

cancer therapy. Although the NY-ESO-1 antigen has been evaluated as a cancer vaccine candidate, few complete humoral and cellular immune responses have been obtained. Indeed, the use of NY-ESO-1 as a therapeutic target is challenging because of the difficulty in designing antigen-binding proteins that target HLA-presented antigens and the need to avoid off-target binding, so as to prevent nonspecific binding that may lead to decreased therapeutic efficacy and/or increased adverse effects (e.g., nonspecific cytotoxicity that diminishes tumor cell killing activity and/or causes side effects in subjects).

[0007] Accordingly, there is an unmet need in the art for new therapeutic strategies to target NY-ESO-1 with high specificity and to treat NY-ESO-1-associated cancers.

BRIEF SUMMARY

[0008] The present disclosure provides antigen-binding proteins that specifically bind to a conformational epitope of an HLA-displayed New York esophageal squamous cell carcinoma 1 (NY-ESO-1) peptide. The antigen-binding proteins of the present disclosure bind with a high degree of specificity to HLA-displayed NY-ESO-1 and do not bind to HLA-displayed peptides that differ by 2, 3, 4, 5 or more amino acids. The antigen-binding proteins of the present disclosure allow for specific targeting of NY-ESO-1 peptide-presenting cells (*i.e.*, cells presenting on their surface a NY-ESO-1 peptide bound to an MHC molecule, *e.g.*, HLA-A2), such as cancer cells expressing NY-ESO-1 and, in some [embodimentexamples](#), stimulating T cell activation, *e.g.*, to stimulate T cell-mediated killing of such cells. Furthermore, when fused to a detectable moiety, the antigen-binding proteins of the present disclosure allow for diagnosis and prognosis of NY-ESO-1-positive diseases or disorders with high sensitivity to changes in the number and distribution of NY-ESO-1 peptide-presenting cells, a more relevant measure of disease progression than circulating NY-ESO-1 levels.

[0009] The antigen-binding proteins of the disclosure may be antibodies, such as full-length (for example, an IgG1 or IgG4 antibody) antibodies, or may comprise only an antigen-binding portion of an antibody (for example, a Fab, F(ab')₂ or scFv fragment), and may be modified to affect functionality, *e.g.*, to eliminate residual effector functions (Reddy *et al.*, 2000, *J. Immunol.* 164:1925-1933). In some [embodimentexamples](#), the antigen-binding proteins of the disclosure may be antibodies, or antigen-binding fragments thereof. In certain [embodimentexamples](#), the antigen-binding proteins may be bispecific.

[\[0009a\]](#) In one aspect, the present disclosure refers to an antigen-binding protein that binds specifically to an HLA-A2:New York esophageal squamous cell carcinoma 1 peptide (NY-ESO-1 peptide) complex at at least one amino acid corresponding to an amino acid selected from the group consisting of M160, W161, and Q164 of SEQ ID NO: 271.

[\[0009b\]](#) In another aspect, the present disclosure refers to an antigen-binding protein that binds specifically to an HLA-A2:NY-ESO-1 157-165 peptide complex, wherein said isolated antigen-binding protein interacts with amino acids M160, W161, and Q164 of said NY-ESO-1 157-165 peptide as determined by X-ray crystallography at a resolution of 4.0 Å or higher.

[\[0009c\]](#) In another aspect, the present disclosure refers to an antigen-binding protein that specifically binds to an HLA-A2:NY-ESO-1 157-165 peptide complex but does not specifically bind to one or more off-target peptides selected from the group consisting of BCL9L 1351-1359 (SEQ ID NO: 273), GRID1 7-15 (SEQ ID NO: 274), ZDHHC1 376-384 (SEQ ID NO: 276), ITCH 807-815 (SEQ ID NO: 277), and URB1

1853-1861 (SEQ ID NO: 280), wherein the ratio of i) said antigen-binding protein binding to cells pulsed with said one or more off-target peptides to ii) unpulsed cells is less than about nine.

[0009d] In another aspect, the present disclosure refers to an antigen-binding protein that competes for binding to an antigen-binding protein as disclosed herein.

[0009e] In another aspect, the present disclosure refers to an antigen-binding protein that binds to the same epitope as an antigen-binding protein as disclosed herein.

[0009f] In another aspect, the present disclosure refers to a pharmaceutical composition comprising an antigen-binding protein that binds to HLA-A2:NY-ESO-1 as disclosed herein and a pharmaceutically acceptable carrier or diluent.

[0009g] In another aspect, the present disclosure refers to an isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a HCVR of an antigen-binding protein as disclosed herein.

[0009h] In another aspect, the present disclosure refers to an isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a LCVR of an antigen-binding protein as disclosed herein.

[0009i] In another aspect, the present disclosure refers to a vector comprising the polynucleotide molecule as disclosed herein.

[0009j] In another aspect, the present disclosure refers to a cell expressing the polynucleotide molecule as disclosed herein, or the vector as disclosed herein.

[0009k] In another aspect, the present disclosure refers to the use of the antigen-binding protein as disclosed herein or the pharmaceutical composition as disclosed herein, or the cell as disclosed herein in the manufacture of a medicament for treating a subject having an NY-ESO-1-associated disease or disorder.

[0009l] In another aspect, the present disclosure refers to an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an extracellular binding domain that specifically binds to a conformational epitope of an HLA-A2 presented New York esophageal squamous cell carcinoma 1 peptide (NY-ESO-1 peptide), a transmembrane domain, and an intracellular signaling domain.

[0009m] In another aspect, the present disclosure refers to a vector comprising the isolated nucleic acid molecule as disclosed herein.

[0009n] In another aspect, the present disclosure refers to an isolated immune effector cell comprising the isolated nucleic acid molecule as disclosed herein or the vector as disclosed herein.

[0009o] In another aspect, the present disclosure refers to the use of the immune effector cell as disclosed herein in the manufacture of a medicament for treating a subject having an NY-ESO-1-associated disease or disorder.

