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DUAL FUNCTION ANTIGEN BINDING MOLECULES

Abstract

Antibodies or antigen binding fragments thereof comprising at least one immunogenic peptide inserted into a variable region of the antibody or antigen binding fragment thereof, wherein the insertion comprises removal of antibody or antigen binding fragment sequence are provided. Dual-function antigen binding molecules comprising an antibody or antigen binding fragment of the invention and a second antibody or antigen binding fragment thereof capable of binding an antigen overexpressed on a target cell are provided. Nucleic acid molecules encoding same, pharmaceutical compositions comprising same and methods of treating cancer by administrating same are also provided. Methods of producing antibodies or antigen binding fragments are also provided.

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Background/Summary

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Israeli Patent Application No. 287372 filed on Oct. 18, 2021, the contents of which are incorporated herein by reference in its entirety.

FIELD OF INVENTION

[0002] The present invention is in the field of anti-cancer immunotherapy.

SUMMARY OF THE INVENTION

[0003] The present invention provides antibodies or antigen binding fragments thereof comprising at least one immunogenic peptide inserted into a variable region of the antibody or antigen binding fragment thereof. Dual-function antigen binding molecules comprising an antibody or antigen binding fragment of the invention and a second antibody or antigen binding fragment thereof capable of binding an antigen overexpressed on a target cell. Nucleic acid molecules encoding same, pharmaceutical compositions comprising same and methods of treating cancer by administrating same are also provided. Methods of producing antibodies or antigen binding fragments are also provided.

[0004] According to a first aspect, there is provided an antibody or antigen binding fragment thereof comprising at least one immunogenic peptide inserted into a variable region of the antibody or antigen binding fragment thereof, and where the insertion comprises removal of antibody or antigen binding fragment sequence.

[0005] According to another aspect, there is provided a dual-function antigen binding molecule comprising: [0006] a. a first antibody or antigen binding fragment thereof comprising at least one immunogenic peptide inserted into a CDR of the antibody or antigen binding fragment thereof, and where the insertion comprises removal of CDR sequence; and [0007] b. a second antibody capable of binding epidermal growth factor receptor (EGFR), wherein the antibody is selected from cetuximab, panitumumab and necitumumab or antibody comprising at least 85% sequence identity thereto.

[0008] According to some embodiments, the antibody or antigen binding fragment thereof binds to a target cell, and wherein the target cell is a cancer cell, a dendritic cell or both. According to some embodiments, the first antibody or antigen binding fragment thereof binds to a target cell, and wherein the target cell is a cancer cell, a dendritic cell or both.

[0009] According to some embodiments, the immunogenic peptide is a cancer specific peptide.

[0010] According to some embodiments, the cancer specific peptide is selected from a peptide sequence provided in Table 1.

[0011] According to some embodiments, the immunogenic peptide is a viral peptide.

[0012] According to some embodiments, the viral peptide is derived from Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), Adenovirus, Human papilloma virus (HPV) or Influenza virus (FLU).

[0013] According to some embodiments, the viral peptide is selected from a peptide sequence provided in Table 2 or Table 3.
[0014] According to some embodiments, the antibody or antigen binding fragment thereof further comprises a cell penetration sequence that targets the antibody or antigen binding fragment thereof to a cytoplasm of a cell binding the antibody or antigen binding fragment thereof. According to some embodiments, the antibody or antigen binding fragment thereof further comprises a cell penetration sequence that targets the first antibody or antigen binding fragment thereof to a cytoplasm of a cell bound by the first antibody or antigen binding fragment thereof.
[0015] According to some embodiments, the cell penetration sequence is an endosomal escape domain (EED).
[0016] According to some embodiments, the antibody devoid of the immunogenic peptide is endocytosed into an endosomal pathway and is delivered to the cytoplasm. According to some embodiments, the first antibody or antigen binding fragment thereof is endocytosed into an endosomal pathway and is delivered to the cytoplasm.
[0017] According to some embodiments, at least one CDR or a portion thereof of the antibody or antigen binding fragment thereof is replaced with the immunogenic peptide. According to some embodiments, at least one CDR or a portion thereof of the first antibody or antigen binding fragment thereof is replaced with the immunogenic peptide.
[0018] According to some embodiments, the CDR is an inert CDR having little or no contribution to binding to a target antigen.
[0019] According to some embodiments, an inert CDR comprises two or fewer amino acids that contact the target antigen.
[0020] According to some embodiments, contact comprises a distance of not more than 5 angstroms between an amino acid of a CDR and an amino acid of the target antigen.
[0021] According to some embodiments, the insertion and removal produces no change or minimal change in the overall conformation of the antibody or antigen binding fragment thereof such that the antibody or antigen binding fragment thereof binds its target antigen at an equivalent affinity to the antibody or antigen binding fragment devoid of the immunogenic peptide. According to some embodiments, the insertion and removal produces no change or minimal change in the overall conformation of the first antibody or antigen binding fragment thereof such that the first antibody or antigen binding fragment thereof binds its target antigen at an equivalent affinity to the first antibody or antigen binding fragment devoid of the immunogenic peptide.
[0022] According to some embodiments, at least one inert CDR of an antigen binding region is replaced with a cell penetration sequence.
[0023] According to some embodiments, the target cell is a dendritic cell and a dendritic cell antigen is selected from CD40, CD205, CD206, CLEC9A, CLEC12A, CD209, and CD207.
[0024] According to some embodiments, the target cell is a malignant immune cell and an immune cell antigen is selected from CD20, CD19, CD21, and CD22.
[0025] According to some embodiments, the target cell is a cancer cell and a cancer cell antigen is selected from HER2, EGFR, EpCAM, PSMA, BCMA, CD123, CD33, CD38, CTLA, LAG-3, ICOS, 4-1BB and PD-L1.
[0026] According to some embodiments, the antibody or antigen binding fragment thereof is devoid of a chemical linker.
[0027] According to some embodiments, the antigen binding region, the immunogenic peptide and the cell penetrating sequence are each separated by a linker. According to some embodiments, within the first antibody or antigen binding fragment thereof the antigen binding region, the immunogenic peptide and the cell penetrating sequence are each separated by a linker.
[0028] According to some embodiments, the immunogenic peptide is recognized by CD4 T cells, CD8 T cells or both.
[0029] According to some embodiments, the antibody before the immunogenic peptide is inserted

is selected from: [0030] a. antibody TMab4 comprising a heavy chain variable region of SEQ ID NO: 1021 and a light chain variable region of SEQ ID NO: 1022; [0031] b. antibody 3E10 comprising a heavy chain variable region of SEQ ID NO: 1023 and a light chain variable region of SEQ ID NO: 1024; and [0032] c. antibody 71F12 comprising a heavy chain variable region of SEQ ID NO: 1026 and a light chain variable region of SEQ ID NO: 1027.

[0033] According to some embodiments, the first antibody before the immunogenic peptide is inserted is selected from: [0034] a. antibody TMab4 comprising a heavy chain variable region of SEQ ID NO: 1021 and a light chain variable region of SEQ ID NO: 1022; [0035] b. antibody 3E10 comprising a heavy chain variable region of SEQ ID NO: 1023 and a light chain variable region of SEQ ID NO: 1024; and [0036] c. antibody 71F12 comprising a heavy chain variable region of SEQ ID NO: 1026 and a light chain variable region of SEQ ID NO: 1027.

[0037] According to some embodiments, at least one of: [0038] a. the immunogenic peptide is inserted into CDRH1, CDRH2, CDRH3 or cDRL3 of the TMab4; [0039] b. the immunogenic peptide is inserted into CDRL1 or CDRL2 of the 3E10; and [0040] c. the immunogenic peptide is inserted into CDRL1 of the 71F12.

[0041] According to some embodiments, the antibody or antigen binding fragment thereof comprises at least one of: [0042] a. a light chain variable region of SEQ ID NO: 1022 and a heavy chain variable region selected from SEQ ID NO: 1028-1040, 1043-1045, 1047-1055, and 1058-1059; [0043] b. a heavy chain variable region of SEQ ID NO: 1021 and a light chain variable region selected from SEQ ID NO: 1041-1042, 1046, and 1056-1057; [0044] c. a heavy chain variable region of SEQ ID NO: 1023 and a light chain variable region selected from SEQ ID NO: 1060-1065; [0045] d. a heavy chain variable region of SEQ ID NO: 1026 and a light chain variable region of SEQ ID NO: 1066; and [0046] e. a light chain variable region of SEQ ID NO: 1027 and a heavy chain variable region of SEQ ID NO: 1067.

[0047] According to some embodiments, the first antibody comprises at least one of: [0048] a. a light chain variable region of SEQ ID NO: 1022 and a heavy chain variable region selected from SEQ ID NO: 1028-1040, 1043-1045, 1047-1055, and 1058-1059; [0049] b. a heavy chain variable region of SEQ ID NO: 1021 and a light chain variable region selected from SEQ ID NO: 1041-1042, 1046, and 1056-1057; [0050] c. a heavy chain variable region of SEQ ID NO: 1023 and a light chain variable region selected from SEQ ID NO: 1060-1065; [0051] d. a heavy chain variable region of SEQ ID NO: 1026 and a light chain variable region of SEQ ID NO: 1066; and [0052] e. a light chain variable region of SEQ ID NO: 1027 and a heavy chain variable region of SEQ ID NO: 1067.

[0053] According to another aspect, there is provided a dual-function antigen binding molecule comprising a first antibody or antigen binding fragment thereof comprising an antibody or antigen binding fragment of the invention and a second antibody or antigen binding fragment thereof capable of binding an antigen overexpressed on a target cancer cell.

[0054] According to some embodiments, the antigen overexpressed on a target cancer cell is EGFR and the second antibody is selected from cetuximab, panitumumab and necitumumab.

[0055] According to some embodiments, the first antibody and the second antibody comprise at least one modification that promotes heterodimerization and inhibit homodimerization.

[0056] According to some embodiments, one of the first and second antibody comprises a heavy chain constant region comprising SEQ ID NO: 1074 and the other antibody comprises a heavy chain constant region comprising SEQ ID NO: 1075.

[0057] According to some embodiments, the dual function antigen binding molecule comprises two heavy chains and two light chains, wherein: [0058] a. the two heavy chains are SEQ ID NO: 1088 and 1080 and the two light chains are SEQ ID NO: 1087 and 1079; [0059] b. the two heavy chains are SEQ ID NO: 1088 and 1082 and the two light chains are SEQ ID NO: 1087 and 1081; [0060] c. the two heavy chains are SEQ ID NO: 1090 and 1080 and the two light chains are SEQ ID NO: 1089 and 1079; [0061] d. the two heavy chains are SEQ ID NO: 1090 and 1082 and the two light

chains are SEQ ID NO: 1089 and 1081; or [0062] e. the two heavy chains are SEQ ID NO: 1088 and 1086 and the two light chains are SEQ ID NO: 1087 and 1085.
[0063] According to another aspect, there is provided a pharmaceutical composition comprising an antibody or antigen binding fragment or the invention or a dual-function antigen binding molecule of the invention and a pharmaceutically acceptable carrier excipient or adjuvant.
[0064] According to another aspect, there is provided a nucleic acid molecule comprising at least one open reading frame, wherein the open reading frame encodes an antibody or antigen binding fragment thereof of the invention or a dual-function antigen binding molecule the invention.
[0065] According to another aspect, there is provided an expression vector comprising at least one regulatory element operatively linked to a nucleic acid molecule of the invention.
[0066] According to another aspect, there is provided a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition of the invention, thereby treating cancer in a subject.
[0067] According to some embodiments, the cancer overexpresses the cancer specific antigen.
[0068] According to some embodiments, the cancer is an EGFR positive cancer.
[0069] According to some embodiments, the dual-function antigen binding molecule is a cancer vaccine and comprises an antigen binding region capable of binding a dendritic cell antigen.
[0070] According to another aspect, there is provided a method of engineering an antibody or antigen binding fragment thereof, the method comprising: [0071] a. selecting an antibody or antigen binding fragment thereof of interest; [0072] b. receiving structural analysis of the selected antibody or antigen binding domain bound to its target; [0073] c. determining at least one CDR of the selected antibody or antigen binding domain that is not required for binding to the target based on the structural analysis; [0074] d. replacing the determined at least one CDR or a portion thereof with an immunogenic peptide;
thereby engineering an antibody or antigen binding fragment thereof.
[0075] According to another aspect, there is provided a method of engineering an antibody or antigen binding fragment thereof, the method comprising: [0076] a. selecting an antibody or antigen binding fragment thereof of interest; [0077] b. receiving a database of immunogenic peptides; [0078] c. performing pairwise alignment of peptides of a variable region of the selected antibody or antigen binding fragment thereof of interest with immunogenic peptides of the database; [0079] d. determining a peptide from the selected antibody or antigen binding fragment thereof and an immunogenic peptide with an alignment score above a predetermined threshold; and [0080] e. replacing the determined peptide from the selected antibody or antigen binding fragment thereof with the determined immunogenic peptide;
thereby engineering an antibody or antigen binding fragment thereof.
[0081] According to some embodiments, the method further comprises optimizing the replacing to produce as little perturbation in the structure of the selected antibody or antigen binding fragment thereof of interest as possible.
[0082] According to some embodiments, the engineered antibody or antigen binding fragment thereof is an immunogenic peptide delivery antibody.
[0083] According to some embodiments, step (a) comprises selecting an antibody or antigen binding fragment thereof that binds to a surface of a target cell.
[0084] According to some embodiments, step (a) comprises selecting an antibody or antigen binding fragment thereof that upon binding to a surface is internalized and delivered to a cytosol of the target cell.
[0085] According to some embodiments, the method further comprises confirming at least one of: delivery of the immunogenic peptide to a cytosol of the target cell, delivery of the immunogenic peptide in complex with an HLA molecule to a surface of the target cell and specific killing of the target cell by an effector cell specific to the immunogenic peptide.
[0086] According to some embodiments, the method further comprises selecting a targeting

antibody that binds to a protein on a surface of a target cell and producing a dual-function antigen binding molecule by combining the engineered antibody and the targeting antibody.
[0087] According to some embodiments, the combining comprises engineering a heavy chain constant region of the targeting antibody and a heavy chain constant region of the engineered antibody to promote heterodimerization and inhibit homodimerization.
[0088] Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

Description

BRIEF DESCRIPTION OF THE DRAWINGS
[0089] FIG. **1**: A schematic showing an antibody engaging its antigen and the presence of inert CDRs not involved in binding.
[0090] FIG. **2**: Images of an anti-PD-L1 antibody with an inert CDR and the Trojan antibody of the invention after the inert CDR is replace with an immunogenic peptide.
[0091] FIG. **3**: A stepwise diagram of dendritic cell vaccination method of the invention.
[0092] FIG. **4**: A stepwise diagram of a B cell targeting CD4+ T cell-mediated method of the invention.
[0093] FIG. **5**: A stepwise diagram of a CD8+ T cell-mediated cancer killing method of the invention.
[0094] FIG. **6**: An overview of embodiments of the Trojan antibody production method.
[0095] FIGS. **7**A-**7**C: Bar charts of specific killing of cancer cells contacted with (**7**A) TAbs containing immunogenic peptides inserted into CDRH1 of TMab4 (P1) antibody, (**7**B) TAbs containing immunogenic peptides inserted into CDRH3 of TMab4 (P1) antibody, and (**7**C) TAbs containing immunogenic peptides inserted into CDRL3 pr CDRH2 of TMab4 (P1) antibody, and then cocultured with peptide specific effector cells.
[0096] FIG. **8**: Bar charts of specific killing of cancer cells contacted with TAbs containing immunogenic peptides inserted into CDRL1 of 3E10 (P2) antibody and then cocultured with peptide specific effector cells.
[0097] FIGS. **9**A-**9**B: Line graphs of apoptotic cancer cells cultured with effector cells alone (Cells only), (**9**A) parental antibody 3E10 (P2) and TAb T2_11, and (**9**B) parental antibody TMab4 (P1) and TAb T18.
[0098] FIG. **10**: Schematics of bi-functional Trojan antibodies and corresponding controls. Bi-TAbs are labeled as FTAbs and control bi-TAbs are labeled as cFTAbs.
[0099] FIG. **11**: Histograms showing binding of bi-TAbs to EGFR on the surface of cancer cells. Therapeutic antibodies are used as the positive control and fluorescently labeled secondary antibodies are used as a negative control. Upper panels show 0.3 nM concentration and lower panels show 3 nM concentration. Bi-TAbs are labeled as FTAbs.
[0100] FIG. **12**: Bar graphs of the percentage of cancer cells displaying the HLA-peptide complex at different time points. Each bi-TAb (white bars) is compared to its negative control (grey bars) with the same killing module but without the immunogenic peptide. Bi-TAbs are labeled as FTAbs and control bi-TAbs are labeled as cFTAbs.
[0101] FIG. **13**: Line graphs of percentage of cancer cells killed by effector cells at various effector to target cell ratios. Each bi-TAb (light grey) is compared to its negative control (black lines). An antibody only point without effector cells is included. Bi-TAbs are labeled as FTAbs and control bi-

TAbs are labeled as cFTAbs.

DETAILED DESCRIPTION OF THE INVENTION

[0102] The present invention, in some embodiments, provides antibodies or antigen binding fragments thereof comprising at least one immunogenic peptide inserted into a variable region of the antibody or antigen binding fragment thereof, wherein the insertion comprises removal of antibody or antigen binding fragment sequence. Dual-function antigen binding molecules comprising an antibody or antigen binding fragment of the invention and a second antibody or antigen binding fragment thereof capable of binding an antigen overexpressed on a target cell. Nucleic acid molecules encoding same, pharmaceutical compositions comprising same and methods of treating cancer by administrating same are also provided. Methods of producing antibodies or antigen binding fragments are also provided.

[0103] The invention is based on the surprising finding that antibodies/antigen binding molecules can be used as a delivery system for immunogenic peptides. That is, a highly immunogenic peptide can be delivered specifically to cancer cells and thereby increase immune surveillance against them. The antibody would in this case have an antigen binding domain to a cancer epitope which would cause the therapeutic molecule to bind the cancer cell. Upon endocytosis of the antibody/antigen binding molecule the immunogenic peptide would be delivered to the cytoplasm. This can be enhanced by the inclusion of a cell penetrating sequence, or specifically an endosomal escape sequence. Endosomal escape however is not essential as some mechanisms, such as receptor-mediated transcytosis of antibodies, deliver the antibody directly to the cytoplasm. The therapeutic molecule would be cleaved, releasing the immunogenic peptide which would then be displayed on the cell surface in complex with an HLA molecule, thus enhancing the immunogenicity of the cancer cell and increasing immune surveillance against the cancer and cancer killing.

[0104] Alternatively, the antigen binding region can bind a dendritic cell antigen, which would deliver the therapeutic molecule comprising a cancer cell antigen to a dendritic cell. The molecule would be endocytosed upon binding and the immunogenic cancer peptide will be cleaved from the rest of the molecule of the invention and displayed on the surface of the dendritic cell by HLA molecules. This will in turn train cytotoxic immune cells (T cell and NK cells) to target this immunogenic peptide and thereby the cancer.

[0105] By a first aspect, there is provided an antigen binding molecule.

[0106] By another aspect, there is provided a composition comprising the antigen binding molecule of the invention.

[0107] By another aspect, there is provided a nucleic acid molecule encoding the antigen binding molecule of the invention.

[0108] By another aspect, there is provided an expression vector comprising the nucleic acid molecule of the invention.

[0109] By another aspect, there is provided a method of expressing a peptide on a surface of a target cell, the method comprising contacting the target cell with an antigen binding molecule of the invention or a pharmaceutical composition of the invention, thereby expressing a peptide on a surface of a target cell.

[0110] By another aspect, there is provided a method of treating cancer in a subject in thereof, the method comprising administering to the subject an antigen binding molecule of the invention or a pharmaceutical composition of the invention, thereby treating cancer in a subject in need thereof.

[0111] By another aspect, there is provided a composition of the invention for use in expressing a peptide on a surface of a target cell. By another aspect, there is provided a composition of the invention for use in treating cancer. By another aspect, there is provided a composition of the invention for use in the production of a medicament for the treating of cancer.

[0112] In some embodiments, the antigen binding molecule is a dual-function molecule. In some embodiments, the first function is binding an antigen. In some embodiments, the second function is

entering a cell. In some embodiments, the second function is delivering a peptide. In some embodiments, the peptide is an immunogenic peptide. In some embodiments, the second function is delivering an immunogenic peptide into a cytoplasm of a cell.

[0113] In some embodiments, the molecule comprises an antigen binding region. In some embodiments, the region is a domain. In some embodiments, the region binds the antigen. In some embodiments, the antigen is on a target cell. In some embodiments, the antigen binding region is capable of binding to a target cell. In some embodiments, the antigen is a cancer antigen. In some embodiments, a cancer antigen is a cancer specific antigen. In some embodiments, a cancer antigen is an antigen on a cancer cell. In some embodiments, the antigen is an immune cell antigen. In some embodiments, the immune cell is selected from a dendritic cell, a B cell, a T cell, a neutrophil, a macrophage and a natural killer (NK) cell. In some embodiments, the immune cell is a dendritic cell. In some embodiments, the antigen is a dendritic cell antigen. In some embodiments, the immune cell is a B cell. In some embodiments, the antigen is a B cell antigen. In some embodiments, an antigen is expressed on a cell. In some embodiments, an antigen is expressed on a cell surface. In some embodiments, an antigen is displayed on the cell surface as an MHC molecule. In some embodiments, an MHC molecule is an MHC class I or class II molecule. In some embodiments, an MHC molecule is a protein complex of the antigen and an HLA protein. In some embodiments, an antigen is a cell surface protein. In some embodiments, a cell surface protein is a cell surface receptor. In some embodiments, the antigen binding region is capable of binding the antigen.

[0114] In some embodiments, a dendritic cell antigen is selected from CD40, CD205, CD206, CLEC9A, CLEC12A, CD209, and CD207. Markers of dendritic cells are well known in the art and any such surface marker may be used as the antigen. In some embodiments, the dendritic cell antigen is CD40. Antigen binding domains that target dendritic cell antigens are well known in the art and any such antigen binding domain may be employed. For a non-limiting example Fab516 binds specifically to CD40.

[0115] In some embodiments, an immune cell antigen is selected from CD20, CD19, CD21, and CD22. In some embodiments, the immune cell antigen is CD20. In some embodiments, the immune cell antigen is a B cell antigen. Markers of immune cells in general, and B cells in particular, are well known in the art and any such surface marker may be used as the antigen. Antigen binding domains that target immune cell antigens are well known in the art and any such antigen binding domain may be employed. For a non-limiting example Arzerra binds specifically to CD20

[0116] In some embodiments, a cancer cell antigen is selected from HER2, EGFR, EpCAM, PSMA, BCMA, CD123, CD33, CD38, CTLA, LAG-3, ICOS, 4-1BB and PD-L1. Markers of cancer cells are well known in the art and any such surface marker may be used as the antigen. In some embodiments, the cancer cell antigen is PD-L1. Antigen binding domains that target cancer cell antigens are well known in the art and any such antigen binding domain may be employed. For a non-limiting example Durvalumab binds specifically to PD-L1.

[0117] In some embodiments, the antigen binding domain is an antigen binding domain of an antibody. In some embodiments, the antigen binding domain is an antibody. In some embodiments, the antigen binding domain is an antibody or antigen binding fragment thereof. In some embodiments, the antibody is a single-chain antibody. In some embodiments, the antibody is a single domain antibody. In some embodiments, the antibody is a full antibody.

[0118] As used herein, the term “antibody” refers to a polypeptide or group of polypeptides that include at least one binding domain that is formed from the folding of polypeptide chains having three-dimensional binding spaces with internal surface shapes and charge distributions complementary to the features of an antigenic determinant of an antigen. An antibody typically has a tetrameric form, comprising two identical pairs of polypeptide chains, each pair having one “light” and one “heavy” chain. The variable regions of each light/heavy chain pair form an

antibody binding site. An antibody may be oligoclonal, polyclonal, monoclonal, chimeric, camelised, CDR-grafted, multi-specific, bi-specific, catalytic, humanized, fully human, anti-idiotypic and antibodies that can be labeled in soluble or bound form as well as fragments, including epitope-binding fragments, variants or derivatives thereof, either alone or in combination with other amino acid sequences. An antibody may be from any species. The term antibody also includes binding fragments, including, but not limited to Fv, Fab, Fab′, F(ab′)2 single stranded antibody (svFC), dimeric variable region (Diabody) and disulphide-linked variable region (dsFv). In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, i.e., molecules that contain an antigen binding site. Antibody fragments may or may not be fused to another immunoglobulin domain including but not limited to, an Fc region or fragment thereof. The skilled artisan will further appreciate that other fusion products may be generated including but not limited to, scFv-Fc fusions, variable region (e.g., VL and VH)˜ Fc fusions and scFv-scFv-Fc fusions.

