	28	0.075

[0305] C-1511 is representative of the antibodies disclosed in Tables 1B, 2B, 3B, and 4B, for which confirmatory test using the same methods is being done. Experiments with C-1511 in a chimeric antigen receptor format are described in Example 8.

[0306] Further rounds of selection and affinity maturation are being performed to identify other antibodies sequences with similar properties.

EXAMPLE 8: Demonstration of fully human antibodies specific for MAGE-$A3_{112-120}$/HLA-A*02:01 complexes in CAR-T cell compositions.

[0307] MAGE-A3-directed cancer therapeutics have had limited clinical success because of neurotoxicity. The safety problems were hypothesized to originate from off-target TCR activity against a closely related MAGE-A12 peptide. Surprisingly, the inventors have

discovered alternative related peptide from EPS8L2 that may be responsible. Given the qualities of MAGE-A3 as an onco-testis antigen widely expressed in tumors and largely absent from normal adult tissues MAGE-A3 remains a putative cancer target. A MAGE-A3 targeting immunotherapy that does not bind the EPS8L2 peptide can overcome limitations of previous MAGE-A3 targeting therapies. Two MAGE-A3 pMHC-directed CARs were isolated, one targeting the same peptide as the clinical TCR. Both CARs have improved selectivity over the EPS8L2 peptide that represents a significant risk for MAGE-A3-targeted therapeutics, showing that there may be other options for MAGE-A3 cell therapy.

[0308] INTRODUCTION

[0309] Off-target toxicity is one of the major risks of investigational therapies. Adoptive transfer of engineered T cells, which reached a milestone recently with the approval of two CD19-directed cancer therapeutics, is no different. Indeed, there are several examples of toxicities—including fatal ones—believed to be caused by therapeutic administration of investigational T cell therapeutics. The origin of the toxicity has been ascribed to homologous peptides that bind the HLA class I allele of the on-target peptide-MHC complex (pMHC) and cross-react with the affinity-enhanced TCR used to create the therapeutic candidate.

[0310] In one of these cases, a TCR-T containing a TCR (A118T) generated by immunization of an HLA-A*2:01 transgenic mouse with a peptide derived from the cancer-testis antigen MAGE-A3 (residues 112–120, KVAELVHFL (SEQ ID NO: 332)) was terminated during Ph 1 clinical study despite early signs of efficacy (Morgan et al. *J. Immunother.* 36:133-151 (2013)). Three of 9 cancer patients treated with the TCR-T developed severe neurotoxicity, and though each patient's history contained complicating factors, the toxicity was classified as treatment-related, and the investigators discontinued the trial for safety reasons. Histopathology supported the view that the TCR-Ts caused T-cell infiltration in the brain.

[0311] To explore the basis for toxicity, the investigators considered a variety of possibilities, foremost among them, TCR cross-reaction with MAGE-family members potentially expressed in the brain. Of these paralogs, MAGE-A12 was considered a prime suspect, as strong TCR cross-reactivity with the homologous peptide that differs by only one residue from the MAGE-A3 peptide had been noted during the original characterization of this TCR

(Chinnasamy et al. *J. Immunol.* 186:685-696 (2011)). The authors conducted a thorough study of MAGE-A12 expression in the brain using a variety of methods, including three different types of mRNA quantification and immunohistochemistry (IHC). They concluded that MAGE-A12 cross-reaction was the best hypothesis for the cause of the neurotoxicity induced by the TCR because: (i) all MAGE-family members, including MAGE-A3, are expressed at low or undetectable levels in the brain, with the exception of MAGE-A12, whose expression in brain samples is low, but detectable at the RNA level (less than ~1 transcript/million, TPM); (ii) IHC using an anti-MAGE-family antibody yielded rare but strongly-positive cells in patient and control brain sections (~1/100 brain cells); and, (iii) the TCR had ~10x higher sensitivity to MAGE-A12112-120, compared to the on-target MAGE-A3 pMHC.

[0312] The present inventors tested the MAGE-A12 hypothesis and propose an alternative. The RNA expression data combined with the IHC experiment published by Morgan et al. predict that MAGE-A12 mRNA is concentrated at high levels in rare cells. The inventors did not detect such rare high-expressing cells in well-controlled RNA fluorescence in situ hybridization (RNA FISH) experiments, evidence that does not conform with a key prediction of the hypothesis. Moreover, an alternative candidate to explain the neurotoxicity was discovered: a related pMHC peptide derived from the EPS8L2 protein. A comparison was performed of the TCR to two novel CARs, one directed at the same MAGE-A3 pMHC, the other against a peptide common to both MAGE-A3 and -A12 (FIG. 8). We show that the CARs have good selectivity in general, and discriminate significantly better against the EPS8L2 peptide than the TCR. These results are important because they provide a path forward to test additional cancer therapeutics directed at the MAGE-A3 peptide known to be displayed in a large population of cancers (Boon et al. *J. Exp. Med.* 183:725-729).

[0313] MATERIALS AND METHODS

[0314] Cell lines and peptides

[0315] A375 cells (CRL-1619), CaSKI (CRM-CRL-1550), PANC.1 (CRL-1469™), SW527(CRL-7940™), PC.3 (CRL-1435™), LNCaP_clone_FGC (CRL-1740™), MCF.7 (HTB-22™), CAPAN-2 (HTB-80™), HEK293T (CRL-11268™), T2 (CTL-1992™), and A375 (CRL-1619) were purchased from ATCC®. VMRC.LCD (JCRB0814) was purchased

from JCRB Cell Bank. Jurkat NFAT-Firefly-Luciferase cells were purchased from BPS Bioscience (#60621). All cell lines were cultured in media as recommended by the vendors. 100 U/mL Penicillin-streptomycin (Gibco 15140163) (1x P/S) was used in all media. Firefly luciferase was recombinantly expressed in MCF-7 and CAPAN-2 cells, and these recombinant cells were cultured their respective culture media supplemented with 400 μg/ml Geneticin. HLA-A2 gene was knocked out in CAPAN-2 cells using CRISPR technology as described previously (Mout et al. *ACS Nano.* 11:2452-2458 (2017)). Suspension cells were maintained below a density of 1E6/mL. Adherent cells lines were passaged at ~80% confluency.

[0316] All peptides were purchased from GenScript by custom order.

[0317] HuTARG™ sort

[0318] HuTARG™ primary libraries were from Innovative Targeting Solutions, Inc. An in vitro V(D)J repertoire with > 1 billion diversity were generated by expression of RAG-1 and TdT in the host cells as described previously (U.S. Pat. No. 8,012,714; Oh et al. *Sci. Rep.* 9:17291 (2019)). pMHC probes were generated as described previously (Xu et al. *Mol. Immunol.* 125:56-64 (2020)). The library was enriched for cells displaying antibodies that bind specifically to target pMHC probes, but not to off-target pMHC probes using a flow sorter device. Multiple enrichment rounds were performed to increase on-target and decrease off-target binding. In the final round, on-target and off-target binding cells were collected. RNA was extracted from these pools and reverse transcribed into cDNA. PCR fragments containing the CDR regions were generated using the cDNAs as template, followed by targeted NGS to determine the frequency of each binder with a unique CDR region. The degree of enrichment/depletion was determined by comparing the output and input NGS counts.

[0319] Target-specific binders from the primary libraries were used in some cases as parents to generate optimization libraries to further improve on-target sensitivity and/or reduce off-target cross-reactivity. Optimization libraries were constructed by diversification of CDR-1, CDR-2 or CDR-3 light chains of parent binders by in vitro RAG-mediated V(D)J recombination. The optimization library was enriched for on-target activity and depleted for

off-target activity as for enrichment of the primary library. NGS was also used to identify binders enriched as described above.

[0320] Molecular cloning

[0321] All CAR constructs were created by fusing an scFv LBD to a hinge, a TM and an intracellular signaling domain. The hinge was derived from CD8, the transmembrane domain from CD28, and the signaling domain from CD28, 4-1BB and CD3. Gene segments were combined using Golden Gate cloning and inserted downstream of a human EF1alpha promoter contained in a lentivirus expression plasmid.

[0322] Jurkat/T2 cell assay

[0323] Jurkat NFAT-Firefly-Luciferase cells were transfected on day 1 with TCR and CAR constructs using standard protocols for the Lonza 4D Nucleofector™ (AAF-1002B) or Neon™ transfection system (ThermoFisher, MPK5000). T2 cells were loaded with peptides listed in Tables 1 and 3. Peptides were resuspended in DMSO, and diluted 16 or 20 times serially 3x per step. Serially diluted peptide solutions were added to T2 cells resuspended in peptide-loading media (RPMI1640 + 1% BSA + 1X P/S). This yielded peptide-loaded T2 cells at approximately 1E6/mL, with peptide concentrations ranging from ~10 fM to 100 μM, including a control at 0 μM. Peptide-loaded T2 cells were incubated overnight at 37°C in 384-well plates (Thermo Scientific AB0781). On day 2, the cells were cocultured in a 384-well plate (Corning 3570). Peptide-loaded T2 cells (10,000 cells/well) were added to CAR/TCR-transfected Jurkat-NFAT-Firefly-Luciferase cells (12,000 cells/well) to a final volume of 20 μL. After a 6-hour incubation at 37°C, the One-Step™ Luciferase assay system (Firefly luciferase, BPS Bioscience, 60690) was used to determine luminescence intensity on a Tecan Infinite® M1000.

[0324] Primary T cell transduction

[0325] Human PBMCs were purified from Leukopaks purchased from Allcells® according to the method described previously (Garcia et al. *J. Immunol. Methods.* 409:99-106 (2014)). Collection protocols and donor informed-consent were approved by an Institutional Review Board (IRB) at Allcells®. Allcells® followed HIPAA compliance and approved protocols (https://www.allcells.com/cell-tissue-procurement/donor-facilities/). Unless otherwise

specified, all LymphoONE™ media (Takara WK552) was supplemented with 1% human AB Serum (GeminiBio 100-512). Human PBMCs were grown in LymphoONE™ and supplemented with TransAct™ (Miltenyi 130-111-160) following the manufacturers guidelines (1:100 dilution) for 24 hours before being transduced with lentivirus encoding a CAR or TCR. 24 hours after transduction, additional LymphoONE™ supplemented with IL-2 (300 IU/ml) was added to transduced cells and cultured for 3 days before transfer to a 24-well G-Rex plate (Wilson Wolf 80192M). Fresh IL-2 (300 IU/ml) was added every 48 hours with a media change every 7 days during expansion in G-Rex plates. Expression and antigen binding of transduced CARs or TCRs in primary T cells was confirmed by flow cytometry as described above. If needed, CAR- or TCR-expressing cells were labeled with protein L-biotin/streptavidin-PE or mTCR-PE, followed by anti-PE microbeads (Miltenyi 130-048-801) according to the manufacturer's protocol, and subsequently enriched using AutoMACS® Pro Separator (Miltenyi). Enriched cells were grown in G-Rex plates until harvest.

[0326] Homogeneous cytotoxicity assay

[0327] Target cells (MCF-7 Renilla-luciferase) were loaded with target peptides as described above in the Jurkat/T2 section, except that LymphoONE™ supplemented with 1% human serum and 1X P/S was used. 24 hours after peptide-loading, a calibration curve was generated using CellTiter-Glo® (Promega G7570) readout to determine the number of target cells seeded per well. T cells were mixed with target cells at 3:1 E:T ratio according to the target cell number determined by calibration. After 48-hour co-culture at 37°C, cytotoxicity of primary T cells was quantified by the bioluminescence using the Renilla Luciferase Assay System (Promega, E2810) on Tecan Infinite® M1000.

[0328] QuIK assay

[0329] QuIK assay method was the same as described previously (Huang et al. *PLoS One*. 2020). Adherent target cells (MCF7) were cultured in standard conditions, and seeded at 2,000 cells per well in LymphoONE™ media containing 1% human serum in 384-well plates (Greiner 781091). Serially diluted peptide solutions were added to the target cells to yield peptide concentrations ranging from ~2 pM to 100 μM. Peptide-loaded target cells were incubated at 37°C overnight. Fresh CMFDA (Invitrogen C7025) working solution at 1 mM was prepared in LymphoONE™ from the solid. The percentages of TCR- and CAR-positive

T cells, determined by flow cytometry, were determined to adjust T cell numbers for co-culture with target cells. T cells were harvested and resuspended in CMFDA working solution at 200,000 cells/mL, and incubated in the dark at 37°C for 30 minutes. After staining, cells were centrifuged and washed 2x with 1 mL LymphoONE™ media containing 1% human serum. Cells were counted and added at an E:T ratio of 1:1 to the peptide-loaded target cells. Annexin V Red (Sartorius 4641) was dissolved according to manufacturer's instructions, and added to the coculture at a final dilution of 1:200; propidium at a final dilution of 1:10,000 (ThermoFisher P3566). Immediately after adding T cells to peptide-loaded target cells, the plate was incubated in an IncuCyteTM S3 and scanned every 2 hours using phase, green, and red channels with 300 ms exposure for the fluorescent channels at 10x magnification.

[0330] Cytotoxicity imaging assay ("inside out" presentation/killing)

[0331] To compare killing of target cells expressing MAGE (on-target) vs. ESP8L2 (off-target), two HLA A*02:01+ target cell lines were selected for a cytotoxicity study (without any peptide loading): A375 (MAGE A3+/MAGE A12+/EPS8L2-) and CAPAN-2 (MAGE A3-/MAGE A12-/EPS8L2+). To provide a control to demonstrate HLA restriction of TCR specificity, CRISPR/Cas9 editing was used to knock-out the HLA A*02:01 locus in CAPAN-2, creating an HLA A*02:01- version of this cell line. Adherent target cells (A375, CAPAN-2 WT or CAPAN-2 A2-) were cultured in standard conditions, and seeded at 2000 cells per well in LymphoOne™ media containing 1% human serum in 384-well plates (Greiner 781091). 2000 target cells were incubated at 37°C overnight. The next day, untransduced and TCR-positive T cells were counted and added to the plated target cells at indicated E:T ratios. Additionally, Annexin V Red (Sartorius 4641) was resuspended according to manufacturer's instructions, and added to the coculture at a final dilution of 1:200. Propidium iodide was added at a final dilution of 1:10,000 (ThermoFisher P3566). Immediately after adding T cells to seeded target cells, the plate was placed in the IncuCyteTM S3 and scanned every 2 hours using phase, and red channels with 300 ms exposure for the fluorescent channels at 10x magnification.

[0332] RNA FISH (BaseScope™)

[0333] The BaseScope™ experiments were performed by ACD (Newark, CA; a Biotechne brand) under a service contract agreement. The probe design used 1 pair of ZZ probes at nucleotide location: 91-127 nt of EPS8L2 (NM_005367.6, FIG. 13). Two human FFPE cell-pellet samples (PC3, and K562), and eight (8) human FFPE brain samples were evaluated by BaseScope™ LS Red ISH. Hs-PPIB was used as a positive control marker for sample QC and to evaluate RNA quality in both cell pellets and tissue samples. The bacterial gene dapB was used as a negative control. Optimization was performed to establish the best signal-to-noise ratio. Optimization of this probe set yielded mild pretreatment assay conditions (Epitope Retrieval 2: 15 minutes at 88°C; Protease III: 15 minutes at 40°C) for the cell pellets and standard pretreatment assay conditions (Epitope Retrieval 2: 15 minutes at 95°C; Protease III: 15 minutes at 40°C) for tissue samples. All samples passed QC with moderate PPIB-positive control staining and little to no dapB background staining. This established BaseScope™ LS Red ISH assay was performed to evaluate two cell pellets, as well as in human brain samples. Specific RNA staining signal was identified as red, punctate dots. Samples were counterstained with Mayer's Hematoxylin.

[0334] pMHC peptide identification from cell lines by mass spectrometry

[0335] Mass spectrometry experiments were conducted by Caprion Biosciences (Montreal, Canada) by contract. PANC.1, A.375, SW527, CaSki, PC.3, LNCaP_clone_FGC, MCF.7, and VMRC.LCD were cultured as described above. They were dissociated using cell dissociation buffer (ThermoFisher 13151014). pMHC complexes were purified from ~300-500 million cells of each cell line by immunoaffinity chromatography using the anti-MHC-I antibody W6/32 from cell lysates. pMHC complexes were disrupted with mild acid. The presented peptides were desalted with a mixed cation exchange matrix (MCX) and analyzed by LC-MS/MS in a single injection using nanoflow reverse-phase liquid chromatography (NanoAcquity UPLC, Waters) coupled to a high-resolution mass spectrometer (Q Exactive, Thermo Scientific). Mass spectrometry data were analyzed using Elucidator software (Rosetta), Mascot software (Matrix Science) and PEAKS software (Bioinformatics Solutions Inc.) to determine peptide sequences and relative quantity in each sample.