[0010] In a first **aspectexample**, the present disclosure provides recombinant antigen-binding proteins that bind specifically to a conformational epitope of an HLA-displayed New York esophageal squamous cell carcinoma 1 (NY-ESO-1) peptide, such as a HLA-displayed peptide comprising amino acid residues 157-165 of NY-ESO-1. In certain **embodimentexamples**, the antigen-binding proteins are antibodies. In some **embodimentexamples**, the antibodies are fully human.

[0011] Exemplary anti-HLA-A2:NY-ESO-1 antigen-binding proteins of the present disclosure

are listed in Tables 1 and 2 herein. Table 1 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs), light chain variable regions (LCVRs), heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) of the exemplary anti-HLA-A2:NY-ESO-1 antibodies. Table 2 sets forth the nucleic acid sequence identifiers of the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2 and LCDR3 of the exemplary anti-HLA-A2:NY-ESO-1 antibodies.

[0012] The present disclosure provides antigen-binding proteins comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100% sequence identity thereto. In some embodimentexamples, an antigen-binding protein with sequence identity less than 100% comprises CDR sequences from an HCVR of Table 1. For example, such an antigen-binding protein can comprise those CDR sequences but have differences in a framework region as compared to the HCVR of Table 1.

[0013] The present disclosure also provides antigen-binding proteins comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100% sequence identity thereto. In some embodimentexamples, an antigen-binding protein with sequence identity less than 100% comprises CDR sequences from an LCVR of Table 1. For example, such an antigen-binding protein can comprise those CDR sequences but have differences in a framework region as compared to the LCVR of Table 1.

[0014] The present disclosure also provides antigen-binding proteins comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1. According to certain embodimentexamples, the present disclosure provides antigen-binding proteins comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary anti-HLA-A2:NY-ESO-1 antigen-binding proteins listed in Table 1. In certain embodimentexamples, the HCVR/LCVR amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 2/10, 22/30, 42/50, 62/70, 82/90, 102/110, 122/130, 142/150, 162/170, 180/186, 196/203, 211/219, and 230/238. In certain embodimentexamples, the HCVR/LCVR amino acid sequence pair is selected from one of SEQ ID NOs: 2/10 (e.g., mAb24955N), 22/30 (e.g., mAb24956N), 42/50

(e.g., mAb24958N), and 62/70 (e.g., mAb24959N).

[0015] In certain embodimentexamples, the present disclosure provides anti-HLA-A2:NY-ESO-1 antigen-binding proteins comprising a HCVR and a LCVR, said HCVR comprising an amino acid sequence listed in Table 1 having no more than five amino acid substitutions, and said LCVR comprising an amino acid sequence listed in Table 1 having no more than five amino acid substitutions. For example, the present disclosure provides anti-HLA-A2:NY-ESO-1 antigen-binding proteins comprising a HCVR and a LCVR, said HCVR comprising an amino acid sequence of SEQ ID NO: 2 having no more than five amino acid substitutions, and said LCVR comprising an amino acid sequence of SEQ ID NO: 10 having no more than five amino acid substitutions. In another exemplary embodimentexample, the present disclosure provides anti-HLA-A2:NY-ESO-1 antigen-binding proteins comprising a HCVR and a LCVR, said HCVR comprising an amino acid sequence of SEQ ID NO: 2 having at least one amino acid substitution, and said LCVR comprising an amino acid sequence of SEQ ID NO: 10 having at least one amino acid substitution.

[0016] The present disclosure also provides antigen-binding proteins comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

[0017] The present disclosure also provides antigen-binding proteins comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

[0018] The present disclosure also provides antigen-binding proteins comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

[0019] The present disclosure also provides antigen-binding proteins comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino

acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

[0020] The present disclosure also provides antigen-binding proteins comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

[0021] The present disclosure also provides antigen-binding proteins comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

[0022] The present disclosure also provides antigen-binding proteins comprising a HCDR3 and a LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 1 paired with any of the LCDR3 amino acid sequences listed in Table 1. According to certain [embodiment examples](#), the present disclosure provides antigen-binding proteins, comprising an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary anti-HLA-A2:NY-ESO-1 antigen-binding proteins listed in Table 1. In certain [embodiment examples](#), the HCDR3/LCDR3 amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 8/16 (*e.g.*, mAb24955N), 28/36 (*e.g.*, mAb24956N), 48/56 (*e.g.*, mAb25958N), and 68/76 (*e.g.*, mAb24959N).

[0023] The present disclosure also provides antigen-binding proteins comprising a HCVR and a LCVR, said HCVR comprising HCDR1 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid, HCDR2 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid, and HCDR3 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid. In certain [embodiment examples](#), the present disclosure provides antigen-binding proteins comprising a HCVR and a LCVR, said LCVR comprising LCDR1 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid, LCDR2 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid, and LCDR3 comprising an amino acid sequence differing from an amino acid sequence listed in Table 1 by 1 amino acid. For example, the present disclosure provides anti-HLA-A2:NY-ESO-1 antigen-binding proteins comprising a HCVR and a LCVR, said HCVR

comprising HCDR1 comprising an amino acid sequence of SEQ ID NO: 4 or an amino acid sequence differing from SEQ ID NO: 4 by 1 amino acid, HCDR2 comprising an amino acid sequence of SEQ ID NO: 6 or an amino acid sequence differing from SEQ ID NO: 6 by 1 amino acid, and HCDR3 comprising an amino acid sequence of SEQ ID NO: 8 or an amino acid sequence differing from SEQ ID NO: 8 by 1 amino acid. In another exemplary

[embodimentexample](#), the present disclosure provides antigen-binding proteins comprising a HCVR and a LCVR, said LCVR comprising LCDR1 comprising an amino acid sequence of SEQ ID NO: 12 or an amino acid sequence differing from SEQ ID NO: 12 by 1 amino acid, LCDR2 comprising an amino acid sequence of SEQ ID NO: 14 or an amino acid sequence differing from SEQ ID NO: 14 by 1 amino acid, and LCDR3 comprising an amino acid sequence of SEQ ID NO: 16 or an amino acid sequence differing from SEQ ID NO: 16 by 1 amino acid.

