

T CELL RECEPTOR-LIKE ANTIBODY AGENTS SPECIFIC FOR EBV LATENT MEMBRANE PROTEIN 2A PEPTIDE PRESENTED BY HUMAN HLA

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of U.S. Provisional Application No. 62/173,330, filed June 9, 2015, the contents of which are hereby incorporated herein in their entirety.

GOVERNMENT SUPPORT

[002] Technical service provided by the Memorial Sloan Kettering Small-Animal Imaging Core Facility and Molecular Cytology Core Facility were supported, in part, by the NIH Cancer Center Support Grant P30 CA008748.

BACKGROUND

[003] T cell based immunotherapy is highly effective for cancer treatment as evidenced by recent reports with chimeric antigen receptors, bispecific antibodies, and checkpoint blockade antibodies. Both chimeric antigen receptors and bispecific antibodies have relied on antibody-based targeting of surface antigens. T cell receptor (TCR)-based approaches, however, have been slower in their development due in part to insufficient clonal frequency or clonal deletion of tumor-specific T cells and dwarfed by the rapid advances in antibody libraries and platform technologies. Unfortunately, clinically approved therapeutic monoclonal antibodies recognize structures of cell surface proteins. Moreover, antibody-based targetable antigens are limited in number. Of the candidates on the National Cancer Institute (NCI) cancer antigen priority list, the majority are found in the cytoplasmic compartment. Development of antibody agents targeting such candidates remains a challenge.

SUMMARY

[004] The present disclosure provides, among other things, human antibody agents (and affinity matured versions thereof) that bind an Epstein-Barr virus (EBV) latent membrane protein 2 (LMP2) peptide in the context of human MHC (e.g., class I) molecules. In some embodiments, provided antibody agents demonstrate high specificity for a center portion (e.g., a center peptide position) of an EBV-LMP2 peptide in a peptide-MHC class I (e.g., an HLA-A02) binding pocket. In some embodiments, provided antibody agents demonstrate high specificity for an end portion (e.g., an end

peptide position) of an EBV-LMP2 peptide in a peptide-MHC class I binding pocket. In some embodiments, provided antibody agents effectively carry out antibody-dependent cell-mediated cytotoxicity (ADCC) of target cells that carry an EBV-LMP2 peptide via HLA-A02 molecules on their surface. In some embodiments, provided antibody agents overcome suboptimal affinity associated with antibodies having specificity for a center portion(s) of a peptide presented in a binding pocket of an MHC molecule (e.g., an MHC class I molecule).

[004a] In one aspect of the present disclosure, there is provided a human antibody agent that binds an EBV-LMP2/HLA peptide complex comprising

(a) heavy chain CDR1 of SEQ ID NO:31, heavy chain CDR2 of SEQ ID NO:33 and heavy chain CDR3 of SEQ ID NO:35; and light chain CDR1 of SEQ ID NO:73, light chain CDR2 of SEQ ID NO:75 and light chain CDR3 of SEQ ID NO:77;

(b) heavy chain CDR1 of SEQ ID NO:37, heavy chain CDR2 of SEQ ID NO:39 and heavy chain CDR3 of SEQ ID NO:41; and light chain CDR1 of SEQ ID NO:79, light chain CDR2 of SEQ ID NO:81 and light chain CDR3 of SEQ ID NO:83;

(c) heavy chain CDR1 of SEQ ID NO:43, heavy chain CDR2 of SEQ ID NO:45 and heavy chain CDR3 of SEQ ID NO:47; and light chain CDR1 of SEQ ID NO:85, light chain CDR2 of SEQ ID NO:87 and light chain CDR3 of SEQ ID NO:89;

(d) heavy chain CDR1 of SEQ ID NO:49, heavy chain CDR2 of SEQ ID NO:51 and heavy chain CDR3 of SEQ ID NO:53; and light chain CDR1 of SEQ ID NO:91, light chain CDR2 of SEQ ID NO:93 and light chain CDR3 of SEQ ID NO:95;

(e) heavy chain CDR1 of SEQ ID NO:55, heavy chain CDR2 of SEQ ID NO:57 and heavy chain CDR3 of SEQ ID NO:59; and light chain CDR1 of SEQ ID NO:97, light chain CDR2 of SEQ ID NO:99 and light chain CDR3 of SEQ ID NO:101;

(f) heavy chain CDR1 of SEQ ID NO:61, heavy chain CDR2 of SEQ ID NO:63 and heavy chain CDR3 of SEQ ID NO:65; and light chain CDR1 of SEQ ID NO:103, light chain CDR2 of SEQ ID NO:105 and light chain CDR3 of SEQ ID NO:107; or

(g) heavy chain CDR1 of SEQ ID NO:67, heavy chain CDR2 of SEQ ID NO:69 and heavy chain CDR3 of SEQ ID NO:71; and light chain CDR1 of SEQ ID NO:109, light chain CDR2 of SEQ ID NO:111 and light chain CDR3 of SEQ ID NO:113.