[0119] Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass.

[0120] An “antigen” is a molecule or a portion of a molecule capable of eliciting antibody formation and being bound by an antibody. Antibody formation can occur in mice, rats, rabbits, pigs and other animals commonly used for generation of antibodies, but of course can also occur in humans as a response to a foreign antigen. An antigen may have one or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

[0121] The term “antigenic determinant” or “epitope” according to the invention refers to the region of an antigen molecule that specifically reacts with particular antibody. Peptide sequences derived from an epitope can be used, alone or in conjunction with a carrier moiety, applying methods known in the art, to immunize animals and to produce additional polyclonal or monoclonal antibodies. Immunoglobulin variable domains can also be analyzed using the IMGT information system (imgt. Cines.fr/) (IMGT®/V-Quest) to identify variable region segments, including CDRs. See, e.g., Brochet, X. et al, Nucl. Acids Res. J6:W503-508 (2008).

[0122] Kabat et al. also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of “Kabat numbering” to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, “Kabat numbering” refers to the numbering system set forth by Kabat et al, U.S. Dept. of Health and Human Services, “Sequence of Proteins of Immunological Interest” (1983).

[0123] The term “antibody” (also referred to as an “immunoglobulin”) is used in the broadest sense and specifically encompasses monoclonal antibodies and antibody fragments so long as they exhibit the desired biological activity. In certain embodiments, the use of a chimeric antibody or a humanized antibody is also encompassed by the invention.

[0124] The basic unit of the naturally occurring antibody structure is a heterotetrameric glycoprotein complex of about 150,000 Daltons, composed of two identical light (L) chains and two identical heavy (H) chains, linked together by both noncovalent associations and by disulfide bonds. Each heavy and light chain also has regularly spaced intra-chain disulfide bridges. Five human antibody classes (IgG, IgA, IgM, IgD and IgE) exist, and within these classes, various subclasses, are recognized based on structural differences, such as the number of immunoglobulin units in a single antibody molecule, the disulfide bridge structure of the individual units, and differences in chain length and sequence. The class and subclass of an antibody is its isotype.

[0125] The amino terminal regions of the heavy and light chains are more diverse in sequence than the carboxy terminal regions, and hence are termed the variable domains. This part of the antibody structure confers the antigen-binding specificity of the antibody. A heavy variable (VH) domain and

a light variable (VL) domain together form a single antigen-binding site, thus, the basic immunoglobulin unit has two antigen-binding sites. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains (Chothia et al., J. Mol. Biol. 186, 651-63 (1985); Novotny and Haber, (1985) Proc. Natl. Acad. Sci. USA 82 4592-4596).
[0126] The carboxy terminal portion of the heavy and light chains form the constant domains i.e., CH1, CH2, CH3, CL. While there is much less diversity in these domains, there are differences from one animal species to another, and further, within the same individual there are several different isotypes of antibody, each having a different function.
[0127] The term “framework region” or “FR” refers to the amino acid residues in the variable domain of an antibody, which are other than the hypervariable region amino acid residues as herein defined. The term “hypervariable region” as used herein refers to the amino acid residues in the variable domain of an antibody, which are responsible for antigen binding. The hypervariable region comprises amino acid residues from a “complementarity determining region” or “CDR”. The CDRs are primarily responsible for binding to an epitope of an antigen. The extent of FRs and CDRs has been precisely defined (see, Kabat et al.). In some embodiments, CDRs are determined using the KABAT system. In some embodiments, CDRs are determined using the Clothia system. In some embodiments, the Clothia system is the enhanced Clothia system (Martin system).
[0128] In some embodiments, the antibody or antigen binding fragment thereof is the antibody or antigen binding fragment thereof devoid or without the immunogenic peptide. In some embodiments, devoid of without the immunogenic peptide is before insertion of the immunogenic peptide. In some embodiments, the antibody or antigen binding fragment thereof binds to a target cell. In some embodiments, the antibody or antigen binding fragment thereof adheres to a surface of a target cell. In some embodiments, the antibody or antigen binding fragment thereof is a cell penetrating antibody. In some embodiments, the antibody or antigen binding fragment thereof is phagocytosed into the cell. In some embodiments, the antibody or antigen binding fragment thereof is endocytosed into the cell. In some embodiments, into the cell is into the endosomal pathway of the cell. In some embodiments, the antibody or antigen binding fragment thereof is brought into the endosomal pathway. In some embodiments, the antibody or antigen binding fragment thereof escapes from the endosomal pathway. In some embodiments, the antibody or antigen binding fragment thereof is delivered into the cytoplasm of a cell that binds it. In some embodiments, the antibody or antigen binding fragment thereof is a DNA binding antibody. In some embodiments, the antibody or antigen binding fragment thereof is a lupus antibody.
[0129] In some embodiments, the antibody or antigen binding fragment thereof is the Tmab4 antibody. In some embodiments, the Tmab4 comprises a heavy chain variable region of SEQ ID NO: 1021 or an analog or homolog comprising at least 85% sequence identity and being capable of binding cells and reaching the cytosol. In some embodiments, the Tmab4 comprises a light chain variable region of SEQ ID NO: 1022 or an analog or homolog comprising at least 85% sequence identity and being capable of binding cells and reaching the cytosol. In some embodiments, the antibody or antigen binding fragment thereof is the 3E10 antibody. In some embodiments, the 3E10 comprises a heavy chain variable region of SEQ ID NO: 1023 or an analog or homolog comprising at least 85% sequence identity and being capable of binding cells and reaching the cytosol. In some embodiments, the 3E10 comprises a light chain variable region of SEQ ID NO: 1024 or an analog or homolog comprising at least 85% sequence identity and being capable of binding cells and reaching the cytosol. In some embodiments, the antibody or antigen binding fragment thereof is the 71F12 antibody. In some embodiments, the 71F12 comprises a heavy chain variable region of SEQ ID NO: 1026 or an analog or homolog comprising at least 85% sequence identity and being capable of binding cells and reaching the cytosol. In some embodiments, the 71F12 comprises a light chain variable region of SEQ ID NO: 1027 or an analog or homolog comprising at least 85% sequence identity and being capable of binding cells and reaching the cytosol.
[0130] In some embodiments, the antigen binding molecule comprises at least one immunogenic

peptide. In some embodiments, the antigen binding molecule comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 immunogenic peptides. Each possibility represents a separate embodiment of the invention. In some embodiments, the immunogenic peptide is an exogenous immunogenic peptide. In some embodiments, the immunogenic peptide is inserted into the antigen binding molecule. In some embodiments, the immunogenic peptide is inserted into a variable region of the antibody or antigen binding fragment thereof. In some embodiments, the immunogenic peptide is not a natural part of the antigen binding molecule. In some embodiments, the peptide is a sequence of amino acids. In some embodiments, the sequence of immunogenic amino acids is inserted into the sequence of the antigen binding molecule. In some embodiments, the immunogenic peptide replaces amino acids of the antigen binding molecule. In some embodiments, insertion of the immunogenic peptide comprises removal of amino acid sequence. In some embodiments, the amino acid sequence is sequence of the antibody or antigen binding fragment thereof. In some embodiments, the immunogenic peptide is not artificially linked to antigen binding molecule. In some embodiments, the antigen binding molecule is a recombinant molecule. In some embodiments, the recombinant molecule comprises an amino acid sequence of the immunogenic peptide. In some embodiments, the immunogenic peptide is not linked by a chemical linkage to the antigen binding molecule. In some embodiments, a chemical linkage is any linkage other than a peptide linkage. In some embodiments, a chemical linkage is any linkage other than an amino acid linkage. In some embodiments, the immunogenic peptide is linked to the antigen binding molecule by a peptide bond, an amino acid linkage or both.

[0131] As used herein, the terms “peptide”, “polypeptide” and “protein” are used interchangeably to refer to a polymer of amino acid residues. In another embodiment, the terms “peptide”, “polypeptide” and “protein” as used herein encompass native peptides, peptidomimetics (typically including non-peptide bonds or other synthetic modifications) and the peptide analogues peptoids and semipeptoids or any combination thereof. In another embodiment, the peptides polypeptides and proteins described have modifications rendering them more stable while in the body or more capable of penetrating into cells. In one embodiment, the terms “peptide”, “polypeptide” and “protein” apply to naturally occurring amino acid polymers. In another embodiment, the terms “peptide”, “polypeptide” and “protein” apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid.

[0132] As used herein, the term “recombinant protein” refers to a protein which is coded for by a recombinant DNA and is thus not naturally occurring. The term “recombinant DNA” refers to DNA molecules formed by laboratory methods of genetic recombination. Generally, this recombinant DNA is in the form of a vector, plasmid or virus used to express the recombinant protein in a cell.

[0133] In some embodiments, the immunogenic peptide comprises at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids. Each possibility represents a separate embodiment of the invention. In some embodiments, the immunogenic peptide comprises at most 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids. Each possibility represents a separate embodiment of the invention. In some embodiments, the immunogenic peptide is between 3 and 20, 3 and 15, 3 and 12, 3 and 11, 3 and 10, 3 and 7, 5 and 20, 5 and 15, 5 and 12, 5 and 11, 5 and 10, 7 and 20, 7 and 15, 7 and 12, 7 and 11, or 7 and 10 amino acids. Each possibility represents a separate embodiment of the invention. In some embodiments, the immunogenic peptide is between 8 and 11 amino acids. In some embodiments, the immunogenic peptide consists of 8 amino acids. In some embodiments, the immunogenic peptide consists of 9 amino acids. In some embodiments, the immunogenic peptide consists of 10 amino acids. In some embodiments, the immunogenic peptide consists of 11 amino acids.

[0134] As used herein, the term “immunogenic peptide” refers to an amino acids sequence that produces an immune response when exposed to the human immune system. In some embodiments, an immunogenic peptide is a non-human peptide. In some embodiments, the immunogenic peptide produces an immune response from an immune cell. In some embodiments, the immunogenic

peptide is recognized an immune response from an immune cell. In some embodiments, recognized is bound by. In some embodiments, an immunogenic peptide produces an immune response from a dendritic cell. In some embodiments, an immunogenic peptide is displayed on the cell surface. In some embodiments, an immunogenic peptide is displayed as an MHC molecule. In some embodiments, an immunogenic peptide is displayed in complex with HLA. In some embodiments, the immunogenic peptide produces an immune response from a T cell. In some embodiments, the immune cell is a dendritic cell. In some embodiments, the immune cell is a T cell. In some embodiments, a T cell is selected from a CD4 T cell and a CD8 T cell. In some embodiments, the T cell is a CD4 T cell. In some embodiments, the T cell is a CD8 T cell. In some embodiments, the immunogenic peptide comprises a CD4 epitope, a CD8 epitope or both. In some embodiments, the immunogenic peptide produces an immune response from an NK cell. In some embodiments, an immune response is an elevated immune response. In some embodiments, immunogenic comprises increase immunogenicity. In some embodiments, increased is as compared to a control peptide. In some embodiments, the control peptide is a human peptide. In some embodiments, a control peptide is a non-cancerous peptide. In some embodiments, a control peptide is a non-immunogenic peptide.

[0135] In some embodiments, the immunogenic peptide is a cancer peptide. In some embodiments, the cancer peptide is a cancer specific peptide. In some embodiments, the cancer peptide is a cancer elevated peptide. In some embodiments, the cancer peptide is a peptide with increased surface expression in cancer cells. In some embodiments, a cancer peptide is a peptide provided in Table 1. In some embodiments, a cancer peptide is selected from a sequence provided in Table 1. In some embodiments, a cancer peptide is selected from the sequences provided in SEQ ID NO: 702-1020. Cancer peptides are well known in the art and can found, for example at the Cancer Antigenic Peptide Database: caped.icp.ucl.ac.be/Peptide/search.

TABLE-US-00001 TABLE 1 Cancer peptides SEQ ID Gene/Protein Peptide Sequence NO: Position p53 SQKTYQGSY 702 99-107 NY-ESO-1/LAGE-2 KEFTVSGNILTI 703 124-135 MAGE-A6 MVKISGGPR 704 290-298 Melan-A/MART-1 AEEAAGIGIL(T) 705 24-33(34) MAGE-A1 DPARYEFLW 706 258-266 MAGE-A10 DPARYEFLW 707 290-298 MAGE-A1 REPVTKAEML 708 120-129 MAGE-A2 REPVTKAEML 709 127-136 MAGE-A3 REPVTKAEML 710 127-136 MAGE-A4 SESLKMIF 711 156-163 MAGE-A6 REPVTKAEML 712 127-136 PBF CTACRWKKACQR 713 499-510 HSDL1 CYMEAVAL 714 20-27 LAGE-1 LAAQERRVPR 715 ORF2 LAGE-1 LAAQERRVPR 716 (18-27) NY-ESO-1/LAGE-2 ASGPGGGAPR 717 53-62 NY-ESO-1/LAGE-2 LAAQERRVPR 718 ORF2 NY-ESO-1/LAGE-2 LAAQERRVPR 719 (18-27) TRP-1/gp75 MSLQRQFLR 720 alt. ORF TRP-2 LLGPGRPYR 721 197-205 TRP-2 LLGPGRPYR 722 197-205 TRP-2 LLGPGRPYR 723 197-205 SNRPD1 SHETVIIEL 724 43739 tyrosinase LHHAFVDSIF 725 388-397 NFYC QQITKTEV 726 275-282 MAGE-A3 WQYFFPVIF 727 143-151 NY-ESO-1/LAGE-2 FATPMEAEL 728 96-104 GAGE-3,4,5,6,7 YYWPRPRRY 729 43374 NA88-A QGQHFLQKV 730 MAGE-A3 MEVDPIGHLY 731 167-176 MAGE-A3 AELVHFLLL 732 114-122 VEGF SRFGGAVVR 733 -i BAGE-1 AARAVFLAL 734 44471 MAGE-A1 SAYGEPRKL 735 230-238 MAGE-A6 ISGGPRISY 736 293-301 K-ras GADGVGKSA 737 43374 K-ras GADGVGKSAL 738 43739 gp100/Pmel17 SNDGPTLI 739 71-78 TRP-2 ANDPIFVVL 740 387-395 tyrosinase QCSGNFMGF 741 90-98 gp100/Pmel17 RTKQLYPEW 742 40-42 and 47-52 Elongation factor 2 ETVSEQSNV 743 581-589 MUM-3 EAFIQPITR 744 322-330 LAGE-1 ELVRRILSR 745 103-111 MAGE-A1 EVYDGREHSA 746 222-231 NY-ESO-1/LAGE-2 TVSGNILTIR 747 127-136 TRP2-INT2 EVISCKLIKR 748 intron 2 gp100/Pmel17 HTMEVTVYHR 749 182-191 MAGE-A1 ITKKVADLVGF 750 102-112 MAGE-C2 ASSTLYLVF 751 42-50 MAGE-A1 SAFPTTINF 752 62-70 NY-ESO-1/LAGE-2 MPFATPMEA 753 94-102 NY-ESO-1/LAGE-2 FATPMEAELAR 754 96-106 CDK12 CILGKLFTK 755 924-932 CDKN2A

AVCPWTWLR 756 125-133 (p14ARF-ORF3) MATN KTLTSVFQK 757 226-234 HERV-E ATFLGSLTWK 758 gp100/Pmel17 ALNFPGSQK 759 87-95 KK-LC-1 RQKRILVNL 760 76-84 BCR-ABL fusion protein (b3a2) GFKQSSKAL 761 922-930 TAG-1 LSRLSNRLL 762 42-50 TAG-2 LSRLSNRLL 763 42-50 RBAF600 RPHVPESAF 764 329-337 SYT-SSX1 or -SSX2 fusion QRPYGYDQIM 765 402-410 protein (SYT) LAGE-1 APRGVRMAV 766 ORF2 LAGE-1 APRGVRMAV 767 (46-54) MAGE-A1 RVRFFFPSL 768 289-298 NY-ESO-1/LAGE-2 APRGPHGGAASGL 769 60-72 gp100/Pmel17 SSPGCQPPA 770 529-537 Intestinal carboxyl esterase SPRWWPTCL 771 alt. ORF RAGE-1 SPSSNRIRNT 772 44136 RU2AS LPRWPPPQL 773 antisense MAGE-A1 SAYGEPRKL 774 230-238 NY-ESO-1/LAGE-2 LAMPFATPM 775 92-100 MUM-2 FRSGLDSYV 776 126-134 GAGE-1,2,8 YRPRPRRY 777 42614 NY-ESO-1/LAGE-2 ARGPESRLL 778 80-88 beta-catenin SYLDSGIHF 779 29-37 KM-HN-1 NYNNFYRFL 780 196-204 KM-HN-1 EYSKECLKEF 781 499-508 KM-HN-1 EYLSLSDKI 782 770-778 LY6K RYCNLEGPPI 783 119-128 MAGE-A2 EYLQLVFGI 784 156-164 MAGE-A3 TFPDLESEF 785 97-105 MAGE-A3 VAELVHFLL 786 113-121 MAGE-A4 NYKRCFPVI 787 143-151 MAGE-A4 NYKRCFPVI 788 143-151 NY-ESO-1/LAGE-2 YLAMPFATPME 789 91-101 SAGE LYATVIHDI 790 715-723 CEA TYACFVSNL 791 652-660 CEA QYSWFVNGTF 792 268-277 gp100/Pmel17 VYFFLPDHL 793 intron 4 OA1 LYSACFWWL 794 126-134 tyrosinase IYMDGTADFSF 795 368-373 and 336-340e tyrosinase AFLPWHRLF 796 206-214 CD45 KFLDALISL 797 556-564 EpCAM RYQLDPKFI 798 173-181 EZH2 KYDCFLHPF 799 291-299 EZH2 KYVGIEREM 800 735-743 glypican-3 EYILSLEEL 801 298-306 HER-2/neu TYLPTNASL 802 63-71 HSPH1 NYGIYKQDL 803 180-188 MUC5AC TCQPTCRSL 804 716-724 PRAME LYVDSLFFLc 805 301-309 PSMA NYARTEDFF 806 178-186 RNF43 NSQPVWLCL 807 721-729 WT1 CMTWNQMNL 808 235-243 CASP-8 FPSDSWCYF 809 476-484 K-ras VVVGAVGVG 810 42186 MAGE-A1 EADPTGHSY 811 161-169 MAGE-A3 EVDPIGHLY 812 168-176 MAGE-A6 EVDPIGHVY 813 168-176 NY-ESO-1/LAGE-2 MPFATPMEAEL 814 94-104 gp100/Pmel17 VPLDCVLYRY 815 471-480 gp100/Pmel17 LPHSSSHWL 816 630-638 Melan-A/MART-1 EAAGIGILTV 817 26-35 tyrosinase LPSSADVEF 818 312-320 tyrosinase TPRLPSSADVEF 819 309-320 M-CSF LPAVVGLSPGEQEY 820 alt. ORF KIAAO205 AEPINIQTW 821 262-270 MUM-1 EEKLIVVLF 822 30-38 MUM-2 SELFRSGLDSY 823 123-133 OS-9 KELEGILLL 824 438-446 MAGE-A1 KEADPTGHSY 825 160-169 MAGE-A3 MEVDPIGHLY 826 167-176 MAGE-C2 SESIKKKVL 827 307-315 tyrosinase SEIWRDIDFD 828 192-200 EFTUD2 KILDAVVAQK 829 668-677 GPNMB TLDWLLQTPK 830 179-188 Myosin class I KINKNPKYK 831 911-919 SIRT2 KIFSEVTLK 832 192-200 MAGE-A1 SLFRAVITK 833 96-104 CEA HLFGYSWYK 834 61-69 gp100/Pmel17 LIYRRRLMK 835 614-622 gp100/Pmel17 IALNFPGSQK 836 86-95 gp100/Pmel17 ALLAVGATK 837 17-25 gp100/Pmel17 RSYVPLAHR 838 195-202 and 191 or 192e gp100/Pmel17 ALNFPGSQK 839 87-95 mammaglobin-A PLLENVISK 840 23-31 FGF5 NTYASPRFKf 841 172-176 and 217-220 HER-2/neu VLRENTSPK 842 754-762 MMP-7 SLFPNSPKWTSK 843 96-107 RGS5 GLASFKSFLK 844 74-83 RhoC RAGLQVRKNK 845 176-185 FLT3-ITD YVDFREYEYY 846 591-600 MART2 FLEGNEVGKTY 847 446-455 N-ras ILDTAGREEY 848 55-64 PPP1R3B YTDFHCQYV 849 172-180 MAGE-A1 EADPTGHSY 850 161-169 MAGE-A3 EVDPIGHLY 851 168-176 MAGE-A4 EVDPASNTY 852 169-177 Sp17 ILDSSEEDK 853 103-111 tyrosinase KCDICTDEY 854 243-251 tyrosinase SSDYVIPIGTY 855 146-156 AIM-2 RSDSGQQARY 856 intron WT1 TSEKRPFMCAY 857 317-327 MAGE-A1 RVRFFFPSL 858 289-298 MAGE-A12 m VRIGHLYIL 859 170-178 MAGE-A12 m VRIGHLYIL 860 170-178 MAGE-A12 m EGDCAPEEK 861 212-220 MAGE-A2 EGDCAPEEK 862 212-220 MAGE-A3 EGDCAPEEK 863 212-220 MAGE-A6

EGDCAPEEK 864 212-220 Melan-A/MART-1 RNGYRALMDKS 865 51-61 alpha-actinin-4 FIASNGVKLV 866 118-127 BCR-ABL fusion protein (b3a2) SSKALQRPV 867 926-934 CASP-5 FLIIWQNTM 868 67-75 CDK4 ACDPHSGHFV 869 23-32 CLPP ILDKVLVHL 870 240-248 CSNK1A1 GLFGDIYLA 871 26-34 ETV6-AML1 fusion protein RIAECILGM 872 334-342 FNDC3B VVMSWAPPV 873 292-300 GAS7 SLADEAEVYL 874 141-150 HAUS3 ILNAMIAKI 875 154-162 hsp70-2 SLFEGIDIYT 876 286-295 ME1 FLDEFMEGV 877 224-232 OGT SLYKFSPFPL 878 28-37 p53 VVPCEPPEV 879 217-225 PRDX5 LLLDDLLVSI 880 163-172 TGF-betaRII RLSSCVPVA 881 131-139 TP53 VVPCEPPEV 882 217-225 CT37/FMRINB YLCSGSSYFV 883 89-98 Cyclin-A1 SLIAAAAFCLA 884 341-351 Cyclin-A1 FLDRFLSCM 885 227-235 GnTV VLPDVFIRC(V) 886 intron HERV-K-MEL MLAVISCAV 887 44440 LAGE-1 MLMAQEALAFL 888 ORF2 LAGE-1 MLMAQEALAFL 889 (1-11) LAGE-1 SLLMWITQC 890 157-165 LRPAP1 FLGPWAAS 891 21-30 MAGE-A1 KVLEYVIKV 892 278-286 MAGE-A1 KVLEYVIKV 893 278-286 MAGE-A10 GLYDGMEHL 894 254-262 MAGE-A12m FLWGPRALV 895 271-279 MAGE-A2 YLQLVFGIEV 896 157-166 MAGE-A3 KVAELVHFL 897 112-120 MAGE-A3 FLWGPRALV 898 271-279 MAGE-A4 GVYDGREHTV 899 230-239 MAGE-A9 ALSVMGVYV 900 223-231 MAGE-C1 ILFGISLREV 901 959-968 MAGE-C1 KVVEFLAML 902 1083-1091 MAGE-C2 ALKDVEERV 903 336-344 MAGE-C2 LLFGLALIEV 904 191-200 NY-ESO-1/LAGE-2 SLLMWITQC 905 157-165 NY-ESO-1/LAGE-2 SLLMWITQC 906 157-165 NY-ESO-1/LAGE-2 SLLMWITQC 907 157-165 NY-ESO-1/LAGE-2 MLMAQEALAFL 908 ORF2 NY-ESO-1/LAGE-2 MLMAQEALAFL 909 (1-11) SSX-2 KASEKIFYV 910 41-49 TAG-1 SLGWLFLLL 911 78-86 XAGE-1b/GAGED2a ROKKIRIQL 912 21-29 CEA YLSGANLNL 913 605-613 CEA GVLVGVALI 914 694-702 CEA IMIGVLVGV 915 691-699 gp100/Pmel17 KTWGQYWQV 916 154-162 gp100/Pmel17 (A)MLGTHTMEV 917 177(8)-186 gp100/Pmel17 KTWGQYWQV 918 154-163 gp100/Pmel17 ITDQVPFSV 919 209-217 gp100/Pmel17 YLEPGPVTA 920 280-288 gp100/Pmel17 VLYRYGSFSV 921 476-485 gp100/Pmel17 LLDGTATLRL 922 457-466 gp100/Pmel17 RLMKQDFSV 923 619-627 gp100/Pmel17 SLADTNSLAV 924 570-579 gp100/Pmel17 RLPRIFCSC 925 639-647 Melan-A/MART-1 ILTVILGVL 926 32-40 Melan-A/MART-1 EAAGIGILTV 927 26(27)-35 NY-BR-1 SLSKILDTV 928 904-912 PAP TLMSAMTNL 929 112-120 PAP ALDVYNGLL 930 299-307 PAP FLFLLFFWL 931 18-26 PSA FLTPKKLQCV 932 165-174 PSA VISNDVCAQV 933 178-187 RAB38/NY-MEL-1 VLHWDPETV 934 50-58 TRP-2 SVYDFFVWL 935 180-188 TRP-2 TLDSQVMSL 936 360-368 tyrosinase MLLAVLYCL 937 44440 tyrosinase CLLWSFQTSA 938 42948 tyrosinase YMDGTMSQV 939 369-377 tyrosinase YMDGTMSQV 940 369-377 adipophilin SVASTITGV 94 129-137 ALDH1A1 LLYKLADLI 942 88-96 alpha-foetoprotein (AFP) GVALQTMKQ 943 542-550 alpha-foetoprotein (AFP) FMNKFIYEI 944 158-166 BCLX (L) YLNDHLEPWI 945 173-182 BING-4 CQWGRLWQL 946 ORF2 CALCA VLLQAGSLHA 947 16-25 CALCA FLALSILVL 948 42979 CALCA LLAALVQDYL 949 50-59 CALCA CMLGTYTQDF 950 91-100 CD274 LLNAFTVTV 95 15-23 CPSF LMLQNALTTM 952 1360-1369 CPSF KVHPVIWSL 953 250-258 cyclin D1 LLGATCMFV 954 101-109 DKK1 ALGGHPLLGV 955 20-29 DKK1 ALGGHPLLGV 956 20-30 ENAH (hMena) TMNGSKSPV 957 502-510 EZH2 FMVEDETVL 958 120-128 EZH2 FINDEIFVEL 959 165-174 G250/MN/CAIX HLSTAFARV 960 254-262 glypican-3 FVGEFFTDV 961 144-152 glypican-3 TIHDSIQYV 962 325-334 HEPACAM RLAPFVYLL 963 16-24 Hepsin SLLSGDWVL 964 191-199 Hepsin GLQLGVQAV 965 229-237 Hepsin PLTEYIQPV 966 268-276 HER-2/neu KIFGSLAFL 967 369-377 HER-2/neu IISAVVGIL 968 654-662 HER-2/neu ALCRWGLLL 969 44329 HER-2/neu ILHNGAYSL 970 435-443 HER-2/neu VVLGVVFGI 971 665-673 HER-2/neu YMIMVKCWMI 972 952-961 HER-2/neu