[0024] The present disclosure also provides antigen-binding proteins comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary antigen-binding proteins listed in Table 1. In certain [embodimentexamples](#), the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequence set is selected from the group consisting of SEQ ID NOs: 4-6-8-12-14-16 (*e.g.*, mAb24955N), 24-26-28-32-34-36 (*e.g.*, mAb24956N), 44-46-48-52-54-56 (*e.g.*, mAb24958N), and 64-66-68-72-74-76 (*e.g.*, mAb24959N).

[0025] In a related [embodimentexample](#), the present disclosure provides antigen-binding proteins comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary antigen-binding proteins listed in Table 1. For example, the present disclosure includes antigen-binding proteins comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10 (*e.g.*, mAb24955N), 22/30 (*e.g.*, mAb24956N), 42/50 (*e.g.*, mAb24958N), and 62/70 (*e.g.*, mAb24959N).

[0026] Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. See, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol.* 273:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA* 86:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antigen-binding protein.

[0027] The present disclosure includes anti-HLA-A2:NY-ESO-1 antigen-binding proteins having a modified glycosylation pattern. In some [embodimentexamples](#), modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield *et al.* (2002) *JBC* 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0028] In certain [embodimentexamples](#), the antigen-binding proteins of the present disclosure are monoclonal antibodies comprising a HCVR and a LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1. In certain [embodimentexample](#), the monoclonal antibodies comprise a Fc domain of an isotype selected from the group consisting of IgA, IgD, IgE, IgG, IgG1, IgG2, IgG3, IgG4, IgM and a variant thereof.

[0029] The present disclosure provides antigen-binding proteins, or antigen-binding fragments thereof, comprising a heavy chain comprising an amino acid sequence selected from any of the HC amino acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto. In some [embodimentexamples](#), an antigen-binding protein with sequence identity less than 100% comprises CDR sequences from an HC of Table 3. For example, such an antigen-binding protein can comprise those CDR sequences but have differences in a framework region as compared to the HC of Table 3.

[0030] The present disclosure also provides antigen-binding proteins, or antigen-binding fragments thereof, comprising a light chain comprising an amino acid sequence selected from any of the LC amino acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto. In some [embodimentexample](#), an antigen-binding protein with sequence identity less than 100% comprises CDR sequences from an LC of Table 3. For example, such an antigen-binding protein can comprise those CDR sequences but have differences in a framework region as compared to the LC of Table 3.

[0031] The present disclosure also provides antigen-binding proteins, or antigen-binding fragments thereof, comprising a HC and a LC amino acid sequence pair (HC/LC) comprising any of the HC amino acid sequences listed in Table 3 paired with any of the LC amino acid sequences listed in Table 3. According to certain [embodimentexamples](#), the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising an HC/LC amino acid sequence

pair contained within any of the exemplary anti-NY-ESO-1 antibodies listed in Table 3. In certain [embodimentexamples](#), the HC/LC amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 18/20, 38/40, 58/60, and 78/80. In certain [embodimentexamples](#), the HC/LC amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 18/20, 38/40, 58/60, and 78/80.

[0032] In one [aspectexample](#), the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that bind to a HLA-peptide complex wherein the antigen-binding protein or antigen-binding fragment thereof contacts at least 60%, at least 70%, at least 80% or at least 90% of the amino acid residues of the peptide that is comprised in the HLA-peptide complex. In certain [embodimentexamples](#), the antigen-binding protein or antigen-binding fragment thereof “covers” or contacts all of the amino acid residues of the peptide comprised in the HLA-peptide complex. In certain [embodimentexamples](#), the antigen-binding protein or antigen-binding fragment thereof binds to a HLA-peptide complex with high affinity and specificity, wherein the antigen-binding protein or antigen-binding fragment thereof contacts the entire length of the displayed peptide. “Contact”, as used herein includes direct or water-mediated hydrogen bonds, charge-charge interactions, or hydrophobic/van der Waals interactions. In one [embodimentexample](#), the antigen-binding protein or antigen-binding fragment thereof binds to HLA-A2-NY-ESO-1 157-165 peptide complex wherein the antigen-binding protein binds to at least 3 of 9 amino acid residues of peptide 157-165 (SEQ ID NO: 269) and to HLA-A2 such that the antigen-binding protein is roughly centered on the peptide in the HLA-A2 peptide-binding groove, thereby “covering” (physically blocking) the HLA-A2-peptide complex. In another [embodimentexample](#), the antigen-binding protein or antigen-binding fragment thereof binds to HLA-A2-NY-ESO-1 157-165 peptide complex wherein the antigen-binding protein binds to at least 3 of 9 amino acid residues of peptide 157-165 (SEQ ID NO: 270 or 291) and to HLA-A2 such that the antigen-binding protein is roughly centered on the peptide in the HLA-A2 peptide-binding groove, thereby “covering” (physically blocking) the HLA-A2-peptide complex. In certain [embodimentexamples](#), the antigen-binding protein or antigen-binding fragment thereof comprises the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1. In one [embodimentexample](#), the antigen-binding protein is fully human. In certain [embodimentexamples](#), the fully human antigen-binding proteins are not obtained using phage display methods and technologies. In some [embodimentexamples](#), the antigen-binding proteins comprise a light chain variable region of the IGKV1-39 sub-type. In some [embodimentexamples](#), the antigen-binding proteins comprise a light chain variable region of the IGKJ1 sub-type.

[0033] In certain [embodimentexamples](#), the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that bind to HLA-A2:NY-ESO-1 157-165 peptide complex, wherein the antigen-binding protein binds to one or more amino acids of SEQ ID NO: 269. In

one embodimentexample, the antigen-binding protein binds to at least 3 amino acids of SEQ ID NO: 269.

[0034] In certain embodimentexamples, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that bind to HLA-A2:NY-ESO-1 157-165 peptide complex, wherein the antigen-binding protein binds to one or more amino acids of SEQ ID NO: 270 or 291. In one embodimentexample, the antigen-binding protein binds to at least 3 amino acids of SEQ ID NO: 270 or 291. In one embodimentexample, the antigen-binding protein binds to one or more amino acids selected from the group consisting of M160, W161, and Q164 of SEQ ID NO: 270.