[0336] RESULTS

[0337] MAGE-A12 expression is very low in the brain

[0338] To add support to the data presented in Morgan et al. that MAGE-A12 expression, averaged across the brain, is extremely low, information was collated from 3 high-quality public RNA-Seq databases (GTEx, Human Protein Atlas (HPA) and FANTOM5). Because of its breadth and depth, focus was on quantification derived from GTEx (Carithers et al. *Biopreserv. Biobank.* 13:311-319 (2015); Lonsdale et al. *Nat. Genet.* 45:580-585 (2013)). RNA-Seq has several advantages compared to other methods of RNA quantification: (i) high sensitivity, constrained mainly by sequencing depth; (ii) high selectivity, based on highly specific DNA sequence calls of current algorithms; and, (iii) linear, large dynamic range (Mortazavi et al. *Nat. Methods* 5:621-628 (2008)). With GTEx, the results of the previous analysis were confirmed, with a median MAGE-A12 expression level of 5.9 TPM in the testis (n=361; FIG. 9 (Lonsdale et al. *Nat. Genet.* 45:580-585 (2013); Wu et al. *Genome Biol.* 10:R130 (2009); Scholtalbers et al. *Genome Med.* 7:118 (2015))). According to GTEx, across multiple regions of the brain the maximum value was 0.3 TPM in basal ganglia (n>151 samples/brain region). For comparison, MAGE-A3 expression was 12.8 TPM in testis, with no reported transcript in any of 13 brain regions examined. Of other MAGE paralogs, the highest brain expression belonged to MAGE-A10 (median <0.1 TPM/brain region), consistent with the evidence that the MAGE family of cancer testis antigens has very low adult normal-tissue expression outside the testis.

[0339] An average mammalian cell contains ~200,000 mRNAs/cells (Shapiro et al. *Nat. Rev. Genet.* 14:618-630 (2013)); a neuron based on its above-average size, presumably more. If this average value is used, it is expected that MAGE-A12 would be present on average at > 0.06 mRNAs/cell. From the histopathology image in Figure 12 (Morgan et al. *J. Immunol.* 180:6116-6131 (2013)), we estimated ~1 in 100 cells stained with the MAGE antibody (6C1) used by the authors (obtained from Santa Cruz Biotechnology, Santa Cruz, CA). Therefore, the stain-positive cells should contain ~100x more MAGE-A12 mRNA, compared to their stain-negative neighbors; thus, >6 transcripts/cell. An experiment was designed to detect the presence of these cells using RNA FISH.

[0340] An RNA FISH probe set was generated and tested that was predicted to be selective for MAGE-A12 over MAGE-A3. Because of the high sequence similarity among MAGE paralogs, it was necessary to maximize identities between the probe and MAGE-A12, and

mismatches with the related off-target mRNAs (FIG. 13; see Methods). These probes were tested on cell lines that expressed either high (K562) or low (PC3) MAGE-A12 levels (FIG. 14). Positive- and negative-control RNA FISH probes were also used in the hybridization. From these initial experiments, the RNA FISH technique was sufficiently sensitive at its limit to perhaps detect the very low level of MAGE-A12 mRNA present in PC3 cells (0.05 TPM), and certainly register transcripts in K562 cells (87 TPM) (Baker et al. *Nat. Commun.* 8:1998 (2017)). A small number of stain dots, statistically higher than observed in the negative-control probe sections, were visible in PC3 cells; ~0.1 dots/cell). In contrast, numerous dots were visible on the K562 sections, averaging 2-3 dots/cell. These results suggest that the RNA FISH technology used in these experiments has high sensitivity—potentially able to detect mRNAs at <1 transcript/cell on average—but is non-linear. Results from the positive-control probe were consistent with this observation.

[0341] Next, brain tissue sections from 8 post-mortem donors (5 normal and 3 cancer patient donors) were tested. In total ~3.2 million brain cells were scanned by eye and ImageJ software. No expression of MAGE-A12 was observed above the detection limit determined by the negative-control probe (FIG. 15). Rare MAGE-A12 high-expressing cells in the brain were not detected, even though they would fall within the dynamic range of the RNA FISH assay. This result, together with the other RNA data, is not consistent with the hypothesis that MAGE-A12 is the underlying cause of neurotoxicity observed with the MAGE-A3 clinical TCR.

[0342] EPS8L2 cross-reactivity is an alternative cause for the observed TCR-T neurotoxicity

[0343] In the GTEx database, EPS8L2 expression was high in several tissues, including the cerebellum (64 TPM, n=241), over 100x higher than MAGE-A12 RNA levels. RNA level correlates with protein level, which in turn correlates with the likelihood of pMHC display (Schwanhausser et al. *Nature*. 473:337-342 (2011)). EPS8L2 protein is detected in brain samples documented by the Human Protein Atlas database (Uhlen et al. *Science*. 347:1260419 (2015)). Previous analysis stated that full-length EPS8L2 expressed exogenously did not activate the MAGE-A3 TCR (Morgan et al. *J. Immunother.* 36:133-151 (2013)). However, the inventors detected the cross-reactive EPS8L2 peptide in a mass spectrometry experiment using a cell line (PANC-1) that expresses 176 TPM endogenous

EPS8L2 (FIG. 21; see Methods). On the other hand, the inventors failed to detect this peptide in 7 other HLA-A*02-positive cell lines that also express EPS8L2 (range 27-393 RPKM). These results were qualitatively similar for MAGE-A3112-120 peptide, where the peptide was observed in only one of three HLA-A*02-positive lines that expressed MAGE-A3 RNA. This suggests that despite high expression, the EPS8L2 and MAGE-A3 peptides are displayed only under certain circumstances, or at the limit of detection of mass spectrometry, which is thought to be less sensitive for pMHC detection than T cell functional assays (Stopfer et al. *Nat. Commun.* 11:2760 (2020)).

[0344] We confirmed the cross-reactivity with MAGE-A12112-120 and EPS8L2$_{339-347}$ using the clinical TCR in Jurkat/T2 cell assays (Aarnoudse et al. *Int. J. Cancer.* 99:7-13 (2002); Salter et al. *EMBO J.* 5:943-949 (1986)) (FIGs. 16-18, ; FIGs. 10-12; see Methods). Compared to the on-target MAGE-A3 peptide, the TCR was ~10x more potent against MAGE-A12 and ~500x less potent against EPS8L2$_{339-347}$.

[0345] We next tested sensitivity and selectivity of the MAGE- A3$_{112-120}$ TCR in primary human T cell assays, using peptide-loaded HLA-A*02-positive MCF7 and HEK293 cells, as these target cell lines do not express endogenous MAGE-A3 or -A12 (FIG. 19; FIG. 9). Not only did the TCR not discriminate between MAGE-A3 and MAGE-A12 peptides but also it displayed significant cross-reactivity with EPS8L2, within ~10x of the on-target EC50. In HEK293 cells, TCR activity was right-shifted ~100x in sensitivity compared to MCF7, perhaps because peptide loading and/or killing are less efficient (FIG. 22). But again, the TCR showed significant cross-reactivity to EPS8L2, comparable to the on-target MAGE-A3 peptide. These results confirm that the TCR is sensitive to EPS8L2339-347 particularly in primary T cell cytotoxicity.

[0346] Lastly, the TCR ability to mediate cytotoxicity through native expression of EPS8L2 protein was tested. The TCR triggered killing of cell lines that express MAGE-A3 (FIG. 20). However, in HLA-A*02-positive CAPAN2 cells, a cell line that expresses EPS8L2 (~450 TPM) but not MAGE-A3 or -A12 (both < 1 TPM), the TCR also mediated significant cytotoxicity. Knock-out of the HLA-A*02 locus demonstrated that killing was dependent on expression of the pMHC complex. Thus, EPS8L2339-347 has properties consistent with causing the toxicity responsible for termination of the A118T TCR in the clinic: (i) It elicits a

strong response from the TCR in carefully controlled peptide-loading experiments; (ii) It is expressed at high levels in the brain—much higher than MAGE-A12; (iii) It is detected by mass spectrometry of immune-precipitated HLA class I pMHCs isolated from an EPS8L2-expressing cell line; And, (iv) in the absence of MAGE-A3 endogenous expression, it provides an explanation for residual HLA-A*02-dependent cytotoxicity displayed by the TCR against cells that express EPS8L2.

[0347] MAGE-A3-directed CAR-Ts as an option for cell therapy

[0348] In principle, binders based on monoclonal antibodies (mAbs) obtained through immunization or in vitro display provide an alternative to TCRs (Dubrovsky et al. *Oncoimmunology.* 5:e1049803 (2015)). To ascertain if scFv binders could be identified with better functional selectivity than the clinical TCR, MAGE-A3-directed CARs were screend with a focus on both MAGE-A12 and EPS8L2 as potential off-target peptides. A mAb-display library was screened in human cells for MAGE-A3 pMHC mAbs that bind the same target as the clinical TCR (HLA-A*02 complexed with MAGE-A3$_{112-120}$) (U.S. Pat. No. 8,012,714; Oh et al. *Sci. Rep.* 9:17291 (2019)). To maximize the chance for selectivity, a counter-screen was performed against off-target pMHCs, including MAGE-A12 and EPS8L2. Screening was also performed for binders against a second MAGE-A3 pMHC presented by HLA-A*02 with identical sequence to the homologous MAGE-A12 proteins (Chinnasamy et al. *J. Immunol.* 186:685-696 (2011)) (MAGE-A3$_{271-279}$; FLWGPRALV). After screening for binders, mAbs were converted to scFv CARs and tested them in Jurkat functional assays, with peptide-loaded T2 cells as stimulus. Two mAbs were focused on, one against each MAGE-A3 pMHC (FIG. 9). Both were selective against many related peptides, including peptides derived from members of the MAGE family (FIG. 16 and FIG. 17). Indeed, both binders exhibited TCR-like selectivity at the level of single amino-acid differences, though the A118T clinical TCR was, in general, more discriminating. This discrimination was also apparent when a panel of off-target peptides was used to compare reactivities at high concentrations. But importantly, the MAGE-A3$_{112-120}$ CAR was ~10x more selective for MAGE-A3 over MAGE-12 compared to the TCR, and at least 6x more selective over EPS8L2339-347 in Jurkat/T2 assays, suggesting that improved selectivity against these potential off-target pMHCs is possible despite their high similarity to the on-

target MAGE- A3$_{112-120}$ peptide. In primary T cell assays, the MAGE-A3$_{112-120}$ CAR retained modest selectivity for the MAGE-A12112-120 peptide, with no detectable activity against EPS8L2339-347 peptide (FIG. 19). Together, these data demonstrate that it is possible to obtain CARs with a selectivity profile similar to TCRs—discriminating among pMHCs that differ at only a single amino acid. Though less potent than the clinical TCR, the MAGE-A3$_{112-120}$ CAR was significantly more selective against EPS8L2339-347, a homologous peptide that represents a risk for therapeutics directed against MAGE-A3$_{112-120}$. Because MAGE-A3 pMHCs, including HLA-A*02/MAGE-A3$_{112-120}$, are such attractive cancer targets, these results suggest it is worth reconsidering selective scFvs (as well as additional TCRs) as options for a MAGE-A3-directed therapeutic.

[0349] DISCUSSION

[0350] Cancer testis antigens are a class of potentially important tumor-selective proteins, so far not targeted by an approved cancer medicine. They are remarkably cancer-specific; expressed in the testis, largely absent from other adult tissues, and overexpressed in a variety of tumor types (Scanlan et al. *Immunol. Rev.* 188:22-32 (2002)). The MAGE family fits this profile (Lee et al. *J. Mol. Biol.* 429:1114-1142 (2017)). Of its paralogs, MAGE-A3 is among the most interesting. It is expressed in small and squamous cell lung, head and neck, bladder, and esophageal carcinomas, as well as melanoma. In the subset of these cancer types that represents the upper quartile of MAGE-A3 mRNA expression, the levels range from 14-179 TPM (see TCGA database). This subset encompasses ~25,000 deaths/yr from cancer in the US. Thus, MAGE-A3 is an attractive cancer target based on its selective expression in tumors and its frequency and level of expression. Only a handful of proteins have these properties.

[0351] MAGE proteins reside inside the cell where they appear to function as modifiers of E3 ligase activity (Lee et al. *J. Mol. Biol.* 429:1114-1142 (2017)). At present, the only known means of targeting them in cancer is via their display as pMHCs. The key challenges are to identify MAGE-A3 pMHCs on the cell surface and develop potent, selective targeting molecules. The A118T TCR was, therefore, an innovative, high-potential therapeutic when it began clinical testing (Morgan et al. *J. Immunother.* 36:133-151 (2013)). However, the occurrence of fatal toxicity compelled termination of this otherwise attractive candidate. The clinical findings raised the possibility of off-target TCR activity which Morgan et al.

investigated. They proposed a reasonable hypothesis for the source of cross-reactivity, MAGE-A12, and provided supporting evidence. Given (i) the high similarity of MAGE-A3 peptide to MAGE-A12 sequence, and the substantial challenge to avoid cross-reactivity with it; and, (ii) the potential high value of MAGE-A3 pMHC as a cancer target, it is sensible to re-examine the strength of the hypothesis.

[0352] Although it is impossible to exclude MAGE-A12 completely as the root cause, the evidence presented here calls into question the hypothesis and provides evidence in favor of an alternative cause of toxicity: EPS8L2. Mindful of the inherent shortcomings of IHC, the present inventors utilized methodologies for mRNA expression that are comparatively more sensitive, quantitative, and less dependent on idiosyncratic reagents like antibodies. Notably, no positive or negative controls for antibody staining were included in the investigators' IHC experiments so the 6C1 antibody may be viewed as a poorly qualified reagent, especially for specific detection of low-level MAGE expression in situ. Moreover, the pattern of rare (1/100), strongly stain-positive cells may be viewed as highly unusual, especially for a protein with no known neural-lineage-specific role. Given the totality of the results presented here and by Morgan et al., it seems more likely that the rare stain-positive cells imaged previously are false positives caused by, for example, spurious aggregation of dye. The inability to detect the rare, strongly MAGE-positive cells in the brain predicted by the hypothesis at the RNA level raises questions about the actual source of toxicity observed in the TCR-T trial.

[0353] The clinical TCR did not react with a variety of cell lines and tissues, at least some of which likely expressed EPS8L2 (Morgan et al. *J. Immunother.* 36:133-151 (2013)). However, in our hands the TCR clearly mediated a response using an EPS2L8-expressing cell line as stimulus. These disparate results may reflect sensitivity differences in the assays used. Based on (i) the ~100x higher expression of EPS8L2 compared to MAGE-A12 in normal tissues; (ii) the detection of EPS8L2339-347 peptide by mass spectrometry among pMHC complexes immunoprecipitated from a cell line; and, (iii) the significant response of the TCR to both EPS8L2339-347 peptide-loaded cell lines and cells expressing full-length EPS8L2 protein, we believe EPS8L2339-347 should be considered as a likely cause of neurotoxicity observed in the clinical study of the A118T TCR.

[0354] Support is provided for a MAGE-A3 therapeutic by demonstrating isolated CARs using two different HLA-A*02-restricted MAGE-A3 peptides. Cross-reactivity of the MAGE-A3$_{112-120}$ CAR is minimized by counter-screening against MAGE-A12. Selectivity is improved compared to the TCR by ~10x. Optimization against ESP8L2 was also successful; the MAGE-A3$_{112-120}$ CAR demonstrated ~10x improvement in selectivity against EPS8L2339-347 compared to the TCR. The two MAGE-A3-directed CARs, like the TCR, were otherwise selective, reacting minimally against a panel of related and unrelated peptides, including other members of the MAGE family.

[0355] The present inventors obtained scFv's with reduced cross-reactivity risks for both potential off-target peptides. A CAR or TCR with such improved selectivity properties and TCR/CARs directed at the other MAGE-A3 pMHC target can be used as safe and effective cancer therapeutics.

CLAIMS

1. A polypeptide, comprising an antibody or antigen-binding fragment thereof that specifically binds to a peptide comprising the sequence of FLWGPRALV (SEQ ID NO: 1) and has an EC_{50} of less than 5 nM.
2. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof binds to the peptide within a peptide-MHC complex.
3. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof is a human antibody or an antigen-binding fragment thereof.
4. The polypeptide of claim 1, wherein the antigen-binding fragment is a single-chain variable fragment (scFv) or a single chain Fab (scFab).
5. The polypeptide of claim 1, wherein the antigen-binding fragment is an scFv and comprises a variable heavy chain (V_H)-linker-variable light chain (V_L) orientation.
6. The polypeptide of claim 1, wherein the antigen-binding fragment is an scFv and comprises a V_L-linker-V_H orientation.
7. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof has an EC_{50} of less than 1 nM.
8. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 221); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 222); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 223); a light chain CDR1 comprising the sequence of RAS [Q/H] [S/P/I/L/F] [-/G/A/S/T] [-F/S/D/L] [-/Y/F/L] [I/V] [S/G] [S/G] YLN (SEQ ID NO: 224); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 225); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 226).
9. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a. a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 5); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 6); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 7); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 8); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 9); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 10);

b. a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 77); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 78); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 79); a light chain CDR1 comprising the sequence of RASQIYVSSYLN (SEQ ID NO: 80); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 81); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 82);

c. a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 85); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 86); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 87); a light chain CDR1 comprising the sequence of RASQSASFVSSYLN (SEQ ID NO: 88); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 89); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 90);

d. a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 93); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 94); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 95); a light chain CDR1 comprising the sequence of RASQPYVSSYLN (SEQ ID NO: 96); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 97);

and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 98);

e. a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 101); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 102); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 103); a light chain CDR1 comprising the sequence of RASQLYVSSYLN (SEQ ID NO: 104); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 105); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 106);

f. a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 109); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 110); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 111); a light chain CDR1 comprising the sequence of RASHPYVSSYLN (SEQ ID NO: 112); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 113); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 114);

g. a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 117); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 118); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 119); a light chain CDR1 comprising the sequence of RASQSTLYVSSYLN (SEQ ID NO: 120); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 121); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 122);

h. a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 125); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 126); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 127); a light chain

CDR1 comprising the sequence of RASQSSDLVSSYLN (SEQ ID NO: 128); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 129); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 130);

i. a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 133); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 134); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 135); a light chain CDR1 comprising the sequence of RASQSTLLVSSYLN (SEQ ID NO: 136); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 137); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 138);

j. a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 141); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 142); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 143); a light chain CDR1 comprising the sequence of RASQSYVSGYLN (SEQ ID NO: 144); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 145); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 146); or

k. a heavy chain CDR1 comprising the sequence of SGGYSWS (SEQ ID NO: 149); a heavy chain CDR2 comprising the sequence of YIYHSGSTYYNPSLKS (SEQ ID NO: 150); a heavy chain CDR3 comprising the sequence of CARDGDVPYWYFDLW (SEQ ID NO: 151); a light chain CDR1 comprising the sequence of RASQFGFFVGGYLN (SEQ ID NO: 152); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 153); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 154).

10. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a. a variable heavy chain comprising the sequence of QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSS (SEQ ID NO: 11) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFG GGTKVEIK (SEQ ID NO: 12);

b. a variable heavy chain comprising the sequence of QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSS (SEQ ID NO: 83) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQIYVSSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTF GGGTKVEIK (SEQ ID NO: 84);

c. a variable heavy chain comprising the sequence of QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSS (SEQ ID NO: 91) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSASFVSSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP LTFGGGTKVEIK (SEQ ID NO: 92);

d. a variable heavy chain comprising the sequence of QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSS (SEQ ID NO: 99) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQPYVSSYLNWYQQKPGKAPKLL

IYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLT FGGGTKVEIK (SEQ ID NO: 100);

e. a variable heavy chain comprising the sequence of QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSS (SEQ ID NO: 107) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQLYVSSYLNWYQQKPGKAPKLL IYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLT FGGGTKVEIK (SEQ ID NO: 108);

f. a variable heavy chain comprising the sequence of QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSS (SEQ ID NO: 115) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASHPYVSSYLNWYQQKPGKAPKLL IYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLT FGGGTKVEIK (SEQ ID NO: 116);

g. a variable heavy chain comprising the sequence of QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSS (SEQ ID NO: 123) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSTLYVSSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP LTFGGGTKVEIK (SEQ ID NO: 124);

h. a variable heavy chain comprising the sequence of QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSS (SEQ ID NO: 131) and a variable

light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSSDLVSSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP LTFGGGTKVEIK (SEQ ID NO: 132);

i. a variable heavy chain comprising the sequence of QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSS (SEQ ID NO: 139) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSTLLVSSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP LTFGGGTKVEIK (SEQ ID NO: 140);

j. a variable heavy chain comprising the sequence of QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSS (SEQ ID NO: 147) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSYVSGYLNWYQQKPGKAPKLL IYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLT FGGGTKVEIK (SEQ ID NO: 148); or

k. a variable heavy chain comprising the sequence of QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSS (SEQ ID NO: 155) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQFGFFVGGYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP LTFGGGTKVEIK (SEQ ID NO: 156).

11. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a. QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAAS SLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGT KVEIK (SEQ ID NO: 246);

b. QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASQIYVSSYLNWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGG GTKVEIK (SEQ ID NO: 247);

c. QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASQSASFVSSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTF GGGTKVEIK (SEQ ID NO: 248);

d. QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASQPYVSSYLNWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGG GTKVEIK (SEQ ID NO: 249);

e. QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASQLYVSSYLNWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGG GTKVEIK (SEQ ID NO: 250);

f. QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASHPYVSSYLNWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGG GTKVEIK (SEQ ID NO: 251);

g. QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASQSTLYVSSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTF GGGTKVEIK (SEQ ID NO: 252);

h. QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASQSSDLVSSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTF GGGTKVEIK (SEQ ID NO: 253);

i. QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASQSTLLVSSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTF GGGTKVEIK (SEQ ID NO: 254);

j. QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASQSYVSGYLNWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGG GTKVEIK (SEQ ID NO: 255);

k. QLQLQESGSGLVKPSQTLSLTCAVSGGSISSGGYSWSWIRQPPGKGLE WIGYIYHSGSTYYNPSLKSRVTISVDRSKNQFSLKLSSVTAADTAVYY CARDGDVPYWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASQFGFFVGGYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTF GGGTKVEIK (SEQ ID NO: 256).

12. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain CDR1 comprising the sequence of ELSMH (SEQ ID NO: 227); a heavy chain CDR2 comprising the sequence of GFDPEDGETIYAQKFQG (SEQ ID NO: 228); a heavy chain CDR3 comprising the sequence of CATDLYSSSWYCDAFDIW (SEQ ID NO: 229); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 230); a light chain CDR2 comprising the sequence of AASSL [Q/H] [-/Y] [-/V] S (SEQ ID NO: 231); and a light chain CDR3 comprising the sequence of QQS [Y/W] [-/A] STPLT (SEQ ID NO: 232).

13. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a. a heavy chain CDR1 comprising the sequence of ELSMH (SEQ ID NO: 13); a heavy chain CDR2 comprising the sequence of GFDPEDGETIYAQKFQG (SEQ ID NO: 14); a heavy chain CDR3 comprising the sequence of CATDLYSSSWYCDAFDIW (SEQ ID NO: 15); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 16); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 17); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 18);

b. a heavy chain CDR1 comprising the sequence of ELSMH (SEQ ID NO: 157); a heavy chain CDR2 comprising the sequence of GFDPEDGETIYAQKFQG (SEQ ID NO: 158); a heavy chain CDR3 comprising the sequence of CATDLYSSSWYCDAFDIW (SEQ ID NO: 159); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 160); a light chain CDR2 comprising the sequence of AASSLHYVS (SEQ ID NO: 161); and a

light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 162); or

c. a heavy chain CDR1 comprising the sequence of ELSMH (SEQ ID NO: 165); a heavy chain CDR2 comprising the sequence of GFDPEDGETIYAQKFQG (SEQ ID NO: 166); a heavy chain CDR3 comprising the sequence of CATDLYSSSWYCDAFDIW (SEQ ID NO: 167); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 168); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 169); and a light chain CDR3 comprising the sequence of QQSWASTPLT (SEQ ID NO: 170).

14. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a. a variable heavy chain comprising the sequence of QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDLYSSSWYCDAFDIWGQGTMVTVSS (SEQ ID NO: 19) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIK (SEQ ID NO: 20);

b. a variable heavy chain comprising the sequence of QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDLYSSSWYCDAFDIWGQGTMVTVSS (SEQ ID NO: 163) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLHYVSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIK (SEQ ID NO: 164); or

c. a variable heavy chain comprising the sequence of QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAV

YYCATDLYSSSWYCDAFDIWGQGTMVTVSS (SEQ ID NO: 171) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSWASTPLT FGGGTKVEIK (SEQ ID NO: 172).

15. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

    a. QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLE WMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAV YYCATDLYSSSWYCDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSG GDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTF GGGTKVEIK (SEQ ID NO: 257);

    b. QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLE WMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAV YYCATDLYSSSWYCDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSG GDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI YAASSLHYVSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP LTFGGGTKVEIK (SEQ ID NO: 258);

    c. QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLE WMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAV YYCATDLYSSSWYCDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSG GDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSWASTPL TFGGGTKVEIK (SEQ ID NO: 259).

16. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain CDR1 comprising the sequence of SSNWWG (SEQ ID NO: 233); a heavy chain CDR2 comprising the sequence of YIYYSGSTYYNPSLKS (SEQ ID NO: 234); a heavy chain CDR3 comprising the sequence of CARIPFGDWWYFDLW (SEQ ID NO: 235); a light chain CDR1 comprising the

sequence of RASQSI [S/G] [-/P] [-/F] [-/L] SYLN (SEQ ID NO: 236); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 237); and a light chain CDR3 comprising the sequence of QQS [Y/W] [S/G] [F/Y/T] [V/P] LT (SEQ ID NO: 238).

17. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a. a heavy chain CDR1 comprising the sequence of SSNWWG (SEQ ID NO: 29); a heavy chain CDR2 comprising the sequence of YIYYSGSTYYNPSLKS (SEQ ID NO: 30); a heavy chain CDR3 comprising the sequence of CARIPFGDWWYFDLW (SEQ ID NO: 31); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 32); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 33); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 34);

b. a heavy chain CDR1 comprising the sequence of SSNWWG (SEQ ID NO: 173); a heavy chain CDR2 comprising the sequence of YIYYSGSTYYNPSLKS (SEQ ID NO: 174); a heavy chain CDR3 comprising the sequence of CARIPFGDWWYFDLW (SEQ ID NO: 175); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 176); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 177); and a light chain CDR3 comprising the sequence of QQSYSFVLT (SEQ ID NO: 178);

c. a heavy chain CDR1 comprising the sequence of SSNWWG (SEQ ID NO: 181); a heavy chain CDR2 comprising the sequence of YIYYSGSTYYNPSLKS (SEQ ID NO: 182); a heavy chain CDR3 comprising the sequence of CARIPFGDWWYFDLW (SEQ ID NO: 183); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 184); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 185); and a light chain CDR3 comprising the sequence of QQSYGYVLT (SEQ ID NO: 186);

d. a heavy chain CDR1 comprising the sequence of SSNWWG (SEQ ID NO: 189); a heavy chain CDR2 comprising the sequence of YIYYSGSTYYNPSLKS (SEQ ID NO: 190); a heavy chain CDR3 comprising the sequence of CARIPFGDWWYFDLW (SEQ ID NO: 191); a light chain CDR1 comprising the sequence of RASQSIGPFLSYLN (SEQ ID NO: 192); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 193); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 194); or

e. a heavy chain CDR1 comprising the sequence of SSNWWG (SEQ ID NO: 197); a heavy chain CDR2 comprising the sequence of YIYYSGSTYYNPSLKS (SEQ ID NO: 198); a heavy chain CDR3 comprising the sequence of CARIPFGDWWYFDLW (SEQ ID NO: 199); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 200); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 201); and a light chain CDR3 comprising the sequence of QQSWGTPLT (SEQ ID NO: 202).

18. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a. a variable heavy chain comprising the sequence of QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARIPFGDWWYFDLWGRGTLVTVSS (SEQ ID NO: 35) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFG GGTKVEIK (SEQ ID NO: 36);

b. a variable heavy chain comprising the sequence of QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARIPFGDWWYFDLWGRGTLVTVSS (SEQ ID NO: 179) and a variable

light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSFVLTFG GGTKVEIK (SEQ ID NO: 180);

c. a variable heavy chain comprising the sequence of QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARIPFGDWWYFDLWGRGTLVTVSS (SEQ ID NO: 187) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYGYVLTF GGGTKVEIK (SEQ ID NO: 188);

d. a variable heavy chain comprising the sequence of QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARIPFGDWWYFDLWGRGTLVTVSS (SEQ ID NO: 195) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSIGPFLSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPL TFGGGTKVEIK (SEQ ID NO: 196); or

e. a variable heavy chain comprising the sequence of QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARIPFGDWWYFDLWGRGTLVTVSS (SEQ ID NO: 203) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSWGTPLTF GGGTKVEIK (SEQ ID NO: 204).

19. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a. QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARIPFGDWWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQM TQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGT KVEIK (SEQ ID NO: 260);

b. QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARIPFGDWWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQM TQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSFVLTFGGGT KVEIK (SEQ ID NO: 261);

c. QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARIPFGDWWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQM TQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYGYVLTFGGGT KVEIK (SEQ ID NO: 262);

d. QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARIPFGDWWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQM TQSPSSLSASVGDRVTITCRASQSIGPFLSYLNWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGG GTKVEIK (SEQ ID NO: 263);

e. QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARIPFGDWWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQM TQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSWGTPLTFGGGT KVEIK (SEQ ID NO: 264).

20. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain CDR1 comprising the sequence of DYAMH (SEQ ID NO: 239); a heavy chain CDR2 comprising the sequence of GISWNSGSIGYADSVKG (SEQ ID NO: 240); a heavy chain CDR3 comprising the sequence of CAKDSTSWVNFPYYMDVW (SEQ ID NO: 241); a light chain CDR1 comprising the sequence of RASQSISS [-/G] [-/L/V] [-/S] YLN (SEQ ID NO: 242); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 243); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 244).

21. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

    a. a heavy chain CDR1 comprising the sequence of DYAMH (SEQ ID NO: 45); a heavy chain CDR2 comprising the sequence of GISWNSGSIGYADSVKG (SEQ ID NO: 46); a heavy chain CDR3 comprising the sequence of CAKDSTSWVNFPYYMDVW (SEQ ID NO: 47); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 48); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 49); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 50);

    b. a heavy chain CDR1 comprising the sequence of DYAMH (SEQ ID NO: 205); a heavy chain CDR2 comprising the sequence of GISWNSGSIGYADSVKG (SEQ ID NO: 206); a heavy chain CDR3 comprising the sequence of CAKDSTSWVNFPYYMDVW (SEQ ID NO: 207); a light chain CDR1 comprising the sequence of RASQSISSGLSYLN (SEQ ID NO: 208); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 209); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 210); or

    c. a heavy chain CDR1 comprising the sequence of DYAMH (SEQ ID NO: 213); a heavy chain CDR2 comprising the sequence of GISWNSGSIGYADSVKG (SEQ ID NO: 214); a heavy chain CDR3 comprising the sequence of CAKDSTSWVNFPYYMDVW (SEQ ID NO: 215); a light chain CDR1 comprising the sequence of RASQSISSGVSYLN

(SEQ ID NO: 216); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 217); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 218).

22. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a. a variable heavy chain comprising the sequence of EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLE WVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL YYCAKDSTSWVNFPYYMDVWGKGTTVTVSS (SEQ ID NO: 51) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFG GGTKVEIK (SEQ ID NO: 52);

b. a variable heavy chain comprising the sequence of EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLE WVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL YYCAKDSTSWVNFPYYMDVWGKGTTVTVSS (SEQ ID NO: 211) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSGLSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPL TFGGGTKVEIK (SEQ ID NO: 212); or

c. a variable heavy chain comprising the sequence of EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLE WVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL YYCAKDSTSWVNFPYYMDVWGKGTTVTVSS (SEQ ID NO: 219) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSGVSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP LTFGGGTKVEIK (SEQ ID NO: 220).

23. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:.

    a. EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLE WVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL YYCAKDSTSWVNFPYYMDVWGKGTTVTVSSGGGGSGGGGSGGGGS GGDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPL TFGGGTKVEIK (SEQ ID NO: 265);

    b. EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLE WVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL YYCAKDSTSWVNFPYYMDVWGKGTTVTVSSGGGGSGGGGSGGGGS GGDIQMTQSPSSLSASVGDRVTITCRASQSISSGLSYLNWYQQKPGKA PKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYS TPLTFGGGTKVEIK (SEQ ID NO: 266);

    c. EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLE WVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAL YYCAKDSTSWVNFPYYMDVWGKGTTVTVSSGGGGSGGGGSGGGGS GGDIQMTQSPSSLSASVGDRVTITCRASQSISSGVSYLNWYQQKPGKA PKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYS TPLTFGGGTKVEIK (SEQ ID NO: 267).

24. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

    a. a heavy chain CDR1 comprising the sequence of DYYMH (SEQ ID NO: 21); a heavy chain CDR2 comprising the sequence of LVDPEDGETIYAEKFQG (SEQ ID NO: 22); a heavy chain CDR3 comprising the sequence of CALLSGWYIDAFDIW (SEQ ID NO: 23); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 24); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 25); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 26);

b. a heavy chain CDR1 comprising the sequence of SYDMH (SEQ ID NO: 37); a heavy chain CDR2 comprising the sequence of AIGTAGDTYYPGSVKG (SEQ ID NO: 38); a heavy chain CDR3 comprising the sequence of CARDLYYDILTGYPDAFDIW (SEQ ID NO: 39); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 40); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 41); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 42);

c. a heavy chain CDR1 comprising the sequence of SSSYYWG (SEQ ID NO: 53); a heavy chain CDR2 comprising the sequence of SIYYSGSTYYNPSLKS (SEQ ID NO: 54); a heavy chain CDR3 comprising the sequence of CARRDGGNWYFDLW (SEQ ID NO: 55); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 56); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 57); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 58);

d. a heavy chain CDR1 comprising the sequence of SSNWWG (SEQ ID NO: 61); a heavy chain CDR2 comprising the sequence of YIYYSGSTYYNPSLKS (SEQ ID NO: 62); a heavy chain CDR3 comprising the sequence of CARNTDPQLEWYFDLW (SEQ ID NO: 63); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 64); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 65); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 66); or

e. a heavy chain CDR1 comprising the sequence of SYSMN (SEQ ID NO: 69); a heavy chain CDR2 comprising the sequence of YISSSSSTIYYADSVKG (SEQ ID NO: 70); a heavy chain CDR3 comprising the sequence of CARDDYSSEYFQHW (SEQ ID NO: 71); a light chain CDR1 comprising the sequence of RASQSISSYLN (SEQ ID NO: 72); a light chain CDR2 comprising the sequence of AASSLQS (SEQ ID NO: 73); and a light chain CDR3 comprising the sequence of QQSYSTPLT (SEQ ID NO: 74).

25. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a. a variable heavy chain comprising the sequence of EVQLVQSGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLE WMGLVDPEDGETIYAEKFQGRVTITADTSTDTAYMELSSLRSEDTAV YYCALLSGWYIDAFDIWGQGTMVTVSS (SEQ ID NO: 27) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFG GGTKVEIK (SEQ ID NO: 28);

b. a variable heavy chain comprising the sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMHWVRQATGKGLE WVSAIGTAGDTYYPGSVKGRFTISRENAKNSLYLQMNSLRAGDTAVY YCARDLYYDILTGYPDAFDIWGQGTMVTVSS (SEQ ID NO: 43) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFG GGTKVEIK (SEQ ID NO: 44);

c. a variable heavy chain comprising the sequence of QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLE WIGSIYYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC ARRDGGNWYFDLWGRGTLVTVSS (SEQ ID NO: 59) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFG GGTKVEIK (SEQ ID NO: 60);

d. a variable heavy chain comprising the sequence of QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARNTDPQLEWYFDLWGRGTLVTVSS (SEQ ID NO: 67) and a variable

light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFG GGTKVEIK (SEQ ID NO: 68); or

e. a variable heavy chain comprising the sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEW VSYISSSSSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC ARDDYSSEYFQHWGQGTLVTVSS (SEQ ID NO: 75) and a variable light chain comprising the sequence of DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFG GGTKVEIK (SEQ ID NO: 76).

26. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof comprises:

a. EVQLVQSGAEVKKPGATVKISCKVSGYTFTDYYMHWVQQAPGKGLE WMGLVDPEDGETIYAEKFQGRVTITADTSTDTAYMELSSLRSEDTAV YYCALLSGWYIDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSGGDI QMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGG GTKVEIK (SEQ ID NO: 268);

b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMHWVRQATGKGLE WVSAIGTAGDTYYPGSVKGRFTISRENAKNSLYLQMNSLRAGDTAVY YCARDLYYDILTGYPDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGS GGDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPL TFGGGTKVEIK (SEQ ID NO: 269);

c. QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLE WIGSIYYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC ARRDGGNWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQMT QSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSL

QSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTK VEIK (SEQ ID NO: 270);

d. QVQLQESGPGLVKPSDTLSLTCAVSGYSISSSNWWGWIRQPPGKGLE WIGYIYYSGSTYYNPSLKSRVTMSVDTSKNQFSLKLSSVTAVDTAVYY CARNTDPQLEWYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSGGDIQ MTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAAS SLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGT KVEIK (SEQ ID NO: 271);

e. EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEW VSYISSSSSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC ARDDYSSEYFQHWGQGTLVTVSSGGGGSGGGGSGGGGSGGDIQMTQ SPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQ SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKV EIK (SEQ ID NO: 272).

27. The polypeptide of claim 1, wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody or antigen-binding fragment thereof.

28. The polypeptide of any one of claims 1-27, wherein the polypeptide is a chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the extracellular antigen-binding domain comprises the antibody or antigen-binding fragment thereof.

29. The polypeptide of claim 28, wherein the transmembrane domain comprises the transmembrane domain of a protein selected from the group consisting of CD8-alpha, CD4, CD28, CD137, CD80, CD86, CD152, and PD1.

30. The polypeptide of claim 28, wherein the intracellular signaling domain comprises an intracellular signaling domain of an immune effector cell.

31. The polypeptide of claim 28, wherein the intracellular signaling domain comprises the intracellular signaling domain from CD3-zeta.

32. The polypeptide of claim 28, wherein the CAR comprises a co-stimulatory domain.

33. The polypeptide of claim 28, wherein the CAR comprises a co-stimulatory domain from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, CD40L, CD3, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, and DAP10.

34. The polypeptide of claim 28, wherein the CAR comprises a hinge domain between the extracellular antigen-binding domain and the transmembrane domain.

35. The polypeptide of claim 28, wherein the CAR comprises a signal peptide.

36. The polypeptide of claim 28, wherein the CAR comprises a signal peptide comprising the sequence of MDMRVPAQLLGLLLLWLRGARC (SEQ ID NO: 245).

37. The polypeptide of claim 28, wherein the CAR exhibits sub-nanomolar potency.

38. The polypeptide of claim 28, wherein the CAR preferentially binds to a peptide-MHC complex containing a peptide having the sequence of SEQ ID NO: 1 over a peptide-MHC complex containing a peptide having a sequence that differs from SEQ ID NO: 1 by one or more amino acids.

39. The polypeptide of claim 28, wherein the hinge, transmembrane, and intracellular domains of the CAR comprise a sequence at least 95%, 99%, or 100% identical to TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDFWVLVVVG GVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPP RDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV KFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR (SEQ ID NO: 273).

40. The polypeptide of any one of claims 1-39, wherein the polypeptide is soluble.

41. A polypeptide, comprising an antibody or antigen-binding fragment thereof that specifically binds to a peptide:MHC (pMHC) complex displaying a MAGE-A3$_{112-120}$ peptide comprising the sequence of KVAELVHFL (SEQ ID NO: 332).

42. The polypeptide of claim 41, wherein the antibody or antigen-binding fragment thereof exhibits at least 1,000-fold lower reactivity (1,000-fold higher EC_{50}) for an

EPS8L2 pMHC complex displaying the EPS8L2 peptide SAAELVHFL (SEQ ID NO: 333) than for the MAGE-A3$_{112-120}$ pMHC complex.

43. The polypeptide of claim 41 or claim 42, wherein the antibody or antigen-binding fragment thereof comprises:

    a. a heavy-chain complementarity-determining region 1 (CDR-H1) comprising the sequence of SEQ ID NO: 287;

    b. a CDR-H2 comprising the sequence of SEQ ID NO: 288;

    c. a CDR-H3 comprising the sequence of SEQ ID NO: 289;

    d. a CDR-L1 comprising the sequence of any one of SEQ ID NOs: 290-292;

    e. a CDR-L2 comprising the sequence of any one of SEQ ID NOs: 293-300;

    f. a CDR-L3 comprising the sequence of any one of SEQ ID NOs: 301-310.

44. The polypeptide of claim 41 or claim 42, wherein the antibody or antigen-binding fragment thereof comprises:

    a. a CDR-H1 comprising the sequence of SEQ ID NO: 287;

    b. a CDR-H2 comprising the sequence of SEQ ID NO: 288;

    c. a CDR-H3 comprising the sequence of SEQ ID NO: 289;

    d. a CDR-L1 comprising the sequence of SEQ ID NO: 290;

    e. a CDR-L2 comprising the sequence of SEQ ID NO: 293;

    f. a CDR-L3 comprising the sequence of SEQ ID NO: 306.

45. The polypeptide of any one of claims 41-44, wherein the polypeptide comprises a heavy-chain sequence according to SEQ ID NOs: 311.

46. The polypeptide of any one of claims 41-43 or 45, wherein the polypeptide comprises a light-chain sequence according to SEQ ID NOs: 312-328.

47. The polypeptide of any one of claims 41-42 or 44-45, wherein polypeptide comprises a light-chain sequence according to SEQ ID NO: 320.

48. The polypeptide of any one of claims 1-47, wherein the polypeptide comprises chimeric antigen receptor comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the extracellular antigen-binding domain comprises the antibody or antigen-binding fragment thereof.

49. The polypeptide of claim 48, wherein the transmembrane domain comprises the transmembrane domain of a protein selected from the group consisting of CD8-alpha, CD4, CD28, CD137, CD80, CD86, CD152, and PD1.

50. The polypeptide of claim 48, wherein the intracellular signaling domain comprises an intracellular signaling domain of an immune effector cell.

51. The polypeptide of claim 48, wherein the intracellular signaling domain comprises the intracellular signaling domain from CD3-zeta.

52. The polypeptide of claim 48, wherein the CAR comprises a co-stimulatory domain.

53. The polypeptide of claim 48, wherein the CAR comprises a co-stimulatory domain from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, CD40L, CD3, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, and DAP10.

54. The polypeptide of claim 48, wherein the CAR comprises a hinge domain between the extracellular antigen-binding domain and the transmembrane domain.

55. The polypeptide of claim 48, wherein the CAR comprises a signal peptide.

56. The polypeptide of claim 48, wherein the CAR comprises a signal peptide comprising the sequence of MDMRVPAQLLGLLLLWLRGARC (SEQ ID NO: 245).

57. The polypeptide of claim 48, wherein the CAR exhibits sub-nanomolar potency.

58. The polypeptide of claim 48, wherein the CAR preferentially binds to a peptide-MHC complex containing a peptide having the sequence of KVAELVHFL (SEQ ID NO: 332) over a peptide-MHC complex containing a peptide having a sequence that differs from KVAELVHFL (SEQ ID NO: 332) by one or more amino acids.

59. The polypeptide of claim 48, wherein the hinge, transmembrane, and intracellular domains of the CAR comprise a sequence at least 95%, 99%, or 100% identical to TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDFWVLVVVG GVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPP RDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV KFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR (SEQ ID NO: 334).

60. The polypeptide of any one of claims 1-47, wherein the polypeptide is a monoclonal antibody.

61. The polypeptide of any one of claims 1-47, wherein the polypeptide is a bispecific antibody.

62. A nucleic acid comprising a polynucleotide sequence encoding the polypeptide of any one of claims 1-61.

63. A vector comprising the nucleic acid of claim 62.

64. A cell comprising the nucleic acid of claim 62.

65. A chimeric antigen receptor, comprising the polypeptide of any one of claims 1-61.

66. A recombinant immune cell expressing the chimeric antigen receptor of claim 65.

67. The recombinant immune cell of claim 66, wherein the immune cell is a T cell.

68. The recombinant immune cell of claim 66, wherein the immune cell is an NK cell.

69. A pharmaceutical composition comprising a polypeptide according to any one of claims 1-61 and a pharmaceutically acceptable carrier, diluent, or excipient.

70. A pharmaceutical composition comprising a recombinant immune cell according to any one of claims 66-68 and a pharmaceutically acceptable carrier, diluent, or excipient.

71. A method for diagnosing a disease involving MAGE-A3 positive cells, the method comprising detecting or measuring MAGE-A3 positive cells using the polypeptide of any one of claims 1-61.

72. A method for treating a cancer that expresses MAGE-A3 in a subject in need thereof, the method comprising administering a polypeptide according to any one of claims 1-61 or a pharmaceutical composition according to claim 70 to the subject.

73. A method for treating a cancer that expresses MAGE-A3 in a subject in need thereof, wherein the method comprises administering a recombinant immune cell according to any one of claims 46-48 or a pharmaceutical composition according to claim 70.

74. The method of claim 72 or 73, wherein the cancer is a hematologic malignancy.

75. The method of claim 72 or 73, wherein the cancer is multiple myeloma.

76. The method of claim72 or 73, wherein the cancer is an epithelial cancer.

77. The method of claim 72 or 73, wherein the cancer is a solid tumor.

78. The method of claim 72 or 73, wherein the cancer is melanoma, head and neck cancer, breast cancer, lung cancer, or synovial sarcoma.

79. A method for treating a cancer that expresses MAGE-A3 in a subject in need thereof, comprising:

    a. selecting a therapeutic composition comprising an antibody, a recombinant immune cell expressing a chimeric antigen receptor, or a recombinant immune cell expressing a chimeric antigen receptor TCR, that:

        i. specifically binds to a peptide:MHC (pMHC) complex displaying a MAGE-A3 peptide; and

ii. exhibits at least 1,000-fold lower reactivity (1,000-fold higher EC_{50}) for an EPS8L2 pMHC complex displaying the EPS8L2 peptide SAAELVHFL (SEQ ID NO: 333) than for the MAGE-$A3_{112-120}$ pMHC complex; and

b. administering an effective amount of the therapeutic composition to the subject.

80. A method for treating a cancer that expresses MAGE-A3 in a subject in need thereof, comprising administering to the subject an effective amount of the therapeutic composition comprising an antibody, a recombinant immune cell expressing a chimeric antigen receptor, or a recombinant immune cell expressing a chimeric antigen receptor TCR, that:

a. specifically binds to a peptide:MHC (pMHC) complex displaying a MAGE-A3 peptide; and/or

b. exhibits at least 1,000-fold lower reactivity (1,000-fold higher EC_{50}) for an EPS8L2 pMHC complex displaying the EPS8L2 peptide SAAELVHFL (SEQ ID NO: 333) than for the MAGE-$A3_{112-120}$ pMHC complex.

81. A method for discovering an antibody useful in treating cancer that expresses MAGE-A3, comprising selecting from an antibody-display library one or more antibodies that:

a. specifically bind to a peptide:MHC (pMHC) complex displaying a MAGE-$A3_{112-120}$ peptide; and

b. exhibit at least 1,000-fold lower reactivity (1,000-fold higher EC_{50}) for an EPS8L2 pMHC complex displaying the EPS8L2 peptide SAAELVHFL(SEQ ID NO: 333) than for the MAGE-$A3_{112-120}$ pMHC complex.

82. A method of making a pharmaceutical composition for treating a cancer that expresses MAGE-A3, comprising:

a. selecting an antibody, or a recombinant immune cell expressing a chimeric antigen receptor, that:

i. specifically binds to a peptide:MHC (pMHC) complex displaying a MAGE-A3 peptide; and

ii. exhibits at least 1,000-fold lower reactivity (1,000-fold higher EC_{50}) for an EPS8L2 pMHC complex displaying the EPS8L2 peptide SAAELVHFL (SEQ ID NO: 333) than for the MAGE-$A3_{112-120}$ pMHC complex; and

b. preparing a pharmaceutical composition comprising the antibody or the recombinant immune cell to the subject.

83. The method of any one of claims 71-82, wherein the therapeutic composition comprises the antibody.

84. The method of any one of claims 71-82, wherein the therapeutic composition comprises the recombinant immune cell expressing a chimeric antigen receptor.

85. The method of any one of claims 71-82, wherein the therapeutic composition comprises the recombinant immune cell expressing a chimeric antigen receptor TCR.

86. The method of any one of claims 71-85, wherein the MAGE-A3 pMHC complex is a MAGE-$A3_{112-120}$ pMHC complex.

87. The method of any one of claims 71-85, wherein the MAGE-A3 pMHC complex is a MAGE-$A3_{271-279}$ pMHC complex.

88. The method of any one of claims 71-87, wherein the cancer is a hematologic malignancy.

89. The method of any one of claims 71-88, wherein the cancer is multiple myeloma.

90. The method of any one of claims 71-87, wherein the cancer is an epithelial cancer.

91. The method of any one of claims 71-87, wherein the cancer is a solid tumor.

92. The method of claim 91, wherein the cancer is melanoma, head and neck cancer, breast cancer, lung cancer, or synovial sarcoma.

FIG. 1

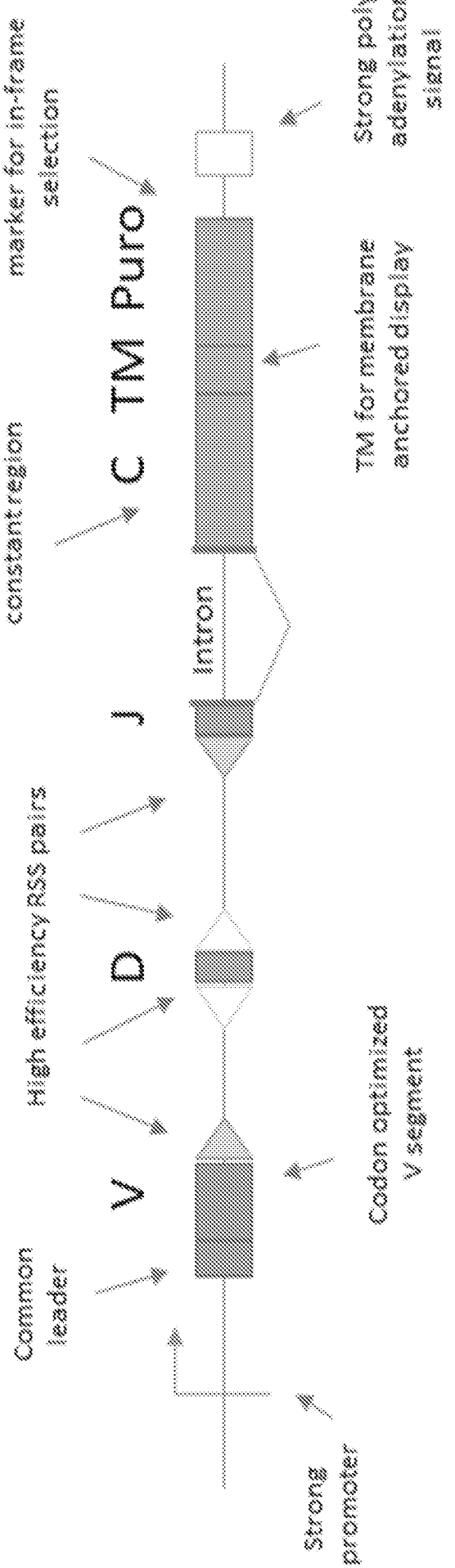

Strong promoter
Common leader
V
High efficiency RSS pairs
D
J
Codon optimized V segment
Intron
Codon optimized constant region
C
TM
Puro
Drug resistance marker for in-frame selection
TM for membrane anchored display
Strong poly adenylation signal