YLVPQQGFFC 973 1023-1032 HER-2/neu RLLQETELV 974 689-697 HER-2/neu HLYQGCQVV 975 48-56 HER-2/neu PLQPEQLQV 976 391-399 HER-2/neu ALIHHNTHL 977 466-474 HER-2/neu TLEEITGYL 978 402-410 HER-2/neu PLTSIISAV 979 650-658 HLA-DOB FLLGLIFLL 980 232-240 HSPH1 RLMNDMTAV 981 169-177 IDO1 ALLEIASCL 982 199-207 IGF2B3 NLSSAEVVV 983 515-523 IL13Ralpha2 WLPFGFILI 984 345-353 Kallikrein 4 FLGYLILGV 985 43770 KIF20A AQPDTAPLPV 986 284-293 KIF20A LLSDDDVVV 987 44166 KIF20A CIAEQYHTV 988 809-817 Lengsin FLPEFGISSA 989 270-279 mdm-2 VLFYLGQY 990 53-60 Meloe TLNDECWPA 991 36-44 Midkine ALLALTSAV 992 13-21 Midkine AQCQETIRV 993 114-122 MMP-2 GLPPDVQRVh 994 560-568 MUC1 STAPPVHNV 995 950-958 MUC1 LLLLTVLTV 996 44166 nectin-4 VLVPPLPSL 997 145-153 p53 RMPEAAPPV 998 65-73 p53 LLGRNSFEV 999 264-272 PAX5 TLPGYPPHV 1000 311-319 PLAC1 VLCSIDWFM 1001 28-36 PRAME VLDGLDVLL 1002 100-108 PRAME ALYVDSLFFL 1003 300-309 PRAME SLYSFPEPEA 1004 142-151 PRAME SLLQHLIGL 1005 425-433 RAGE-1 LKLSGVVRL 1006 352-360 RAGE-1 PLPPARNGGLg 1007 32-40 RGS5 LAALPHSCL 1008 41395 RNF43 ALWPWLLMA(T) 1009 11-19(20) Secernin 1 KMDAEHPEL 1010 196-204 SOX10 AWISKPPGV 1011 332-340 SOX10 SAWISKPPGV 1012 331-340 STEAP1 MIAVFLPIV 1013 292-300 STEAP1 HQQYFYKIPILVINK 1014 102-116 survivin ELTLGEFLKL 1015 95-104 survivin ELTLGEFLKL 1016 95-104 Telomerase ILAKFLHWLE 1017 540-548 Telomerase RLVDDFLLV 1018 865-873 TPBG RLARLALVL 1019 17-25 IGF2B3 RLLVPTQFV 1020 199-207

[0136] In some embodiments, the immunogenic peptide is a non-human peptide. In some embodiments, the immunogenic peptide is a viral peptide. In some embodiments, the immunogenic peptide is a bacterial peptide. Viral peptides are well known in the art and any such peptide may be employed. Such peptides can found, for example, at the Immune Epitope Database (IEDB) and VDJDB database: iedb.org and vdjdb.cdr3.net. In some embodiments, the viral peptide is derived from a virus selected from Cytomegalovirus (CMV), Epstein-Barr virus (EBV) or Influenza virus (FLU). In some embodiments, the viral peptide is derived from a virus selected from CMV, EBV, FLU, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), Adenovirus and Human Papilloma virus (HPV). In some embodiments, the virus is CMV. In some embodiments, the virus is EBV. In some embodiments, the virus is FLU. In some embodiments, the virus is SARS-CoV2. In some embodiments, the virus is Adenovirus. In some embodiments, the virus is HPV. In some embodiments, the viral peptide is a peptide provided in Table 2. In some embodiments, the viral peptide is selected from a sequence provided in Table 2. In some embodiments, the viral peptide is a peptide provided in Table 3. In some embodiments, the viral peptide is selected from a sequence provided in Table 3. In some embodiments, the viral peptide is selected from SEQ ID NO: 1-701. In some embodiments, the viral peptide is selected from SEQ ID NO: 1-695. In some embodiments, the viral peptide is selected from SEQ ID NO: 1-11. In some embodiments, the viral peptide is selected from SEQ ID NO: 1-9. In some embodiments, the viral peptide is selected from SEQ ID NO: 1-5. In some embodiments, the viral peptide is SEQ ID NO: 1. In some embodiments, the viral peptide is SEQ ID NO: 2. In some embodiments, the viral peptide is SEQ ID NO: 3. In some embodiments, the viral peptide is SEQ ID NO: 4. In some embodiments, the viral peptide is SEQ ID NO: 5. In some embodiments, the viral peptide is SEQ ID NO: 6. In some embodiments, the viral peptide is SEQ ID NO: 7. In some embodiments, the viral peptide is SEQ ID NO: 8. In some embodiments, the viral peptide is SEQ ID NO: 9. In some embodiments, the viral peptide is SEQ ID NO: 10. In some embodiments, the viral peptide is SEQ ID NO: 11. In some embodiments, the viral peptide is selected from SEQ ID NO: 1, 2, 3, 4, 5, 151, 197, 471, 677, 696, 697, 698, 699, 700, and 701.

TABLE-US-00002 TABLE 2 Viral peptides Peptide SEQ ID NO: source NLVPMVATV 1 CMV GLCTLVAML 197 EBV CLGGLLTMV 2 EBV CTELKLSDY 677 FLU CLGGLLTMV,

GLCTLVAML, NLVPMVATV, 2, 197, 1, 3 EBV, CMV, GILGFVFTL FLU DYNFVKQLF, GLCTLVAML, TYPVLEEMF, 696, 197, 697, EBV, CMV, RYSIFFDYM, AVFDRKSDAK, TYSAGIVQI, 698, 4, 699, FLU IVTDFSVIK, SSCSSCPLSK, AYAQKIFKIL, 151, 629, 471, VYALPLKML, QYDPVAALF, NLVPMVATV, 700, 5, 1, 701 GPISGHVLK,

TABLE-US-00003 TABLE 3 Full list of viral peptides SEQ ID Peptide NO: NLVPMVATV 1 CLGGLLTMV 2 GILGFVFTL 3 AVFDRKSDAK 4 QYDPVAALF 5 TPSVSSSISSL 6 LPFNDGVYF 7 FLGERVTLT 8 LLALHRSYL 9 STDVASLNY 10 TLGIVCPI 11 RYSIFFDY 12 CPLSKILL 13 SLGYITTV 14 RAKFKQLL 15 TNKIKEQL 16 QTRQKFHL 17 EMRLRMIL 18 MAREKNDL 19 CCKCDSTL 20 HDIILECV 21 LPQGFSAL 22 NLTTRTQL 23 AEHVNNSY 24 AEVQIDRL 25 EPLVDLPI 26 FPREGVFV 27 NITRFQTL 28 QPYRVVVL 29 RLQSLQTY 30 SPRRARSV 31 VYYPDKVF 32 YAWNRKRI 33 FLPFFSNV 34 APHGVVFL 35 GVYHKNNK 36 TYFNLGNKF 37 TDLGQNLLY 38 TLLYVLFEV 39 VLAWTRAFV 40 YVAGFLALY 41 ALMGAVTSL 42 MMLRDRWSL 43 AVLCLYLLY 44 FLWEDQTLL 45 FIPQYLSAV 46 FLIAYQPLL 47 SVYPYDEFV 48 VYMSPFYGY 49 FLGDDPSPA 50 RLTGYPAGI 51 APASVYQPA 52 CPRRPAVAF 53 VVRGPTVSL 54 LIDGIFLRY 55 SQLAHLVYV 56 FLGAGALAV 57 FLGGHVAVA 58 TLRGLFFSV 59 KYFYCNSLF 60 EYQRLYATF 61 APRIGGRRA 62 APRTWCRLL 63 ALMLRLLRI 64 RILGVLVHL 65 YMESVFQMY 66 ILIEGIFFA 67 YMANQILRY 68 VPRPDDPVL 69 VYTPSPYVF 70 AYLPRPVEF 71 AILTQYWKY 72 ATDSLNNEY 73 AYVSVLYRW 74 LASDPHYEY 75 LLAYVSVLY 76 RLNELLAYV 77 SIVHHHAQY 78 ALATVTLKY 79 ALLDRDCRV 80 FLADAVVRL 81 FTAPEVGTY 82 RLLGFADTV 83 RSSLGSLLY 84 ALHTALATV 85 TLLELVVSV 86 VPGWSRRTL 87 ALLAKMLFY 88 RMLGDVMAV 89 TMLEDHEFV 90 ALLGLTLGV 91 FVLATGDFV 92 GIFEDRAPV 93 LLTTPKFTV 94 NLLTTPKFT 95 TMYYKDVTV 96 YLANGGFLI 97 ALSALLTKL 98 FLTCTDRSV 99 DRLDNRLQL 100 KSRRPLTTF 101 RRAQMAPKR 102 GPHETITAL 103 PAWSRRTLL 104 ASDSLNNEY 105 FLVDAIVRV 106 GLADTVVAC 107 RPRGEVRFL 108 SAPLPSNRV 109 SLPRSRTPI 110 ALWALPHAA 111 ILIEGIFFV 112 VTEHDTLLY 113 ELNRKMIYM 114 IPSINVHHY 115 TRATKMQVI 116 QIKVRVKMV 117 DELRRKMMY 118 EEAIVAYTL 119 ELKRKMMYM 120 QIKVRVDMV 121 VLEETSVML 122 ARAKKDELK 123 ARAKKDELR 124 DELKRKMIY 125 FMDILTTCV 126 KEWAYCVEM 127 LITGRLAAL 128 LLLNCLWSV 129 TMLDIQPED 130 LPRWYFYYL 131 KLWHYCSTL 132 LLIEGIFFI 133 AFLGERVTL 134 KLGPGEEQV 135 RFIAQLLLL 136 TLTSYWRRV 137 VEDLFGANL 138 WQWEHIPPA 139 LPCVLWPVL 140 RAKFKQLLQ 141 SENDRLRLL 142 TLDTKPLSV 143 FMVFLQTHI 144 HPVGEADYF 145 VLKDAIKDL 146 AYSSWMYSY 147 QAKWRLQTL 148 RLRAEAQVK 149 SVRDRLARL 150 IVTDFSVIK 151 VSFIEFVGW 152 EGGVGWRHW 153 QPRAPIRPI 154 RRIYDLIEL 155 RPPIFIRLL 156 YPLHEQHGM 157 LMIIPLINV 158 SLVIVTTFV 159 TLFIGSHVV 160 YLQQNWWTL 161 ALLVLYSFA 162 GLGTLGAAL 163 LLSAWILTA 164 LLWTLVVLL 165 MGSLEMVPM 166 YLLEMLWRL 167 VLQWASLAV 168 FLYALALLL 169 IEDPPFNSL 170 RRRWRRLTV 171 TYGPVFMCL 172 FLRGRAYGL 173 LVLILYLCV 174 LLNGWRWRL 175 IGLITVLFL 176 DAAPAIQHI 177 KQYLGVYIW 178 TYATFLVTW 179 KENIAAYKF 180 KTNNWHAGW 181 APKTATSSW 182 QTTGRITNR 183 VEDINRVFL 184 CYDHAQTHL 185 TLDYKPLSV 186 YRSGIIAVV 187 RPPIFIRRL 188 FLDKGTYTL 189 ARYAYYLQF 190 RRRKGWIPL 191 LQHYREVAA 192 AENAGNDAC 193 RVRAYTYSK 194 YVLDHLIVV 195 DEVEFLGHY 196 GLCTLVAML 197 KDTWLDARM 198 VLFGLLCLL 199 ELRRKMMYM 200 ELKRKMIYM 201 EFKSKFSTL 202 EGRDRILTV 203 FEKERFLFL 204 EGRERILTV 205 NVKHKKNPL 206 VVKGKVLSI 207 EARRRLAEM 208 RSKPRHMCV 209 DFKSKYLTL 210 SPRSRLQQL 211 VLATAVREL 212 FQANTPPAV 213 IPYTAAVQV 214 KLAKLIIDL 215 RLPREKLKK 216 SPKAGLLSL 217 SLQQEITLL 218 ISDYFHNTY 219 KVLIRCYLC 220 SIDQLCKTF 221 HNNGICWGN 222 CYEQLGDSS 223 ITIRCIICQ 224

KTLEERVKK 225 MRGDKATIK 226 PYGVCIMCL 227 QLGDSSDEE 228 RLQCVQCKK 229 VYKFLFTDL 230 YYYAGSSRL 231 LQFIFQLCK 232 MTLCAEVKK 233 QYRVFRIKL 234 RIKLPDPNK 235 ILIRCIICQ 236 KCLNEILIR 237 KVCLRLLSK 238 ATEVRTLQQ 239 CTIVCPSCA 240 LCINSTATE 241 AVPDDLYIK 242 KYTFWEVNL 243 RVRLPDPNK 244 TSESQLFNK 245 YTFWEVNLK 246 YLTAPTGCI 247 DSAPILTAF 248 KSAIVTLTY 249 LAVSKNKAL 250 LQDVSLEVY 251 NPCHTTKLL 252 NTTPIVHLK 253 QVILCPTSV 254 RLECAIYYK 255 SPEIIRQHL 256 TLYTAVSST 257 VVEGQVDYY 258 YRFKKHCTL 259 ALQAIELQL 260 TLQDVSLEV 261 YIIFVYIPL 262 FAFRDLCIV 263 IILECVYCK 264 TTLEQQYNK 265 VCDKCLKFY 266 CPEEKQRHL 267 YGTTLEQQY 268 EYRHYCYSL 269 VYDFAFQDL 270 GTLGIVCPI 271 IVCPICSQK 272 QAEPDRAHY 273 RAHYNIVTF 274 TLHEYMLDL 275 LQPETTDLY 276 TPTLHEYML 277 LLMGTLGIV 278 TLGIVCPIC 279 IHSMNSTIL 280 ISEYRHYCY 281 KFYSKISEY 282 KLPQLCTEL 283 VYDFAFRDL 284 TIHDIILEC 285 YMLDLQPET 286 IHSMNSSIL 287 NVFPIFLQM 288 KLPDLCTEL 289 NLLIRCLRC 290 FQQLFLNTL 291 LFLNTLSFV 292 LLLGTLNIV 293 TIDQLCKTF 294 EADVQQWLT 295 FYTPLADQF 296 GLCPHCINV 297 GLENNVLYH 298 LHTDFEQVM 299 LLHTDFEQV 300 SESSFFNLI 301 SSHSGSFQI 302 TEADVQQWL 303 VQQWLTWCN 304 TAKSRVHPL 305 LLLIWFRPV 306 AVDTVLAKK 307 EPLVWIDCY 308 AITEVECFL 309 LLMWEAVTV 310 RARRELPRF 311 YLEKESIYY 312 NPKASLLSL 313 QVMLRWGVL 314 GILGFVFTL 315 SRYWAIRTR 316 NMLSTVLGV 317 LPFERATVM 318 LPFERATIM 319 LPFDKSTVM 320 LPFEKSTIM 321 LPFDKPTIM 322 LPFEKSTVM 323 CVNGSCFTV 324 LPFDRTTIM 325 KTGGPIYRR 326 LPFDRPTIM 327 GLDNHTILL 328 ILMWEAVTL 329 SITEVECFL 330 NLNESLIDL 331 RLDKVEAEV 332 VYDPLQPEL 333 TLKSFTVEK 334 HADQLTPTW 335 NATNVVIKV 336 VGYLQPRTF 337 YFQPRTFLL 338 FQFCNDPFL 339 ASVYAWNRK 340 GYLQPRTFL 341 YLQLRTFLL 342 YLQPRIFLL 343 AEVQIDRLI 344 ALNTLVKQL 345 CVADYSVLY 346 GSFCTQLNR 347 GVVFLHVTY 348 LLFNKVTLA 349 LLQYGSFCT 350 RLQSLQTYV 351 RVDFCGKGY 352 SVLNDILSR 353 VLNDILSRL 354 FVFKNIDGY 355 GTHWFVTQR 356 GTITSGWTF 357 KEIDRLNEV 358 LEPLVDLPI 359 LPPAYTNSF 360 NGVEGENCY 361 NQKLIANQF 362 RISNCVADY 363 RLFRKSNLK 364 TLDSKTQSL 365 TPINLVRDL 366 YFPLQSYGF 367 FQPTNGVGY 368 RFDNPVLPF 369 TSNQVAVLY 370 GLTVLPPLL 371 SIIAYTMSL 372 YLQPRTFLL 373 EPVLKGVKL 374 LTDEMIAQY 375 QYIKWPWYI 376 VLKGVKLHY 377 VTYVPAQEK 378 KCYGVSPTK 379 SPRRARSVA 380 YEQYIKWPW 381 AEIRASANL 382 GVYFASTEK 383 GVYYHKNNK 384 MIAQYTSAL 385 NSASFSTFK 386 NYNYLYRLF 387 ADAGFIKQY 388 ALDPLSETK 389 AYSNNSIAI 390 DAVRDPQTL 391 EILPVSMTK 392 ETKCTLKSF 393 EVFAQVKQI 394 EYVSQPFLM 395 FAMQMAYRF 396 FASVYAWNR 397 FERDISTEI 398 FPQSAPHGV 399 FTISVTTEI 400 FVIRGDEVR 401 FVSNGTHWF 402 GAAAYYVGY 403 GEVFNATRF 404 HLMSFPQSA 405 HVSGTNGTK 406 HWFVTQRNF 407 IAIPTNFTI 408 IANQFNSAI 409 INITRFQTL 410 IPFAMQMAY 411 IPTNFTISV 412 IYQTSNFRV 413 KIADYNYKL 414 KIYSKHTPI 415 KSNLKPFER 416 KVFRSSVLH 417 LGAENSVAY 418 LPFNDGVYF 419 LPLVSSQCV 420 LPPLLTDEM 421 NASVVNIQK 422 NATRFASVY 423 NSFTRGVYY 424 NSIAIPTNF 425 QELGKYEQY 426 QIAPGQTGK 427 QLTPTWRVY 428 QPTESIVRF 429 QTNSPRRAR 430 RSVASQSII 431 RVYSTGSNV 432 SANNCTFEY 433 SFKEELDKY 434 SVYAWNRKR 435 TLADAGFIK 436 VASQSIIAY 437 VFAQVKQIY 438 VFKNIDGYF 439 VGGNYNYLY 440 WFVTQRNFY 441 WTAGAAAYY 442 WTFGAGAAL 443 YGFQPTNGV 444 YNYLYRLFR 445 YQDVNCTEV 446 YYHKNNKSW 447 TQDLFLPFF 448 FVFLVLLPL 449 LVKNKCVNF 450 STQDLFLPF 451 NIADYNYKL 452 NYNYRYRLF 453 TIADYNYKL 454 LLYANSAHAL 455 SSGVVFGTWY 456 EYVHARWAAF 457 HTDLHPNNTY 458 AYLGAFLSVL 459 FVYTPSPYVF 460 AYSLLFPAPF 461 RPTERPRAPA 462 FTDALGIDEY 463 SALPTNADLY 464 LYPDAPPLRL 465 GFLIAYQPLL 466 FLVDAIVRVA 467 PHSVVNPFVK 468 MILIEGIFFV 469 TPRVTGGGAM 470 AYAQKIFKIL 471 YILEETSVML