[0035] In certain embodimentexamples, the present disclosure provides antigen-binding protein that binds specifically to a conformational epitope of an HLA-displayed New York esophageal squamous cell carcinoma 1 (NY-ESO-1) peptide, wherein the conformational epitope comprises one or more amino acids of SEQ ID NO: 269.

[0036] In certain embodimentexamples, the present disclosure provides antigen-binding protein that binds specifically to a conformational epitope of an HLA-displayed New York esophageal squamous cell carcinoma 1 (NY-ESO-1) peptide, wherein the conformational epitope comprises one or more amino acids of SEQ ID NO: 270 or 291. In the certain embodimentexamples, the conformational epitope comprises one or more amino acids selected from the group consisting of M160, W161, and Q164 of SEQ ID NO: 270 or 291.

[0037] The present disclosure also provides for antigen-binding proteins that compete for specific binding to HLA-A2:NY-ESO-1 with an antigen-binding protein comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1.

[0038] The present disclosure also provides antigen-binding proteins that cross-compete for binding to HLA-A2:NY-ESO-1 with a reference antigen-binding protein comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1.

[0039] The present disclosure also provides antigen-binding proteins that bind to the same epitope as a reference antigen-binding protein comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1. In certain embodimentexamples, the present disclosure provides antigen-binding proteins that bind to the same epitope as a reference antigen-binding protein comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR is selected from the group consisting of SEQ ID NOs: 2, 22, 42, and 62, and the LCVR is selected from the group consisting of SEQ ID Nos: 10, 30, 50, and 70.

[0040] In one embodimentexample, the disclosure provides a recombinant antigen-binding protein that binds specifically to a conformational epitope of an HLA-A2 presented New York esophageal squamous cell carcinoma 1 (NY-ESO-1) peptide, wherein the antigen-binding protein has a

property selected from the group consisting of: (a) binds monomeric HLA-A2:NY-ESO-1 157-165 peptide complex (C165 or V165) with a binding dissociation equilibrium constant (K_D) of less than about 1nM as measured in a surface plasmon resonance assay at 25°C; (b) binds to HLA-A2:NY-ESO-1 157-165 peptide complex expressing cells with an EC_{50} less than about 10nM and does not bind to cells expressing predicted off-target peptides as determined by flow cytometry assay; and (c) the epitope comprises one or more amino acids of SEQ ID NO: 269, 270, or 291. As disclosed elsewhere herein, an “off-target peptide” refers to a peptide that differs by 2, 3, 4, 5 or more amino acids from a target peptide (e.g., NY-ESO-1 157-165 peptide (SEQ ID NO: 269 and/or SEQ ID NO:270 or 291)).

[0041] In a second [aspectexample](#), the present disclosure provides nucleic acid molecules encoding anti-HLA-A2:NY-ESO-1 antigen-binding proteins. For example, the present disclosure provides nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 1; in certain [embodimentexamples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto.

[0042] The present disclosure also provides nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 1; in certain [embodimentexamples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto.

[0043] The present disclosure also provides nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 1; in certain [embodimentexamples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto.

[0044] The present disclosure also provides nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 1; in certain [embodimentexamples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at

least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto.

[0045] The present disclosure also provides nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 1; in certain embodimentexamples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto.

[0046] The present disclosure also provides nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 1; in certain embodimentexamples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto.

[0047] The present disclosure also provides nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 1; in certain embodimentexamples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto.

[0048] The present disclosure also provides nucleic acid molecules encoding any of the LCDR3 amino acid sequences listed in Table 1; in certain embodimentexamples the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto.

[0049] The present disclosure also provides nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3), wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary anti-HLA-A2:NY-ESO-1 antigen-binding proteins listed in Table 1.

[0050] The present disclosure also provides nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary anti-

HLA-A2:NY-ESO-1 antigen-binding proteins listed in Table 1.

[0051] The present disclosure also provides nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino acid sequences listed in Table 1, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 1. In certain embodimentexamples, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto, and a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity thereto. In certain embodimentexamples according to this aspect of the disclosure, the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same anti-HLA-A2:NY-ESO-1 antigen-binding protein listed in Table 1.

[0052] The present disclosure provides nucleic acid molecules encoding any of the heavy chain amino acid sequences listed in Table 3. The present disclosure also provides nucleic acid molecules encoding any of the light chain amino acid sequences listed in Table 3.

[0053] The present disclosure also provides nucleic acid molecules encoding both heavy chain (HC) and a light chain (LC), wherein the HC comprises an amino acid sequence of any of the HC amino acid sequences listed in Table 3, and wherein the LC comprises an amino acid sequence of any of the LC amino acid sequences listed in Table 3.

[0054] In a related aspectexample, the present disclosure provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy and/or or light chain variable region of an anti-HLA-A2:NY-ESO-1 antigen-binding protein. For example, the present disclosure includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 1. The present disclosure also provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy and/or light chain of an anti-HLA-A2:NY-ESO-1 antigen-binding protein. For example, the present disclosure includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the heavy chain or light chain sequences as set forth in Table 2. Also included within the scope of the present disclosure are host cells into which such vectors have been introduced, as well as methods of producing the

antigen-binding proteins by culturing the host cells under conditions permitting production of the antigen-binding proteins, and recovering the antigen-binding proteins so produced.

[0055] In a third [aspectexample](#), the present disclosure provides a pharmaceutical composition comprising a therapeutically effective amount of a recombinant antigen-binding protein that binds specifically to a conformational epitope of an HLA-A2 presented NY-ESO-1 peptide (e.g., a peptide comprising amino acid residues 157-165 of NY-ESO-1), and a pharmaceutically acceptable carrier. In a related [aspectexample](#), the disclosure features a composition which is a combination of an anti-HLA-A2:NY-ESO-1 antigen-binding protein and a second therapeutic agent. In one [embodimentexample](#), the second therapeutic agent is any agent that is advantageously combined with an anti-HLA-A2:NY-ESO-1 antigen-binding protein. Exemplary agents that may be advantageously combined with an anti-HLA-A2:NY-ESO-1 antigen-binding protein include, without limitation, other agents that modulate immune cell activation. Additional therapies that can be used in combination with the anti-HLA-A2:NY-ESO-1 antigen-binding proteins of the present disclosure are disclosed elsewhere herein.