FIG. 2

FIG. 2A

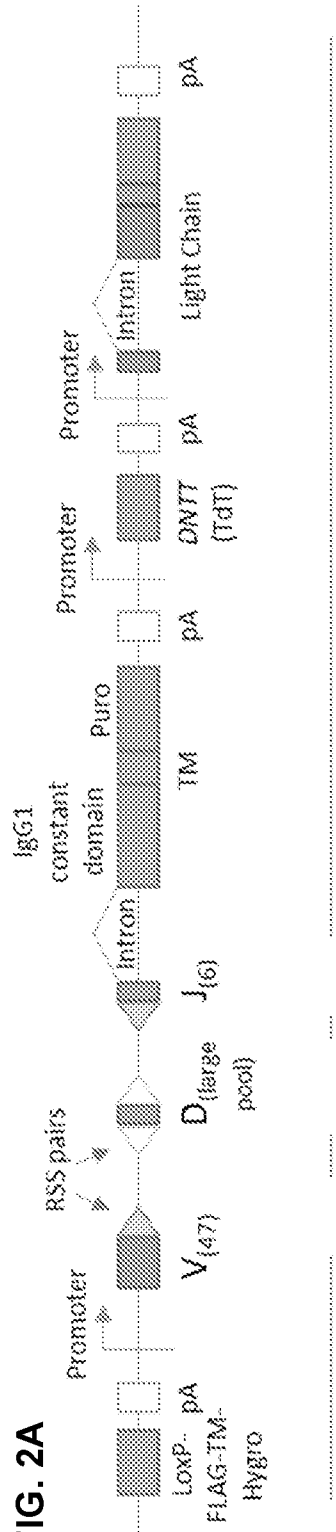


FIG. 2B

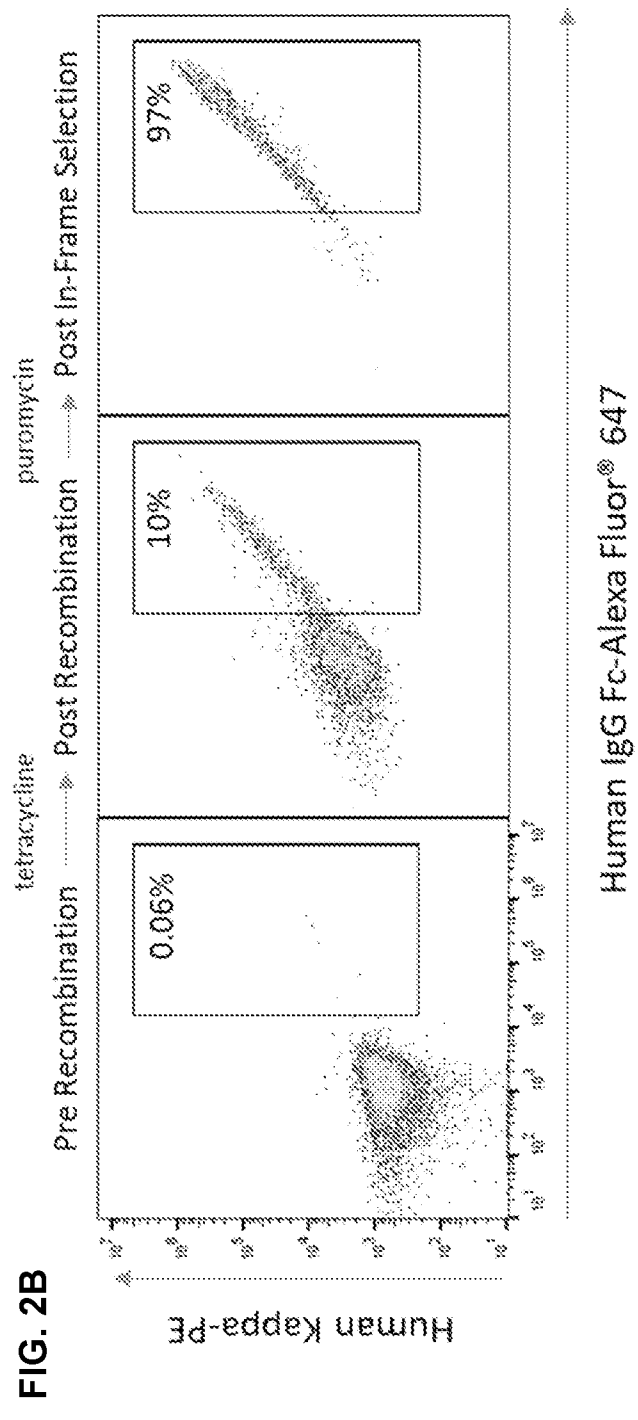


FIG. 3

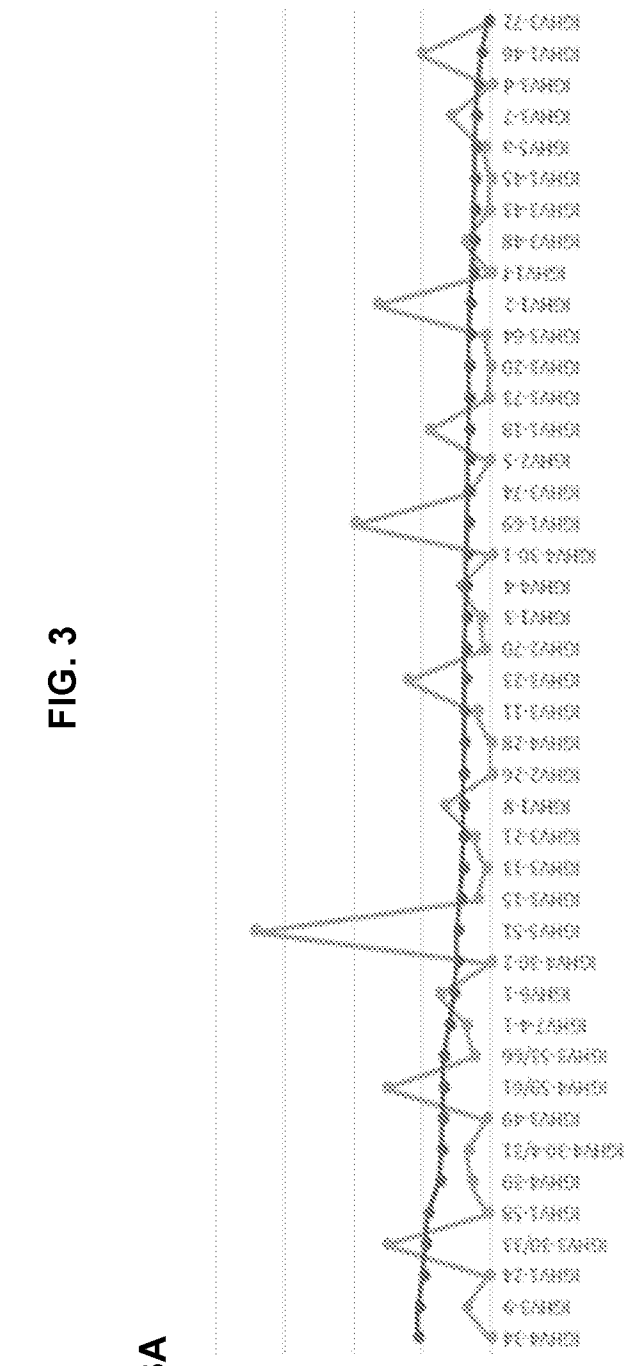


FIG. 3A

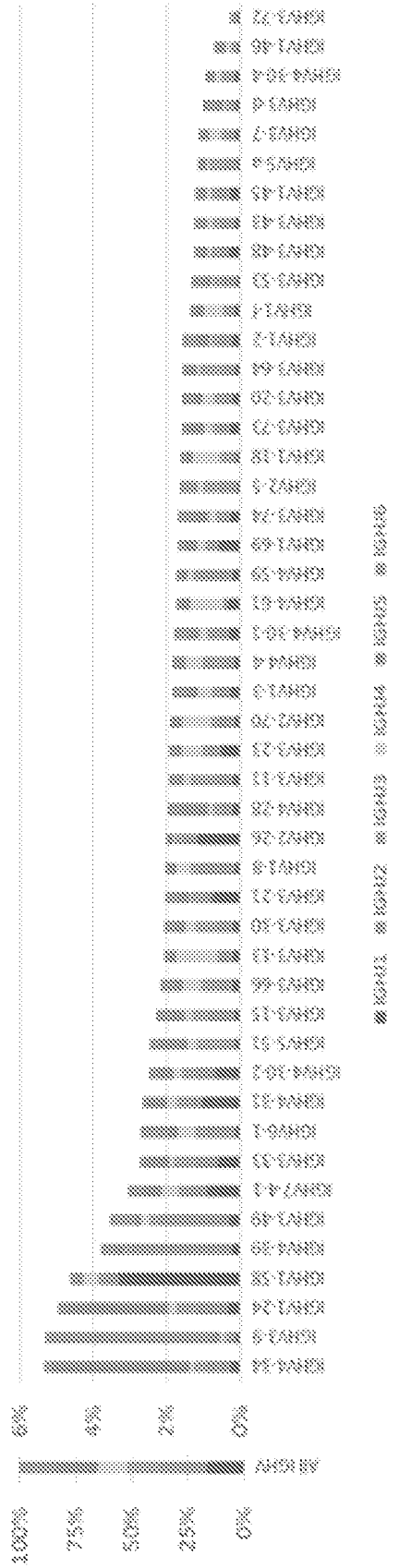


FIG. 3B

FIG. 3C

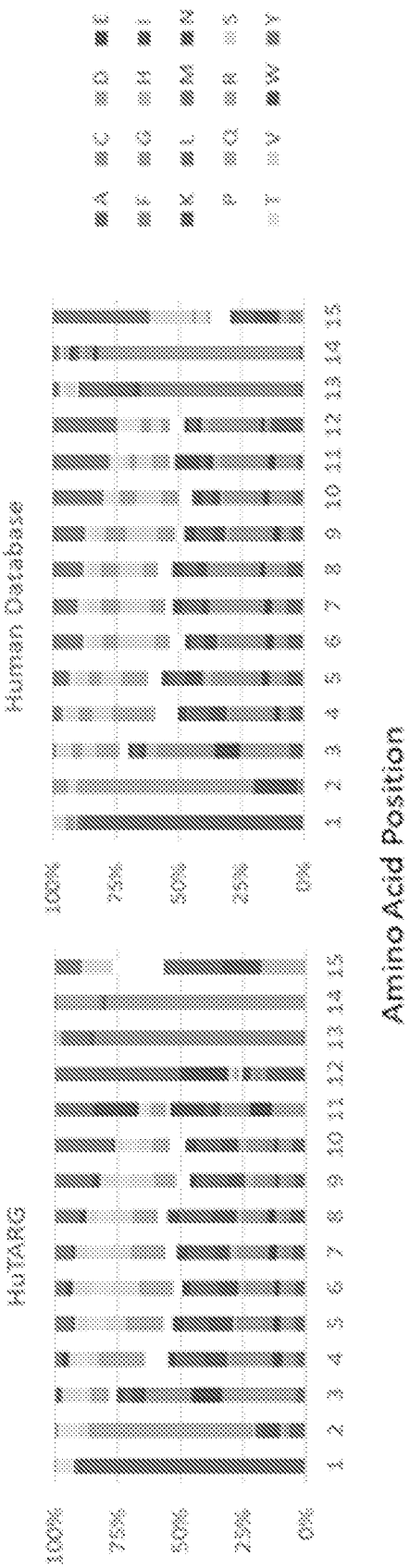

HuTARG
Human Database
100%
75%
50%
25%
0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
A C D E
F G H I
K L M N
P Q R S
T V W Y
Amino Acid Position

FIG. 3D

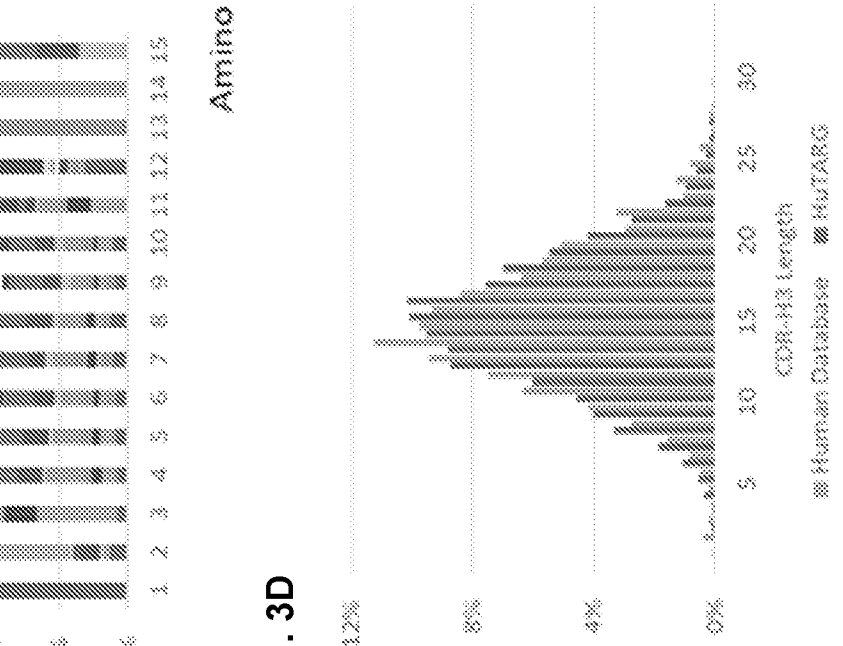

12%
8%
4%
0%
5 10 15 20 25 30
CDR-H3 Length
Human Database
HuTARG

FIG. 4

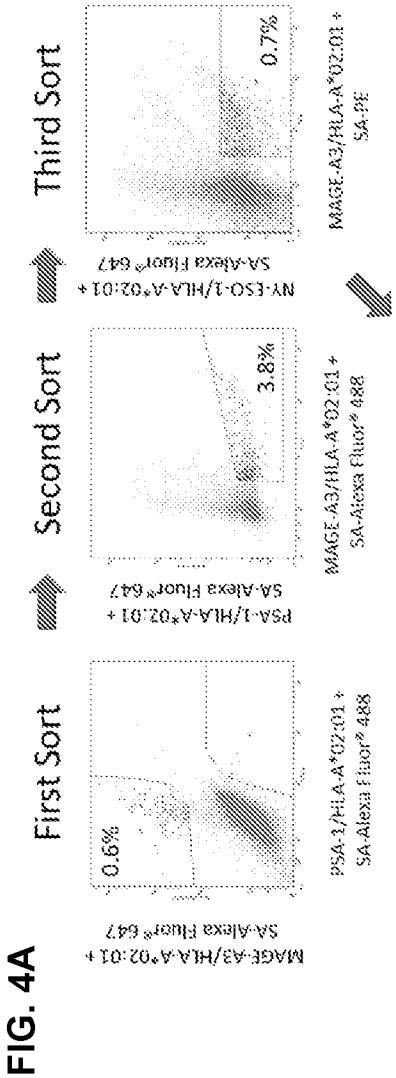


FIG. 4A

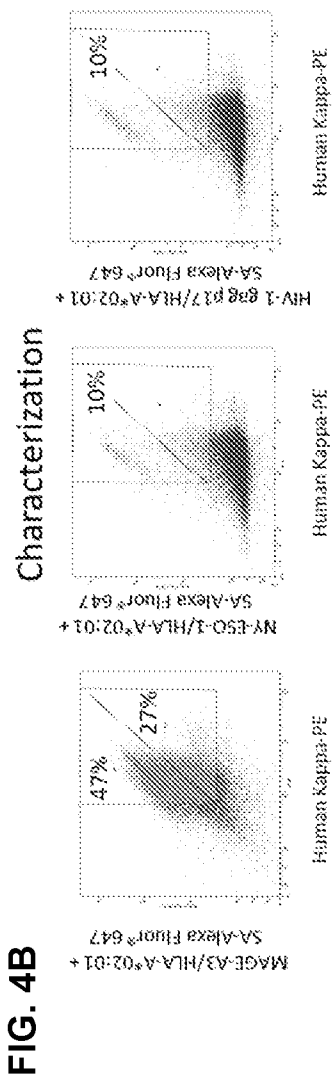
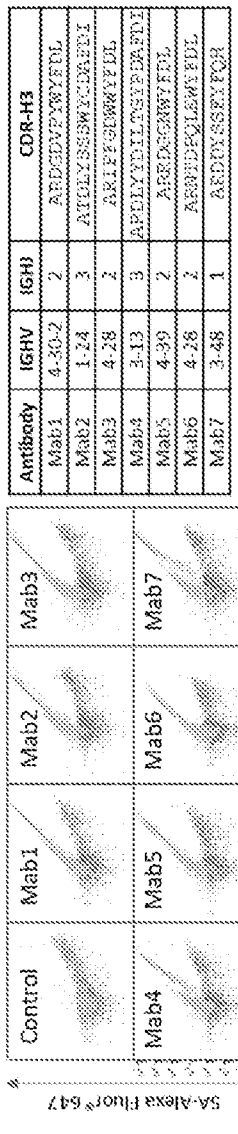


FIG. 4B



Antibody	IGHV	IGHJ	CDR-H3
Mab1	4-30-2	2	ARDGDVPYSWYFDL
Mab2	1-24	3	ATDLYSSSWYCDAFDI
Mab3	4-28	2	ARIFFSSMWYFDL
Mab4	3-13	3	APDLYYDILTSYPDAFDI
Mab5	4-39	2	ARRIASGNWFFDL
Mab6	4-28	2	ARNIDFGLEWIFDL
Mab7	3-48	1	AEDDYSSEYFQH