[004b] In another aspect of the present disclosure, there is provided a bispecific antibody comprising a first antigen-binding site that binds an EBV-LMP2/HLA peptide complex and a second antigen-binding site, wherein the first antigen-binding site comprises

(a) heavy chain CDR1 of SEQ ID NO:31, heavy chain CDR2 of SEQ ID NO:33 and heavy chain CDR3 of SEQ ID NO:35; and light chain CDR1 of SEQ ID NO:73, light chain CDR2 of SEQ ID NO:75 and light chain CDR3 of SEQ ID NO:77;

(b) heavy chain CDR1 of SEQ ID NO:37, heavy chain CDR2 of SEQ ID NO:39 and heavy chain CDR3 of SEQ ID NO:41; and light chain CDR1 of SEQ ID NO:79, light chain CDR2 of SEQ ID NO:81 and light chain CDR3 of SEQ ID NO:83;

(c) heavy chain CDR1 of SEQ ID NO:43, heavy chain CDR2 of SEQ ID NO:45 and heavy chain CDR3 of SEQ ID NO:47; and light chain CDR1 of SEQ ID NO:85, light chain CDR2 of SEQ ID NO:87 and light chain CDR3 of SEQ ID NO:89;

(d) heavy chain CDR1 of SEQ ID NO:49, heavy chain CDR2 of SEQ ID NO:51 and heavy chain CDR3 of SEQ ID NO:53; and light chain CDR1 of SEQ ID NO:91, light chain CDR2 of SEQ ID NO:93 and light chain CDR3 of SEQ ID NO:95;

(e) heavy chain CDR1 of SEQ ID NO:55, heavy chain CDR2 of SEQ ID NO:57 and heavy chain CDR3 of SEQ ID NO:59; and light chain CDR1 of SEQ ID NO:97, light chain CDR2 of SEQ ID NO:99 and light chain CDR3 of SEQ ID NO:101;

(f) heavy chain CDR1 of SEQ ID NO:61, heavy chain CDR2 of SEQ ID NO:63 and heavy chain CDR3 of SEQ ID NO:65; and light chain CDR1 of SEQ ID NO:103, light chain CDR2 of SEQ ID NO:105 and light chain CDR3 of SEQ ID NO:107; or

(g) heavy chain CDR1 of SEQ ID NO:67, heavy chain CDR2 of SEQ ID NO:69 and heavy chain CDR3 of SEQ ID NO:71; and light chain CDR1 of SEQ ID NO:109, light chain CDR2 of SEQ ID NO:111 and light chain CDR3 of SEQ ID NO:113.

[004c] In a further aspect of the present disclosure, there is provided a human antibody agent that binds an EBV-LMP2/HLA peptide complex comprising

(i) heavy chain CDR1 of SEQ ID NO:43, heavy chain CDR2 of SEQ ID NO:45 or a sequence that differs from SEQ ID NO:45 in an I51V substitution, and heavy chain CDR3 of SEQ ID NO:47; and

(ii) light chain CDR1 of SEQ ID NO:85, light chain CDR2 of SEQ ID NO:87 or a sequence that differs from SEQ ID NO:87 in one or both of a S52G and P55H substitution, and light chain CDR3 of SEQ ID NO:89 or a sequence that differs from SEQ ID NO:89 in a N95I substitution.

[004d] In another aspect of the present disclosure, there is provided a bispecific antibody comprising a first antigen-binding site that binds an EBV-LMP2/HLA peptide complex and a second antigen-binding site, wherein the first antigen-binding site comprises

(i) heavy chain CDR1 of SEQ ID NO:43, heavy chain CDR2 of SEQ ID NO:45 or a sequence that differs from SEQ ID NO:45 in an I51V substitution, and heavy chain CDR3 of SEQ ID NO:47; and

(ii) light chain CDR1 of SEQ ID NO:85, light chain CDR2 of SEQ ID NO:87 or a sequence that differs from SEQ ID NO:87 in one or both of a S52G and P55H substitution, and light chain CDR3 of SEQ ID NO:89 or a sequence that differs from SEQ ID NO:89 in a N95I substitution.

[005] In some embodiments, the present disclosure also provides human antibody agents that bind an EBV-LMP2/HLA-A02 complex. In some embodiments, provided antibody agents bind a center portion (e.g., position 5) in an EBV-LMP2 peptide in an EBV-LMP2/HLA-A02 complex. In some embodiments, provided antibody agents bind position 1 (P1), position 5 (P5), position 8 (P8), and/or combinations thereof, of an EBV-LMP2 peptide in an EBV-LMP2/HLA-A02 complex. In some embodiments, provided antibody agents bind positions 1-5 (P1-P5), positions 4-7 (P4-P7) or positions 4-8 (P4-P8) of an EBV-LMP2 peptide in an EBV-LMP2/HLA-A02 complex.

[006] The present disclosure also provides, multi-specific binding agents that include binding moieties that interact with a particular target. In many embodiments, such binding moieties are or comprise antibody components. In some embodiments, multi-specific binding agents are bispecific binding agents (e.g., bispecific antibodies). In some embodiments, multi-specific binding agents of the present disclosure comprise antibody components that have high specificity for a center and/or end portion of an EBV-LMP2 peptide in a peptide-MHC (e.g., a MHC class I) binding pocket. In some embodiments, multi-specific binding agents of the present disclosure comprise a first binding moiety based on an antibody component that has high specificity for an EBV-LMP2 peptide in a peptide-MHC class I binding pocket (e.g., an HLA-A02 binding

pocket) and a second binding moiety that interacts with an immune effector cell (e.g., a T cell). In some embodiments, provided multi-specific binding agents have a high potency in cytotoxicity assays against EBV⁺ and tumor cell lines. In some embodiments, provided multi-specific binding agents overcome low affinity barriers associated with TCR-like monoclonal antibodies. Such provided agents have improved functional characteristics as compared to parental binding agents that lack components as described herein.