[0056] In a fourth [aspectexample](#), the disclosure provides methods to treat a subject having a NY-ESO-1-associated disease or disorder, such as a NY-ESO-1-positive cancer. The methods include administering a therapeutically effective amount of an anti-HLA-A2:NY-ESO-1 antigen-binding protein of the disclosure or a pharmaceutical composition of the disclosure to the subject in need thereof. The disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented by the antigen-binding proteins and compositions provided herein. In certain [embodimentexamples](#), the antigen-binding protein (or pharmaceutical composition) of the disclosure is administered in combination with a second therapeutic agent to the subject in need thereof. The second therapeutic agent may be selected from the group consisting of an antibody to a T cell co-inhibitor, an antibody to a tumor cell antigen, an antibody to a T cell receptor, a cytotoxic agent, an anti-cancer drug, an anti-inflammatory drug (e.g., corticosteroids), chemotherapeutic agent, surgery, radiation therapy, an immunosuppressant and any other drug or therapy known in the art. In certain [embodimentexamples](#), the second therapeutic agent may be an agent that helps to counteract or reduce any possible side effect(s) associated with antigen-binding protein of the disclosure, if such side effect(s) should occur.

[0057] In certain [embodimentexamples](#), the present disclosure provides methods for suppressing growth of a NY-ESO-1-associated cancer. For example, the present disclosure provides methods to suppress tumor growth due to a primary tumor or a metastatic tumor in a subject. In certain [embodimentexamples](#), the present disclosure provides methods to enhance survival (e.g., progression-free survival or overall survival) of a subject with a NY-ESO-1-associated cancer. Examples of cancer include, but are not limited to, a liposarcoma, a neuroblastoma, a myeloma, a metastatic melanoma, a synovial sarcoma, a bladder cancer, an esophageal cancer, a hepatocellular cancer, a head and neck cancer, a non-small cell lung cancer, an ovarian cancer,

a prostate cancer, a breast cancer, astrocytic tumor, glioblastoma multiforme, anaplastic astrocytoma, brain tumor, fallopian tube cancer, ovarian epithelial cancer, primary peritoneal cavity cancer, advanced solid tumors, soft tissue sarcoma, melanoma, a sarcoma, myelodysplastic syndrome, acute myeloid leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, Hodgkin disease, multiple myeloma, synovial sarcoma, metastatic solid tumors, esophageal cancer, rhabdomyosarcoma, advanced myxoid, round cell liposarcoma, metastatic melanoma, or recurrent non-small cell lung cancer.

[0058] In certain [embodimentexamples](#), the present disclosure provides methods for inhibiting or suppressing growth of established tumors. The methods comprise administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of an antigen-binding protein of the present disclosure. In certain [embodimentexamples](#), the antigen-binding protein is administered in combination with a second therapeutic agent.

[0059] The antigen-binding protein, *e.g.*, antibody, or antigen-binding fragment thereof, may be administered subcutaneously, intravenously, intradermally, intraperitoneally, orally, intramuscularly, or intracranially. The antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof, may be administered at a dose of about 0.1 mg/kg of body weight to about 100 mg/kg of body weight of the subject.

[0060] In a fifth [aspectexample](#), the present disclosure provides an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), or a cell expressing such a CAR (*e.g.*, a cell expressing the CAR on its surface). The CAR may include an extracellular binding domain that specifically binds to a conformational epitope of an HLA-displayed New York esophageal squamous cell carcinoma 1 (NY-ESO-1) peptide, *e.g.*, amino acid residues 157-165 of NY-ESO-1, a transmembrane domain, and an intracellular signaling domain. In one [embodimentexample](#), the extracellular binding domain is an anti-HLA-A2:NY-ESO-1 antigen-binding protein or an antigen-binding fragment thereof. Exemplary anti-HLA-A2:NY-ESO-1 antigen-binding proteins of the present disclosure are any of the antigen-binding proteins described herein.

[0061] For example, in certain [embodimentexamples](#), the antigen-binding protein suitable for use in the CARs of the disclosure comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences listed in Table 1; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences listed in Table 1.

[0062] In other [embodimentexamples](#), the antigen-binding protein suitable for use in the CARs of the disclosure comprises a HCVR having an amino acid sequence selected from the group consisting of HCVR sequences listed in Table 1; and/or a LCVR having an amino acid sequence selected from the group consisting of LCVR sequences listed in Table 1.

[0063] In some [embodimentexamples](#), the antigen-binding protein suitable for use in the CARs of the

disclosure comprises (a) a HCVR having an amino acid sequence selected from the group consisting of HCVR sequences listed in Table 1; and (b) a LCVR having an amino acid sequence selected from the group consisting of LCVR sequences listed in Table 1.

[0064] In one ~~embodiment~~example, the antigen-binding protein suitable for use in the CARs of the disclosure comprises (a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 24, 44, 64, 84, 104, 124, 144, 164, 213, and 232; (b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 26, 46, 66, 86, 106, 126, 146, 166, 182, 199, 215, and 234; (c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 28, 48, 68, 88, 108, 128, 148, 168, 184, 201, 217, and 236; (d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 32, 52, 72, 92, 112, 132, 152, 172, 188, 221, and 240; (e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 34, 54, 74, 94, 114, 134, 154, 174, and 242; and (f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 36, 56, 76, 96, 116, 136, 156, 190, 205, 224, and 244.

[0065] In further ~~embodiment~~example, the antigen-binding protein suitable for use in the CARs of the disclosure comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 22/30, 42/50, 62/70, 82/90, 102/110, 122/130, 142/150, 162/170, 180/186, 196/ 203, 211/219, and 230/238.

[0066] In some ~~embodiment~~examples the antigen-binding protein for use in the CARs of the present disclosure is an scFv.

[0067] In other ~~aspects~~examples, the present disclosure provides vectors comprising the isolated CAR nucleic acid molecules; and immune effector cells comprising such vectors.