FIG. 4C

FIG. 5

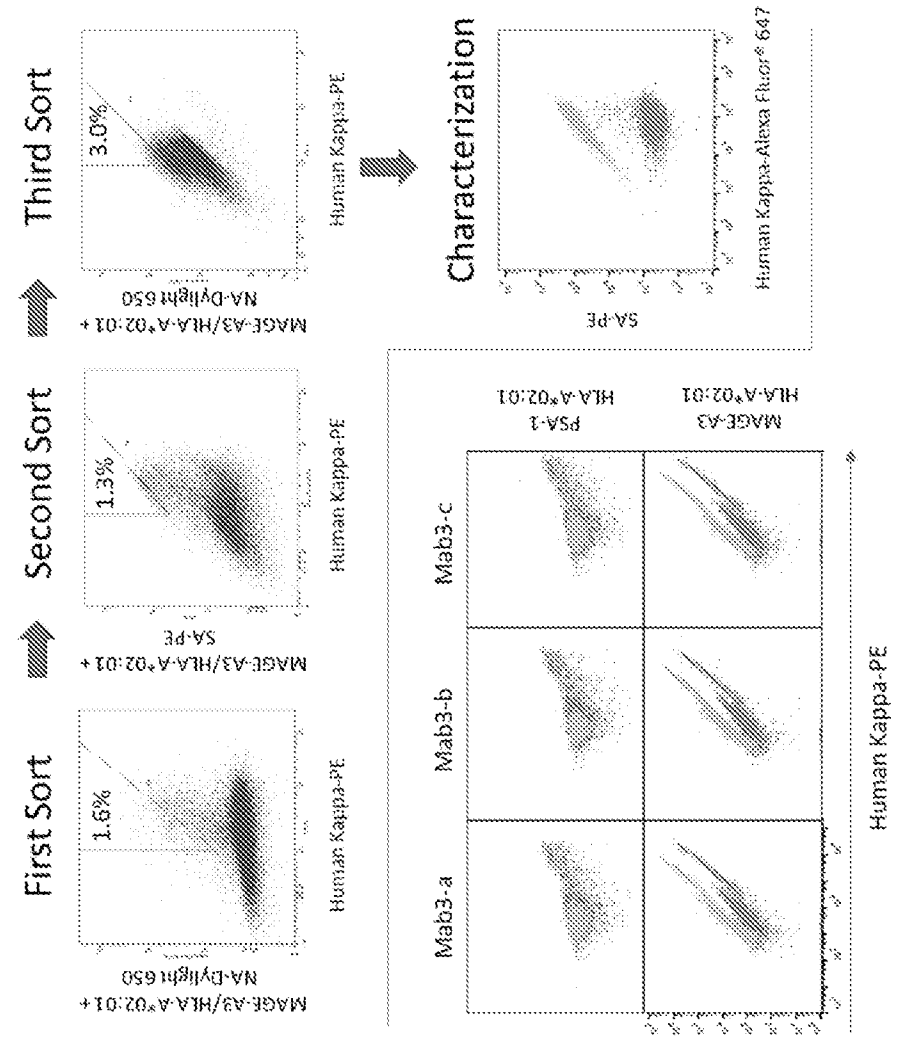


FIG. 5A

FIG. 5B

Construct	CDR-L1	CDR-L2	CDR-L3
Mab3	RASQSLI-----SSYLN	AASSLQS	QQSYSTPLT
Mab3-a	RASQSLI-----SSYLN	AASSLQS	QQSYSPYLT
Mab3-b	RASQSFI-----SSYLN	AASSLQS	QQSYSYYLT
Mab3-c	RASQSIGSSLSYLN	AASSLQS	QQSYSTPLT

FIG. 6

Construct	Average EC50 (nM)	SD EC50 (nM)	n	Fold Improved
CAR3	2.238	-	1	-
CAR3-a	0.0018	0.0004	5	1571
CAR3-b	0.0014	0.0016	6	3228
CAR3-c	0.0007	0.0002	3	4085

FIG. 6A

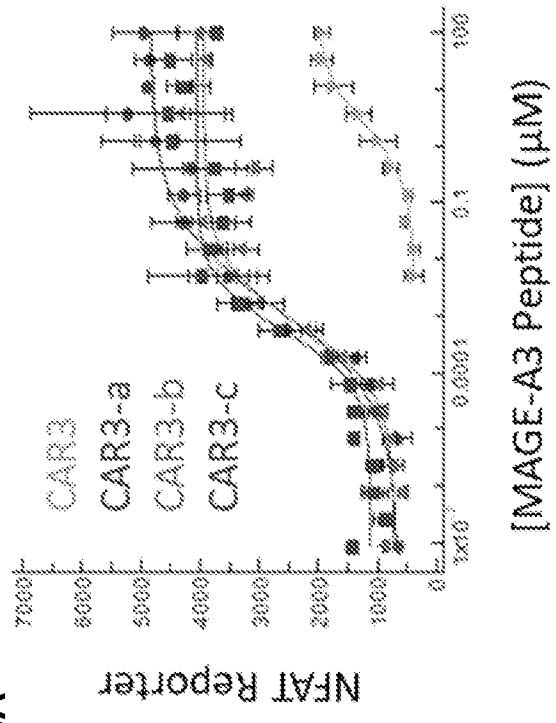
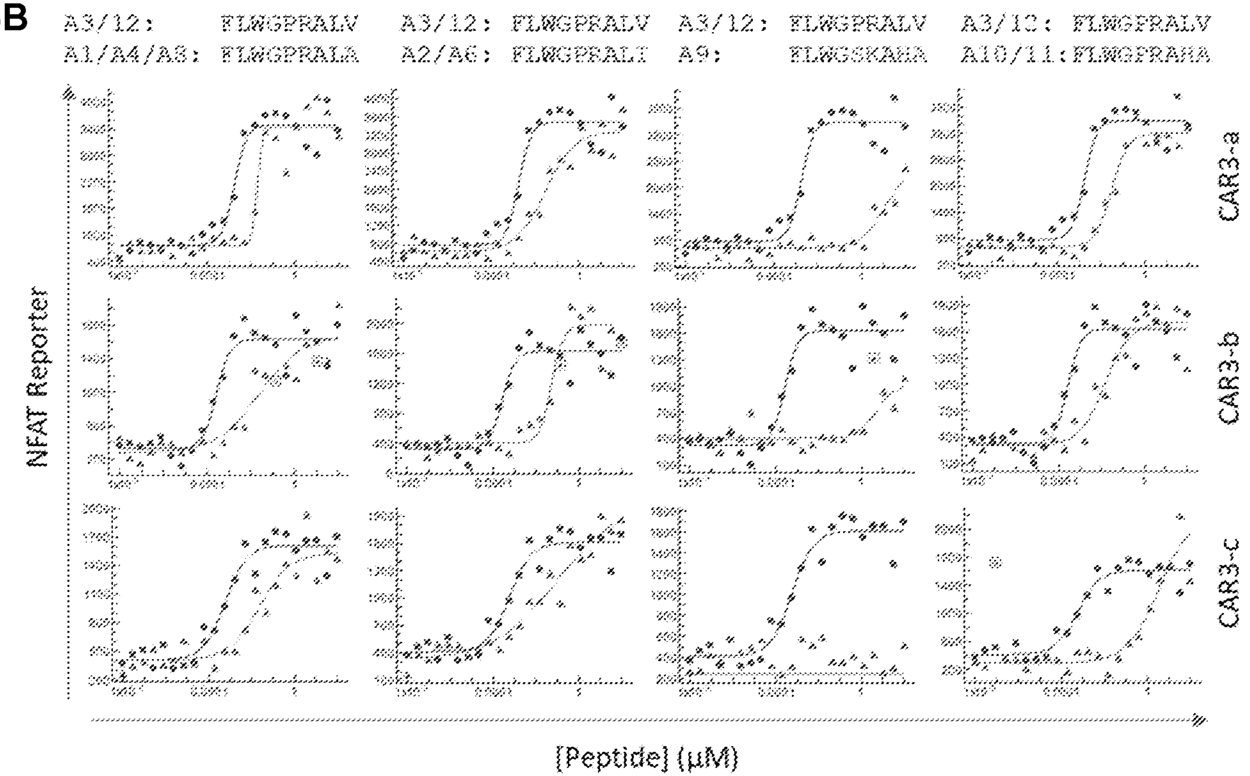


FIG. 6B



Peptide Sequence	MAGE family member	CAR3-a EC_{50} (nM)	CAR3-b EC_{50} (nM)	CAR3-c EC_{50} (nM)
FLWGPRALV	A3/A12 (target)	1.7	0.3	0.6
FLWGPRALA	A1/A4/A8	19.7	23.4	23.2
FLWGPRALI	A2/A6	28.1	52.2	96.9
FLWGSKAHA	A9	10809.7	4922.4	>100000
FLWGPRAHA	A10/A11	29.8	14.0	3353.5

FIG. 7

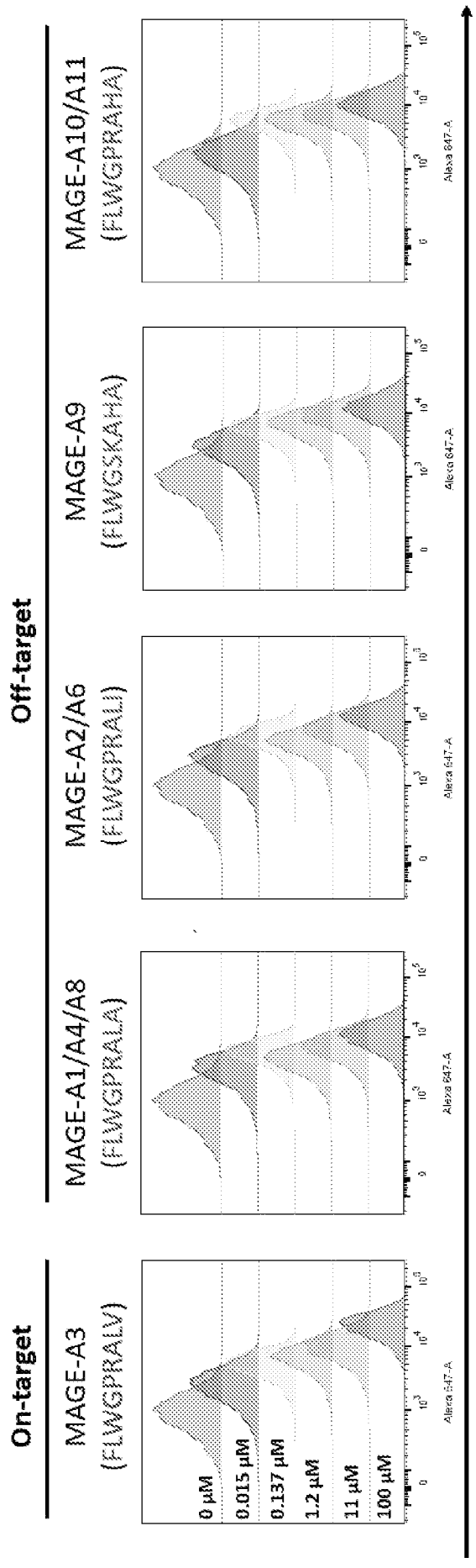

On-target
Off-target
MAGE-A3 (FLWGPRALV)
MAGE-A1/A4/A8 (FLWGPRALA)
MAGE-A2/A6 (FLWGPRALI)
MAGE-A9 (FLWGSKAHA)
MAGE-A10/A11 (FLWGPRAHA)
0 µM
0.015 µM
0.137 µM
1.2 µM
11 µM
100 µM
Alexa 647-A
anti-HLA antibody binding

FIG. 8

Peptide name	Position	Sequence	Constructs
MAGE-A3$_{271-279}$	271-279	FLWGPRALV	CT139 (TCR) C564 (CAR)
MAGE-A3$_{112-120}$	112-120	KVAELVHFL	CT138 (TCR) C1511(CAR)

FIG. 9

Genes	Testis (TPM)[1]	Brain region (TPM)[1]			HEK293[2]	MCF7 (RPKM)[3]	CAPAN-2 (RPKM)[3]	A375 (RPKM)[3]	PC3 (RPKM)[3]	K562 (RPKM)[3]
		All (avg.)	Basal ganglia[4]	Cerebellum[4]						
MAGE-A3	12.8	ND	ND	ND	no/low	0.03	0.1	175	0.8	74
MAGE-A12	5.9	0.2	0.3	0.1	no/low	0.04	0.1	245	0.05	87
MAGE –A10	3.8	0	ND	ND	no/low	0	0.07	74	0.04	0.3
EPS8L2	13.0	12.6	5.4	64.0	no/low	109	453	27	151	12

[1]Highest expression region for MAGE family members; GTEx (Nat Genet. 2013 June ; 45(6): 580–585. doi:10.1038/ng.2653)
[2]BioGPS (Wu et al., 2009); Below median of >60 cell lines on the U133A Affimatrix chip
[3]TRON (Scholtalbers et al., 2015)
[4]Highest expression region in the brain for the target gene (average of putamen and nucleus accumbens
ND, no TPM reported

FIG. 10

		MAGE-A3$_{112-120}$ TCR (CT138)				MAGE-A3$_{112-120}$ CAR (C1511)			
Peptide name	**Peptide seq**	**MAGE-A3$_{112-120}$ EC50 (μM)**	**Selectivity window (EC50 ratio)**	**MAGE-A3$_{112-120}$ ECmin (μM)**	**Selectivity window (ECmin ratio)**	**MAGE-A3$_{112-120}$ EC50 (μM)**	**Selectivity window**	**MAGE-A3$_{112-120}$ ECmin (μM)**	**Selectivity window ECmin/ECmin**
MAGE-A1	KVADLVGFL	>100	>10000	N/D	N/D	100	>2000	N/D	N/D
MAGE-A2	KMVELVHFL	53.4	5340	N/D	N/D	1.5	29	N/D	N/D
MAGE-A3$_{112-120}$ (on target)	KVAELVHFL	0.01	N/A	0.0003	N/A	0.05	N/A	0.0004	N/A
MAGE-A4	KVDELAHFL	>100	>10000	N/D	N/D	27	532	N/D	N/D
MAGE-A5	KVADLIHFL	>100	>10000	N/D	N/D	1.50	30	N/D	N/D
MAGE-A6	KVAKLVHFL	>100	>10000	N/D	N/D	33.8	676	N/D	N/D
MAGE-A8	KVAELVRFL	>100	>10000	N/D	N/D	33.5	670	N/D	N/D
MAGE-A10	KVTDLVQFL	>100	>10000	N/D	N/D	>100	>2000	N/D	N/D
MAGE-A11	KIIDLVHLL	>100	>10000	N/D	N/D	60.7	1214	N/D	N/D
MAGE-A12	KMAELVHFL	0.001	0.1	0.00002	0.067	0.007	0.14	0.0006	1.5
EPS8L2$_{339-347}$	SAAELVHFL	2.47	250	0.14	467	>100	>2000	1.23	3075
EPS8	SAADLVHFL	>100	>10000	N/D	N/D	25.3	506	N/D	N/D
MAGE-C3	KVAELVQFL	>100	>10000	N/D	N/D	24.4	488	N/D	N/D
MAGE-F1	TVAELVQFV	>100	>10000	N/D	N/D	8.50	170	N/D	N/D
MAGE-B18	KVVSLVHFL	>100	>10000	N/D	N/D	N/D	N/D	N/D	N/D
MRVI1	KLEELVHFL	>100	>10000	N/D	N/D	15.2	304	N/D	N/D
DDX28	KVAELVHIL	13.2	1320	N/D	N/D	N/D	N/D	N/D	N/D
PPP2R1B	GIAELVHFS	>100	>10000	N/D	N/D	67.2	1344	N/D	N/D

FIG. 11

		MAGE-A3$_{271-279}$ TCR (CT139)		MAGE-A3$_{271-279}$ CAR (C564)	
Peptide name	**Peptide seq**	**MAGE-A3$_{271-279}$ EC50 (μM)**	**Selectivity window**	**MAGE-A3$_{271-279}$ EC50 (μM)**	**Selectivity window**
1MAGE-A3$_{271-279}$ (on target)	FLWGPRALV	0.001	N/A	0.0007	N/A
A1/A4/A8	FLWGPRALA	3.22	3200	0.35	500
A2/A6	FLWGPRALI	1.43	1400	0.39	557
A9	FLWGSKAHA	>100	>100000	20.3	29000
A10/11	FLWGPRAHA	15.8	15800	0.53	757
Pan-MAGE A	FLWGPRAL	8.20	8200	0.18	257
MAGE-H1	FFWGPRAHV	>100	>100000	11.5	16428
EPS8L2	SAAELVHFL	>100	>100000	>100	>142857
MAGE-E2 pep1	FLWGSRAHRE	>100	>100000	>100	>142857
MAGE-E2 pep2	FLWGSRAHR	>100	>100000	>100	>142857
PCSK1Na	LLWGPRAGGV	>100	>100000	0.14	200
PCSK1Nb	LLWGPRAGG	>100	>100000	32.0	45714
IMMP2L-pep1	FLWFIVVLG	>100	>100000	>100	>142857
IMMP2L-pep2	FLWFIVVL	>100	>100000	>100	>142857
IgG VH	FAYWGPRAL	>100	>100000	12.4	17714

FIG. 12

Peptide	Cell lines	Assay	EC50 (μM)	
			MAGE-A3$_{112-120}$ CAR (C1511)	MAGE-A3$_{112-120}$ TCR (CT138)
KVAELVHFL (MAGE-A3$_{112-120}$)	MCF-7	Homogeneous	1.1	0.0007
	HEK293	QuIK	30	1.6
SAAELVHFL (EPS8L2)	MCF-7	Homogenous	>100	0.005
	HEK293	QuIK	>100	1.4
KMAELVHFL (MAGE-A12)	MCF-7	Homogeneous	4.0	0.0003
	HEK293	QuIK	36	0.27