[007] The present disclosure also provides, methods of generating, selecting and/or identifying antibody agents and/or multi-specific binding agents that bind an EBV-LMP2 peptide in a peptide-MHC binding pocket (e.g., an MHC class I binding pocket). In many embodiments, methods of the present disclosure include generating, selecting and/or identifying antibody agents and/or multi-

specific binding agents that bind a center and/or end portion of an EBV-LMP2 peptide presented by a human MHC class I molecule (e.g., an HLA-A02 molecule).

[008] In some embodiments, methods include selecting antibody agents based on binding (or lack of binding) peptides having non-native amino acid substitutions at a center and/or end portion(s) of a peptide presented by an MHC class I molecule. In some embodiments, methods include selecting antibody agents based on lack of binding peptides having non-native amino acid substitutions at a center and/or end portion and further having extra native flanking residues at the N- and/or C-termini of the peptide presented by an MHC class I molecule. In some embodiments, methods include negatively selecting antibody agents based on binding a peptide at a position(s) outside of a center portion of the peptide (e.g., position 5). In some embodiments, peptide positions outside a center portion of the peptide are mutated. In some embodiments, peptides are nonamer or decamer peptides. In many embodiments, an MHC class I molecule is a human HLA-A02 molecule.

[009] In some embodiments, the present disclosure provides a human antibody agent that binds an EBV-LMP2/HLA peptide complex, wherein the human antibody agent interacts directly with amino acid residues at one or more positions selected from the group consisting of position 1, position 5, position 8, and combinations thereof, in the EBV-LMP2 peptide.

[0010] In some embodiments, the present disclosure provides a human antibody agent that binds an EBV-LMP2/HLA peptide complex, wherein the human antibody agent has a K_D of about 2.0 to about 170 nM.

[0011] In some embodiments, an EBV-LMP2 peptide has an amino acid sequence that is or comprises CLGGLLTMV (SEQ ID NO:1).

[0012] In some embodiments, a human antibody agent interacts directly with amino acid residues at one or more positions selected from the group consisting of position 1, 2, 3, 4, 5, 6, 7, 8, and combinations thereof, in the EBV-LMP2 peptide.

[0013] In some embodiments, a human antibody agent comprises heavy chain CDR1 of SEQ ID NO:31, CDR2 of SEQ ID NO:33 and CDR3 of SEQ ID NO:35; and wherein the human antibody agent comprises light chain CDR1 of SEQ ID NO:73, CDR2 of SEQ ID NO:75 and CDR3 of SEQ ID NO:77. In some embodiments, a human antibody agent comprises heavy chain CDR1 of SEQ ID NO:37, CDR2 of SEQ ID NO:39 and CDR3 of SEQ ID NO:41; and wherein the human antibody agent comprises light chain CDR1 of SEQ ID NO:79, CDR2 of SEQ ID NO:81 and CDR3 of SEQ ID NO:83. In some embodiments, a human antibody agent comprises heavy chain CDR1 of

human antibody agent comprises the light chain variable region of SEQ ID NO:7 and the heavy chain variable region of SEQ ID NO:9. In some embodiments, a human antibody agent comprises the light chain variable region of SEQ ID NO:11 and the heavy chain variable region of SEQ ID NO:13. In some embodiments, a human antibody agent comprises the light chain variable region of SEQ ID NO:15 and the heavy chain variable region of SEQ ID NO:17. In some embodiments, a human antibody agent comprises the light chain variable region of SEQ ID NO:19 and the heavy chain variable region of SEQ ID NO:21. In some embodiments, a human antibody agent comprises the light chain variable region of SEQ ID NO:23 and the heavy chain variable region of SEQ ID NO:25. In some embodiments, a human antibody agent comprises the light chain variable region of SEQ ID NO:27 and the heavy chain variable region of SEQ ID NO:29.

[0017] In some embodiments, a human antibody agent is a human monoclonal antibody that is defucosylated and/or glycosylated with terminal mannose, N-acetylglucose or glucose.

[0018] In some embodiments, the present disclosure provides a human antibody agent that binds an EBV-LMP2/HLA peptide complex, which human antibody agent comprises one or more amino acid substitutions that increase affinity to the EBV-LMP2/HLA peptide complex, and wherein the human antibody agent is characterized by (a) a KA of at least about 1.6 higher affinity than affinity to a reference peptide/HLA complex, and/or (b) no cross-reactivity to a reference peptide/HLA complex. In some embodiments, a human antibody agent is characterized by (a) a KA of at least about 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, or at least about 9.0 higher affinity than affinity to a reference peptide/HLA complex, and/or (b) no cross-reactivity to a reference peptide/HLA complex.

[0019] In some certain embodiments, a human antibody agent comprises a light chain variable region that comprises one or more amino acid substitutions at any of amino acid positions 48, 52, 55, 66, 95 and combinations thereof. In some embodiments, one or more amino acid substitutions is I48V/S52G, P55H, K66R or N95I.

[0020] In some certain embodiments, a human antibody agent comprises a heavy chain variable region that comprises one or more amino acid substitutions at any of amino acid positions 5, 10, 26, 51, 78 and combinations thereof. In some embodiments, one or more amino acid substitutions is V5E, E10D, G26E/I51V or V78A.

[0021] In various embodiments, a human antibody agent is a human monoclonal antibody or a fragment thereof. In some embodiments, a human monoclonal antibody is an IgG1, IgG2, IgG3 or IgG4 antibody. In some certain embodiments, a human monoclonal antibody is an IgG1. In some embodiments, a human monoclonal antibody fragment is an scFv.