472 RRKMMYMCYR 473 TYSQKIFKIL 474 RPPIFIRRLH 475 VEITPYKPTW 476 EENLLDFVRF 477 LLDFVRFMGV 478 PYLFWLAAIA 479 VMSNTLLSAW 480 QNGALAINTF 481 DTPLIPLTIF 482 ATVKTGNIKL 483 AERQGSPTPA 484 KPQGQRLIEV 485 QTDTYTLLGY 486 ILIEGIFFVS 487 HVFPHRFISF 488 RIRLVVPSAL 489 SLILIGITTL 490 KVEGEQHVIK 491 IAPYAGLIMI 492 KPAVGVYHIV 493 FSECNALGSY 494 STELNYNHLY 495 FLTEAIVHSV 496 YVLDLQPEAT 497 FACYDLCIVY 498 FAFSDLYVVY 499 FAFKDLCIVY 500 VAFTEIKIVY 501 YILDLQPETT 502 FVFADLRIVY 503 FAFSDLCIVY 504 ILTAFNSSHK 505 LTAPTGCIKK 506 TLKCLRYRFK 507 YYVHEGIRTY 508 YICEEASVTV 509 LLIRCINCOK 510 AVCDKCLKFY 511 CVYCKQQLLR 512 NPYAVCDKCL 513 RPRKLPQLCT 514 YAVCDKCLKF 515 QYNKPLCDLL 516 GIVCPICSQK 517 HGDTPTLHEY 518 HYNIVTFCCK 519 YMLDLQPETT 520 TIHDIILECV 521 HNIRGRWTGR 522 FAFRDLCIVY 523 QERPRKLPQL 524 RWTGRCMSCC 525 SSRTRRETQL 526 HPAATHTKAV 527 TLLQQYCLYL 528 GLCPHCINVG 529 HAKALKERMV 530 IDTCISATFR 531 LLHTDFEQVM 532 QSALKLAIYK 533 SALKLAIYKA 534 KLYQNPTTYI 535 RLYQNPTTYI 536 KFLPDLYDYK 537 VLRGFLILGK 538 GILGFVFTLT 539 RMVLASTTAK 540 SFSFGGFTFK 541 KNIDGYFKIY 542 CMTSCCSCLK 543 DSFKEELDKY 544 QPYRVVVLSF 545 SEPVLKGVKL 546 CALDPLSETK 547 EILDITPCSF 548 FTISVTTEIL 549 IGAEHVNNSY 550 ILPDPSKPSK 551 IYSKHTPINL 552 KLPDDFTGCV 553 KTSVDCTMYI 554 LSSTASALGK 555 STQDLFLPFF 556 SVLNDILSRL 557 TEKSNIIRGW 558 WIFGTTLDSK 559 YHLMSFPQSA 560 TQLNRALTGI 561 NESLIDLQEL 562 DGVYFASTEK 563 DSKVGGNYNY 564 EVFAQVKQIY 565 FDEDDSEPVL 566 FERDISTEIY 567 FEYVSQPFLM 568 FLPFFSNVTW 569 GVFVSNGTHW 570 GVYYPDKVFR 571 GYLQPRTFLL 572 HVTYVPAQEK 573 IHADQLTPTW 574 KFLPFQQFGR 575 KVGGNYNYLY 576 LPIGINITRF 577 NTSNQVAVLY 578 NVYADSFVIR 579 QIPFAMQMAY 580 QYIKWPWYIW 581 RASANLAATK 582 RFASVYAWNR 583 RVYSTGSNVF 584 SETKCTLKSF 585 STGSNVFQTR 586 SVASQSIIAY 587 SWMESEFRVY 588 TECSNLLLQY 589 TPCSFGGVSV 590 VFVSNGTHWF 591 VYSSANNCTF 592 YTNSFTRGVY 593 IYKTPPIKDF 594 LLTDEMIAQY 595 SYFIASFRLF 596 EITDTIDKFGK 597 LPEGMDPFAEK 598 ARLCDLPATPK 599 LQRGPQYSEHP 600 PSQEPMSIYVY 601 QEFFWDANDIY 602 QYDPVAALFFF 603 RLTVSGLAWTR 604 RNLVPMVATVQ 605 TPRVTGGGAMA 606 VFPTKDVALRH 607 VLCPKNMIIKP 608 YSEHPTFTSQY 609 YYTSAFVFPTK 610 QYTPDSTPCHR 611 RPHERNGFTVL 612 AKARAKKDELR 613 CYVLEETSVML 614 EFCRVLCCYVL 615 EQVTEDCNENP 616 ESLKTFEQVTE 617 FPKTTNGCSQA 618 GKSTHPMVTRS 619 KDELRRKMMYM 620 KNSAFPKTTNG 621 KQIKVRVDMVR 622 MAYAQKIFKIL 623 NIEFFTKNSAF 624 SVMKRRIEEIC 625 YPRNPTEQGNI 626 HPVGEADYFEY 627 QPRAPIRPIPT 628 SSCSSCPLSKI 629 EPLPQGOLTAY 630 AVYENPLSVEK 631 TEADVQQWLTW 632 FFAVGGDPLEM 633 IAVGLLLYCKA 634 TIAMELIRMIK 635 EILDITPCSFG 636 AQALNTLVKQL 637 FCNDPFLGVYY 638 FPQSAPHGVVF 639 KSWMESEFRVY 640 LQIPFAMQMAY 641 NYNYLYRLFRK 642 YEQYIKWPWYI 643 IPIGAGICASY 644 KPFERDISTEI 645 QEVFAQVKQIY 646 RFPNITNLCPF 647 YECDIPIGAGI 648 YENQKLIANQF 649 YYVGYLQPRTF 650 SFELLHAPATV 651 TYVPAQEKNFT 652 HPVGDADYFEY 653 HPVGQADYFEY 654 RVAGDSGFAAY 655 EFFWDANDIY 656 RPHERNGFTV 657 LPRRSGAAGA 658 ALQIPFAMQM 659 QELIRQGTDY 660 RARSVASQSI 661 RLITGRLQSL 662 TLATHGLAAV 663 TTDPSFLGRY 664 VENPHLMGWD 665 YPDKVFRSSV 666 AYAQKIFKI 667 CRVLCCYVL 668 CVETMCNEY 669 DEEDAIAAY 670 FRCPRRFCF 671 KLGGALQAK 672 MLNIPSINV 673 VMAPRTLIL 674 YILEETSVM 675 MLDLQPETT 676 CTELKLSDY 677 FEDLRVLSF 678 ALSKGVHFV 679 ALWEIQQVV 680 DTDFVNEFY 681 FIAGLIAIV 682 FTSDYYQLY 683 KQIYKTPPI 684 KTFPPTEPK 685 LLLDRLNQL 686 LLYDANYFL 687 MLAKALRKV 688 MQLFFSYFA 689

PTDNYITTY 690 SPRWYFYYL 691 VYFLQSINF 692 VYIGDPAQL 693 FPTKDVAL 694 NEGVKAAW 695 DYNFVKQLF 696 TYPVLEEMF 697 RYSIFFDYM 698 TYSAGIVQI 699 VYALPLKML 700 GPISGHVLK 701

[0137] In some embodiments, the immunogenic peptide is inserted into a complementarity determining region (CDR) of the antibody or antigen binding fragment thereof. In some embodiments, insertion of the immunogenic peptide comprises removal of CDR sequence. In some embodiments, at least one CDR of the antibody or antigen binding fragment thereof is replaced with the immunogenic peptide. In some embodiments, the CDR is the whole CDR. In some embodiments, the CDR is at least a portion of the CDR. In some embodiments, CDR sequence comprises sequence of at least a portion of the CDR. In some embodiments, a portion is at least 4 amino acids. In some embodiments, a portion is at least 5 amino acids. In some embodiments, a portion is at least 6 amino acids. In some embodiments, a portion is at least 7 amino acids. In some embodiments, a portion is at least 8 amino acids. In some embodiments, a portion is at least 9 amino acids. In some embodiments, insertion of the immunogenic peptide comprises removal of the CDR. In some embodiments, insertion of the immunogenic peptide comprises removal of at least a portion of the CDR.

[0138] In some embodiments, replacement of the CDR or a portion of the CDR also comprises replacement of at least one amino acid flanking the CDR. In some embodiments, flanking is N-terminal to the CDR. In some embodiments, flanking is C-terminal to the CDR. In some embodiments, at least one amino acid is at least the 1, 2, 3, 4 or 5 amino acids directly flanking the CDR. Each possibility represents a separate embodiment of the invention. In some embodiments, at least one amino acid is 4-5 amino acids. In some embodiments, at least one amino acid is 4 amino acids. In some embodiments, at least one amino acid is 5 amino acids. In some embodiments, the flanking region is not more than 5, 6, 7, 8, 9 or 10 amino acids. Each possibility represents a separate embodiment of the invention. In some embodiments, the flanking region is not more than 5 amino acids. In some embodiments, the flanking region is not more than 10 amino acids. In some embodiments, a flanking region comprises a stem of the CDR loop. It will be understood by a skilled artisan that in order to preserve antibody structure and to produce as little perturbation as possible it may be necessary to also replace a portion of the CDR flanking regions along with the CDR itself (or a portion thereof).

[0139] In some embodiments, the CDRH1 of TMab4 comprises or consists of amino acid 26-33 of SEQ ID NO: 1021. In some embodiments, the CDRH2 of TMab4 comprises or consists of amino acid 51-58 of SEQ ID NO: 1021. In some embodiments, the CDRH3 of TMab4 comprises or consists of amino acid 97-109 of SEQ ID NO: 1021. In some embodiments, the CDRL1 of TMab4 comprises or consists of amino acid of 27-38SEQ ID NO: 1022. In some embodiments, the CDRL2 of TMab4 comprises or consists of amino acid 56-58 of SEQ ID NO: 1022. In some embodiments, the CDRL3 of TMab4 comprises or consists of amino acid 95-103 of SEQ ID NO: 1022. In some embodiments, the CDRH1 of 3E10 comprises or consists of amino acid 26-33 of SEQ ID NO: 1023. In some embodiments, the CDRH2 of 3E10 comprises or consists of amino acid 51-58 of SEQ ID NO: 1023. In some embodiments, the CDRH3 of 3E10 comprises or consists of amino acid 97-105 of SEQ ID NO: 1023. In some embodiments, the CDRL1 of 3E10 comprises or consists of amino acid 27-36 of SEQ ID NO: 1024. In some embodiments, the CDRL2 of 3E10 comprises or consists of amino acid 54-56 of SEQ ID NO: 1024. In some embodiments, the CDRL3 of 3E10 comprises or consists of amino acid 93-101 of SEQ ID NO: 1024. In some embodiments, the CDRH1 of 71F12 comprises or consists of amino acid 26-33 of SEQ ID NO: 1026. In some embodiments, the CDRH2 of 71F12 comprises or consists of amino acid 51-57 of SEQ ID NO: 1026. In some embodiments, the CDRH3 of 71F12 comprises or consists of amino acid 96-105 of SEQ ID NO: 1026. In some embodiments, the CDRL1 of 71F12 comprises or consists of amino acid 26-34 of SEQ ID NO: 1027. In some embodiments, the CDRL2 of 71F12 comprises or consists of amino acid 52-54 of SEQ ID NO: 1027. In some embodiments, the

CDRL3 of 71F12 comprises or consists of amino acid 91-100 of SEQ ID NO: 1027.
[0140] In some embodiments, the antigen binding molecule comprises a cell penetration sequence. In some embodiments, the cell penetration sequence is a cell penetration domain. In some embodiments, the cell penetration sequence is a cell penetration peptide. In some embodiments, the cell penetration sequence targets the molecule to the inside of the cell. In some embodiments, the cell penetration sequence delivers the molecule to the inside of the cell. In some embodiments, the cell penetration sequence enables entrance of the molecule to the inside of the cell. In some embodiments, the inside of the cell is an endosome. In some embodiments, the inside of the cell is the cytoplasm. In some embodiments, delivery to the cytoplasm comprises exit from an endosome. In some embodiments, delivery to the cytoplasm comprises escape from an endosome. In some embodiments, an endosome is the endosomal pathway. In some embodiments, the cell penetration sequence is a peptide transduction domain (PTD). In some embodiments, the cell penetration sequence is a cell penetrating peptide (CPP). In some embodiments, the cell penetration sequence is an endosomal escape domain (EED). Peptide sequences that allow entrance into the cytoplasm and in particular escape from the endosomal pathway after endocytosis are well known in the art and any such peptide sequence may be employed.
[0141] It is known in the art that the CDRL1 and CDRL3 of Tmab4 are both play a role in antibody penetration (see Kim et al., “Endosomal acidic pH-induced conformational changes of a cytosol-penetrating antibody mediate endosomal escape”, J Control Release, 2016, 10; 235:165-175 and Choi et al., “A general strategy for generating intact, full-length IgG antibodies that penetrate into the cytosol of living cells”, Mabs, 2014; 6(6):1402-14, herein incorporated by reference in their entirety. In some embodiments, the cell penetration sequence comprises the CDRL1 of Tmab4. In some embodiments, the cell penetration sequence consists of the CDRL1 of Tmab4. In some embodiments, the cell penetration sequence comprises the CDRL3 of Tmab4. In some embodiments, the cell penetration sequence consists of the CDRL3 of Tmab4. Methods of grafting the cell penetration sequence into other antibodies are known in the art, such as for example, the method provided in Choi et al.
[0142] In some embodiments, the antigen binding molecule is a bi-specific antibody. In some embodiments, the antigen binding molecule is a bi-specific antibody fragment of an antibody. In some embodiments, the antigen binding molecule comprises a plurality of antigen binding regions. In some embodiments, the antigen binding molecule comprises at least two antigen binding regions. In some embodiments, the antigen binding molecule comprises two antigen binding regions. In some embodiments, at least one of the antigen binding regions is the antigen binding region that is capable of binding to a target cell. In some embodiments, at least one of the antigen binding regions is mutated to comprises the immunogenic peptide. In some embodiments, at least one of the antigen binding regions is mutated to comprises the cell penetration sequence. In some embodiments, at least one of the antigen binding regions is mutated to comprises the immunogenic peptide, the cell penetration sequence or both. In some embodiments, the mutation is mutation of a complementarity determining region (CDR). In some embodiments, a CDR of an antigen binding region is mutated. In some embodiments, mutated is replaced. In some embodiments, replaced is replaced with the immunogenic peptide. In some embodiments, replaced is replaced with the cell penetration sequence.
[0143] In some embodiments, the CDR is an inert CDR. In some embodiments, an inert CDR does not contribute to binding to a target antigen. In some embodiments, binding is binding of the antigen binding region comprising the CDR to the target antigen. In some embodiments, an inert CDR comprises little or no contribution to binding. In some embodiments, an inert CDR comprises two or fewer amino acids that contact the target antigen. In some embodiments, an inert CDR comprises fewer than 2 amino acids that contact the target antigen. In some embodiments, an inert CDR comprises 2, 1 or 0 amino acids that contact the target antigen. In some embodiments, an inert CDR comprises 2 amino acid that contacts the target antigen. In some embodiments, an inert CDR

comprises 1 amino acid that contacts the target antigen. In some embodiments, an inert CDR does not comprise an amino acid that contacts the target antigen. In some embodiments, an inert CDR does not contact the target antigen. In some embodiments, contact comprises a distance of not more than 3, 5, 7, 9 or 10 angstroms. Each possibility represents a separate embodiment of the invention. In some embodiments, contact comprises a distance of not more than 5 angstroms.

[0144] In some embodiments, the distance is between the amino acid of the CDR and an amino acid of the target antigen. In some embodiments, the distant is the distance present when the antigen binding domain is bound to the antigen. In some embodiments, the distance is the distance during crystallography studies of the binding.

[0145] In some embodiments, mutation or replacement of an inert CDR does not diminish binding. In some embodiments, not diminishing binding is not significantly diminishing binding. In some embodiments, mutation or replacement of an inert CDR does not abrogate binding. In some embodiments, a significant diminishment is a reduction in binding of more than 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 75, 80, 90, 95 or 99%. Each possibility represents a separate embodiment of the invention. In some embodiments, a significant diminishment is a reduction in binding of more than 10%. In some embodiments, a significant diminishment is a reduction in binding of more than 20%. In some embodiments, mutation or replacement of an inert CDR does not reduce binding by 100%.

[0146] In some embodiments, mutation or replacement of an inert CDR does not diminish cell penetrance. In some embodiments, not diminishing cell penetrance is not significantly diminishing cell penetrance. In some embodiments, mutation or replacement of an inert CDR does not abrogate cell penetrance. In some embodiments, a significant diminishment is a reduction in penetrance of more than 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 75, 80, 90, 95 or 99%. Each possibility represents a separate embodiment of the invention. In some embodiments, a significant diminishment is a reduction in penetrance of more than 10%. In some embodiments, a significant diminishment is a reduction in penetrance of more than 20%. In some embodiments, mutation or replacement of an inert CDR does not reduce penetrance by 100%. In some embodiments, mutation or replacement of an inert CDR does not reduce penetrance to the level of a control antibody. In some embodiments, a control antibody is an antibody that does not enter a cell. Antibodies that do not enter cells are well known in the art, and include for example adalimumab and muromonab. In some embodiments, not diminishing cell penetrance comprises retaining penetrance that is substantially equal to the binding of the antibody or antigen binding fragment thereof devoid of or without the immunogenic peptide.

[0147] In some embodiments, insertion of the immunogenic peptide and removal of CDR sequence produces no change in the conformation of the antibody or antigen binding fragment thereof. In some embodiments, insertion of the immunogenic peptide and removal of CDR sequence produces minimal change in the conformation of the antibody or antigen binding fragment thereof. In some embodiments, conformation is overall conformation. In some embodiments, conformation is 3D structure. In some embodiments, conformation is tertiary structure. In some embodiments, change is perturbation. In some embodiments, minimal change is without a loss of a bond. In some embodiments, minimal change is a change of less than 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50%. Each possibility represents a separate embodiment of the invention. In some embodiments, minimal change comprises binding of a target antigen at an equivalent affinity to the antibody or antigen binding fragment thereof devoid or without the immunogenic peptide. In some embodiments, equivalent is with no reduction in affinity. In some embodiments, equivalent is with a reduction in affinity of not more than 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50%. Each possibility represents a separate embodiment of the invention. In some embodiments, equivalent is with a reduction in affinity of not more than 10%. In some embodiments, equivalent is with a reduction in affinity of not more than 20%

[0148] In some embodiments, the mutated CDR is in the antigen binding region capable of binding

the target cell. In some embodiments, the mutated CDR is not in the antigen binding region capable of binding the target cell. In some embodiments, the mutated CDR is in an antigen binding region other than the antigen binding region capable of binding the target cell. In some embodiments, the immunogenic peptide replaces a CDR in the antigen binding region capable of binding the target cell. In some embodiments, the cell penetrating sequence replaces a CDR in an antigen binding region that is not the antigen binding region that binds the target cell. In some embodiments, the immunogenic peptide and the cell penetration sequence are in the same antigen binding region. In some embodiments, the immunogenic peptide and the cell penetration sequence are in different antigen binding regions.

[0149] In some embodiments, the target cell is a dendritic cell, the dendritic cell antigen is CD40, the antigen binding region capable of binding to CD40 is Fab516 and light chain CDR L2 is replaced with said cell penetrating sequence, said immunogenic peptide or both.

[0150] In some embodiments, the target cell is a B cell, the B cell antigen is CD20, the antigen binding region capable of binding to CD20 is Arzerra and at least one of heavy chain CDR H1, light chain CDR L1 and light chain CDR L2 is replaced with said cell penetrating sequence, said immunogenic peptide or both. In some embodiments, replaced is mutated to include the peptide or sequence. In some embodiments, heavy chain CDR H1 is replaced. In some embodiments, the light chain CDR L1 is replaced. In some embodiments, the light chain CDR L2 is replaced.

[0151] In some embodiments, the target cell is a cancer cell, the cancer cell antigen is PD-L1, the antigen binding region capable of binding to PD-L1 is Durvalumab and at least one of heavy chain CDR H1, and light chain CDR L2 is replaced with said cell penetrating sequence, said immunogenic peptide or both. In some embodiments, replaced is mutated to include the peptide or sequence. In some embodiments, heavy chain CDR H1 is replaced. In some embodiments, the light chain CDR L2 is replaced.

[0152] In some embodiments, the antibody is TMab4 and the immunogenic peptide is inserted into any one of CDRH1, CDRH2, CDRH3 and CDRL3. In some embodiments, the antibody is TMab4 and the immunogenic peptide is inserted into CDRH1. In some embodiments, the antibody is TMab4 and the immunogenic peptide is inserted into CDRH2. In some embodiments, the antibody is TMab4 and the immunogenic peptide is inserted into CDRH3. In some embodiments, the antibody is TMab4 and the immunogenic peptide is inserted into CDRL3. In some embodiments, SEQ ID NO: 1 is inserted into CDRH1 of TMab4. In some embodiments, SEQ ID NO: 2 is inserted into CDRH1 of TMab4. In some embodiments, SEQ ID NO: 3 is inserted into CDRH1 of TMab4. In some embodiments, SEQ ID NO: 4 is inserted into CDRH1 of TMab4. In some embodiments, SEQ ID NO: 5 is inserted into CDRH1 of TMab4. In some embodiments, SEQ ID NO: 1 is inserted into CDRH2 of TMab4. In some embodiments, SEQ ID NO: 2 is inserted into CDRH2 of TMab4. In some embodiments, SEQ ID NO: 3 is inserted into CDRH2 of TMab4. In some embodiments, SEQ ID NO: 4 is inserted into CDRH2 of TMab4. In some embodiments, SEQ ID NO: 5 is inserted into CDRH2 of TMab4. In some embodiments, SEQ ID NO: 1 is inserted into CDRH3 of TMab4. In some embodiments, SEQ ID NO: 2 is inserted into CDRH3 of TMab4. In some embodiments, SEQ ID NO: 3 is inserted into CDRH3 of TMab4. In some embodiments, SEQ ID NO: 4 is inserted into CDRH3 of TMab4. In some embodiments, SEQ ID NO: 5 is inserted into CDRH3 of TMab4. In some embodiments, SEQ ID NO: 1 is inserted into CDRL3 of TMab4. In some embodiments, SEQ ID NO: 2 is inserted into CDRL3 of TMab4. In some embodiments, SEQ ID NO: 3 is inserted into CDRL3 of TMab4. In some embodiments, SEQ ID NO: 4 is inserted into CDRL3 of TMab4. In some embodiments, SEQ ID NO: 5 is inserted into CDRL3 of TMab4. In some embodiments, SEQ ID NO: 6 is inserted into CDRH1 of TMab4. In some embodiments, SEQ ID NO: 7 is inserted into CDRH23 of TMab4. In some embodiments, SEQ ID NO: 8 is inserted in place of amino acids 14-22 of the light chain of TMab4. In some embodiments, into CDRH1 comprises replacing amino acids 25-33 of the heavy chain. In some embodiments, into CDRH1 comprises replacing amino acids 26-33 of the heavy chain. In some embodiments, into CDRH1 comprises replacing amino

acids 26-32 of the heavy chain. In some embodiments, into CDRH1 comprises replacing amino acids 27-33 of the heavy chain. In some embodiments, into CDRH1 comprises replacing amino acids 28-33 of the heavy chain. In some embodiments, into CDRH1 comprises replacing amino acids 22-30 of the heavy chain. In some embodiments, into CDRH1 comprises replacing amino acids 22-29 of the heavy chain. In some embodiments, into CDRH1 comprises replacing amino acids 26-31 of the heavy chain. In some embodiments, into CDRH1 comprises replacing amino acids 23-32 of the heavy chain. In some embodiments, into CDRH1 comprises replacing amino acids 23-31 of the heavy chain. In some embodiments, into CDRH1 comprises replacing amino acids 28-35 of the heavy chain. In some embodiments, into CDRH3 comprises replacing amino acids 100-108 of the heavy chain. In some embodiments, into CDRH3 comprises replacing amino acids 99-106 of the heavy chain. In some embodiments, into CDRH3 comprises replacing amino acids 99-105 of the heavy chain. In some embodiments, into CDRH3 comprises replacing amino acids 100-106 of the heavy chain. In some embodiments, into CDRH3 comprises replacing amino acids 100-105 of the heavy chain. In some embodiments, into CDRH3 comprises replacing amino acids 100-104 of the heavy chain. In some embodiments, into CDRH3 comprises replacing amino acids 99-107 of the heavy chain. In some embodiments, into CDRH2 comprises replacing amino acids 52-59 of the heavy chain. In some embodiments, into CDRH2 comprises replacing amino acids 52-60 of the heavy chain. In some embodiments, into CDRL3 comprises replacing amino acids 97-103 of the light chain. In some embodiments, into CDRL3 comprises replacing amino acids 98-103 of the light chain. In some embodiments, into CDRL3 comprises replacing amino acids 96-104 of the light chain. In some embodiments, into CDRL3 comprises replacing amino acids 98-104 of the light chain.

[0153] In some embodiments, the antibody is 3E10 and the immunogenic peptide is inserted into any one of CDRL1 and CDRL2. In some embodiments, the antibody is 3E10 and the immunogenic peptide is inserted into CDRL1. In some embodiments, the antibody is 3E10 and the immunogenic peptide is inserted into CDRL2. In some embodiments, SEQ ID NO: 1 is inserted into CDRL1 of 3E10. In some embodiments, SEQ ID NO: 2 is inserted into CDRL1 of 3E10. In some embodiments, SEQ ID NO: 3 is inserted into CDRL1 of 3E10. In some embodiments, SEQ ID NO: 4 is inserted into CDRL1 of 3E10. In some embodiments, SEQ ID NO: 5 is inserted into CDRL1 of 3E10. In some embodiments, SEQ ID NO: 1 is inserted into CDRL2 of 3E10. In some embodiments, SEQ ID NO: 2 is inserted into CDRL2 of 3E10. In some embodiments, SEQ ID NO: 3 is inserted into CDRL2 of 3E10. In some embodiments, SEQ ID NO: 4 is inserted into CDRL2 of 3E10. In some embodiments, SEQ ID NO: 5 is inserted into CDRL2 of 3E10. In some embodiments, SEQ ID NO: 6 is inserted into CDRL1 of 3E10. In some embodiments, SEQ ID NO: 9 is inserted into CDRL2 of 3E10. In some embodiments, into CDRL1 comprises replacing amino acids 27-35 of the light chain. In some embodiments, into CDRL1 comprises replacing amino acids 28-36 of the light chain. In some embodiments, into CDRL2 comprises replacing amino acids 50-58 of the light chain.

[0154] In some embodiments, the antibody is 71F12 and the immunogenic peptide is inserted into CDRL1. In some embodiments, SEQ ID NO: 1 is inserted into CDRL1 of 71F12. In some embodiments, SEQ ID NO: 2 is inserted into CDRL1 of 71F12. In some embodiments, SEQ ID NO: 3 is inserted into CDRL1 of 71F12. In some embodiments, SEQ ID NO: 4 is inserted into CDRL1 of 71F12. In some embodiments, SEQ ID NO: 5 is inserted into CDRL1 of 71F12. In some embodiments, into CDRL1 comprises replacing amino acids 28-36 of the light chain. In some embodiments, into CDRL1 comprises replacing amino acids 26-34 of the light chain. In some embodiments, into CDRL2 comprises replacing amino acids 50-58 of the light chain.