[0068] In yet other ~~aspects~~examples of the present disclosure, methods for treating a subject having a NY-ESO-1-associated disease or disorder, such as a NY-ESO-1-positive cancer, *e.g.*, a liposarcoma, a neuroblastoma, a myeloma, a metastatic melanoma, a synovial sarcoma, a bladder cancer, an esophageal cancer, a hepatocellular cancer, a head and neck cancer, a non-small cell lung cancer, an ovarian cancer, a prostate cancer, a breast cancer, astrocytic tumor, glioblastoma multiforme, anaplastic astrocytoma, brain tumor, fallopian tube cancer, ovarian epithelial cancer, primary peritoneal cavity cancer, advanced solid tumors, soft tissue sarcoma, melanoma, a sarcoma, myelodysplastic syndrome, acute myeloid leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, Hodgkin disease, multiple myeloma, synovial sarcoma, metastatic solid tumors, esophageal cancer, rhabdomyosarcoma, advanced myxoid, round cell liposarcoma, metastatic melanoma, or recurrent non-small cell lung cancer, are provided. The methods include administering to the subject a population of immune effector cells comprising a CAR of the disclosure.

[0069] In some ~~aspects~~examples, the present disclosure provides methods for detecting NY-ESO-1-

positive cells, e.g., in a subject or in a sample obtained from a subject. The methods include contacting a cell, such as a cell sample obtained from a subject, or administering to a subject, an antigen-binding protein of the disclosure comprising a detectable moiety, and detecting the presence of the detectable moiety.

[0070] Other embodiment examples will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0071] FIG. 1A depicts diagrams of three different CAR constructs, including promoters and vector elements.

[0072] FIG. 1B depicts tumor volumes in mice treated with T cells that express either a non-binding control BB/z CAR (Control CAR T), the anti-HLA-A2/NY-ESO-1₁₅₇₋₁₆₅ BB/z CAR, or the anti-HLA-A2/NY-ESO-1₁₅₇₋₁₆₅ 28/z CAR over days 0 to 21. Left panel: average tumor volume; right panel: tumor volumes for individual mice.

[0073] FIG. 1C depicts tumor volumes in mice treated with T cells that express either a non-binding control BB/z CAR (Control CAR T), the anti-HLA-A2/NY-ESO-1₁₅₇₋₁₆₅ BB/z CAR, or the anti-HLA-A2/NY-ESO-1₁₅₇₋₁₆₅ 28/z CAR over days 0 to 38. Left panel: average tumor volume; right panel: tumor volumes for individual mice.

DETAILED DESCRIPTION

[0074] Before the present methods are described, it is to be understood that this disclosure is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

[0075] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

[0076] The terms “NY-ESO-1,” “NY-ESO-1,” “New York esophageal squamous cell carcinoma 1,” and “CTAG1B” refer to the well-known cancer-testis antigen (CTA) that is re-expressed in numerous cancer types and encoded by the *CTAG1B* gene.

[0077] The amino acid sequence of full-length NY-ESO-1 is provided in GenBank as accession number NP_001318.1 (SEQ ID NO:271). The term “NY-ESO-1” includes recombinant NY-ESO-1 or a fragment thereof. The term also encompasses NY-ESO-1 or a fragment thereof

Claims

What is claimed is:

1. An antigen-binding protein that binds specifically to an HLA-A2:New York esophageal squamous cell carcinoma 1 peptide (NY-ESO-1 peptide) complex at at least one amino acid corresponding to an amino acid selected from the group consisting of M160, W161, and Q164 of SEQ ID NO: 271.
2. The antigen-binding protein of claim 1, wherein said isolated antigen-binding protein specifically binds to a conformational epitope of the HLA-A2 presented NY-ESO-1 polypeptide.
3. The antigen-binding protein of claim 1 or 2, wherein said at least one amino acid is at least two amino acids.
4. The antigen-binding protein of claim 3, wherein said at least one amino acid is three amino acids.
5. An antigen-binding protein that binds specifically to an HLA-A2:NY-ESO-1 157-165 peptide complex, wherein said isolated antigen-binding protein interacts with amino acids M160, W161, and Q164 of said NY-ESO-1 157-165 peptide as determined by X-ray crystallography at a resolution of 4.0 Å or higher.
6. The antigen-binding protein of claim 5, wherein said resolution is 3.5 Å or higher.
7. The antigen-binding protein of claim 6, wherein said resolution is 3.3 Å or higher.
8. An antigen-binding protein that specifically binds to an HLA-A2:NY-ESO-1 157-165 peptide complex but does not specifically bind to one or more off-target peptides selected from the group consisting of BCL9L 1351-1359 (SEQ ID NO: 273), GRID1 7-15 (SEQ ID NO: 274), ZDHHC1 376-384 (SEQ ID NO: 276), ITCH 807-815 (SEQ ID NO: 277), and URB1 1853-1861 (SEQ ID NO: 280), wherein the ratio of i) said antigen-binding protein binding to cells pulsed with said one or more off-target peptides to ii) unpulsed cells is less than about nine.
9. The antigen-binding protein of claim 8, wherein said ratio is less than about 8.

10. The antigen-binding protein of claim 9, wherein said ratio is less than about 7.
11. The antigen-binding protein of claim 10, wherein said ratio is less than about 6.
12. The antigen-binding protein of claim 11, wherein said ratio is less than about 5.
13. The antigen-binding protein of claim 12, wherein said ratio is less than about 4.
14. The antigen-binding protein of claim 13, wherein said ratio is less than about 3.
15. The antigen-binding protein of claim 14, wherein said ratio is less than about 2.
16. The antigen-binding protein of any one of claims 1-15, wherein said antigen-binding protein binds to a monomeric HLA-A2:NY-ESO-1 157-165 peptide complex with a binding dissociation equilibrium constant (K_D) of less than about 1nM as measured in a surface plasmon resonance assay at 25°C.
17. The antigen-binding protein of any one of claims 1-16, wherein said NY-ESO-1 peptide comprises the amino acid sequence of SLLMWITQC (SEQ ID NO: 269) or SLLMWITQV (SEQ ID NO: 291).
18. The antigen-binding protein of any one of claims 1-17, wherein the antigen-binding protein is a full-length antibody, a Fab, a Fab', a (Fab')₂, an Fv, a single chain Fv (scFv), a T-body construct, or a Chimeric Antigen Receptor (CAR).
19. The antigen-binding protein of claim 19, wherein the antigen-binding protein is a CAR.
20. The CAR of claim 19, wherein the CAR comprises a heavy chain variable region (LCVR), a light chain variable region (LCVR), a hinge region, a transmembrane domain, a costimulatory domain, and a signaling domain.
21. The CAR of claim 20, wherein the costimulatory domain is a 4-1BB costimulatory domain.
22. The CAR of claim 20, wherein the costimulatory domain is a CD28 costimulatory domain.