FIG. 13

MAGE-A12 probes have multiple mismatches with most of the MAGE-A family members

```
A9:   TG---------ACCA-------GGAGG-----ACAGGAGCCC-CAA
A1:   CA---------ACCC-------AGAGG-----ACAGGATTCC-CTG
A3:   TG---------ACCT-------GGAGG-----A--------C-CAG
A4:   C----------AACC--------GGAGG-----ACAGGATTCC-CTG
A5:   TGACCAGGATCACCA-------GGAAGCTC-CAGAGGATCCC-CAG
A6:   TG---------ACGT-------GGAGG---------AA-----CAG
A10:  AG---------AGCA-------AGAGG-----TCAAGAGCTG-TGG
A11:  CA---------ACCT-------GGAGG-----ACAGGAGTCC-CAG
A12:  GG---------ACCTTTTCTTCAGAGGGTGACTCAGGTCAACACAG
```

MAGE-A family members predicted to cross-react with probe have minimum expression in the brain

```
A2:       TGACCTTGTTTTCAGAAGGTGACTCAGGTCAACACAG
A2B:      TGACCTTGTTTTCAGAAGGTGACTCAGGTCAACACAG
A10:      GAACCTTGCTCTCAGAGGGTGACTCAAGTCAACACAG
A12:      GGACCTTTTCTTCAGAGGGTGACTCAGGTCAACACAG
```

MAGE-A expression in the brain

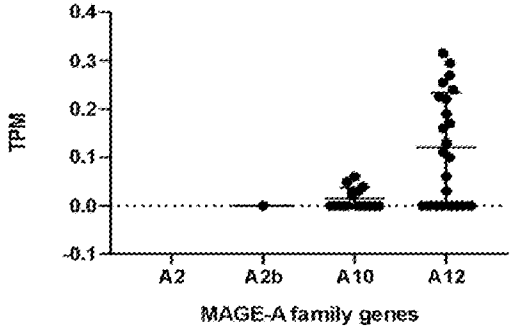


FIG. 14

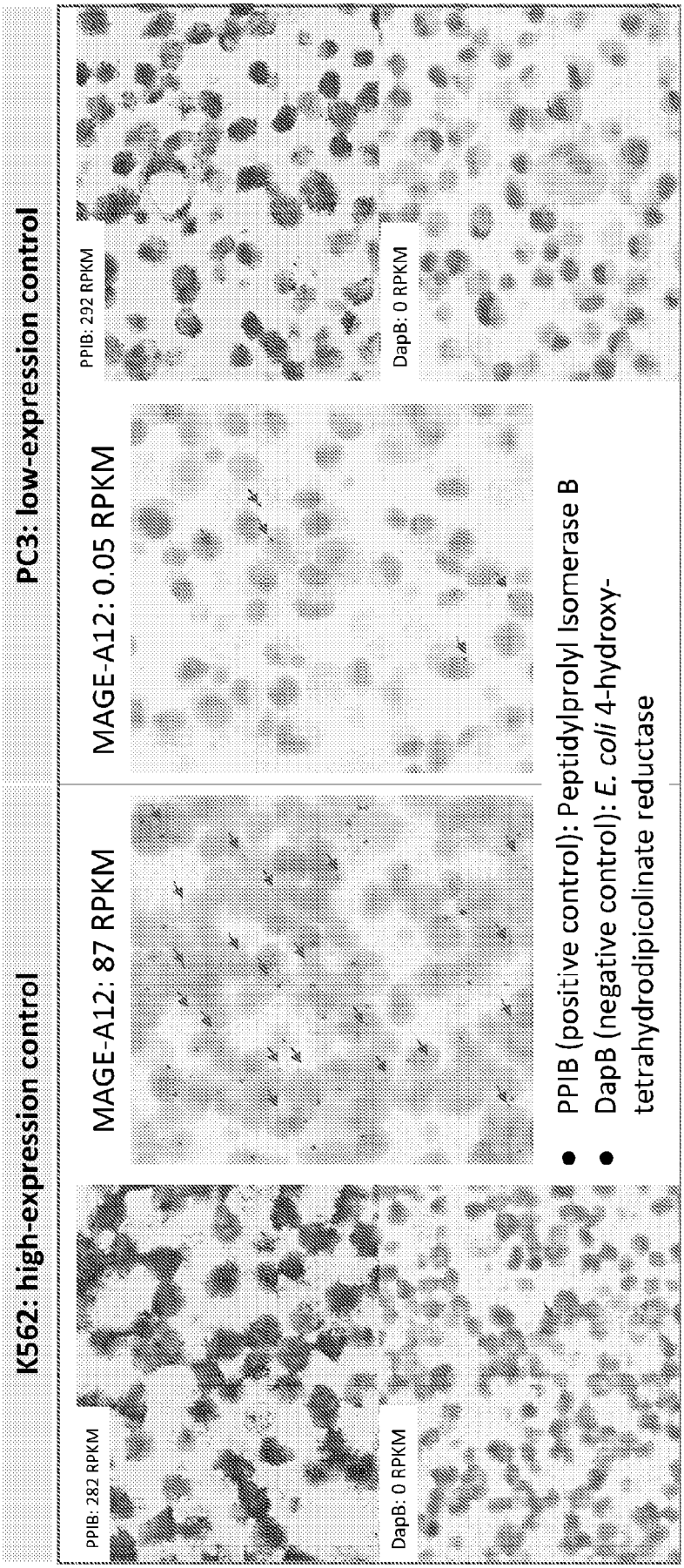
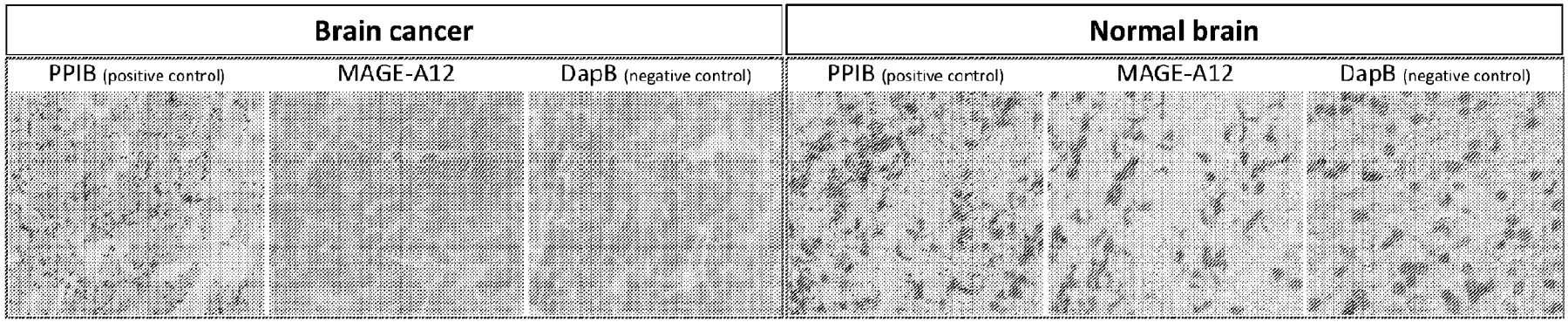

K562: high-expression control
PC3: low-expression control
PPIB: 282 RPKM
DapB: 0 RPKM
MAGE-A12: 87 RPKM
MAGE-A12: 0.05 RPKM
PPIB: 292 RPKM
DapB: 0 RPKM
PPIB (positive control): Peptidylprolyl Isomerase B
DapB (negative control): E. coli 4-hydroxy-tetrahydrodipicolinate reductase

FIG. 15



Sample types	Total donors	Total number of cells imaged	PPIB	MAGE-A12	DapB
Normal brain (cerebral cortex) or brain cancer	8	~3.2 million	GTEx: ~107 TPM	GTEx: 0.2 TPM	0 TPM
			RNA FISH: >4 dots/cell	RNA FISH: <1 dot/20 cells	RNA FISH: <1 dot/20 cells

FIG. 16

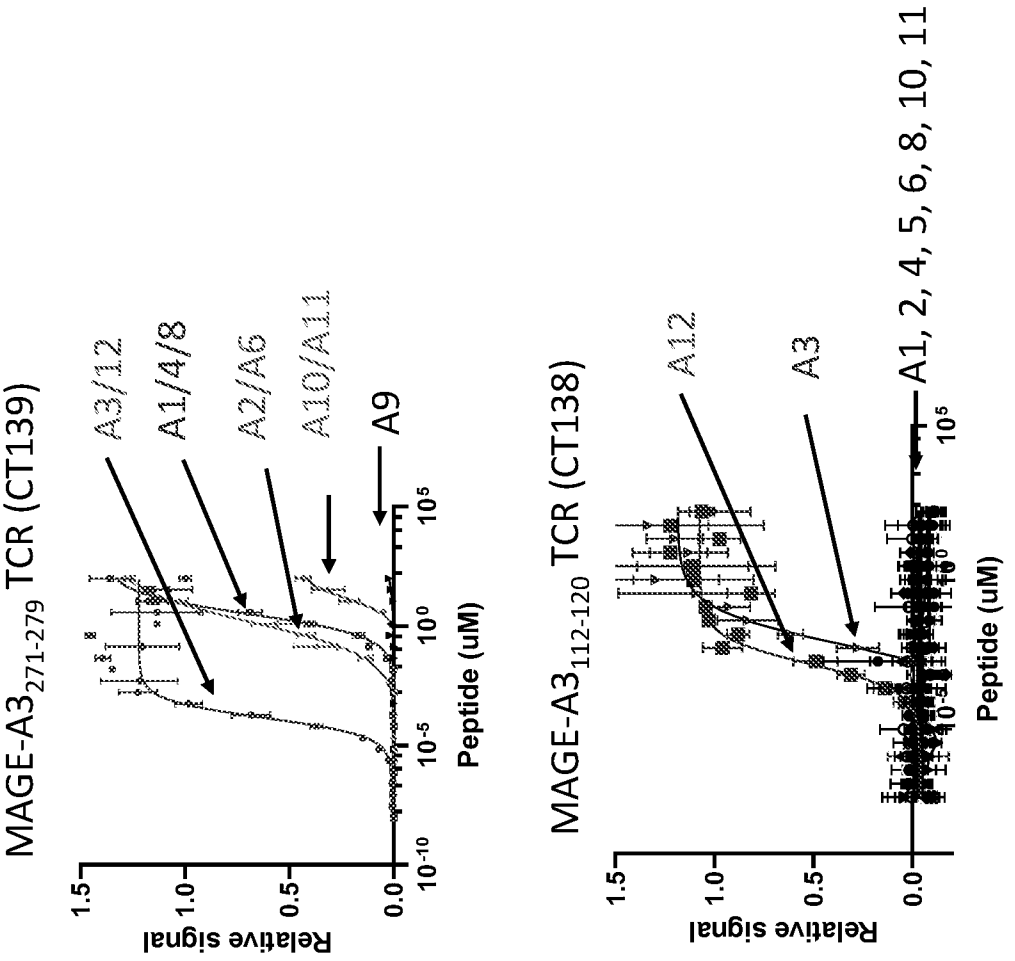

MAGE-A3271-279 TCR (CT139)
A3/12
A1/4/8
A2/A6
A10/A11
A9
Relative signal
Peptide (uM)
MAGE-A3112-120 TCR (CT138)
A12
A3
A1, 2, 4, 5, 6, 8, 10, 11
Relative signal
Peptide (uM)
MAGE-A3271-279 CAR (C564)
A3/A12
A1/4/8
A10/A11
A9
Relative signal
Peptide (uM)
MAGE-A3112-120 CAR (C1511)
A12
A3
A5
A2
A8
A6
A1, 4, 10,
Relative signal
Peptide (uM)

FIG. 17

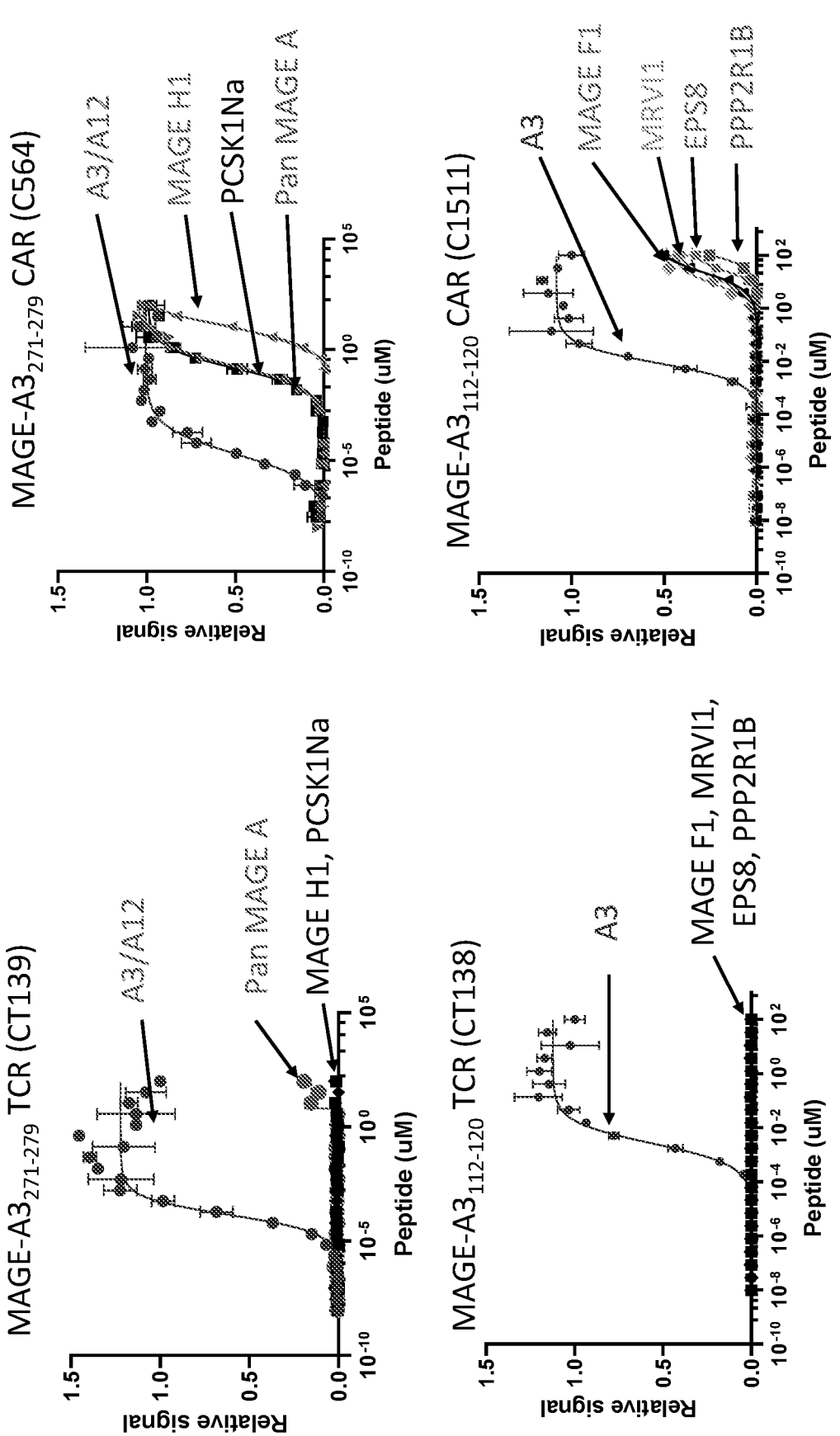

MAGE-A3271-279 CAR (C564)
A3/A12
MAGE H1
PCSK1Na
Pan MAGE A
Relative signal
Peptide (uM)
MAGE-A3112-120 CAR (C1511)
A3
MAGE F1
MRVI1
EPS8
PPP2R1B
MAGE-A3271-279 TCR (CT139)
A3/A12
Pan MAGE A
MAGE H1, PCSK1Na
MAGE-A3112-120 TCR (CT138)
A3
MAGE F1, MRVI1, EPS8, PPP2R1B

FIG. 18

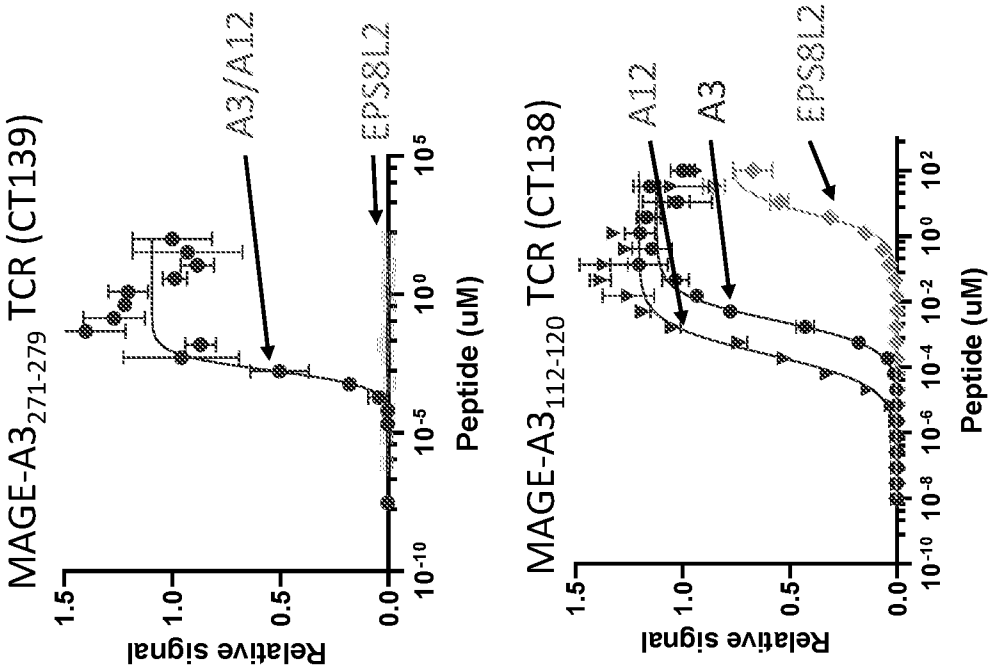

MAGE-A3271-279 TCR (CT139)
A3/A12
EPS8L2
Relative signal
Peptide (uM)
MAGE-A3112-120 TCR (CT138)
A12
A3
EPS8L2
Relative signal
Peptide (uM)
MAGE-A3271-279 CAR (C564)
A3/A12
EPS8L2
Relative signal
Peptide (uM)
MAGE-A3112-120 CAR (C1511)
A12
A3
EPS8L2
Relative signal
Peptide (uM)