[0155] In some embodiments, the antibody is a commercially available antibody. In some embodiments, the antibody penetrates into a bound cell at a level comparable to any one of TMab4, 3E10 and 71F12. In some embodiments, the antibody penetrates into a bound cell at a level comparable to TMab4. In some embodiments, the antibody penetrates into a bound cell at a level

comparable to 3E10. In some embodiments, the antibody penetrates into a bound cell at a level comparable to 71F12. In some embodiments, comparable is with a penetrance that is at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 92, 95, 97, 99 or 100% of the original antibody. Each possibility represents a separate embodiment of the invention. In some embodiments, comparable is with a penetrance that is at least 80% of the original antibody. In some embodiments, comparable is with a penetrance that is at least 90% of the original antibody.

[0156] In some embodiments, the antigen binding region and the immunogenic peptide are part of the same amino acid chain. In some embodiments, the antigen binding region and the cell penetration sequence are part of the same amino acid chain. In some embodiments, the immunogenic peptide and the cell penetration sequence are part of the same amino acid chain. In some embodiments, the antigen binding molecule of the invention is a single fusion protein. In some embodiments, the antigen binding molecule of the invention is a single amino acid chain.

[0157] In some embodiments, the antigen binding region and the immunogenic peptide are separated by a linker. In some embodiments, the antigen binding region and the cell penetrating sequence are separated by a linker. In some embodiments, the immunogenic peptide and the cell penetrating sequence are separated by a linker. In some embodiments, the linker is not a chemical linker. In some embodiments, the linker is not an artificial linker. In some embodiments, the linker is a peptide linker. In some embodiments, the linker is an amino acid linker. In some embodiments, the linker comprises or consists of at least 1, 2, 3, 4 or 5 amino acids. Each possibility represents a separate embodiment of the invention. In some embodiments, the linker comprises or consists of at most 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids. Each possibility represents a separate embodiment of the invention. In some embodiments, the linker comprises or consists of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids. Each possibility represents a separate embodiment of the invention.

[0158] In some embodiments, the antibody is selected from antibodies T1-T19, T1_30-T1_35, T1_39-T1_45 and T1_47. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising a sequence selected from SEQ ID NO: 1028-1040, 1043-1045, 1047-1055, and 1058-1059 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, an analog or homolog comprises the immunogenic peptide. In some embodiments, an analog or homolog is capable of binding cells. In some embodiments, an analog or homolog is delivered to the cytosol upon cell binding. In some embodiments, reaching the cytosol comprises displaying the immunogenic peptide on the surface of the bound cell. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1028 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1029 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1030 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1031 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1032 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1033 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising

SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1034 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1035 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1036 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1037 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1038 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1039 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1040 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1043 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1044 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1045 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1047 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1048 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1049 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1050 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1051 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1052 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1053 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1054 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the

antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1055 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1058 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a light chain variable region comprising SEQ ID NO: 1022 and a heavy chain variable region comprising SEQ ID NO: 1059 or analogs or homologs comprising at least 85% sequence identity.

[0159] In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1021 and a light chain variable region comprising a sequence selected from SEQ ID NO: 1041-1042, 1046, and 1056-1057 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1021 and a light chain variable region comprising SEQ ID NO: 1041 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1021 and a light chain variable region comprising SEQ ID NO: 1042 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1021 and a light chain variable region comprising SEQ ID NO: 1046 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1021 and a light chain variable region comprising SEQ ID NO: 1056 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1021 and a light chain variable region comprising SEQ ID NO: 1057 or analogs or homologs comprising at least 85% sequence identity.

[0160] In some embodiments, the antibody is selected from antibodies T2_6, T2_11-T2_13, T2_20 and T2_23. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1023 and a light chain variable region comprising a sequence selected from SEQ ID NO: 1060-1065 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1023 and a light chain variable region comprising SEQ ID NO: 1060 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1023 and a light chain variable region comprising SEQ ID NO: 1061 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1023 and a light chain variable region comprising SEQ ID NO: 1062 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1023 and a light chain variable region comprising SEQ ID NO: 1063 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1023 and a light chain variable region comprising SEQ ID NO: 1064 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1023 and a light chain variable region comprising SEQ ID NO: 1065 or analogs or homologs comprising at least 85% sequence identity.

[0161] In some embodiments, the antibody is selected from antibodies T4_1 and T4_3. In some

embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1026 and a light chain variable region comprising SEQ ID NO: 1066 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO: 1027 and a light chain variable region comprising SEQ ID NO: 1067 or analogs or homologs comprising at least 85% sequence identity.

[0162] In some embodiments, at least 85% identity is at least 90, 92, 95, 97, 99 or 100% identity. Each possibility represents a separate embodiment of the invention. It will be understood by a skilled artisan that while certain regions in the antibodies are required for function, such as the antigen binding CDRs, the immunogenic peptide and the cell penetrating moiety, other regions may bear alterations without altering function. Other inert CDRs are such a region as are many inter-CDR sequences as well as sequences within the constant region of an antibody. Analogs and homologs that retain function but contain alterations with these other regions are also encompassed within the invention.

[0163] In some embodiments, the antigen biding region is a single-chain antibody. In some embodiments, the antigen binding molecule of the invention comprises a first single-chain antibody and a second single chain antibody. In some embodiments, the first single-chain antibody is capable of binding the antigen of the target cell. In some embodiments, the second single-chain antibody is not functional. In some embodiments, the second single-chain antibody comprises the cell penetrating sequence. In some embodiments, the first single-chain antibody comprises the immunogenic peptide. In some embodiments, the second single-chain antibody comprises the immunogenic peptide. In some embodiments, the first single-chain antibody comprises an inert CDR replaced with the immunogenic peptide. In some embodiments, the first single-chain antibody comprises an inert CDR replaced with the cell penetrating sequence. In some embodiments, the first single-chain antibody and the second single-chain antibody are separated by a linker. In some embodiments, the linker is a peptide linker or an amino acid linker. In some embodiments, the peptide linker is an amino acid linker. In some embodiments, a peptide linker is a peptide bond.

[0164] In some embodiments, the antigen biding molecule is an antibody. In some embodiments, the antigen biding molecule comprises a first heavy chain and a first light chain. In some embodiments, the first antigen binding region comprises a first heavy chain and a first light chain. In some embodiments, the first heavy chain and the first light chain are capable of binding the antigen on a target cell. In some embodiments, a CDR of the first heavy chain is inert. In some embodiments, a CDR of the first light chain is inert. In some embodiments, an inert CDR of the first heavy chain is replaced with the immunogenic peptide. In some embodiments, an inert CDR of the first light chain is replaced with the immunogenic peptide. In some embodiments, an inert CDR of the first heavy chain is replaced with the cell penetration sequence. In some embodiments, an inert CDR of the first light chain is replaced with the cell penetration sequence.

[0165] In some embodiments, the antigen biding molecule comprises a second heavy chain. In some embodiments, the antigen biding molecule comprises a second light chain. In some embodiments, the antigen biding molecule comprises a second heavy chain and a second light chain. In some embodiments, the second antigen binding region comprises a second heavy chain. In some embodiments, the second antigen binding region comprises a second light chain. In some embodiments, the second antigen binding region comprises a second heavy chain and a second light chain. In some embodiments, a CDR of the second heavy chain is inert. In some embodiments, a CDR of the second light chain is inert. In some embodiments, the second heavy chain comprises the immunogenic peptide. In some embodiments, the second light chain comprises the immunogenic peptide. In some embodiments, the second heavy chain comprises the cell penetrating sequence. In some embodiments, the second light chain comprises the cell penetrating sequence. In some embodiments, a CDR of the second light chain is replaced. In some

embodiments, a CDR of the second heavy chain is replaced. In some embodiments, an inert CDR of the second heavy chain is replaced with the immunogenic peptide. In some embodiments, an inert CDR of the second light chain is replaced with the immunogenic peptide. In some embodiments, an inert CDR of the second heavy chain is replaced with the cell penetration sequence. In some embodiments, an inert CDR of the second light chain is replaced with the cell penetration sequence.

[0166] In some embodiments, the antigen binding molecule is a dual-function antigen binding molecule. In some embodiments, the composition comprises a dual-function antigen binding molecule. In some embodiments, the dual-function antigen binding molecule comprises an antibody or antigen binding fragment of the invention. In some embodiments, the dual-function antigen binding molecule is a bi-specific antibody. In some embodiments, the dual function antigen binding molecule comprises a first antibody or antigen binding molecule and a second antibody or antigen binding molecule. In some embodiments, the first antibody is a first heavy chain and a first light chain and the second antibody and a second heavy chain and a second light chain. In some embodiments, the heavy chain and light chain are hybridized between the CH1 domain of the heavy chain and the CL domain of the light chain. In some embodiments, hybridized is bonded. In some embodiments, hybridized comprises disulfide bonds. In some embodiments, the second antibody or antigen binding fragment thereof is capable of binding an antigen overexpressed on a target cell. In some embodiments, the target cell is a cancer cell. In some embodiments, the antigen is a cancer antigen.

[0167] Examples of cancer antigens include but are not limited to epidermal growth factor (EGFR), Receptor tyrosine-protein kinase erbB2 (HER2), Nectin cell adhesion molecule 4 (NECTIN-4), Tumor-associated calcium signal transducer 2 (TROP-2/TACSTD2), Tissue Factor (TF/F3), B-cell maturation antigen (BCMA/TNFRSF17), Programmed death-ligand 1 (PDL-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), Epithelial cell adhesion molecule (EpCAM), TNF receptor superfamily member 8 (CD3/TNFRSF8), B-lymphocyte antigen CD19 (CD19), cluster of differentiation-22 (CD22), Siglec-3 (CD33), cluster of differentiation 38 (CD38), Cluster of differentiation 79 (CD79), Lymphocyte-activation gene 3 (LAG-3), C-C MotifChemokine Receptor 4 (CCR4), vascular endothelial growth factor receptor 2 (VEGFR2/KDR), Folate receptor 1 (FOLR1), CAMPATH-1 antigen (CD52), platelet-derived growth factor receptor A (PDGFRα), disialoganglio side GD2, mono sialodihexo sylganglio side (GM3), insulin-like growth factor 1 (IGF-1) receptor (IGF1R), SLAM family member 7 (SLAMF7), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Antibodies that bind to these cancer antigens are well known in the art and any such antibody can be used as the targeting module of the invention. In some embodiments, the cancer antigen targeted by the targeting molecule is selected from HER2, EGFR, EpCAM, BCMA, CD33, CD38, CTLA, LAG-3, and PD-L1. Examples of antibodies that can be used for the targeting moiety are provided in Table 4.

TABLE-US-00004 TABLE 4 targeting moiety antibodies International Non-Proprietary Name Target Omburtamab B7-H3 (CD276) Belantamab mafodotin (belantamab mafodotin-blmf) BCMA Teclistamab BCMA, CD3 Mogamulizumab (mogamulizumab-kpkc) CCR4 Loncastuximab tesirine CD19 Tafasitamab (tafasitamab-cxix) CD19 Blinatumomab CD19, CD3 Ibritumomab tiuxetan CD20 Obinutuzumab CD20 Ofatumumab CD20 Ripertamab CD20 Rituximab CD20 Tositumomab-I131 CD20 Mosunetuzumab CD20, CD3 Glofitamab CD20, CD3e Inotuzumab ozogamicin CD22 Moxetumomab pasudotox CD22 (moxetumomab pasudotox-tdfk) Brentuximab vedotin CD30 Gemtuzumab ozogamicin CD33 Daratumumab CD38 Isatuximab (isatuximab-irfc) CD38 Alemtuzumab CD52 Polatuzumab vedotin (polatuzumab vedotin-piiq) CD79 Ipilimumab CTLA-4 Tremelimumab CTLA-4 Cetuximab EGFR Necitumumab EGFR Nimotuzumab EGFR Panitumumab EGFR Amivantamab EGFR, cMET Edrecolomab EpCAM Oportuzumab monatox EpCAM Catumaxomab EPCAM, CD3 Mirvetuximab soravtansine FOLR1 Dinutuximab GD2 Naxitamab-gqgk GD2 Racotumomab GM3 [fam-]trastuzumab deruxtecan, HER2 (fam-

trastuzumab deruxtecan-nxki) Ado-trastuzumab emtansine HER2 Disitamab vedotin HER2 Inetetamab HER2 Margetuximab-cmkb HER2 Pertuzumab HER2 Trastuzumab HER2 Teprotumumab (teprotumumab-trbw) IGF-1R Relatlimab LAG-3 Enfortumab vedotin (enfortumab vedotin-ejfv) Nectin-4 Olaratumab PDGFRα Adebrelimab PD-L1 Atezolizumab PD-L1 Avelumab PD-L1 Durvalumab PD-L1 Envafolimab PD-L1 Socazolimab PD-L1 Sugemalimab PD-L1 Tagitanlimab PD-L1 Elotuzumab SLAMF7 Sacituzumab govitecan (sacituzumab govitecan-hziy) TROP-2 Ramucirumab VEGFR2

[0168] In some embodiments, the antigen is epidermal growth factor receptor (EGFR). In some embodiments, the second antibody is selected from: cetuximab, panitumumab and necitumumab. In some embodiments, the second antibody is cetuximab. In some embodiments, the second antibody is panitumumab. In some embodiments, the second antibody is necitumumab. In some embodiments, cetuximab comprises a heavy chain comprising SEQ ID NO: 1069 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, cetuximab comprises a light chain comprising SEQ ID NO: 1068 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, panitumumab comprises a heavy chain comprising SEQ ID NO: 1071 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, panitumumab comprises a light chain comprising SEQ ID NO: 1070 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, necitumumab comprises a heavy chain comprising SEQ ID NO: 1073 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, necitumumab comprises a light chain comprising SEQ ID NO: 1072 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the analog or homolog retains target binding function. In some embodiments, the analog or homolog retains the CDRs of the antibody. In some embodiments, the CDRs are all of the CDRs that are not inert.

[0169] In some embodiments, at least one inert CDR of the second antibody is replaced with an immunogenic peptide. In some embodiments, the second antibody is a targeting antibody. In some embodiments, CDRH1 of cetuximab is replaced. In some embodiments, CDRL1 of cetuximab is replaced. In some embodiments, CDRL2 of cetuximab is replaced. In some embodiments, CDRL1 of panitumumab is replaced. In some embodiments, CDRL2 of panitumumab is replaced. In some embodiments, CDRL1 of necitumumab is replaced. In some embodiments, CDRL2 of necitumumab is replaced. In some embodiments, the CDR is replaced with a peptide selected from those provided in Tables 1-3. In some embodiments, the CDR is replaced with a peptide selected from those provided in Table 1. In some embodiments, the CDR is replaced with a peptide selected from those provided in Table 2. In some embodiments, the CDR is replaced with a peptide selected from those provided in Table 3. In some embodiments, the CDR is replaced with a peptide selected from SEQ ID NO: 1-11. In some embodiments, the CDR is replaced with a peptide selected from SEQ ID NO: 1-5. In some embodiments, the CDR is replaced with SEQ ID NO: 1. In some embodiments, the second antibody comprises an immunogenic peptide in place of amino acid sequence from an inert CDR. In some embodiments, the inert CDRs are provided in Table 14.

[0170] In some embodiments, the first antibody comprises at least one modification that promotes heterodimerization. In some embodiments, the first antibody comprises at least one modification that inhibits homodimerization. In some embodiments, the second antibody comprises at least one modification that promotes heterodimerization. In some embodiments, the second antibody comprises at least one modification that inhibits homodimerization. In some embodiments, the modification are knob-in-holes modifications. In some embodiments, the modification is a mutation. In some embodiments, the modification is in the constant region. In some embodiments, the constant region is the Fc region.

[0171] In some embodiments, the Fc region comprises an Ig CH2 domain. In some embodiments, the Fc region comprises an Ig heavy chain CH2 domain. In some embodiments, the Fc region comprises an Ig CH3 domain. In some embodiments, the Fc region comprises an Ig heavy chain

CH3 domain. In some embodiments, the Fc region comprises or consists of both an Ig CH2 domain and Ig CH3 domain. In some embodiments, the Fc region comprises or consists of both an Ig heavy chain CH2 and an Ig heavy chain CH3 domain. In some embodiments, the first chain comprises a first portion of an Fc region and the second chain comprises a second portion of the Fc region. In some embodiments, the first portion comprises a CH2 domain, a CH3 domain or both. In some embodiments, the second portion comprises a CH2 domain, a CH3 domain or both. In some embodiments, interface of the first portion of an Fc region and the second portion of an Fc region produces a functional Fc region. In some embodiments, interface comprises contact. In some embodiments, interface comprises adjacent positioning. In some embodiments, interface comprises formation of the protein complex of the invention. In some embodiments, interface comprises dimerization of the first and second dimerization domains. In some embodiments, the CH2 domain is an Ig CH2 domain. In some embodiments the CH2 domain is a heavy chain CH2 domain. In some embodiments, the CH3 domain is an Ig CH3 domain. In some embodiments, the CH3 domain is a heavy chain CH3 domain.

[0172] In some embodiments, a CH2 domain comprises the amino acid sequence SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK (SEQ ID NO: 1097) or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the CH2 domain consists of SEQ ID NO: 1097. In some embodiments, SEQ ID NO: 1097 is the IgG1 CH2 domain.

[0173] In some embodiments, a CH3 domain comprises the amino acid sequence GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1098) or analogs or homologs comprising at least 85% sequence identity. In some embodiments, a CH3 domain comprises the amino acid sequence GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1099) or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the CH3 domain consists of SEQ ID NO: 1098. In some embodiments, the CH3 domain consists of SEQ ID NO: 1099. In some embodiments, SEQ ID NO: 1098 is the IgG1 CH3 domain. In some embodiments, SEQ ID NO: 1099 is the IgG1 CH3 domain. In some embodiments, the SEQ ID NO: 1098 sequence is the sequence found predominantly is humans of European and American descent. In some embodiments, SEQ ID NO: 1099 is the sequence found predominantly in humans of Asian descent.

[0174] In some embodiments, a CH3 domain comprises a mutation. In some embodiments, the first CH3 domain comprises a first mutation. In some embodiments, the second CH3 domain comprises a second mutation. In some embodiments, a CH2 domain comprises a mutation. In some embodiments, the first CH2 domain comprises a first mutation. In some embodiments, the second CH2 domain comprises a second mutation. In some embodiments, the CH2 and CH3 domains both comprise mutations. In some embodiments, the first CH2 domain and first CH3 domains each comprise a first mutation. In some embodiments, the second CH2 domain and the second CH3 domain each comprise a second mutation. In some embodiments, the mutations inhibit homodimerization of the first polypeptide chain. In some embodiments, the first mutation inhibits homodimerization of the first polypeptide chain. In some embodiments, the mutations inhibit homodimerization of the second polypeptide chain. In some embodiments, the second mutation inhibits homodimerization of the second polypeptide chain. In some, embodiments, the mutations permit heterodimerization. In some embodiments, the mutations permit heterodimerization of the first and second chains. In some embodiments, permitting is promoting. In some embodiments, permitting is enhancing.

[0175] Mutations that promote heavy chain heterodimerization and/or inhibit homodimerization are

well known in the art. Any such mutations or alterations may be used for constructing the polypeptides of the invention. In some embodiments, a region from an IgG is replaced with a region from an IgA. In some embodiments, a region from a TCRa is inserted into the first CH3 domain and a region from TCRb is inserted in to the second CH3 domain. In some embodiments, the mutation is insertion of a region from a TCR. In some embodiments, the TCR is selected from TCRa and TCRb. In some embodiments, the mutation is insertion of a region from a different Ig. Examples of these mutations can be found in Table 5. In some embodiments, the mutation is selected from a mutation in Table 5. In some embodiments, the first mutation is selected from a group of mutation provided in a row and the second column of Table 5 and the second mutation is the group of mutations provided in that same row of Table 5 in the third column. The mutations in Table 5 are provided with the Kabat numbering for IgG1 unless otherwise stated; corresponding mutations can be made in other IGs and specifically in other IgGs. In some embodiments, the first mutation is T366Y, and the second mutation is Y407T. In some embodiments, the first mutation is S354C and T366W and the second mutation is Y349C, T366S, L368A, and Y407V. In some embodiments, the first mutation is S364H and F405A and the second mutation is Y349T and T392F. In some embodiments, the first mutation is T350V, L351Y, F405A, and Y407V and the second mutation is T350V, T366L, K392L, and T394W. In some embodiments, the first mutation is K392D, and K409D and the second mutation is E356K, and D399K. In some embodiments, the first mutation is D221E, P228E, and L368E and the second mutation is D221R, P228R, and K409R. In some embodiments, the first mutation is K360E, and K409W and the second mutation is Q347R, D399V, and F405T. In some embodiments, the first mutation is K360E, K409W, and Y349C and the second mutation is Q347R, D399V, F405T, and S354C. In some embodiments, the first mutation is F405L and the second mutation is K409R. In some embodiments, the first mutation is K360D, D399M, and Y407A and the second mutation is E345R, Q347R, T366V, and K409V. In some embodiments, the first mutation is Y349S, K370Y, T366M, and K409V and the second mutation is E356G, E357D, S364Q, and Y407A. In some embodiments, the first mutation is T366K, and the second mutation is selected from C351D, Y349E, Y349D, L368E, L368D, Y349E and R355E, Y349E and R355D, Y349D and R355E, and Y349D and R355D. In some embodiments, the first mutation is T366K and C351K and the second mutation is selected from C351D, Y349E, Y349D, L368E, L368D, Y349E and R355E, Y349E and R355D, Y349D and R355E, and Y349D and R355D. In some embodiments, the first mutation is L351D and L368E and the second mutation is L351K and T366K. In some embodiments, the first mutation is L368D and K370S and the second mutation is E357Q and S364K. In some embodiments, the first mutation is T366W, and the second mutation is T366S, L368A and Y407V. In some embodiments, the Ig is IgG2, and the first mutation is C223E, P228E, and L368E and the second mutation is C223R, E225R, P228R, and K409R. In some embodiments, the first mutation is S354C or T366W and the second mutation is Y349C, T366S, L368A, or Y407V. In some embodiments, the first mutation is S364H or F405A and the second mutation is Y349T or T392F. In some embodiments, the first mutation is T350V, L351Y, F405A, or Y407V and the second mutation is T350V, T366L, K392L, or T394W. In some embodiments, the first mutation is K392D, or K409D and the second mutation is E356K, or D399K. In some embodiments, the first mutation is D221E, P228E, or L368E and the second mutation is D221R, P228R, or K409R. In some embodiments, the first mutation is K360E, or K409W and the second mutation is Q347R, D399V, or F405T. In some embodiments, the first mutation is K360E, K409W, or Y349C and the second mutation is Q347R, D399V, F405T, or S354C. In some embodiments, the first mutation is K360D, D399M, or Y407A and the second mutation is E345R, Q347R, T366V, or K409V. In some embodiments, the first mutation is Y349S, K370Y, T366M, or K409V and the second mutation is E356G, E357D, S364Q, or Y407A. In some embodiments, the first mutation is L351D or L368E and the second mutation is L351K or T366K. In some embodiments, the first mutation is L368D or K370S and the second mutation is E357Q or S364K. In some embodiments, the first mutation is T366W, and the second mutation is T366S,

L368A or Y407V. In some embodiments, the Ig is IgG2, and the first mutation is C223E, P228E, or L368E and the second mutation is C223R, E225R, P228R, or K409R. In some embodiments, the first heavy chain constant region comprises or consists of SEQ ID NO: 1074 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the second heavy chain constant region comprises or consists of SEQ ID NO: 1074. In some embodiments, the first heavy chain constant region comprises or consists of SEQ ID NO: 1075 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the second heavy chain constant region comprises or consists of SEQ ID NO: 1075. It will be understood that SEQ ID NO: 1074 and SEQ ID NO: 1075 heterodimerize with each other, but inhibit homodimerization. In some embodiments, the CL domain comprises or consists of SEQ ID NO: 1076 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the light chain constant region comprises or consists of SEQ ID NO: 1076. In some embodiments, the analog or homolog retains the mutations that promote heterodimerization and inhibit homodimerization.