[0022] In some embodiments, the present disclosure provides a bispecific antibody comprising a first antigen-binding site that binds an EBV-LMP2/HLA peptide complex and a second antigen-binding site.

[0023] In some embodiments, a bispecific antibody is characterized by an EC₅₀ of about 0.2 to about 135 pM in T2 cells pulsed with EBV-LMP2 peptide CLGGLTMV (SEQ ID NO:1). In some embodiments, a bispecific antibody has a K_D of about 10 to about 130 nM.

[0024] In some embodiments, first and/or second antigen-binding sites are selected from the group consisting of an immunoglobulin molecule, scFv, scFab, Fab, Fv or a combination thereof. In some embodiments, first and second antigen-binding sites are configured such that they form a single polypeptide chain. In some embodiments, first and second antigen-binding sites are each scFvs. In some embodiments, first and second antigen-binding sites are linked by a peptide linker. In some embodiments, a second antigen-binding site is linked to the C-terminal end of a first antigen-binding site.

[0025] In some embodiments, a second antigen-binding site binds an immunological cell selected from the group consisting of a T cell, B cell, NK cell, dendritic cell, monocyte, macrophage, neutrophil, mesenchymal stem cell and neural stem cell. In some certain embodiments, a second antigen-binding site binds CD3 on T cells. In some embodiments, a second antigen-binding site is or comprises SEQ ID NO:124.

[0026] In some embodiments, the present disclosure provides a bispecific antibody comprising a first scFv that binds to EBV-LMP2 peptide CLGGLTMV (SEQ ID NO:1) presented by an HLA-A02 molecule, and a second scFv that binds CD3 on T cells, wherein the bispecific antibody is characterized by an EC₅₀ of about 0.2 to about 135 pM in T2 cells pulsed with the EBV-LMP2 peptide.

[0027] In some embodiments, a first scFv comprises (a) the light chain variable region of SEQ ID NO:3 and the heavy chain variable region of SEQ ID NO:5; (b) the light chain variable region of SEQ ID NO:7 and the heavy chain variable region of SEQ ID NO:9; (c) the light chain variable region of SEQ ID NO:11 and the heavy chain variable region of SEQ ID NO:13; (d) the light chain variable region of SEQ ID NO:15 and the heavy chain variable region of SEQ ID NO:17; (e) the

light chain variable region of SEQ ID NO:19 and the heavy chain variable region of SEQ ID NO:21; (f) the light chain variable region of SEQ ID NO:23 and the heavy chain variable region of SEQ ID NO:25; or (g) the light chain variable region of SEQ ID NO:27 and the heavy chain variable region of SEQ ID NO:29.

[0028] In some embodiments, a second scFv is linked to the C-terminal end of a first scFv. In some certain embodiments, a second scFv is or comprises SEQ ID NO:124.

[0029] In some embodiments, a first scFv of a bispecific antibody interacts directly with amino acid residues at one more positions selected from the group consisting of position 1, 2, 3, 4, 5, 6, 7, 8, and combinations thereof, in the EBV-LMP2 peptide. In some embodiments, a first scFv of the bispecific antibody interacts directly with at least position 1, at least position 5, or at least position 8 of the EBV-LMP2 peptide.

[0030] In some embodiments, the present disclosure provides a chimeric antigen receptor comprising an antigen-binding site of a human antibody agent that binds an EBV-LMP2/HLA peptide complex.

[0031] In some embodiments, an antigen-binding site is an scFv. In some embodiments, an antigen-binding site is expressed by an immune effector cell. In some embodiments, an immune effector cell is a T cell.

[0032] In some embodiments, the present disclosure provides an isolated nucleic acid molecule encoding, in whole or in part, a human antibody agent, a bispecific antibody, or a chimeric antigen receptor as described herein.

[0033] In some embodiments, the present disclosure provides a recombinant vector encoding a nucleic acid molecule as described herein.

[0034] In some embodiments, the present disclosure provides a host cell comprising a recombinant vector as described herein or a nucleic acid molecule as described herein. In some embodiments, a host cell is selected from a bacterial, yeast, insect or mammalian cell. In some embodiments, a host cell is selected from the group consisting of *E.coli*, *P.pastoris*, Sf9, COS, HEK293, CHO and a mammalian lymphocyte. In some certain embodiments, a mammalian lymphocyte is a human lymphocyte.

[0035] In some embodiments, the present disclosure provides a method for producing a human antibody agent, bispecific antibody, or chimeric antigen receptor as described herein, comprising culturing a host cell as described herein in a culture medium under conditions suitable for expression of the human antibody agent, bispecific antibody, or chimeric antigen receptor and

recovering the human antibody agent, bispecific antibody, or chimeric antigen receptor (or a host cell expressing the chimeric antigen receptor) from the culture medium.

[0036] In some embodiments, the present disclosure provides a composition comprising a human antibody agent, bispecific antibody, or chimeric antigen receptor as described herein.

[0037] In some embodiments, the present disclosure provides a pharmaceutical composition comprising a human antibody agent, bispecific antibody, or chimeric antigen receptor as described herein, or a composition as described herein, and further comprising a pharmaceutically acceptable carrier or diluent.

[0038] In some embodiments, the present disclosure provides a kit comprising a human antibody agent, bispecific antibody, or chimeric antigen receptor as described herein.