23. The antigen-binding protein of any one of claims 1-22, wherein the antigen-binding protein is a human monoclonal antibody or an antigen-binding fragment thereof.

24. The antigen-binding protein of any one of claims 1-23, comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences listed in Table 1, and three light chain CDRs (LCDR1, LCDR2, and LCDR3) contained within any one of the light chain variable region (LCVR) sequences listed in Table 1.

25. The antigen-binding protein of claim 24, wherein said HCVR sequences are selected from the group consisting of SEQ ID NOs: 2, 22, 42, 142, and 250.

26. The antigen-binding protein of claim 24, wherein said LCVR sequences are selected from the group consisting of SEQ ID NOs: 10, 30, 50, 150, and 260.

27. The antigen-binding protein of claim 24, wherein said HCVR/LCVR sequences are selected from the group consisting of SEQ ID NOs: 2/10, 22/30, 42/50, 62/70, 82/90, 102/110, 122/130, 142/150, 162/170, 180/186, 196/203, 211/219, 230/238, and 250/258.

28. The antigen-binding protein of claim 24, wherein said HCVR/LCVR sequences are selected from the group consisting of SEQ ID NOs: 2/10, 22/30, 42/50, 142/150, and 250/258.

29. The antigen-binding protein of any one of claims 1-25, comprising a HCVR having an amino acid sequence selected from the group consisting of the HCVR sequences listed in Table 1.

30. The antigen-binding protein of claim 29, wherein said HCVR sequences are selected from the group consisting of SEQ ID NOs: 2, 22, 42, 142, and 250.

31. The antigen-binding protein of any one of claims 1-30, comprising a LCVR having an amino acid sequence selected from the group consisting of the LCVR sequences listed in Table 1.

32. The antigen-binding protein of claim 31, wherein said LCVR sequences are selected from the group consisting of SEQ ID NOs: 10, 30, 50, 150, and 260.

33. The antigen-binding protein of any one of claims 1-32, comprising: (a) a HCVR having an amino acid sequence selected from the group consisting of the HCVR sequences listed in Table 1; and (b) a LCVR having an amino acid sequence selected from the group consisting of the LCVR sequences listed in Table 1.

34. The antigen-binding protein of claim 33, wherein said HCVR/LCVR sequences are selected from the group consisting of SEQ ID NOs: 2/10, 22/30, 42/50, 62/70, 82/90, 102/110, 122/130, 142/150, 162/170, 180/186, 196/203, 211/219, 230/238, and 250/258.

35. The antigen-binding protein of claim 34, wherein said HCVR/LCVR sequences are selected from the group consisting of SEQ ID NOs: 2/10, 22/30, 42/50, 142/150, and 250/258.

36. The antigen-binding protein of any one of claims 1-35, comprising:

- (a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 24, 44, 64, 84, 104, 124, 144, 164, 213, 232, and 252;
- (b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 26, 46, 66, 86, 106, 126, 146, 166, 182, 199, 215, 234, and 254;
- (c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 28, 48, 68, 88, 108, 128, 148, 168, 184, 201, 217, 236, and 256;
- (d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 32, 52, 72, 92, 112, 132, 152, 172, 188, 221, 240, and 260;
- (e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 34, 54, 74, 94, 114, 134, 154, 174, 242, and 262; and
- (f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 36, 56, 76, 96, 116, 136, 156, 190, 205, 224, 244, and 264.

37. The antigen-binding protein of any one of claims 1-28 and 36, wherein said antigen-binding protein comprises an HCVR comprising at least 80% sequence identity to an HCVR sequence listed in Table 1.

38. The antigen-binding protein of claim 37, wherein said antigen-binding protein comprises an HCVR comprising at least 90% sequence identity to an HCVR sequence listed in Table 1.

39. The antigen-binding protein of claim 38, wherein said isolated antigen-binding protein comprises an HCVR comprising at least 95% sequence identity to an HCVR sequence listed in Table 1.

40. The antigen-binding protein of any one of claims 1-28 and 36-39, wherein said antigen-binding protein comprises an LCVR comprising at least 80% sequence identity to an LCVR sequence listed in Table 1.

41. The antigen-binding protein of claim 40, wherein said isolated antigen-binding protein comprises an LCVR comprising at least 90% sequence identity to an LCVR sequence listed in Table 1.

42. The antigen-binding protein of claim 38, wherein said antigen-binding protein comprises an LCVR comprising at least 95% sequence identity to an LCVR sequence listed in Table 1.

43. An antigen-binding protein that competes for binding to an antigen-binding protein of any one of claims 1-42.

44. An antigen-binding protein that binds to the same epitope as an antigen-binding protein of any one of claims 1-42.

45. The antigen-binding protein of any one of claims 1-44, comprising a detectable moiety.

46. A pharmaceutical composition comprising an antigen-binding protein that binds to HLA-A2:NY-ESO-1 according to any one of claims 1-45 and a pharmaceutically acceptable carrier or diluent.

47. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a HCVR of an antigen-binding protein as set forth in any one of claims 1-45.

48. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a LCVR of an antigen-binding protein as set forth in any one of claims 1-45.