TABLE-US-00005 TABLE 5 Mutations for enhancing heterodimerization and inhibiting homodimerization of CH3 domains. Strategy CH3 domain Chain 1 CH3 domain Chain 2 1 Knobs-into-holes (Y-T) T366Y Y407T 2 Knobs-into-holes (CW- S354C, T366W Y349C, T366S, L368A, CSAV) Y407V 3 HA-TF S364H, F405A Y349T, T394F 4 ZW1 (VYAV-VLLW) T350V, L351Y, F405A, T350V, T366L, K392L, Y407V T394W 5 CH3 charge pairs (DD- K392D, K409D E356K, D399K KK) 6 Hinge/CH3 charge (EEE- D221E, P228E, L368E D221R, P228R, K409R RRR) 7 EW-RVT K360E, K409W, Q347R, D399V, F405T 8 EW-RVTS-S K360E, K409W, Y349C Q347R, D399V, F405T, S354C 9 (L-R) F405L K409R 10 7.8.60 (DMA-RRVV) K360D, D399M, E345R, Q347R, T366V, Y407A K409V 11 20.8.34 (SYMV-GDQA) Y349S, K370Y, E356G, E357D, S364Q, T366M, K409V Y407A 12 Electrostatic steering 366K or 366K+ C351K C351D or E or D at 349, effects 368, 349, or 349 +355 13 “DEKK” L351D and L368E L351K and T366K 14 XmAb L368D/K370S E357Q/S364K 15 KiH T366W T366S/L368A/Y407V 16 IgG2 hinge/CH3 charge IgG2: C223E, P228E, IgG2: C223R, E225R, (EEE-RRRR) L368E P228R, K409R 17 SEEDbody IgG/A chimera IgG/A chimera 18 BEAT residues from TCRa residues from TCRb interface interface

[0176] In some embodiments, the dual-function antigen binding molecule comprises two heavy chains and two light chains. In some embodiments, the two heavy chains are SEQ ID NO: 1088 and 1080 and the two light chains are SEQ ID NO: 1087 and 1079 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the two heavy chains are SEQ ID NO: 1088 and 1082 and the two light chains are SEQ ID NO: 1087 and 1081 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the two heavy chains are SEQ ID NO: 1090 and 1082 and the two light chains are SEQ ID NO: 1089 and 1079 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the two heavy chains are SEQ ID NO: 1090 and 1082 and the two light chains are SEQ ID NO: 1089 and 1081 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the two heavy chains are SEQ ID NO: 1088 and 1086 and the two light chains are SEQ ID NO: 1087 and 1085 or analogs or homologs comprising at least 85% sequence identity. It will be understood that analogs or homologs will target to the target protein by the targeting module, bind the cell and be delivered to the cytosol by the killing module and the will contain the immunogenic peptide which is displayed on the cell surface in complex with HLA.

[0177] In some embodiments, the composition is a pharmaceutical composition. In some embodiments, the composition comprises a pharmaceutically acceptable carrier, excipient or adjuvant. In some embodiments, the composition if formulated for administration to a subject. In some embodiments, the composition is formulated for systemic administration. In some embodiments, the composition if formulated for administration to a tumor. In some embodiments, the composition is formulated for intravenous administration. In some embodiments, the composition is formulated for administration to a subject. In some embodiments, the subject is a

human.

[0178] As used herein, the term “carrier,” “excipient,” or “adjuvant” refers to any component of a pharmaceutical composition that is not the active agent. As used herein, the term “pharmaceutically acceptable carrier” refers to non-toxic, inert solid, semi-solid liquid filler, diluent, encapsulating material, formulation auxiliary of any type, or simply a sterile aqueous medium, such as saline. Some examples of the materials that can serve as pharmaceutically acceptable carriers are sugars, such as lactose, glucose and sucrose, starches such as corn starch and potato starch, cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt, gelatin, talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol, polyols such as glycerin, sorbitol, mannitol and polyethylene glycol; esters such as ethyl oleate and ethyl laurate, agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline, Ringer's solution; ethyl alcohol and phosphate buffer solutions, as well as other non-toxic compatible substances used in pharmaceutical formulations. Some non-limiting examples of substances which can serve as a carrier herein include sugar, starch, cellulose and its derivatives, powered tragacanth, malt, gelatin, talc, stearic acid, magnesium stearate, calcium sulfate, vegetable oils, polyols, alginic acid, pyrogen-free water, isotonic saline, phosphate buffer solutions, cocoa butter (suppository base), emulsifier as well as other non-toxic pharmaceutically compatible substances used in other pharmaceutical formulations. Wetting agents and lubricants such as sodium lauryl sulfate, as well as coloring agents, flavoring agents, excipients, stabilizers, antioxidants, and preservatives may also be present. Any non-toxic, inert, and effective carrier may be used to formulate the compositions contemplated herein. Suitable pharmaceutically acceptable carriers, excipients, and diluents in this regard are well known to those of skill in the art, such as those described in The Merck Index, Thirteenth Edition, Budavari et al., Eds., Merck & Co., Inc., Rahway, N.J. (2001); the CTFA (Cosmetic, Toiletry, and Fragrance Association) International Cosmetic Ingredient Dictionary and Handbook, Tenth Edition (2004); and the “Inactive Ingredient Guide,” U.S. Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) Office of Management, the contents of all of which are hereby incorporated by reference in their entirety. Examples of pharmaceutically acceptable excipients, carriers and diluents useful in the present compositions include distilled water, physiological saline, Ringer's solution, dextrose solution, Hank's solution, and DMSO. These additional inactive components, as well as effective formulations and administration procedures, are well known in the art and are described in standard textbooks, such as Goodman and Gillman's: The Pharmacological Bases of Therapeutics, 8th Ed., Gilman et al. Eds. Pergamon Press (1990); Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, Pa. (1990); and Remington: The Science and Practice of Pharmacy, 21st Ed., Lippincott Williams & Wilkins, Philadelphia, Pa., (2005), each of which is incorporated by reference herein in its entirety. The presently described composition may also be contained in artificially created structures such as liposomes, ISCOMS, slow-releasing particles, and other vehicles which increase the half-life of the peptides or polypeptides in serum. Liposomes include emulsions, foams, micelies, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. Liposomes for use with the presently described peptides are formed from standard vesicle-forming lipids which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally determined by considerations such as liposome size and stability in the blood. A variety of methods are available for preparing liposomes as reviewed, for example, by Coligan, J. E. et al, Current Protocols in Protein Science, 1999, John Wiley & Sons, Inc., New York, and see also U.S. Pat. Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

[0179] The carrier may comprise, in total, from about 0.1% to about 99.99999% by weight of the pharmaceutical compositions presented herein.

[0180] As used herein, the terms “administering,” “administration,” and like terms refer to any method which, in sound medical practice, delivers a composition containing an active agent to a subject in such a manner as to provide a therapeutic effect. One aspect of the present subject matter provides for intravenous administration of a therapeutically effective amount of a composition of the present subject matter to a patient in need thereof. Other suitable routes of administration can include parenteral, subcutaneous, oral, intramuscular, intratumoral or intraperitoneal.
[0181] In some embodiments, the nucleic acid molecule comprises an open reading frame. In some embodiments, the open reading frame encodes the antigen binding molecule of the invention. In some embodiments, the nucleic acid molecule comprises a plurality of open reading frames which collectively encode the antigen binding molecule of the invention.
[0182] In some embodiments, the vector is an expression vector. In some embodiments, the vector comprises at least one regulatory element operatively linked to a nucleic acid molecule of the invention. In some embodiments, the vector comprises at least one regulatory element operatively linked to an open reading frame encoding the antigen binding molecule of the invention. In some embodiments, the vector comprises a plurality of regulatory elements each operatively linked to an open reading frame which collectively encode the antigen binding molecule of the invention. In some embodiments, a composition comprises a plurality of vectors each comprising at least one regulatory element operatively linked to an open reading frame wherein the plurality of open reading frames collectively encodes the antigen binding molecule of the invention.
[0183] The term “expression” as used herein refers to the biosynthesis of a gene product, including the transcription and/or translation of said gene product. Thus, expression of a nucleic acid molecule may refer to transcription of the nucleic acid fragment (e.g., transcription resulting in mRNA or other functional RNA) and/or translation of RNA into a precursor or mature protein (polypeptide).
[0184] Expressing of a gene within a cell is well known to one skilled in the art. It can be carried out by, among many methods, transfection, viral infection, or direct alteration of the cell's genome. In some embodiments, the gene is in an expression vector such as plasmid or viral vector.
[0185] A vector nucleic acid sequence generally contains at least an origin of replication for propagation in a cell and optionally additional elements, such as a heterologous polynucleotide sequence, expression control element (e.g., a promoter, enhancer), selectable marker (e.g., antibiotic resistance), poly-Adenine sequence.
[0186] The vector may be a DNA plasmid delivered via non-viral methods or via viral methods. The viral vector may be a retroviral vector, a herpesviral vector, an adenoviral vector, an adeno-associated viral vector or a poxviral vector. The promoters may be active in mammalian cells. The promoters may be a viral promoter.
[0187] In some embodiments, the gene is operably linked to a promoter. The term “operably linked” is intended to mean that the nucleotide sequence of interest is linked to the regulatory element or elements in a manner that allows for expression of the nucleotide sequence (e.g. in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell).
[0188] In some embodiments, the vector is introduced into the cell by standard methods including electroporation (e.g., as described in From et al., Proc. Natl. Acad. Sci. USA 82, 5824 (1985)), Heat shock, infection by viral vectors, high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., Nature 327. 70-73 (1987)), and/or the like.
[0189] The term “promoter” as used herein refers to a group of transcriptional control modules that are clustered around the initiation site for an RNA polymerase i.e., RNA polymerase II. Promoters are composed of discrete functional modules, each consisting of approximately 7-20 bp of DNA, and containing one or more recognition sites for transcriptional activator or repressor proteins.
[0190] In some embodiments, nucleic acid sequences are transcribed by RNA polymerase II

(RNAP II and Pol II). RNAP II is an enzyme found in eukaryotic cells. It catalyzes the transcription of DNA to synthesize precursors of mRNA and most snRNA and microRNA.

[0191] In some embodiments, mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1 (±), pGL3, pZeoSV2(±), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, pNMT81, which are available from Invitrogen, pCI which is available from Promega, pMbac, pPbac, pBK-RSV and pBK-CMV which are available from Strategene, pTRES which is available from Clontech, and their derivatives.

[0192] In some embodiments, expression vectors containing regulatory elements from eukaryotic viruses such as retroviruses are used by the present invention. SV40 vectors include pSVT7 and pMT2. In some embodiments, vectors derived from bovine papilloma virus include pBV-1MTHA, and vectors derived from Epstein Bar virus include pHEBO, and p2O5. Other exemplary vectors include pMSG, pAV009/A+, pMTO10/A+, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV-40 early promoter, SV-40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

[0193] In some embodiments, recombinant viral vectors, which offer advantages such as lateral infection and targeting specificity, are used for in vivo expression. In one embodiment, lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. In one embodiment, the result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. In one embodiment, viral vectors are produced that are unable to spread laterally. In one embodiment, this characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

[0194] Various methods can be used to introduce the expression vector of the present invention into cells. Such methods are generally described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega et al., Gene Targeting, CRC Press, Ann Arbor Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988) and Gilboa et at. [Biotechniques 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

[0195] In one embodiment, plant expression vectors are used. In one embodiment, the expression of a polypeptide coding sequence is driven by a number of promoters. In some embodiments, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV [Brisson et al., Nature 310:511-514 (1984)], or the coat protein promoter to TMV [Takamatsu et al., EMBO J. 6:307-311 (1987)] are used. In another embodiment, plant promoters are used such as, for example, the small subunit of RUBISCO [Coruzzi et al., EMBO J. 3:1671-1680 (1984); and Brogli et al., Science 224:838-843 (1984)] or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B [Gurley et al., Mol. Cell. Biol. 6:559-565 (1986)]. In one embodiment, constructs are introduced into plant cells using Ti plasmid, Ri plasmid, plant viral vectors, direct DNA transformation, microinjection, electroporation and other techniques well known to the skilled artisan. See, for example, Weissbach & Weissbach [Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463 (1988)]. Other expression systems such as insects and mammalian host cell systems, which are well known in the art, can also be used by the present invention.

[0196] It will be appreciated that other than containing the necessary elements for the transcription and translation of the inserted coding sequence (encoding the polypeptide), the expression

construct of the present invention can also include sequences engineered to optimize stability, production, purification, yield or activity of the expressed polypeptide.

[0197] In some embodiments, the method is a method of treating cancer. In some embodiments, the antigen binding molecule of the invention is for use in treating cancer. In some embodiments, the dual function antigen binding molecule of the invention is for use in treating cancer. In some embodiments, the composition of the invention is for use in treating cancer. In some embodiments, the cancer is a cancer that expresses the cancer specific antigen. In some embodiments, the cancer is a cancer that overexpresses the cancer specific antigen. In some embodiments, overexpresses is expresses at a level higher than in non-cancerous cells. In some embodiments, the non-cancerous cells are of the same cell type or tissue as the cancerous cells. In some embodiments, the cancer is a cancer that expresses the immunogenic cancer peptide. In some embodiments, the cancer is a hematopoietic cancer. In some embodiments, the cancer comprises a malignant immune cell. In some embodiments, the immune cell is a B cell. In some embodiments, the cancer is a solid cancer. In some embodiments, the cancer is a PD-L1 positive cancer. In some embodiments, the cancer is an EGFR positive cancer. In some embodiments, the cancer is an EGFR overexpressing cancer.

[0198] As used herein “cancer” refers to diseases associated with cell proliferation. Non-limiting types of cancer include carcinoma, sarcoma, lymphoma, leukemia, blastoma and germ cells tumors. In one embodiment, carcinoma refers to tumors derived from epithelial cells including but not limited to breast cancer, prostate cancer, lung cancer, pancreas cancer, and colon cancer. In one embodiment, sarcoma refers of tumors derived from mesenchymal cells including but not limited to sarcoma botryoides, chondrosarcoma, ewings sarcoma, malignant hemangioendothelioma, malignant schwannoma, osteosarcoma and soft tissue sarcomas. In one embodiment, lymphoma refers to tumors derived from hematopoietic cells that leave the bone marrow and tend to mature in the lymph nodes including but not limited to hodgkin lymphoma, non-hodgkin lymphoma, multiple myeloma and immunoproliferative diseases. In one embodiment, leukemia refers to tumors derived from hematopoietic cells that leave the bone marrow and tend to mature in the blood including but not limited to acute lymphoblastic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, hairy cell leukemia, T-cell prolymphocytic leukemia, large granular lymphocytic leukemia and adult T-cell leukemia. In one embodiment, blastoma refers to tumors derived from immature precursor cells or embryonic tissue including but not limited to hepatoblastoma, medulloblastoma, nephroblastoma, neuroblastoma, pancreatoblastoma, pleuropulmonary blastoma, retinoblastoma and glioblastoma-multiforme. In one embodiment, germ cell tumors refers to tumors derived from germ cells including but not limited to germinomatous or seminomatous germ cell tumors (GGCT, SGCT) and nongerminomatous or nonseminomatous germ cell tumors (NGGCT, NSGCT). In one embodiment, germinomatous or seminomatous tumors include but not limited to germinoma, dysgerminoma and seminoma. In one embodiment, nongerminnomatous or nonseminomatous tumors refers to pure and mixed germ cells tumors including but not limited to embryonal carcinoma, endodermal sinus tumor, choriocarcinoma, tearoom, polyembryoma, gonadoblastoma and teratocarcinoma.

[0199] In some embodiments, the antigen binding molecule of the invention is a cancer vaccine. In some embodiments, cancer vaccine is an antigen binding molecule comprising an antigen binding region capable of binding a dendritic cell antigen. It will be understood by a skilled artisan that upon entrance into a dendritic cell, the immunogenic cancer peptide will be cleaved from the rest of the molecule of the invention and displayed on the surface of the dendritic cell by HLA molecules. This will in turn train cytotoxic immune cells (T cell and NK cells) to target this immunogenic peptide and thereby the cancer.

[0200] In some embodiments, treating further comprises administering effector cells specific to the immunogenic peptide to the subject. In some embodiments, an effector cell is an immune cell. In some embodiments, an effector cell is a cytotoxic cell. In some embodiments, an effector cell is a lymphocyte. In some embodiments, an effector cell is a CD8 T cell. In some embodiments, an

effector cell is a natural killer (NK) cell. In some embodiments, the effector cell has been exposed to the peptide. In some embodiments, the effector cell has been exposed to an antigen presenting cell presenting the peptide in complex with an HLA.

[0201] In some embodiments, the treating further comprises providing a vaccine comprising the immunogenic peptide. In some embodiments, the subject has previously received a vaccine comprising the immunogenic peptide. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human. In some embodiments, the subject suffers from cancer. In some embodiments, the subject is suitable to be treated by a method of the invention. In some embodiments, the subject has previously been exposed to the immunogenic peptide. In some embodiments, the subject has previously been infected by the pathogen from which the immunogenic peptide originates. In some embodiments, a pathogen is a virus. In some embodiments, the subject is capable of mounting an immune response against the peptide. In some embodiments, the subject comprises T cells comprising a TCR that recognizes the peptide or a portion thereof. In some embodiments, the subject comprises memory B cells comprising a BCR that recognizes the peptide or a portion thereof.

[0202] In some embodiments, the method is a method of producing surface display of the peptide in a target cell. In some embodiments, the surface display is display of HLA complexed with the peptide. In some embodiments, expressing is surface display. In some embodiments, expressing is expressing in an HLA complex.

[0203] By another aspect, there is provided a method of engineering an antibody or antigen binding fragment thereof, the method comprising: [0204] a. selecting an antibody or antigen binding fragment thereof of interest; [0205] b. determining at least one CDR of the selected antibody or antigen binding fragment thereof is not required for binding to a target; [0206] c. replacing the determined at least one CDR or a portion thereof with a peptide;

thereby engineering an antibody or antigen binding fragment thereof.

[0207] By another aspect, there is provided a method of engineering an antibody or antigen binding fragment thereof, the method comprising: [0208] a. selecting an antibody or antigen binding fragment thereof of interest; [0209] b. receiving a database of peptides; [0210] c. performing alignment of peptides of a variable region of the selected antibody or antigen binding fragment thereof with peptides of the database; [0211] d. determining a peptide from the selected antibody of antigen binding fragment thereof and a peptide from the database with an alignment score above a predetermined threshold; and [0212] e. replacing the determined peptide from the selected antibody or antigen binding fragment thereof with the determined peptide from the database;

thereby engineering an antibody or antigen binding fragment thereof.

[0213] In some embodiments, the engineered antibody or antigen binding fragment thereof is an antibody or antigen binding fragment of the invention. In some embodiments, the engineered antibody or antigen binding fragment thereof is an antigen binding molecule of the invention. In some embodiments, the engineered antibody or antigen binding fragment thereof is a dual-function antigen binding molecule of the invention. In some embodiments, the engineered antibody or antigen binding fragment thereof is an immunogenic peptide delivery antibody. In some embodiments, the engineered antibody or antigen binding fragment thereof is for use in a method of the invention. In some embodiments, the antibody or antigen binding fragment thereof before engineering is a cell penetrating antibody. In some embodiments, the selecting is selecting an antibody that penetrates into cells to which it binds. In some embodiments, the antibody or antigen binding fragment thereof before engineering is a commercially available antibody.

[0214] In some embodiments, the antibody or antigen binding fragment thereof before engineering binds to the surface of a target cell. In some embodiments, the target cell is a cancer cell. In some embodiments, step (a) comprises selecting an antibody or antigen binding fragment thereof that binds to a surface of a target cell. In some embodiments, binding the surface is binding a surface antigen. In some embodiments, antibody or antigen binding fragment thereof before engineering is

a DNA binding antibody. In some embodiments, antibody or antigen binding fragment thereof before engineering is endocytosed into the endocytic pathway of a cell. In some embodiments, antibody or antigen binding fragment thereof before engineering is transported into the cytoplasm of a cell to which it binds. In some embodiments, antibody or antigen binding fragment thereof before engineering is capable of endosomal escape. In some embodiments, antibody or antigen binding fragment thereof before engineering is delivered to the cytosol of a cell to which it binds. In some embodiments, the method further comprises inserting into the antibody or antigen binding fragment thereof a cell penetrating moiety. In some embodiments, the cell penetrating moiety is inserted before the immunogenic peptide. In some embodiments, the cell penetrating moiety is inserted after the immunogenic peptide. In some embodiments, the cell penetrating moiety is inserted concomitantly to the insertion of the immunogenic peptide. In some embodiments, step (a) comprises selecting an antibody or antigen binding fragment thereof that upon binding to a surface of a target cell is internalized and delivered to a cytosol of the target cell.

[0215] In some embodiments, the antibody or antigen binding fragment thereof before engineering penetrates/internalizes into a bound cell at a level comparable to any one of TMab4, 3E10 and 71F12. In some embodiments, the antibody or antigen binding fragment thereof before engineering penetrates/internalizes into a bound cell at a level comparable to TMab4. In some embodiments, the antibody or antigen binding fragment thereof before engineering penetrates/internalizes into a bound cell at a level comparable to 3E10. In some embodiments, the antibody or antigen binding fragment thereof before engineering penetrates/internalizes into a bound cell at a level comparable to 71F12. In some embodiments, comparable is with a penetrance that is at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 92, 95, 97, 99 or 100% of the original antibody. Each possibility represents a separate embodiment of the invention. In some embodiments, comparable is with a penetrance that is at least 80% of the original antibody. In some embodiments, comparable is with a penetrance that is at least 90% of the original antibody.

[0216] In some embodiments, the determining is determining at least one inert CDR. In some embodiments, the determining is determining at least one CDR not involved in transport into the cell. In some embodiments, the determining is determining at least one CDR not involved in endosomal escape. In some embodiments, the determining is based on structural analysis of the antibody or antigen binding fragment thereof bound to its target. In some embodiments, the structural analysis is in silico analysis. In some embodiments, the structural analysis is crystallographic analysis. In some embodiments, the method further comprises receiving the structural analysis. In some embodiments, the method further comprises performing the structural analysis. In some embodiments, the analysis provides the distance of each amino acid of the antibody or antigen binding fragment thereof to an amino acid of the target. In some embodiments, the analysis provides the distance of each amino acid of a CDR of the antibody or antigen binding fragment thereof to an amino acid of the target. In some embodiments, the determining comprises determining the distance of each amino acid of a CDR to the target. In some embodiments, a CDR with two or fewer amino acids in contact with the antigen is determined as not required for binding. In some embodiments, the determining comprises determining the distance of each amino acid of a CDR to the target. In some embodiments, a CDR with one or fewer amino acids in contact with the antigen is determined as not required for binding. In some embodiments, a CDR not required for binding is an inert CDR. In some embodiments, a CDR not required for binding is a CDR in which mutations have been shown not to effect antibody function. In some embodiments, the function is binding.

[0217] In some embodiments, the peptide is an immunogenic peptide. In some embodiments, the peptide comprises at least 5, 6, 7, 8, 9, 10, or 11 amino acids. Each possibility represents a separate embodiment of the invention. In some embodiments, the peptide comprises at least 7 amino acids. In some embodiments, the peptide comprises at least 8 amino acids. In some embodiments, the peptide comprises between 8 and 11 amino acids. In some embodiments, the peptide is an

immunogenic peptide provided hereinabove. In some embodiments, peptide is from the variable region of the antibody or antigen binding fragment thereof. In some embodiments, peptide is from a CDR of the antibody or antigen binding fragment thereof. Methods of determining the position of CDRs are well known in the art and the Clothia and Kabat systems may be used. Given the sequence of a variable domain of an antibody a skilled artisan will be readily able to identify the CDRs.

[0218] In some embodiments, the at least one CDR is removed. In some embodiments, a portion of the at least one CDR is removed. In some embodiments, the removed amino acids are replaced with the immunogenic peptide. In some embodiments, the replacing is optimized. In some embodiments, the method further comprises optimizing the replacement. In some embodiments, optimizing is structural optimization. In some embodiments, optimizing comprises performing an optimization algorithm. In some embodiments, optimizing comprises producing as little perturbation in the structure of the selected antibody or antigen binding fragment thereof as possible. In some embodiments, optimizing comprises in silico analysis or the insertion of the immunogenic peptide into the at least one determined CDR with removal of all possible portions of the CDR and selected the insertion and removal of a portion that produces the least perturbation. In some embodiments, optimization comprise insertion of at least one filler amino Acid. some embodiments, optimization comprise producing at least one compensatory mutation outside the determined CDR. In some embodiments, the optimization algorithm is a minimal perturbation replacement (MBR) algorithm. In some embodiments, optimization comprises optimization of CDR flanking sequence. In some embodiments, optimization of flanking sequence comprises antibody stem preservation. In some embodiments, the structure of the stems flanking the CDRs are maintained. In some embodiments, the stems are the ends of the beta sheets flanking the CDR. In some embodiments, the end comprises the 1, 2, 3, 4, or 5 amino acids directly adjacent to the CDR. Each possibility represents a separate embodiment of the invention.