[0039] In some embodiments, the present disclosure provides a method of treating a medical condition in a subject characterized by expression of an EBV-LMP2/HLA peptide complex, comprising a step of administering a therapeutically effective amount of a human antibody agent, bispecific antibody, chimeric antigen receptor (or an immune effector cell expressing the chimeric antigen receptor) as described herein, a composition as described herein, or a pharmaceutical composition as described herein.

[0040] In some embodiments, a medical condition is Hodgkin's disease, non-Hodgkin's disease or infectious mononucleosis. In some embodiments, a medical condition is selected from Burkitt's lymphoma, immunosuppressive lymphoma, diffuse large B-cell lymphoma, diffuse large B-cell lymphoma associated with chronic inflammation, lymphomatoid granulomatosis, plasmablastic lymphoma, primary effusion lymphoma, post-transplant lymphoproliferative disorder, nasopharyngeal carcinoma, gastric adenocarcinoma, lymphoepithelioma-associated carcinoma, and immunodeficiency-related leiomyosarcoma.

[0041] In some embodiments, the present disclosure provides use of a human antibody agent as, bispecific antibody or chimeric antigen receptor (or an immune effector cell expressing the chimeric antigen receptor) as described herein for the treatment or detection of a condition related to EBV infection.

[0042] In some embodiments, the present disclosure provides a method of directing T cells to kill target cells expressing an EBV-LMP2/HLA peptide complex, the method comprising a step of contacting one or more target cells expressing an EBV-LMP2/HLA peptide complex with a bispecific antibody as described herein, the contacting being performed under conditions and for a time sufficient that T cells to which the bispecific antibody has bound mediates killing of the target

cells. In some embodiments, a bispecific antibody comprises a first antigen-binding site that binds an EBV-LMP2/HLA peptide complex and a second antigen-binding site that binds CD3 on T cells.

[0043] In some embodiments, an EBV-LMP2 peptide is or comprises CLGGLTMV (SEQ ID NO:1). In some embodiments, a first antigen-binding site of a bispecific antibody interacts directly with amino acid residues at one or more positions selected from the group consisting of position 1, 2, 3, 4, 5, 6, 7, 8, and combinations thereof, in the EBV-LMP2 peptide.

[0044] In some embodiments, the present disclosure provides a method of selecting an antibody agent that binds a central residue of a peptide presented by a human HLA class I molecule of interest from a population, the method comprising the steps of (a) selecting one or more antibody agents from a population that bind a peptide-HLA class I complex of interest; and (b) screening the one or more antibody agents from (a) for binding a peptide-HLA class I complex that includes a peptide having one or more amino acid substitutions at a residue selected from the group consisting of position 1, position 5, position 8, and combinations thereof; wherein loss of binding of an antibody agent to a peptide-HLA class I complex that includes a peptide having a substitution at position 5 indicates an antibody agent that binds a central residue of a peptide presented by a human HLA class I molecule.

[0045] In some embodiments, the present disclosure provides a method of selecting an antibody agent that binds a central residue of a peptide presented by a human HLA class I molecule of interest from a population, the method comprising the steps of (a) selecting one or more antibody agents from a population that do not bind a first peptide-HLA class I complex, which first peptide-HLA class I complex includes a peptide having an amino acid substitution at position 5 and/or one or more additional amino acids at the N- and/or C-terminus of the peptide; and (b) screening the one or more antibody agents from (a) for binding a second peptide-HLA class I complex, which second peptide-HLA class I complex includes a peptide having a wild-type sequence of interest; wherein binding of an antibody agent to the second peptide-HLA class I complex indicates an antibody agent that binds a central residue of a peptide presented by a human HLA class I molecule.

[0046] In some embodiments, a peptide of a peptide/HLA class I complex is an Epstein-Barr virus (EBV)-related peptide. In some embodiments, a peptide/HLA class I complex is an EBV-LMP2/HLA-A02 complex.

[0047] In some embodiments, an antibody agent is a human monoclonal antibody. In some embodiments, an antibody agent is a human monoclonal antibody fragment.

[0048] In some embodiments, in a method of providing a high affinity human monoclonal antibody that binds a peptide/HLA complex, the present disclosure provides an improvement comprising providing a human monoclonal antibody that interacts directly with or prefers one or more amino acids at the center of a peptide in a peptide/HLA complex.

[0049] In some embodiments, in a method of providing a high affinity human monoclonal antibody that binds a peptide/HLA complex, the present disclosure provides an improvement comprising providing a human monoclonal antibody that interacts directly with or prefers one or more amino acids at the center of a peptide in an HLA binding pocket, wherein the peptide/HLA complex is not generated using an *in silico* prediction method.

[0050] In some embodiments, in a method of providing a high affinity bispecific antibody composition comprising a bispecific antibody that includes first and second antigen-binding sites, which first antigen-binding site binds a peptide/HLA complex, the present disclosure provides an improvement comprising providing at least one of such first antigen-binding sites as a human monoclonal antibody or fragment thereof that interacts directly with one or more amino acids at the center of a peptide in a peptide/HLA complex.

[0051] In some embodiments, in a method of providing a chimeric antigen receptor composition comprising one or more antigen binding sites, wherein at least one of the one or more antigen binding sites binds a peptide/HLA complex, the present disclosure provides an improvement comprising providing an antigen-binding site of a human antibody agent that interacts directly with one or more amino acids at the center of a peptide in an HLA binding pocket. In some certain embodiments, a chimeric antigen receptor composition is expressed by an immune effector cell. In some embodiments, an immune effector cell is a T cell.