49. A vector comprising the polynucleotide molecule of claim 47 or 48.

50. A cell expressing the polynucleotide molecule of claim 47 or 48, or the vector of claim 49.

51. Use of the antigen-binding protein as set forth in any one of claims 1-45 or the pharmaceutical composition of claim 46, or the cell of claim 50 in the manufacture of a medicament for ~~A method of~~ treating a subject having an NY-ESO-1-associated disease or disorder, ~~comprising administering to the subject a therapeutically effective amount of the antigen-binding protein as set forth in any one of claims 1-45 or the pharmaceutical composition of claim 46, or the cell of claim 50, thereby treating the subject.~~

52. The method-use of claim 51, wherein the NY-ESO-1-associated disease or disorder is an NY-ESO-1-associated cancer.

53. The method-use of claim 52, wherein the NY-ESO-associated cancer is selected from the group consisting of: a liposarcoma, a neuroblastoma, a myeloma, a metastatic melanoma, a synovial sarcoma, a bladder cancer, an esophageal cancer, a hepatocellular cancer, a head and neck cancer, a non-small cell lung cancer, an ovarian cancer, a prostate cancer, a breast cancer, astrocytic tumor, glioblastoma multiforme, anaplastic astrocytoma, brain tumor, fallopian tube cancer, ovarian epithelial cancer, primary peritoneal cavity cancer, advanced solid tumors, soft tissue sarcoma, melanoma, a sarcoma, myelodysplastic syndrome, acute myeloid leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, Hodgkin disease, multiple myeloma, synovial sarcoma, metastatic solid tumors, esophageal cancer, rhabdomyosarcoma, advanced myxoid, round cell liposarcoma, metastatic melanoma, or recurrent non-small cell lung cancer.

54. The method-use of any one of claims 51-53, wherein the antigen-binding protein is to be administered to the subject in combination with a second therapeutic agent.

55. The method-use of claim 54, wherein the second therapeutic agent is selected from the group consisting of a PD-1 inhibitor, a CTLA-4 inhibitor, an antibody to a tumor specific antigen, an antibody to a virally-infected-cell antigen, a PD-L1 inhibitor, a CD20 inhibitor, a bispecific antibody against CD20 and CD3, a dietary supplement such as an antioxidant, a VEGF antagonist, a chemotherapeutic agent, a cytotoxic agent, surgery, radiation, a NSAID, a corticosteroid, and any other therapy useful for ameliorating at least one symptom associated with the disease or disorder.

56. The ~~method-use~~ of any one of claims 51-55, wherein the antigen-binding protein is to be administered subcutaneously, intravenously, intradermally, intraperitoneally, orally, intramuscularly or intracranially.

57. The ~~method-use~~ of any one of claims 51-56, wherein the antigen-binding protein is to be administered at a dose of about 0.1 mg/kg of body weight to about 100 mg/kg of body weight of the subject.

58. An isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an extracellular binding domain that specifically binds to a conformational epitope of an HLA-A2 presented New York esophageal squamous cell carcinoma 1 peptide (NY-ESO-1 peptide), a transmembrane domain, and an intracellular signaling domain.

59. The isolated nucleic acid molecule of claim 58, wherein the extracellular binding domain is an anti-HLA-A2:NY-ESO-1 antigen-binding protein.

60. The isolated nucleic acid molecule of claim 59, wherein the antigen-binding protein comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences listed in Table 1; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences listed in Table 1.

61. The isolated nucleic acid molecule of any one of claims 58-60, wherein the antigen-binding protein comprises a HCVR having an amino acid sequence selected from the group consisting of the HCVR sequences listed in Table 1.

62. The isolated nucleic acid molecule of any one of claims 58-61, wherein the antigen-binding protein comprises a LCVR having an amino acid sequence selected from the group consisting of the LCVR sequences listed in Table 1.

63. The isolated nucleic acid molecule of any one of claims 58-62, wherein the antigen-binding protein comprises (a) a HCVR having an amino acid sequence selected from the group consisting of HCVR sequences listed in Table 1; and (b) a LCVR having an amino acid sequence selected from the group consisting of LCVR sequences listed in Table 1.

64. The isolated nucleic acid molecule of any one of claims 58-63, wherein the antigen-binding protein comprises

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 24, 44, 64, 84, 104, 124, 144, 164, 213, 232, and 252;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 26, 46, 66, 86, 106, 126, 146, 166, 182, 199, 215, 234, and 254;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 28, 48, 68, 88, 108, 128, 148, 168, 184, 201, 217, 236, and 256;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 32, 52, 72, 92, 112, 132, 152, 172, 188, 221, 240, and 260;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 34, 54, 74, 94, 114, 134, 154, 174, 242, and 262; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 36, 56, 76, 96, 116, 136, 156, 190, 205, 224, 244, and 264.

65. The isolated nucleic acid molecule of any one of claims 58-64, wherein the antigen-binding protein comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 22/30, 42/50, 62/70, 82/90, 102/110, 122/130, 142/150, 162/170, 180/186, 196/203, 211/219, 230/238, and 250/258.

66. The isolated nucleic acid molecule of any one of claims 58-65, wherein the antigen-binding protein comprises an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 22/30, 42/50, 142/150, and 250/258

67. The isolated nucleic acid molecule of any one of claims 58-66, comprising any one of the sequences listed in Table 2.

68. The isolated nucleic acid molecule of any one of claims 58-67, wherein the antigen-binding protein is an scFv.

69. A vector comprising the isolated nucleic acid molecule of any one of claims 58-68.

70. An isolated immune effector cell comprising the isolated nucleic acid molecule of any one of claims 58-68 or the vector of claim 69.

71. The isolated immune effector cell of claim 70, which is a T-body.

72. The isolated immune effector cell of claim 70 or 71, wherein said cell expresses said CAR.

73. Use of the immune effector cell of any one of claims 70-72 in the manufacture of a medicament for ~~A method of~~ treating a subject having an NY-ESO-1-associated disease or disorder, ~~comprising administering to the subject the immune effector cell of any one of claims 70-72.~~

74. The ~~method-use~~ of claim 73, wherein the NY-ESO-1-associated disease or disorder is NY-ESO-1-associated cancer.

75. The ~~method-use~~ of claim 73 or 74, wherein the antigen-binding protein is to be administered to the subject in combination with a second therapeutic agent.