[0219] In some embodiments, the method further comprises confirming binding of the engineered antibody or antigen binding fragment thereof to the same target that was bound by the selected antibody or antigen binding fragment thereof. In some embodiments, the method further comprises measuring binding of the engineered antibody or antigen binding fragment thereof to the same target that was bound by the selected antibody or antigen binding fragment thereof. In some embodiments, the method further comprises determining binding of the engineered antibody or antigen binding fragment thereof to the same target that was bound by the selected antibody or antigen binding fragment thereof. In some embodiments, binding is equivalent to the binding of the selected antibody or antigen binding fragment thereof. In some embodiments, the measuring further comprises determining that the binding is not significantly reduced as compared to the binding of the selected antibody or antigen binding fragment thereof. In some embodiments, significantly is statistically significantly. In some embodiments, significantly reduced comprises a greater than 10% reduction. In some embodiments, significantly reduced comprises a greater than 20% reduction. In some embodiments, significantly reduced comprises a greater than 50% reduction. In some embodiments, significantly reduced is abolished.

[0220] In some embodiments, the method further comprises measuring levels of peptide in the cytosol of the target cell. In some embodiments, the method further comprises determining delivery of the peptide to the cytosol. In some embodiments, the method further comprises measuring delivery of the peptide to the cytosol.

[0221] In some embodiments, the method further comprises confirming delivery of the peptide to a cytosol of the target cell. In some embodiments, the method further comprises measuring levels of peptide in the cytosol of the target cell. In some embodiments, the method further comprises determining delivery of the peptide to the cytosol. In some embodiments, the method further comprises measuring delivery of the peptide to the cytosol.

[0222] In some embodiments, the method further comprises confirming delivery of the peptide to

the surface of the target cell. In some embodiments, delivery is surface display of the peptide. In some embodiments, delivery is delivery of the peptide in complex with an HLA molecule to the surface of the target cell. In some embodiments, the method further comprises measuring levels of peptide on the surface of the target cell. In some embodiments, levels is levels of the peptide in complex with HLA. In some embodiments, the method further comprises determining delivery of the peptide to the surface of the target cell. In some embodiments, the method further comprises measuring delivery of the peptide to the surface of the target cell.

[0223] In some embodiments, the method further comprises confirming killing of the target cell by effector cells. In some embodiments, killing is specific killing. In some embodiments, effector cells are specific to the peptide. In some embodiments, effector cells are immune cells. In some embodiments, the method further comprises measuring killing of the target cell by effector cells. In some embodiments, the method further comprises determining killing of the target cell by effector cells. In some embodiments, the method is an in vitro method. In some embodiments, the confirming, measuring and determining is performed in vitro. In some embodiments, in vitro is ex vivo. Examples of methods of performing the confirming, measuring and determining are provided hereinbelow, but any assay known in the art for such measuring/confirming/determining may be used.

[0224] Databases of peptides and specifically immunogenic peptides are known in the art and any such database may be used. In some embodiments, the peptide is a cancer peptide. In some embodiments, database comprises or consists of Table 3. In some embodiments, the alignment is a pairwise alignment. In some embodiments, the alignment is alignment of one peptide from the selected antibody or antigen binding fragment thereof and one peptide of the database to produce an alignment pair. In some embodiments, an alignment pair with an alignment score above a predetermined threshold is used for replacement in step (e)

[0225] In some embodiments, the method further comprises inserting the engineered antibody into a dual-function antigen binding molecule. In some embodiments, the dual-function antigen binding molecule is a molecule of the invention. In some embodiments, the method further comprises selecting a targeting antibody that binds to a protein on a target cell. In some embodiments, on a target cell is on the surface of a target cell. In some embodiments, the target cell is a cancer cell. In some embodiments, the protein is a receptor. In some embodiments, the protein is a cancer antigen. In some embodiments, the protein is a protein only expressed on cancer cells and not counterpart healthy cell. In some embodiments, the protein is a protein overexpressed on cancer cells as compared to counterpart healthy cells. In some embodiments, counterpart cell are cells of the same tissue or cell type as the cancer cells. In some embodiments, counterpart cells are control cells. In some embodiments, the selected targeting antibody is combined with the engineered antibody. In some embodiments, the combining produces the dual-function antigen binding molecule. In some embodiments, a dual-function antigen binding molecule is a bi-specific antibody.

[0226] In some embodiments, the engineered antibody comprises one heavy chain and one light chain. In some embodiments, the targeting antibody or antigen binding fragment comprises one heavy chain and one light chain. In some embodiments, the engineered antibody is a single chain antibody. In some embodiments, the targeting antibody is a single chain antibody. In some embodiments, a single chain antibody comprises a heavy chain variable domain and a light chain variable domain linked in a single polypeptide by an amino acid linker. In some embodiments, the heavy chain constant region of the targeting antibody is engineered to promote heterodimerization and/or inhibit homodimerization. In some embodiments, the heavy chain constant region of the engineered antibody is engineered to promote heterodimerization and/or inhibit homodimerization. In some embodiments, engineered is modified. In some embodiments, modified is mutated. In some embodiments, the method comprises engineering the constant regions of the engineered antibody and/or the targeting antibody. In some embodiments, the engineering is producing a set of mutations provided in Table 5. In some embodiments, the heavy chain constant regions used

comprise or consist of SEQ ID NO: 1074 and 1075 or analogs or homologs comprising at least 85% sequence identity. In some embodiments, the analogs or homologs retain the knob-in-holes mutations.

[0227] As used herein, the term “about” when combined with a value refers to plus and minus 10% of the reference value. For example, a length of about 1000 nanometers (nm) refers to a length of 1000 nm+−100 nm.

[0228] It is noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a polynucleotide” includes a plurality of such polynucleotides and reference to “the polypeptide” includes reference to one or more polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

[0229] In those instances where a convention analogous to “at least one of A, B, and C, etc.” is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., “a system having at least one of A, B, and C” would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase “A or B” will be understood to include the possibilities of “A” or “B” or “A and B.”

[0230] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0231] Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

[0232] Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

[0233] Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, “Molecular Cloning: A laboratory Manual” Sambrook et al., (1989); “Current Protocols in Molecular Biology” Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., “Current Protocols in Molecular Biology”, John Wiley and Sons, Baltimore, Maryland (1989); Perbal, “A Practical Guide to Molecular Cloning”, John Wiley & Sons, New York (1988); Watson et al., “Recombinant DNA”, Scientific American Books, New York; Birren et al. (eds) “Genome Analysis: A Laboratory Manual Series”, Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; “Cell Biology: A

Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells—A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Strategies for Protein Purification and Characterization—A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference. Other general references are provided throughout this document.

Materials and Methods

[0234] Cell penetration assay: The penetration ability of all Abs is screened by intracellular antibody detection followed by flow cytometry analysis. A fluorescent labeled anti-Fc-FITC is used to detect intracellular antibody. Briefly, breast cancer cells are seeded in 24 well plates and incubated for 6 hours with 0.5-1 uM of either control antibody or Trojan Ab. Next, cells are washed with PBS and low pH buffer (Glycine pH=2.5) to remove antibodies that are bound on the cell surface. The cells are then fixed, permeabilized and intracellularly fluorescently labeled by anti-Fc-FITC antibody (0.1% Saponin 1% BSA in PBS two hours at room temperature). Labeled cells are analyzed by flow cytometry for FITC and unlabeled cells are used as a negative control. To determine the level of proteasomal degradation of intracellular Trojan Abs a proteasomal inhibitor MG132 is added to cells prior to Trojan antibody addition.

[0235] Luciferase killing assay: Luciferase expressing target cancer cells (T) are seeded on 96-well plates and treated with the Trojan-Abs at various concentrations (0.5-4 μM). After 3-24 hours antigen-specific effector cells (E), specific to the relevant peptide within the Trojan-Ab, are added at various E:T ratios. Cells are then cocultured for 18-24 hours, followed by Luciferase substrate (Bio-Glo; Promega) addition. To quantify the number of viable cells, luminescence intensity (lum) is recorded. Killing is assessed by calculating % Killing=1−lum (Treated Target+Effectors)/Lum (Control non-Treated), this can also be calculated vs. other controls.

[0236] Image based killing assay (IncuCyte Imaging system): Target cells (T) are first labeled with a cytosolic red dye (IncuCyte® Cytolight Rapid Red) enabling tracking of target cell proliferation on the IncuCyte imager. Labeled target cell are then seeded on clear 96-well plates and treated with the Trojan-Abs at various concentrations (0.5-4 PM). After 3-24 hours antigen-specific effector cells (E), specific to the relevant peptide within the Trojan-Ab, are added at various E:T ratios. Next, plates are placed in the IncuCyte (37° C.; 5% CO2) and imaged at 2-hour intervals for 48 hours. Alternatively, a Caspase3/7 green dye was added to the media to enable apoptosis assessment of the target cells. Data is analyzed by the IncuCyte software analysis tool comparing two main parameters: Proliferation: The confluence of the red dye normalized to time 0 h; and Apoptosis: (Red+Green area)/Red-area normalized to time 0 h.

[0237] EGFR binding assay: To assess the ability of full Trojan antibodies (FTAbs) to bind EGFR on the cancer cell surface, a flow cytometry binding assay was established. Pre-plated cells are trypsinized, washed and then incubated on ice for 1 hour with 0.3 or 3 nM FTAb or EGFR bivalent therapeutic antibodies serving as a positive control. Then cells are washed with 2% FBS in PBS buffer three times and labeled with a fluorescent secondary antibody (anti-human Fc-FITC) for 30 minutes on ice. Cells are then washed with the same buffer three times and analyzed by flow cytometry. FTAbs binding is proportional to the FITC fluorescence level.

[0238] Presentation of HLA-peptide complex on the cancer cell surface: To quantify the level of presentation of HLA-peptide complex on the cancer cells upon incubation with FTAbs, TCR-like antibodies that are able bind specific HLA-peptide complex were used. Briefly, cells are seeded in 24 well plates and incubated for different time durations (4 h, 8 h and 10 h) with 1 M of FTAb or cFTAb (control FTAb) at 37C, 5% CO2. Next, cells are incubated on ice for 30 minutes with C1-17 antibody (see Lee et al. "Affinity Maturation of a T-Cell Receptor-Like Antibody Specific for a Cytomegalovirus pp65-Derived Peptide Presented by HLA-A*02:01" Int. J. Mol. Sci. 2021, 22, 2349, herein incorporated by reference in its entirety.) that is conjugated to fluorescein (FITC) and

analyzed by flow cytometry.

Example 1: Trojan Antibody Design

[0239] It is known that immunogenic peptides that are presented by cancer cells can lead to immune cell activation and cancer cell killing. Lymphocytes within the subject recognize the immunogenic peptide and the subject's immune system is brought to bear against the cancer cell. This mechanism can be harnessed by actively delivering the immunogenic peptide into cancer cells, who intern display the immunogenic peptide on their cell surface. Two classes of immunogenic peptides can be employed: 1) non-self-peptides/peptides not found in humans such as viral or bacterial peptides and 2) modified self-peptides which are human peptides that are mutated or otherwise altered to provide cancer specificity so that the immune system is not activated against healthy cells.

[0240] It has previously been hypothesized that the immunogenic peptide can be delivered via an antibody. Sefrin et al., 2019 “Sensitization of tumors for attack by virus-specific CD8+ T-cells through antibody-mediated delivery of immunogenic T cell epitopes”, Front. Immunol., August 21; 10:1962 made use of a full-sized antibody conjugated to a peptide via a disulfide bond. However, this construct may have very low stability, resulting in high rates of de-conjugation in serum. This in turn may lead to poor delivery to the target cells, reduced potency and high off target effects (side effects). Gaston et al., 2019, “Intracellular delivery of therapeutic antibodies into specific cells using antibody-peptide fusions”, Sci. Rep., December 10; 9(1):18688 employed a fusion protein construct in which the peptide was integrated into an end of the amino acid chain of the heavy or light chain or was placed next to the hinge domain within the chain. This method led to decreased production yield, increased aggregation and reduced in vitro stability.

[0241] To alleviate the problems inherent to these methods, rather than insert the immunogenic peptide at the end of the chain or between structures the immunogenic peptide is inserted into the antibody variable region. In particular, the immunogenic peptide is use to replace all or part of “inert”, non-essential CDRs within the heavy or light chain of the antibody (FIG. **1**). In silico modeling is used to identify inert CDR loops in known therapeutic antibodies. The inert CDR is then replaced by the immunogenic peptide (FIG. **2**). In this conformation the engineered antibody maintains high stability and antigen binding affinity. If the immunogenic peptide is shorter than the CDR filler amino acids can be added so as not to change the antibody conformation. Similarly, if the CDR is longer than the immunogenic peptide only a portion of the CDR is removed. Flanking regions next to the inert CDRs can also be replaced but their overall structure should be conserved as these regions are often important for overall antibody conformation. These engineered antibodies are referred to as Trojan antibodies.

Example 2: Trojan Antibody Uses

[0242] One use of the Trojan antibodies of the invention is dendritic cell vaccination. An antibody against a dendritic cell surface marker (e.g., CD40) is used for engineering. In this case anti-human CD40 antibody Fab516 is employed, and a modified self-antigen expressed by the target cancer is inserted into an inert CDR. After antibody binding to CD40, the antibody is digested and short peptides, including the immunogenic peptide, are displayed on the cell surface by MHCI molecules. CD8+ T cells are activated by these dendritic cells and become tumor specific leading to increased tumor cell killing (FIG. **3**).

[0243] The Trojan antibodies of the invention are also used for treating B cell malignancies, such as leukemia. In this instance an antibody against a B cell surface marker (e.g., CD20) is used. For example, the Arzerra antibody has an inert CDR replaced with a viral epitope (e.g., a CD4 viral epitope/epitope recognized by CD4 T cells). After endocytosis, the antibody is processed and presented on the B cell's surface by MHCII molecules. Cytotoxic T cells recognize the viral epitope and kill the malignant cells (FIG. **4**).

[0244] The Trojan antibodies of the invention are also used for treating solid tumors or general cancers. Antibodies that bind cancer specific epitopes such as HER2, EGFR, EpCAM and PSMA

are used and in particular bi-specific antibodies are employed. In the case of a bi-specific molecule only one of the antigen binding domains is required to bring the agent to the cancer cell; the other can be substituted with one or more peptides recognized by CD8+ T cells and/or with a cell penetrating component. It will of course be understood that a cell penetrating component can also be inserted into a non-bispecific antibody if there are two inert CDRs (one for the penetrating component and one for the immunogenic peptide). Alternatively, an antibody can be used which is known to be internalized, so long as insertion of the immunogenic peptide does not interfere with internalization. After binding the target protein, the antibody is digested and released to the cytosol (endosomal escape). From there it is displayed on the cancer cell's surface via MHCII molecules (FIG. **5**).

Example 3: Trojan Antibody Pipeline with Inert CDR Identification (FIG. **6**, Right Pathway)

[0245] Trojan antibodies are designed by first selecting an antibody of interest. Structural analysis of the binding of the antibody to its target is undertaken and inert CDRs are identified. An inert CDR is one that does not directly contact the protein target (a distance of at great than 5 angstroms from the protein target). A suitable immunogenic peptide that can replace the CDR is identified and inserted into the engineered antibody. The immunogenic peptide can be preselected, or an immunogenic peptide can be selected based on its similarity to the CRD being removed. Computational modeling is used to select the suitable peptide and confirm correct antibody conformation. This computational modeling is referred to as the minimal perturbation replacement (MBR) and it can be applied to optimize the replacement location within the CDR. For example, if the CDR is longer than the immunogenic peptide, filling amino acids may need to be added for minimal perturbation of antigen binding. Similarly, if the CDR is shorter than the immunogenic peptide, amino acids from the inter-CDR regions may also be removed. Flanking amino acids are often also involved in binding and antibody confirmation and structural similarity/minimal perturbation in the flanking regions is also desirous. The MBR optimizes the positioning of the immunogenic peptide.

[0246] If an antibody is selected without an obvious inert CDR, the MBR is applied to rank possible replacement locations within the antibody and specifically the CDRs. If a location can be found that does not perturb binding (or perturbs it by less than a predetermined threshold) than the immunogenic peptide is inserted in this location.

[0247] The MBR analysis takes into account the canonical structure of the antibody and selects positions/replacement location that most closely maintains this structure. Antibody stem preservation is highly conserved. The stem refers to the section of the beta-sheet directly adjacent to the CDR loop. This area is also referred to as the CDR flanking region. These 1-5 amino acids are important for loop position and tend to be highly rigid, whereas the loop itself, especially an inert CDR loop, may be less ordered. Stem preservation is thus important during MBR analysis. The MBR also takes into account the position frequency matrix of other known antibodies. The computational model has imputed hundreds of known antibodies and the frequency of each amino acid at each position is considered. Peptide replacement that most closely conforms to the positional frequency of the known antibodies is preferred. Finally, the MBR outputs the optimal location within the antibody for peptide replacement, or if there is more than one acceptable location it outputs a hierarchy positions.

Example 4: Trojan Antibody Pipeline by Sequence Similarity (FIG. **6**, Left Pathway)

[0248] Trojan antibodies can alternatively be generated based on sequence similarity. A target antibody is selected, as is a list of immunogenic peptides (see for example Tables 1-3). Pairwise sequence alignment is done between the various viral peptides and the antibody. Overlapping peptides within the antibody of 8-11 amino acids are compared with the immunogenic peptides and an alignment score is given for each pair (1 peptide from the antibody and one immunogenic peptide). The score was calculated based on blosum62 and a palanty of −3 per gap opening and per gap extension. A threshold was set for acceptable alignment (for example >25 was used in the

below example) Immunogenic peptides and the peptides in the antibody to be replaced that meet the threshold for alignment score are selected and the MBR is applied to optimize replacement position.

Example 5: Trojan Antibody Functional Confirmation (Inert CDRs)

[0249] Replacement based on sequence similarity is not limited to CDRs but can be done anywhere in the antibody. Because of the similarity in sequence, minimal perturbation is expected. Nevertheless, this method and the inert CDR replacement method still requires confirmation that the antibody has retained its functionality. In particular, it must be determined that the antibody will still bind to its target and is still internalized into cells. If binding/internalization is abolished than the new antibody is of no use.

[0250] The first antibody selected for insertion of an immunogenic peptide was a DNA hydrolyzing antibody 3D8, also known as TMab4. The mouse antibody 3D8 was first disclosed in Kim et al., "Heavy and Light Chain Variable Single Domains of an Anti-DNA Binding Antibody Hydrolyze Both Double- and Single-stranded DNAs without Sequence Specificity", J Biol Chem., 2006, June 2; 281(22):15287-9 and its humanized form was provided in International Patent Application Wo2019/244086. TMab4 was shown to bind to and penetrate into cancer cells and reach the cytoplasm via endosomal escape, thus it was selected for conversion into a Trojan antibody. It was found that CDRs 1, 2 and 3 of the heavy chain (CDRH1, CDRH2, CDRH3) and CDR 3 of the light chain (CDRL3) were inert CDRs.

[0251] Initially, the ability to replace CDRH1 of TMab4 was tested. Two peptides from CMV, two peptides from EBV and one peptide from influenza were inserted into various positions in CDRH1 (see Table 6). Penetration superior to that of the negative control (Adalimumab) was retained for all inserted peptides regardless of positioning within CDRH1. However, some insertions showed superior penetrance, while others diminished penetrance as compared to the parental TMab4. Several of the constructs included the replacement of a flanking amino acids next to the CDR. This was done as these additional replacements were predicted to reduce perturbation of the overall antibody conformation. Specifically, in construct T1, the "S" directly N-terminal to the CDR was also replaced. In constructs T 32, T_33 and T_34 the "CAAS" directly N-terminal to the CDR was also replaced. In constructs T 42 and T_43 the "AAS" directly N-terminal to the CDR was also replaced. One construct, T1_34, included a compensatory mutation (T30S) in the heavy chain that was predicted to reduce perturbation of the antibody. Thus, though overall alteration of the inert CDR did not abolish penetrance, as expected, it did have an impact on the overall ability to enter the cell.

TABLE-US-00006 TABLE 6 Trojan antibodies with insertion into CDRH1 of TMab4. (PC)-positive control; (NC)-Negative control; (Tab)-Trojan antibody; (The relative penetration of N1 is given as an average of all replicates.) PC/ Location of Sequence of NC/ Peptide replaced replaced Relative Ab name TAb (SEQ ID NO:) Location fragment fragment penetration P1 (TMab4) PC — — — — 100.00% N1 NC — — — — — 50.72% (Adalimumab) T1 TAb CMV CDRH1 VH 25-33 SGYTFTSYV 104.54% NLVPMVATV (1) T2 TAb CMV CDRH1 VH 26-33 GYTFTSYV 84.06% NLVPMVATV (1) T3 TAb CMV CDRH1 VH 26-32 GYTFTSY 96.92% NLVPMVATV (1) T4 TAb CMV CDRH1 VH 27-33 YTFTSYV 81.45% NLVPMVATV (1) T5 TAb CMV CDRH1 VH 28-33 TFTSYV 83.25% NLVPMVATV (1) T1_32 TAb EBV CDRH1 VH 22-30 CAASGYTFT 89.85% CLGGLLTMV (2) T1_33 TAb EBV CDRH1 VH 22-29 CAASGYTF 155.93% CLGGLLTMV (2) T1_34 TAb EBV CDRH1 VH 22-29 CAASGYTF 106.10% CLGGLLTMV (2) T1_35 TAb FLU CDRH1 VH 26-31 GYTFTS NA GILGFVFTL (3) T1_42 TAb EBV CDRH1 VH 23-32 AASGYTFTSY 115.37% AVFDRKSDAK (4) T1_43 TAb EBV CDRH1 VH 23-31 AASGYTFTS 101.66% AVFDRKSDAK (4) T1_46 TAb CMV CDRH1 VH 26-32 GYTFTSY 81.46% QYDFVAALF (5)

[0252] Several of the generated antibodies were tested for their ability to kill target cells in the

presence of effector cells specific to the immunogenic peptide present in the Trojan antibody. As can be seen in FIG. **7**A, though all antibodies were still able to penetrate the cell, the ability to induce specific killing was highly variable. Of the five insertions of the NLVPMVATV (SEQ ID NO: i) peptide two produced no specific killing and while the other three did With respect to peptide CLGGLLTMV (SEQ ID NO: 2) the two insertions that produced superior penetration also produced superior killing.

[0253] Next, insertion of SEQ ID NO: 1 and SEQ ID NO: 3 into CDRH3 was tested (Table 7). SEQ ID NO: 3 showed some structural similarity to the CDR it is replacing, in particular the “G” near the beginning of the CDR was maintained, and so it was tested along with SEQ ID NO: 1. It had previously been reported that CDRH3 was completely dispensable for antibody binding and cell penetration (see Lee et al., “Functional Consequences of Complementarity-determining Region Deactivation in a Multifunctional Anti-nucleic Acid Antibody”, J Biol Chem. 2013 Dec. 13; 288(50):35877-85, hereby incorporated by reference in its entirety.). However, insertion of SEQ ID NO: 1 greatly impaired penetrance such that it was only slightly superior to the negative control antibody. In contrast, insertion of SEQ ID NO: 3 invariably produced penetration with two of the insertion positions as good if not better than the parental antibody. This all suggests that selection of the immunogenic peptide to match the insertion location is highly important.

TABLE-US-00007 TABLE 7 Trojan antibodies with insertion into CDRH3 of TMab4. (PC)-positive control; (NC)-Negative control; (Tab)-Trojan antibody; (The relative penetration of N1 is given as an average of all replicates.) PC/ Location Sequence of NC/ Peptide of replaced replaced Relative Ab name TAb (SEQ ID NO:) Location fragment fragment penetration P1 (TMab4) PC — — — — 100.00% N1 NC — — — — 50.72% (Adalimumab) T7 TAb CMV CDRH3 VH 100-108 AYKRGYAMD 84.45% NLVPMVATV (1) T8 TAb CMV CDRH3 VH 99-106 GAYKRGYA 60.39% NLVPMVATV (1) T9 TAb CMV CDRH3 VH 99-105 GAYKRGY 53.97% NLVPMVATV (1) T10 TAb CMV CDRH3 VH 100-105 AYKRGY 59.90% NLVPMVATV (1) T11 TAb CMV CDRH3 VH 100-106 AYKRGYA 62.05% NLVPMVATV (1) T12 TAb CMV CDRH3 VH 101-105 YKRGY 51.82% NLVPMVATV (1) T13 TAb CMV CDRH3 VH 101-106 YKRGYA 57.99% NLVPMVATV (1) T14 TAb CMV CDRH3 VH 101-104 YKRG 63.08% NLVPMVATV (1) T1_39 TAb FLU CDRH3 VH 99-107 GAYKRGYAM 75.15% GILGFVFTL (3) T1_40 TAb FLU CDRH3 VH 99-106 GAYKRGYA 148.88% GILGFVFTL (3) T1_41 TAb FLU CDRH3 VH 99-105 GAYKRGY 94.74% GILGFVFTL (3)

[0254] Interestingly, when specific killing was tested with the Trojan antibodies containing SEQ ID NO: 1 inserted into CDRH3 are surprising result was observed. The two Tabs with the lowest measured penetrance demonstrated specific killing (FIG. **7**B). It may be that the low detection within the cell was due to a rapid antibody processing and display on the cell surface leading to effective killing.