[0052] In various embodiments, an HLA is a class I HLA molecule.

[0053] In various embodiments, a peptide is a nonamer peptide.

[0054] In various embodiments, a peptide is a decamer peptide.

[0055] In various embodiments, a peptide is an Epstein-Barr virus (EBV)-related peptide.

[0056] In various embodiments, a peptide/HLA complex is an EBV-LMP2/HLA-A02 complex.

[0057] In various embodiments, an HLA of an EBV-LMP2/HLA peptide complex is an HLA class I molecule. In various embodiments, an HLA class I molecule is HLA-A02. In various embodiments, an HLA-A02 is selected from HLA-A*02:01, HLA-A*02:02, HLA-A*02:03, HLA-A*02:04, HLA-A*02:05 and HLA-A*02:06.

[0058] In some embodiments, the present disclosure provides use of a human antibody agent, bispecific antibody, or chimeric antigen receptor (or an immune effector cell expressing the chimeric antigen receptor) as described herein in the manufacture of a medicament for use in medicine.

[0059] In some embodiments, the present disclosure provides use of a human antibody agent, bispecific antibody, or chimeric antigen receptor (or an immune effector cell expressing the chimeric antigen receptor) as described herein in the manufacture of a medicament for use in a diagnostic test or assay.

[0060] In some embodiments, the present disclosure provides use of a human antibody agent, bispecific antibody, or chimeric antigen receptor (or an immune effector cell expressing the chimeric antigen receptor) as described herein in the manufacture of a medicament for use in the diagnosis or treatment of cancer.

[0061] In some embodiments, the present disclosure provides use of a human antibody agent, bispecific antibody, or chimeric antigen receptor (or an immune effector cell expressing the chimeric antigen receptor) as described herein in the manufacture of a medicament for use in the diagnosis or treatment of a medical condition characterized by expression of an EBV-LMP2/HLA peptide complex.

BRIEF DESCRIPTION OF THE DRAWING

[0062] The Drawing included herein, which is composed of the following Figures, is for illustration purposes only not for limitation.

[0063] *Figure 1* shows a graph depicting average per residue van der Waals interaction energies of T cell receptor:nonamer-peptide/MHC class I complexes. The peptide position (P1, P2, etc.) is indicated on the x-axis of the graph.

[0064] *Figure 2* shows a graph depicting average per residue van der Waals interaction energies of T cell receptor:decamer-peptide/MHC class I complexes. The peptide position (P1, P2, etc.) is indicated on the x-axis of the graph.

[0065] *Figure 3* shows a size exclusion chromatogram depicting the various species observed during purification of monomeric EBV-LMP2(CLG)/HLA-A*02:01 peptide complex.

[0066] *Figure 4* shows an image of SDS-PAGE of monomeric EBV-LMP2(CLG)/HLA-A*02:01 peptide complex under reducing (left) and non-reducing (right) conditions. Identity of each sample is indicated for each column as well as the location of the monomer (EBV-

Claims:

1. A human antibody agent that binds an EBV-LMP2/HLA peptide complex comprising
 - (a) heavy chain CDR1 of SEQ ID NO:31, heavy chain CDR2 of SEQ ID NO:33 and heavy chain CDR3 of SEQ ID NO:35; and light chain CDR1 of SEQ ID NO:73, light chain CDR2 of SEQ ID NO:75 and light chain CDR3 of SEQ ID NO:77;
 - (b) heavy chain CDR1 of SEQ ID NO:37, heavy chain CDR2 of SEQ ID NO:39 and heavy chain CDR3 of SEQ ID NO:41; and light chain CDR1 of SEQ ID NO:79, light chain CDR2 of SEQ ID NO:81 and light chain CDR3 of SEQ ID NO:83;
 - (c) heavy chain CDR1 of SEQ ID NO:43, heavy chain CDR2 of SEQ ID NO:45 and heavy chain CDR3 of SEQ ID NO:47; and light chain CDR1 of SEQ ID NO:85, light chain CDR2 of SEQ ID NO:87 and light chain CDR3 of SEQ ID NO:89;
 - (d) heavy chain CDR1 of SEQ ID NO:49, heavy chain CDR2 of SEQ ID NO:51 and heavy chain CDR3 of SEQ ID NO:53; and light chain CDR1 of SEQ ID NO:91, light chain CDR2 of SEQ ID NO:93 and light chain CDR3 of SEQ ID NO:95;
 - (e) heavy chain CDR1 of SEQ ID NO:55, heavy chain CDR2 of SEQ ID NO:57 and heavy chain CDR3 of SEQ ID NO:59; and light chain CDR1 of SEQ ID NO:97, light chain CDR2 of SEQ ID NO:99 and light chain CDR3 of SEQ ID NO:101;
 - (f) heavy chain CDR1 of SEQ ID NO:61, heavy chain CDR2 of SEQ ID NO:63 and heavy chain CDR3 of SEQ ID NO:65; and light chain CDR1 of SEQ ID NO:103, light chain CDR2 of SEQ ID NO:105 and light chain CDR3 of SEQ ID NO:107; or
 - (g) heavy chain CDR1 of SEQ ID NO:67, heavy chain CDR2 of SEQ ID NO:69 and heavy chain CDR3 of SEQ ID NO:71; and light chain CDR1 of SEQ ID NO:109, light chain CDR2 of SEQ ID NO:111 and light chain CDR3 of SEQ ID NO:113.
2. The human antibody agent of claim 1, wherein the human antibody agent has a K_D of about 2.0 to about 170 nM.
3. The human antibody agent of claim 1, wherein the EBV-LMP2 peptide has an amino acid sequence that is or comprises CLGGLLTMV (SEQ ID NO:1).