FIG. 19

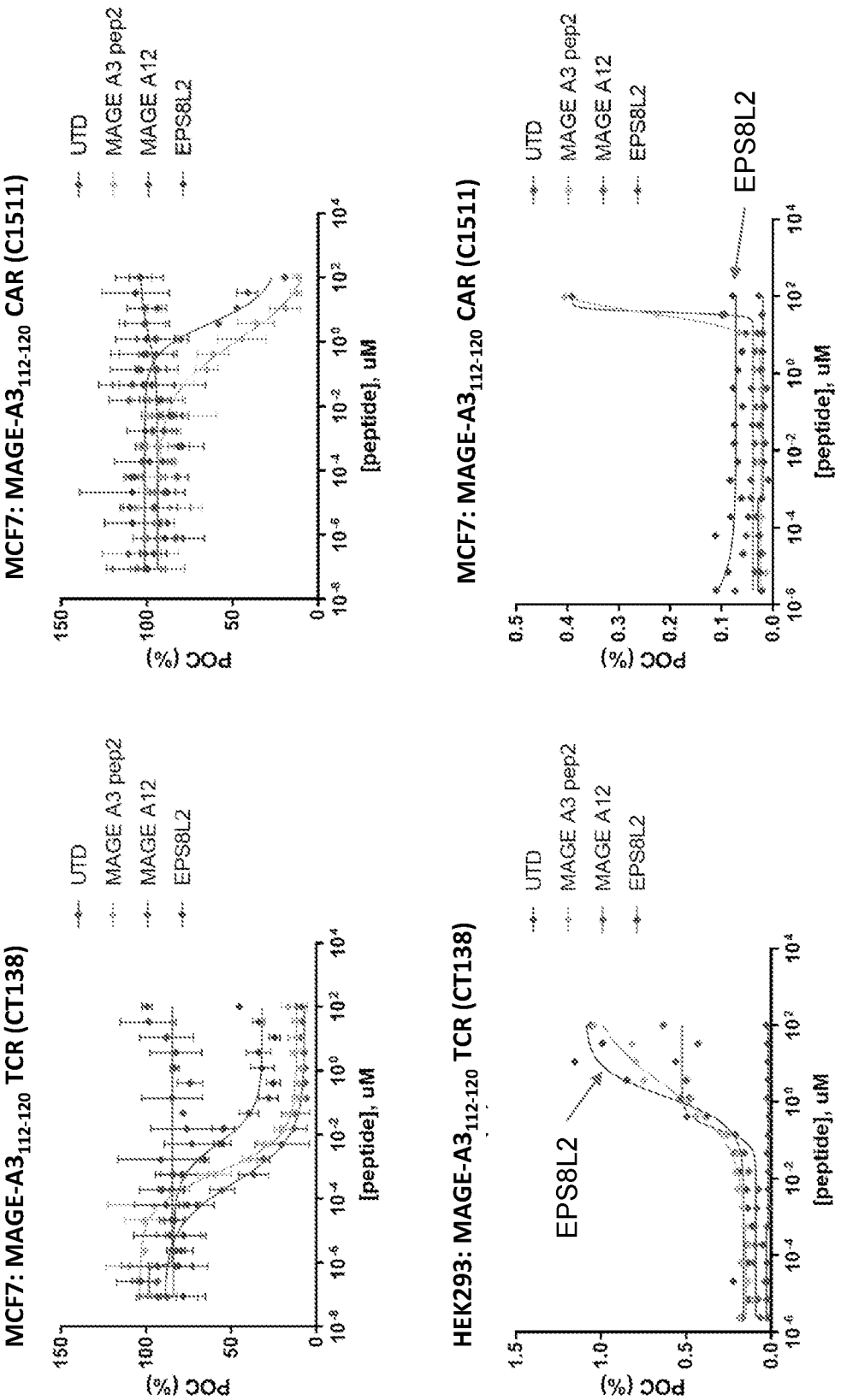

MCF7: MAGE-A3112-120 CAR (C1511)
UTD
MAGE A3 pep2
MAGE A12
EPS8L2
PDC (%)
[peptide], uM
MCF7: MAGE-A3112-120 TCR (CT138)
HEK293: MAGE-A3112-120 TCR (CT138)
EPS8L2

FIG. 20

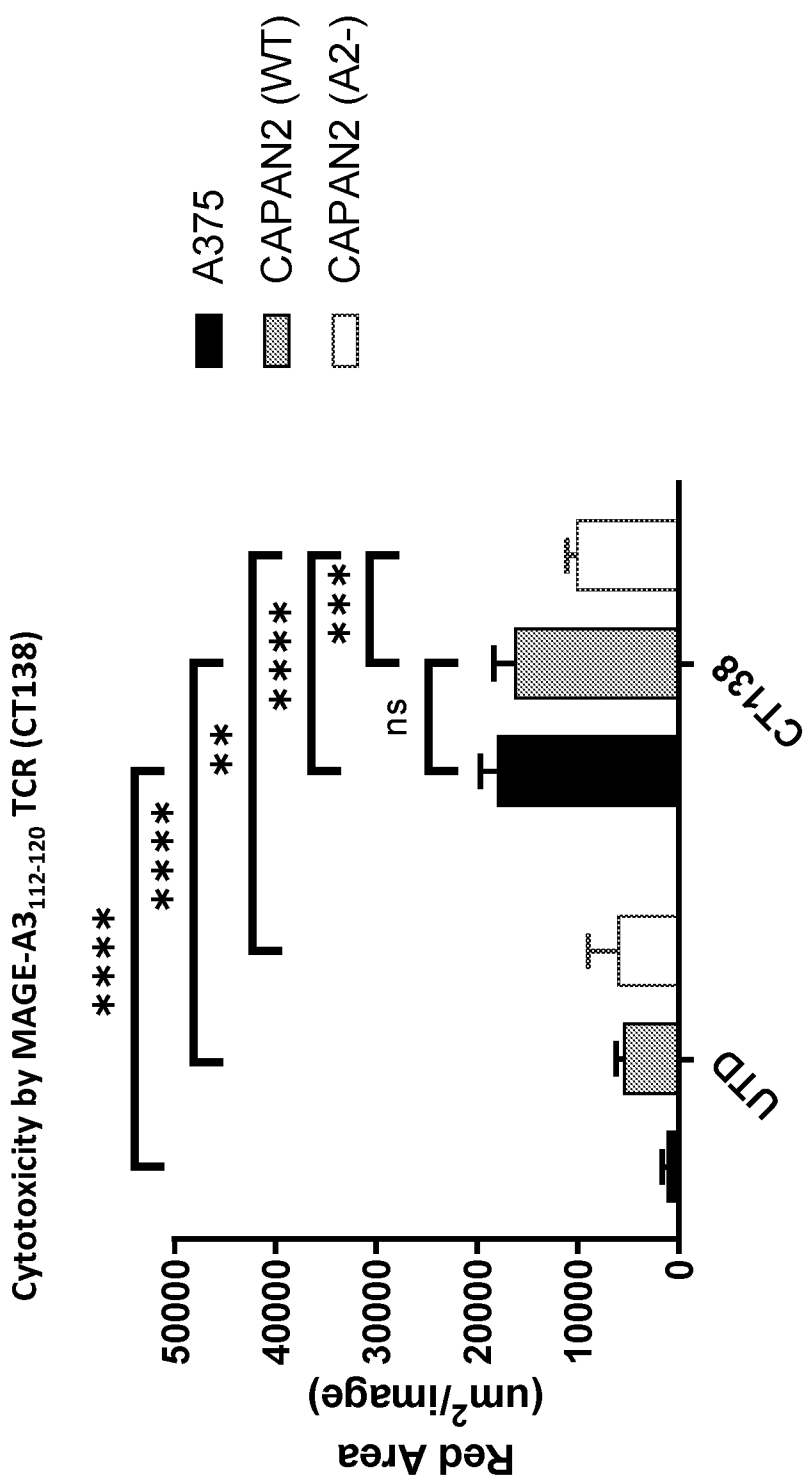

Cytotoxicity by MAGE-A3112-120 TCR (CT138)
A375
CAPAN2 (WT)
CAPAN2 (A2-)
Red Area (um2/image)
0
10000
20000
30000
40000
50000
****
****
**
****
ns
***
UTD
CT138

FIG. 21

		PANC.1	A.375[2]	SW527	CaSki	PC.3	LNCaP_clone_FGC	MCF.7	VMRC.LCD
	HLA-A[1]	A2/A11	A1/A2	A2/A24	A2/A3	A1/A24	A1/A2	A2/A2	A2/A2
FPKM[1]	MAGE-A3	0.2	174.6	0.02	0.04	0.8	98.9	0.03	176.4
	EPS8L2	176.2	27.2	122.3	393.4	151.4	151.5	109.7	78.7
Identified by mass spec?	MAGE-A3 pep1	No	No	No	N/D[3]	No	No	No	No
	MAGE-A3 pep2	No	No	No	N/D[3]	No	No	No	yes
	EPS8L2	Yes	No	No	No	No	No	No	No

[1]TRON database

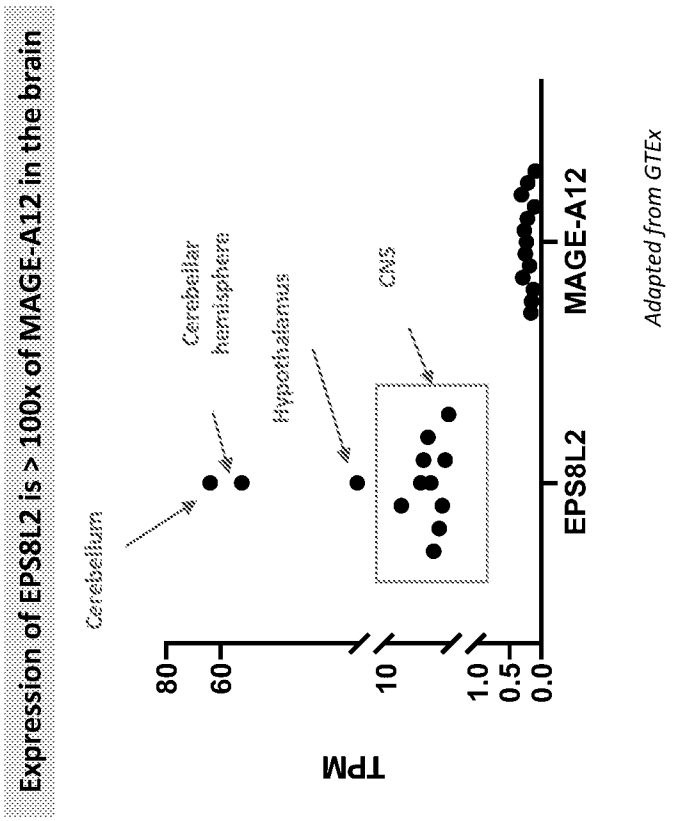
[2]500 nM MAGE-A3$_{271-279}$ peptide was loaded on 500 million cells

[3]A mixture of MAGE-A3$_{271-279}$ and MAGE-A3$_{112-120}$ peptides were spiked in Caski cell pellet as controls

FIG. 22

	Peptide name	Peptide seq	MAGE-A3 TCR EC50 (uM)	MAGE-A3 CAR EC50 (uM)
T2	MAGE-A3/12 pep 1	FLWGPRALV	0.0006	0.0007
	MAGE-A3 pep 2	KVAELVHFL	0.01	0.06
MCF7	MAGE-A3/12 pep 1	FLWGPRALV	0.72	0.22
	MAGE-A3 pep 2	KVAELVHFL	1.88	N/A
HEK	MAGE-A3/12 pep 1	FLWGPRALV	0.047	0.011
	MAGE-A3 pep 2	KVAELVHFL	0.4	1.03

FIG. 23


Expression of EPS8L2 is > 100x of MAGE-A12 in the brain
Cerebellum
Cerebellar hemisphere
Hypothalamus
CNS
80
60
10
1.0
0.5
0.0
TPM
EPS8L2
MAGE-A12
Adapted from GTEx

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/019420

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/019420

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-40(completely); 60-78, 83-92(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/019420

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/30 A61K39/00 C07K14/725
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2018/097951 A1 (US HEALTH [US]) 31 May 2018 (2018-05-31) claims 1-3	1-40
A	WO 2012/091563 A1 (APO T B V [NL]; WILLEMSEN RALPH ALEXANDER [NL]; RENES JOHAN [NL]) 5 July 2012 (2012-07-05) example 1; sequences 5,8	1-40
A	WO 2012/163769 A1 (CT ATLANTIC LTD [CH]; UNIV ZUERICH [CH] ET AL.) 6 December 2012 (2012-12-06) the whole document	1-40

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 May 2021	23/07/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Siaterli, Maria

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2012/163771 A1 (CT ATLANTIC LTD [CH]; UNIV ZUERICH [CH] ET AL.) 6 December 2012 (2012-12-06) claims 1-4; figure 3; examples 1,8; table 1; sequences 106,107	1-40
A	WO 2012/010635 A1 (GLAXOSMITHKLINE BIOLOG SA [BE]; BERGERON ALAIN [CA] ET AL.) 26 January 2012 (2012-01-26) claims 1-29; table 6	1-40
X	WO 2019/235915 A1 (APO T B V [NL]) 12 December 2019 (2019-12-12) claims 1-20; figure 4; examples 3-5	1-40, 60-78, 83-92
X	JULYUN OH ET AL: "Single variable domains from the T cell receptor [beta] chain function as mono- and bifunctional CARs and TCRs", SCIENTIFIC REPORTS, vol. 9, no. 1, 21 November 2019 (2019-11-21), pages 1-12, XP055732916, DOI: 10.1038/s41598-019-53756-4 the whole document page 17292, paragraph 5	1-7, 27-38, 40, 60-78, 83-92
X	BERNARDEAU KARINE ET AL: "Assessment of CD8 involvement in T?cell clone avidity by direct measurement of HLA-A2/Mage3 complex density using a high-affinity TCR like monoclonal antibody", EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 35, no. 10, 1 October 2005 (2005-10-01), pages 2864-2875, XP055792882, Weinheim ISSN: 0014-2980, DOI: 10.1002/eji.200526307 page 2865, right-hand column, last paragraph; figures 1B, 2A	1-40, 60-78, 83-92
X	WO 2013/105856 A1 (APO T B V [NL]) 18 July 2013 (2013-07-18) figure 6; examples 2,5	1-40, 60-78, 83-92
X	WO 2012/054825 A1 (US HEALTH [US]; MORGAN RICHARD A [US] ET AL.) 26 April 2012 (2012-04-26) the whole document claims 1-47; example 1	1-40, 60-78, 83-92

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/019420

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018097951 A1	31-05-2018	NONE	
WO 2012091563 A1	05-07-2012	CA 2822938 A1	05-07-2012
		DK 2658872 T3	12-10-2020
		EP 2658872 A1	06-11-2013
		EP 3778642 A1	17-02-2021
		US 2014205599 A1	24-07-2014
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		WO 2012091563 A1	05-07-2012
WO 2012163769 A1	06-12-2012	EP 2714743 A1	09-04-2014
		JP 2014524891 A	25-09-2014
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		JP 2014518889 A	07-08-2014
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WO 2012010635 A1	26-01-2012	BR 112013001637 A2	24-05-2016
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		US 2013123472 A1	16-05-2013
		WO 2012010635 A1	26-01-2012
WO 2019235915 A1	12-12-2019	AU 2019283639 A1	07-01-2021
		CA 3102254 A1	12-12-2019
		EP 3802614 A1	14-04-2021
		WO 2019235915 A1	12-12-2019
WO 2013105856 A1	18-07-2013	AU 2013208364 A1	07-08-2014
		AU 2018200442 A1	08-02-2018
		CA 2860914 A1	18-07-2013
		EP 2802356 A1	19-11-2014
		EP 3470434 A1	17-04-2019
		JP 2015504895 A	16-02-2015
		JP 2018083814 A	31-05-2018
		JP 2020097579 A	25-06-2020
		SG 10201705698P A	30-08-2017
		SG 11201404007W A	28-08-2014
		US 2013183307 A1	18-07-2013
		US 2015056198 A1	26-02-2015
		US 2015202318 A1	23-07-2015
		US 2018154013 A1	07-06-2018
		US 2021205465 A1	08-07-2021
		WO 2013105856 A1	18-07-2013
		ZA 201405109 B	23-12-2015
WO 2012054825 A1	26-04-2012	NONE	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-40(completely); 60-78, 83-92(partially)

   A polypeptide, comprising an antibody or antigen-binding fragment thereof that specifically binds to MAGE-A3 271-279 peptide comprising the sequence of FLWGPRALV (SEQ ID NO: 1

   ---

2. claims: 41-59, 79-82(completely); 60-78, 83-92(partially)

   A polypeptide, comprising an antibody or antigen-binding fragment thereof that specifically binds to a peptide: MHC (pMHC) complex displaying a MAGE-A3112 -120 peptide comprising the sequence of KVAELVHFL (SEQ ID NO: 332).