4. The human antibody agent of claim 3, wherein the human antibody agent interacts directly with amino acid residues at one or more positions selected from the group consisting of position 1, 2, 3, 4, 5, 6, 7, 8, and combinations thereof, in the EBV-LMP2 peptide.
5. The human antibody agent of claim 1, wherein the human antibody agent interacts directly with amino acid residues at one or more positions selected from the group consisting of position 1, position 5, position 8, and combinations thereof, in the EBV-LMP2 peptide.
6. The human antibody agent of claim 1, wherein the human antibody agent comprises
 - (a) a heavy chain variable region sequence as set forth in any one of SEQ ID NOs: 5, 9, 13, 17, 21, 25, and 29, and
 - (b) a light chain variable region sequence as set forth in any one of SEQ ID NOs: 3, 7, 11, 15, 19, 23, and 27.
7. The human antibody agent of claim 1, wherein the human antibody agent comprises
 - (a) the light chain variable region of SEQ ID NO:3 and the heavy chain variable region of SEQ ID NO:5;
 - (b) the light chain variable region of SEQ ID NO:7 and the heavy chain variable region of SEQ ID NO:9;
 - (c) the light chain variable region of SEQ ID NO:11 and the heavy chain variable region of SEQ ID NO:13;
 - (d) the light chain variable region of SEQ ID NO:15 and the heavy chain variable region of SEQ ID NO:17;
 - (e) the light chain variable region of SEQ ID NO:19 and the heavy chain variable region of SEQ ID NO:21;
 - (f) the light chain variable region of SEQ ID NO:23 and the heavy chain variable region of SEQ ID NO:25; or
 - (g) the light chain variable region of SEQ ID NO:27 and the heavy chain variable region of SEQ ID NO:29.
8. The human antibody agent of claim 1, wherein the human antibody agent is a human monoclonal antibody that is defucosylated and/or glycosylated with terminal mannose, N-acetylglucose or glucose.

9. The human antibody agent of claim 1, which human antibody agent comprises one or more amino acid substitutions that increase affinity to the EBV-LMP2/HLA peptide complex, wherein the one or more amino acid substitutions is selected from a light chain I48V substitution, a light chain K66R substitution, a heavy chain V5E substitution, a heavy chain E10D substitution, a heavy chain G26E substitution, and a heavy chain V78A substitution.
10. The human antibody agent of claim 9, which human antibody agent that comprises one or more amino acid substitutions is characterized by
- (a) a K_A of at least about 1.6 higher affinity than affinity to a reference peptide/HLA complex, and/or
 - (b) no cross-reactivity to a reference peptide/HLA complex.
11. The human antibody agent of claim 1, wherein the human antibody agent is a human monoclonal antibody or a fragment thereof.
12. The human antibody agent of claim 11, wherein the human monoclonal antibody is an IgG1.
13. The human antibody agent of claim 11, wherein the human monoclonal antibody fragment is an scFv.
14. A bispecific antibody comprising a first antigen-binding site that binds an EBV-LMP2/HLA peptide complex and a second antigen-binding site, wherein the first antigen-binding site comprises
- (a) heavy chain CDR1 of SEQ ID NO:31, heavy chain CDR2 of SEQ ID NO:33 and heavy chain CDR3 of SEQ ID NO:35; and light chain CDR1 of SEQ ID NO:73, light chain CDR2 of SEQ ID NO:75 and light chain CDR3 of SEQ ID NO:77;
 - (b) heavy chain CDR1 of SEQ ID NO:37, heavy chain CDR2 of SEQ ID NO:39 and heavy chain CDR3 of SEQ ID NO:41; and light chain CDR1 of SEQ ID NO:79, light chain CDR2 of SEQ ID NO:81 and light chain CDR3 of SEQ ID NO:83;
 - (c) heavy chain CDR1 of SEQ ID NO:43, heavy chain CDR2 of SEQ ID NO:45 and heavy chain CDR3 of SEQ ID NO:47; and light chain CDR1 of SEQ ID NO:85, light chain CDR2 of SEQ ID NO:87 and light chain CDR3 of SEQ ID NO:89;

(d) heavy chain CDR1 of SEQ ID NO:49, heavy chain CDR2 of SEQ ID NO:51 and heavy chain CDR3 of SEQ ID NO:53; and light chain CDR1 of SEQ ID NO:91, light chain CDR2 of SEQ ID NO:93 and light chain CDR3 of SEQ ID NO:95;

(e) heavy chain CDR1 of SEQ ID NO:55, heavy chain CDR2 of SEQ ID NO:57 and heavy chain CDR3 of SEQ ID NO:59; and light chain CDR1 of SEQ ID NO:97, light chain CDR2 of SEQ ID NO:99 and light chain CDR3 of SEQ ID NO:101;

(f) heavy chain CDR1 of SEQ ID NO:61, heavy chain CDR2 of SEQ ID NO:63 and heavy chain CDR3 of SEQ ID NO:65; and light chain CDR1 of SEQ ID NO:103, light chain CDR2 of SEQ ID NO:105 and light chain CDR3 of SEQ ID NO:107; or

(g) heavy chain CDR1 of SEQ ID NO:67, heavy chain CDR2 of SEQ ID NO:69 and heavy chain CDR3 of SEQ ID NO:71; and light chain CDR1 of SEQ ID NO:109, light chain CDR2 of SEQ ID NO:111 and light chain CDR3 of SEQ ID NO:113.

15. The bispecific antibody of claim 14, wherein the bispecific antibody is characterized by an EC₅₀ of about 0.2 to about 135 pM in T2 cells pulsed with EBV-LMP2 peptide CLGGLLTMV (SEQ ID NO:1).

16. The bispecific antibody of claim 14, wherein the first and/or second antigen-binding sites are selected from the group consisting of an immunoglobulin molecule, scFv, scFab, Fab, Fv or a combination thereof.

17. The bispecific antibody of claim 14, wherein the first and second antigen-binding sites are configured such that they form a single polypeptide chain.

18. The bispecific antibody of claim 17, wherein the first and second antigen-binding sites are each scFvs.

19. The bispecific antibody of claim 14, wherein the second antigen-binding site binds an immunological cell selected from the group consisting of a T cell, B cell, NK cell, dendritic cell, monocyte, macrophage, neutrophil, mesenchymal stem cell and neural stem cell.

20. The bispecific antibody of claim 19, wherein the second antigen-binding site binds CD3 on T cells.

21. The bispecific antibody of claim 14, comprising
a first scFv that binds to EBV-LMP2 peptide CLGGLLTMV (SEQ ID NO:1) presented by an HLA-A02 molecule, and
a second scFv that binds CD3 on T cells,
wherein the bispecific antibody is characterized by an EC₅₀ of about 0.2 to about 135 pM in T2 cells pulsed with the EBV-LMP2 peptide.
22. The bispecific antibody of claim 21, wherein the first scFv comprises
(a) the light chain variable region of SEQ ID NO:3 and the heavy chain variable region of SEQ ID NO:5;
(b) the light chain variable region of SEQ ID NO:7 and the heavy chain variable region of SEQ ID NO:9;
(c) the light chain variable region of SEQ ID NO:11 and the heavy chain variable region of SEQ ID NO:13;
(d) the light chain variable region of SEQ ID NO:15 and the heavy chain variable region of SEQ ID NO:17;
(e) the light chain variable region of SEQ ID NO:19 and the heavy chain variable region of SEQ ID NO:21;
(f) the light chain variable region of SEQ ID NO:23 and the heavy chain variable region of SEQ ID NO:25; or
(g) the light chain variable region of SEQ ID NO:27 and the heavy chain variable region of SEQ ID NO:29.
23. The bispecific antibody of claim 21, wherein the HLA-A02 molecule is selected from HLA-A*02:01, HLA-A*02:02, HLA-A*02:03, HLA-A*02:04, HLA-A*02:05 and HLA-A*02:06.
24. The bispecific antibody of claim 21, wherein the first scFv of the bispecific antibody interacts directly with at least position 5 of the EBV-LMP2 peptide.
25. A human antibody agent that binds an EBV-LMP2/HLA peptide complex comprising

(i) heavy chain CDR1 of SEQ ID NO:43, heavy chain CDR2 of SEQ ID NO:45 or a sequence that differs from SEQ ID NO:45 in an I51V substitution, and heavy chain CDR3 of SEQ ID NO:47; and

(ii) light chain CDR1 of SEQ ID NO:85, light chain CDR2 of SEQ ID NO:87 or a sequence that differs from SEQ ID NO:87 in one or both of a S52G and P55H substitution, and light chain CDR3 of SEQ ID NO:89 or a sequence that differs from SEQ ID NO:89 in a N95I substitution.

26. The human antibody agent of claim 25, comprising

(i) a light chain variable region sequence that comprises a sequence of SEQ ID NO: 11 or a sequence that differs from SEQ ID NO:11 in one or more amino acid substitutions selected from I48V, S52G, P55H, K66R and N95I; and

(ii) a heavy chain variable region that comprises a sequence of SEQ ID NO: 13 or a sequence that differs from SEQ ID NO:13 in one or more amino acid substitutions selected from V5E, E10D, G26E, I51V and V78A.

27. A bispecific antibody comprising a first antigen-binding site that binds an EBV-LMP2/HLA peptide complex and a second antigen-binding site, wherein the first antigen-binding site comprises

(i) heavy chain CDR1 of SEQ ID NO:43, heavy chain CDR2 of SEQ ID NO:45 or a sequence that differs from SEQ ID NO:45 in an I51V substitution, and heavy chain CDR3 of SEQ ID NO:47; and

(ii) light chain CDR1 of SEQ ID NO:85, light chain CDR2 of SEQ ID NO:87 or a sequence that differs from SEQ ID NO:87 in one or both of a S52G and P55H substitution, and light chain CDR3 of SEQ ID NO:89 or a sequence that differs from SEQ ID NO:89 in a N95I substitution.

28. The bispecific antibody of claim 27, wherein the first antigen-binding site comprises

(i) a light chain variable region sequence that comprises a sequence of SEQ ID NO: 11 or a sequence that differs from SEQ ID NO:11 in one or more amino acid substitutions selected from I48V, S52G, P55H, K66R and N95I; and

(ii) a heavy chain variable region that comprises a sequence of SEQ ID NO: 13 or a sequence that differs from SEQ ID NO:13 in one or more amino acid substitutions selected from V5E, E10D, G26E, I51V and V78A.