[0255] Insertion of SEQ ID NO: 1 and SEQ ID NO: 5 into CDRL3 and insertion of SEQ ID NO: 1 into CDRH2 was also tested (Table 8). SEQ ID NO: 5 had very high sequence alignment with CDRL3 and so was selected for testing. Several of the constructs included the replacement of a flanking amino acids next to the CDR. This was done as these additional replacements were predicted to reduce perturbation of the overall antibody conformation. Specifically, in constructs T1_44 and T1_45, the “F” directly C-terminal to the CDR was also replaced. In constructs T18 the “Y” directly C-terminal to the CDR was also replaced. All of the tested insertions produced good penetrance essentially equivalent to the parental antibody. This result is highly important as CDRL3 has been reported to be important for cell penetration, and this function was not perturbed by replacement of this CDR with the tested immunogenic peptides.

TABLE-US-00008 TABLE 8 Trojan antibodies with insertion into CDRL3 and CDRH2 of TMab4. (PC)- positive control; (NC)-Negative control; (Tab)-Trojan antibody; (The relative penetration of N1 is given as an average of all

replicates.) PC/ Location of Sequence of NC/ Peptide replaced replaced Relative Ab name TAb (SEQ ID NO:) Location fragment fragment penetration P1 (TMab4) PC — — — — 100.00% N1 NC — — — — 50.72% (Adalimumab) T15 TAb CMV CDRL3 VL 97-103 YYYHMYT 103.87% NLVPMVATV (1) T16 TAb CMV CDRL3 VL 98-103 YYHMYT 92.94% NLVPMVATV (1) T1_44 TAb CMV CDRL3 VL 96-104 QYYYHMYTF 100.63% QYDFVAALF (5) T1_45 TAb CMV CDRL3 VL 98-104 YYHMYTF 94.69% QYDFVAALF (5) T18 TAb CMVNLVPMVATV CDRH2 VH 52-59 NPYNDGNY 111.90% (1)

[0256] Specific killing was tested for the Trojan antibodies containing SEQ ID NO: 1 and in this case all of the antibodies were found to induce killing (FIG. **7**C). This indicates that this insertion produces highly measured penetrance and high levels of specific killing. Trojan antibodies are also generating containing a combination of inert CDR replacements. As 4 CDRs are inert, combinations of 2, 3 and 4 immunogenic peptides are generated. The inserted peptides can be repeats of the same peptide or different peptides.

[0257] A second DNA binding antibody capable of penetrating into cells was also tested. Antibody 3E10 (see Weisbart et al., "DNA-dependent targeting of cell nuclei by a lupus autoantibody", Sci Rep. 2015 Jul. 9; 5:12022, hereby incorporated by reference in its entirety) was examined for inert CDRs and it was determined that CDRL1 and CDRL2 both did not engage the antigen during binding. SEQ ID NO: 1 was inserted into either CDRL1 or CDRL2 and SEQ ID NO: 3 was also inserted into CDRL1 (Table 9). One of the constructs included the replacement of a flanking amino acids next to the CDR. This was done as the additional replacements were predicted to reduce perturbation of the overall antibody conformation. Specifically, in construct T2_13, the "LLIK" directly N-terminal to the CDR and "YL" directly C-terminal to the CDR were also replaced. All of the SEQ ID NO: 1 insertions produced penetration above the negative control, although once again the levels of penetration varied.

TABLE-US-00009 TABLE 9 Trojan antibodies with insertion into CDRL1 and CDRL2 of 3E10. (PC)- positive control; (NC)-Negative control; (Tab)-Trojan antibody. PC/ Location of Sequence of NC/ Peptide replaced replaced Relative Ab name TAb (SEQ ID NO:) Location fragment fragment penetration P2 (3E10) PC — — — — 100.00% N2 NC — — — — 54.70% (Muromonab) T2_6 TAb CMV CDRL1 VL 27-35 KSVSTSSYS 80.61% NLVPMVATV (1) T2_11 TAb CMV CDRL1 VL 28-36 SVSTSSYSY 98.50% NLVPMVATV (1) T2_12 TAb FLU CDRL1 VL 28-36 SVSTSSYSY X GILGFVFTL (3) T2_13 TAb CMV CDRL2 VL 50-58 LLIKYASYL 72.17% NLVPMVATV (1)

[0258] Killing with peptide specific effector cells was also tested for cancer cells treated with the Trojan antibodies derived from 3E10 and including SEQ ID NO: 1. Both tested TAbs produced robust specific killing (FIG. **8**). These results demonstrate the universality of the Trojan system. Immunogenic peptides can be inserted into a variety of antibodies that can penetrate into cells and produce peptide specific killing.

[0259] All TAbs generated are cultured with cancer cells and penetrance is determined. Penetration is observed for all peptides which are determined to retain antibody conformation upon replacement of inert CDRs. All TAbs are tested for their ability to induce specific cancer cell killing in the presence of peptide specific effector cells. It will be understood by a skilled artisan that the specific type of cancer tested is not important as all cancer cells are able to bring HLA complex up to the cell surface and thereby display the immunogenic peptides of the TAbs. TAbs are able to produce enhanced specific cell killing above that of the parental antibody that does not have an immunogenic peptide. Trojan antibodies are also generating containing a combination of inert CDR replacements. As 2 CDRs are inert, combinations of two immunogenic peptides are generated. The inserted peptides can be repeats of the same peptide or different peptides.

Example 6: Trojan Antibody Functional Confirmation (Sequence Similarity)

[0260] Replacement based on sequence similarity was also performed for the TMab4 antibody. A screen of immunogenic peptides (see Table 3) found only 0.0057% of tested peptides were above

the alignment score threshold (>25) for possible replacement. The three with the most significant similarity to the TMab4 antibody were selected for replacement. SEQ ID NO: 6 from EBV corresponded to amino acids 28-35 within the heavy chain of the antibody. This includes two amino acids (“MH”) flanking the CDRH1 on its C-terminus. A compensatory mutation in the heavy chain (Y27D) was also made to reduce perturbation of the antibody. SEQ ID NO: 7 from SARS-CoV2 corresponded to amino acids 52-60 within the heavy chain of the antibody. This includes two amino acids (“YY”) flanking the CDRH2 on its C-terminus. SEQ ID NO: 8 from EBV corresponds to amino acids 14-22 in the first framework region on the light chain of the antibody. These three TAbs were generated, and penetrance was tested as before (Table 10). Although all three showed penetrance that was above that of the negative control SEQ ID NO: 7 and SEQ ID NO: 8 produced penetrance that was as good or better than the parental antibody, while SEQ ID NO: 6 produced worse penetrance.

TABLE-US-00010 TABLE 10 Trojan antibodies with insertion based on sequence similarity into TMab4. (PC)-positive control; (NC)-Negative control; (Tab)-Trojan antibody; (The relative penetration of N1 is given as an average of all replicates.) PC/ Location of Sequence of NC/ Peptide replaced replaced Relative Ab name TAb (SEQ ID NO:) Location fragment fragment penetration P1 (TMab4) PC — — — — 100.00% N1 NC — — — — 50.72% (Adalimumab) T19 TAb EBV CDRH1 VH 28-35 TFTSYVMH 53.97% SVSSSISSL (6) T1_30 TAb Cov2 CDRH2 VH 52-60 NPYNDGNYY 117.01% LPFNDGVYF (7) T1_31 TAb EBV FW1L VL 14-22 SVGDRVTIT 115.18% FLGERVTLT (8)

[0261] The sequence similarity method was also used to modify the 3E10 antibody. A screen of immunogenic peptides (see Table 3) found only 0.0093% of tested peptides were above the alignment score threshold (>25) for possible replacement. SEQ ID NO: 6 from EBV corresponded to amino acids 28-36 within CDRLS of the antibody. SEQ ID NO: 9 from SARS-CoV2 corresponded to amino acids 50-58 within the light chain of the antibody. This includes four amino acids (“LLIK”) flanking the CDRL2 on its N-terminus and two amino acids (“YL”) flanking the CDRL2 on its C-terminus. Both TAbs were generated and were found to have penetrance into cells that was comparable to the parental antibody (Table 11).

TABLE-US-00011 TABLE 11 Trojan antibodies with insertion based on sequence similarity into 3E10. (PC)-positive control; (NC)-Negative control; (Tab)-Trojan antibody PC/ Location of Sequence of NC/ Peptide replaced replaced Relative Ab name TAb (SEQ ID NO:) Location fragment fragment penetration P2 (3E10) PC — — — — 100.00% N2 NC — — — — 54.70% (Muromonab) T2_20 TAb EBV CDRL1 VL 28-36 SVSTSSYSY 99.64% SVSSSISSL (6) T2_23 TAb COV2 CDRL2 VL 50-58 LLIKYASYL 98.32% LLALHRSYL (9)

[0262] Finally, a third antibody DNA binding antibody, 71F12 (see Sakakibara et al., “Clonal evolution and antigen recognition of anti-nuclear antibodies in acute systemic lupus erythematosus”, Sci Rep. 2017; 7: 16428, hereby incorporated by reference in its entirety), was analyzed for inert CDRs and it was determined that CDRL1 was inert. Substitutions were made based on sequence similarity and SEQ ID NO: 10 from Adenovirus corresponded to amino acids 26-34 within CDRL1 of the antibody (called T4_1). SEQ ID NO: 11 from HPV corresponds to amino acids 17-24 in the first framework region on the heavy chain of the antibody (called T4_3). Both TAbs are generated and are found to have penetrance into cells that was at least comparable to the parental antibody.

[0263] All generated TAbs are also tested for specific cell killing as described hereinabove. TAbs that successfully enter the cell are able to induce cell killing that is superior to the parental antibody that does not include killing.

Example 7: Further Confirmation of Trojan Antibody Killing

[0264] Killing by the Trojan antibodies was confirmed by a second method. An IncuCyte Imaging

system was used to follow the cancer cells and quantify apoptosis by the presence of caspace-3/7 dye (see Materials and Methods). TAbs derived from both the TMab4 and 3E10 antibodies (T18 and T2_11) were selected for further investigation as both had demonstrated good killing. As can be seen in FIGS. **9**A-**9**B both TAbs produced specific cell killing well above that produced by the effector cells alone or by effector cells cocultured with cell treated with the parental antibody. This shows that the immunogenic peptides are not only transferred into the target cells, but are processed and displayed on the cell surface which enables specific killing by effector cells.

[0265] The other TAbs are also tested using the IncuCyte Imaging assay. Specific killing above that induced by the effector cells alone and/or the parental antibody is observed with the TAbs of the invention.

[0266] The sequences of the various TAbs produced, and the parental antibodies are summarized in Table 12. It will be understood that when a heavy chain contains the immunogenic peptide the light chain of the parent is used to make the TAb and when the light chain contains the immunogenic peptide the heavy chain of the parent is used to make the TAb. Notably, the 3E10 antibody has two variants (called P2 and P3) with different light chains. Either can be used for generating TAbs.

TABLE-US-00012 TABLE 12 Antibody sequences SEQ SEQ Name Chain ID NO: Name Chain ID NO: TMab04- P1 VH 1021 T1_30 VH 1045 TMab04- P1 VL 1022 T1_31 VL 1046 3E10-P2/P3 VH 1023 T1_32 VH 1047 3E10-P2 VL 1024 T1_33 VH 1048 3E10-P3 VL 1025 T1_34 VH 1049 71F12-P4 VH 1026 T1_35 VH 1050 71F12-P4 VL 1027 T1_39 VH 1051 T1 VH 1028 T1_40 VH 1052 T2 VH 1029 T1_41 VH 1053 T3 VH 1030 T1_42 VH 1054 T4 VH 1031 T1_43 VH 1055 T5 VH 1032 T1_44 VL 1056 T7 VH 1033 T1_45 VL 1057 T8 VH 1034 T1_46 VH 1058 T9 VH 1035 T1_47 VH 1059 T10 VH 1036 T2_20 VL 1060 T11 VH 1037 T2_23 VL 1061 T12 VH 1038 T2_6 VL 1062 T13 VH 1039 T2_11 VL 1063 T14 VH 1040 T2_12 VL 1064 T15 VL 1041 T2_13 VL 1065 T16 VL 1042 T4_1 VL 1066 T18 VH 1043 T4_3 VH 1067 T19 VH 1044

[0267] Various known therapeutic antibodies were examined for inert CDRs. Crystal structure analysis for each CDR in contact with its antigen was performed and the number of amino acids contacting the antigen was counted. A distance of less than 5 angstroms between an amino acid of the CDR and an amino acid of the antigen was considered contact. More than 2 amino acids in contact with the antigen indicates a CDR involved in binding. Fewer than 2 indicates a definitely inert CDR. Exactly 2 indicates a CDR that is unlikely to be involved in binding or lowly involved. For purposes of evaluation all such CDRs are considered inert. Numerous other antibodies were found that contained inert CDRs which could be replaced with immunogenic peptides such as those provided in Tables 1-3. The MBR is used to optimize positioning. Alternatively, TAbs are designed by identifying peptides from Table 3 with sequence homology to known antibodies and specifically those with inert CDRs. Peptides with high homology are swapped into the antibody to produce TAbs. All produced Tabs are checked for cell penetrance and penetrance is retained. TAbs are also check for specific cell killing as described hereinabove and levels of killing above those produced by the parental antibody are observed.

Example 8: Trojan Antibody Combined with a Targeting Antibody (Bispecific Trojan, Bi-TAb)

[0268] The TAbs described hereinabove can be considered killing modules that they have been shown to enter into target cells and induce specific killing by effector cells primed against the immunogenic peptide. However, in order to increase the specificity of these molecules, target them to cancer cells and reduce off-target effects a targeting module was added. Three EGFR antibodies were selected to use as the targeting module: Cetuximab, Panitumumab and Necitumumab. All three are known to specifically bind to EGFR and to target to EGFR overexpressing cancers. Antibodies targeting to other cancer specific/overexpressed molecules are also possible. As this is merely the targeting module, and is distinct from the killing module, any known cancer targeting antibody can be used. EGFR is used merely as a proof of concept.

[0269] The T18 and T2_11 TAbs were used for the generation of bi-TAbs as a proof of principle. Control bi-functional antibodies were also generated using the TMab4 or 3E10 parental antibodies.

The bispecific molecules were generated using the known knob-in-holes approach for restricting heavy chain homodimerization. The unique modifications are made in the constant region of the heavy chain of both the killing module and the targeting module (see Table 13, see the method provided in Shatz et al., “Knob-into-holes antibody production in mammalian cell lines reveals that asymmetric afucosylation is sufficient for full antibody-dependent cellular cytotoxicity”, 2013, mAbs 5:6, 872-881, herein incorporated by reference in its entirety). This promotes heterodimerization between the different heavy chains and discourages homodimerization. The produced bi-Tabs were found to have at least 90% purity for the desired dual-function molecule. Schematics of the final bi-TAbs and their controls are shown in FIG. **10** and the full sequences are provided in Table 13.

TABLE-US-00013 TABLE 13 Sequences of targeting arms and bifunctional antibodies. SEQ ID Name Chain NO: cFTAb1_arm1_P2 LC 1077 cFTAb1_arm1_P2 HC 1078 cFTAb1_arm2_Cetuximab LC 1079 cFTAb1_arm2_Cetuximab HC 1080 cFTAb2_arm1_P2 LC 1077 cFTAb2_arm1_P2 HC 1078 cFTAb2_arm2_Panitumumab LC 1081 cFTAb2_arm2_Panitumumab HC 1082 cFTAb3_arm1_P1 LC 1083 cFTAb3_arm1_P1 HC 1084 cFTAb3_arm2_Cetuximab LC 1079 cFTAb3_arm2_Cetuximab HC 1080 cFTAb4_arm1_P1 LC 1083 cFTAb4_arm1_P1 HC 1084 cFTAb4_arm2_Panitumumab LC 1081 cFTAb4_arm2_Panitumumab HC 1082 cFTAb5_arm1_P2 LC 1077 cFTAb5_arm1_P2 HC 1078 cFTAb5_arm2_Necitumumab LC 1085 cFTAb5_arm2_Necitumumab HC 1086 FTAb1_arm1_T2_11 LC 1087 FTAb1_arm1_T2_11 HC 1088 FTAb1_arm1_Cetuximab LC 1079 FTAb1_arm1_Cetuximab HC 1080 FTAb2_arm1_T2_11 LC 1087 FTAb2_arm1_T2_11 HC 1088 FTAb2_arm2_Panitumumab LC 1081 FTAb2_arm2_Panitumumab HC 1082 FTAb3_arm1_T18 LC 1089 FTAb3_arm1_T18 HC 1090 FTAb3_arm2_Cetuximab LC 1079 FTAb3_arm2_Cetuximab HC 1080 FTAb4_arm1_T18 LC 1089 FTAb4_arm1_T18 HC 1090 FTAb4_arm2_Panitumumab LC 1081 FTAb4_arm2_Panitumumab HC 1082 FTAb5_arm1_T2_11 LC 1087 FTAb5_arm1_T2_11 HC 1088 FTAb5_arm2_Necitumumab LC 1085 FTAb5_arm2_Necitumumab HC 1086

[0270] The bi-TAbs were tested for their ability to bind surface EGFR on breast cancer cells that express high levels of EGFR. It is predicted that the additional killing module should not impact surface binding of the targeting module and indeed that is what is observed (FIG. **11**). The bi-TAbs at either a concentration of 0.3 nM or 3 nM successfully bound surface EGFR and at a level comparable to the control original anti-EGFR antibody. Thus, the bi-TAbs can effectively target to cancer cells based on the targeting module selected.

[0271] Next, the bi-TAbs ability to bring the immunogenic peptide on to the surface of the cancer cells in complex with an HLA molecule was tested. HLA-peptide display was measured as described hereinabove (Materials and Methods). A time course experiment was performed to monitor surface display over time. All five of the bi-TAbs successfully brought the peptide to the cell surface by 10 hours of incubation (FIG. **12**). All bi-Tabs were superior to their control counterparts.

[0272] Finally, cancer cell killing was examined at various effector cell concentration. Bi-TAbs 3-5 each with a different targeting module were tested at effector:target cell (E:T) concentrations of 1:1, 3:1 and 6:1. At a ratio of 6:1 all three antibodies induced specific killing above what is observed with the control antibodies (FIG. **13**). Bi-TAbs 4 and 5 (one which contains the P1 derived TAb and one which contains the P2 derived TAb) also showed increased killing at ratios of 3:1 and even 1:1. A culture without effector cells was used as a control. These results demonstrate the high level of effectiveness of the bi-TAb as it combines cancer cell targeting with high levels of specific killing.

[0273] The three anti-EGFR antibodies used themselves contain inert CDRs. A summary of these inert CDRs is provided in Table 14. CDRH1, CDRL1 and CDRL2 of Cetuximab, CDRL1 and CDRL2 of Panitumumab and CDRL1 and CDRL2 of Necitumumab were all found to be inert.

These CDRs are also replaced with immunogenic peptides. The targeting of the replaced antibodies is confirmed and the ability to bind to EGFR and target to EGFR overexpressing cancer cells is retained. The combination of the modified targeting module to the killing module increases the number of immunogenic peptides delivered. The peptide used in the killing module and the peptide used in the targeting module can be the same or different.

TABLE-US-00014 TABLE 14 Inert CDRs in anti-EGFR antibodies. CDRH1 CDRH2 CDRH3 CDRL1 CDRL2 CDRL3 Inert Antibody score score score score score score CDRs Cetuximab 1 >2 >2 2 1 >2 H1, L1, L2 Panitumumab >2 >2 >2 2 1 >2 L1, L2 Necitumumab >2 >2 >2 2 0 >2 L1, L2

[0274] Bi-TAbs are generated with the other killing modules and various targeting modules. The bi-Tabs are evaluated as above to cancer cell targeting, HLA-peptide surface display and for specific killing. Bi-Tabs are superior to the control bi-functional molecule and are able to target to cancers, induce peptide display and ultimately enhance killing of the target cells by effector cells specific to the immunogenic peptide.

[0275] In vivo validation is also performed. The engineered bi-Tabs are injected into immune competent mice expressing a tumor targetable by the targeting module. Control bi-functional molecules lacking the immunogenic peptide and/or binding a non-cancer related target are also administered. Animal survival over time is monitored as is tumor size. The Trojan antibodies are found to shrink tumors and/or extend survival time in a statistically significant manner, indicating that they activate the immune system against the cancer. Mice may be vaccinated with the immunogenic peptide beforehand.

[0276] Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

Claims

1. A dual-function antigen binding molecule comprising: a. a first antibody or antigen binding fragment thereof comprising at least one immunogenic peptide inserted into a CDR of said antibody or antigen binding fragment thereof, and where said insertion comprises removal of CDR sequence; and b. a second antibody capable of binding epidermal growth factor receptor (EGFR), wherein said antibody is selected from cetuximab, panitumumab and necitumumab or antibody comprising at least 85% sequence identity thereto.

2. The dual function antigen binding molecule of claim 1, wherein said first antibody or antigen binding fragment thereof and said second antibody comprise at least one modification that promotes heterodimerization and inhibit homodimerization, and wherein one of said first and second antibody comprises a heavy chain constant region comprising SEQ ID NO: 1074 and the other antibody comprises a heavy chain constant region comprising SEQ ID NO: 1075.

3. (canceled)

4. The dual function antigen binding molecule of claim 1, wherein said first antibody or antigen binding fragment thereof binds to a target cell, and wherein said target cell is a cancer cell and a cancer cell antigen is selected from HER2, EGFR, EpCAM, PSMA, BCMA, CD123, CD33, CD38, CTLA, LAG-3, ICOS, 4-1BB and PD-L1, a dendritic cell and a dendritic cell antigen is selected from CD40, CD205, CD206, CLEC9A, CLEC12A, CD209, and CD207 or both.

5. The dual function antigen binding molecule of claim 1, wherein said immunogenic peptide is a cancer specific peptide, is a viral peptide, is a peptide recognized by CD4 T cells, CD8 T cells or a combination thereof.

6. The dual function antigen binding molecule of claim 5, wherein said peptide is a cancer specific peptide and is selected from a peptide sequence provided in Table 1.

7. (canceled)
8. The dual function antigen binding molecule of claim 5, wherein said peptide is a viral peptide is-derived from Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), Adenovirus, Human papilloma virus (HPV) or Influenza virus (FLU) and is selected from a peptide sequence provided in Table 2 or Table 3.
9. (canceled)
10. The dual function antigen binding molecule of claim 1, further comprising a cell penetration sequence that targets said first antibody or antigen binding fragment thereof to a cytoplasm of a cell bound by said first antibody or antigen binding fragment thereof and wherein said cell penetration sequence is an endosomal escape domain (EED).
11. (canceled)
12. (canceled)
13. The dual function antigen binding molecule of claim 1, wherein said CDR is an inert CDR having little or no contribution to binding to a target antigen.
14. The dual function antigen binding molecule of claim 13, wherein an inert CDR comprises two or fewer amino acids that contact said target antigen and wherein contact comprises a distance of not more than 5 angstroms between an amino acid of a CDR and an amino acid of said target antigen.
15. (canceled)
16. The dual function antigen binding molecule of claim 1, wherein said insertion and removal produces no change or minimal change in the overall conformation of said first antibody or antigen binding fragment thereof such that said first antibody or antigen binding fragment thereof binds its target antigen at an equivalent affinity to said first antibody or antigen binding fragment devoid of said immunogenic peptide.
17. The dual function antigen binding molecule of claim 1, wherein at least one inert CDR of an antigen binding region is replaced with a cell penetration sequence.
18. (canceled)
19. (canceled)
20. (canceled)
21. (canceled)
22. The dual function antigen binding molecule of claim 1, wherein within said first antibody or antigen binding fragment thereof said antigen binding region, said immunogenic peptide and said cell penetrating sequence are each separated by a linker, wherein said dual function antigen binding molecule is devoid of a chemical linker, or both.
23. (canceled)
24. The dual function antigen binding molecule of claim 1, wherein said first antibody before said immunogenic peptide is inserted is selected from: a. antibody TMab4 comprising a heavy chain variable region of SEQ ID NO: 1021 and a light chain variable region of SEQ ID NO: 1022; b. antibody 3E10 comprising a heavy chain variable region of SEQ ID NO: 1023 and a light chain variable region of SEQ ID NO: 1024; and c. antibody 71F12 comprising a heavy chain variable region of SEQ ID NO: 1026 and a light chain variable region of SEQ ID NO: 1027; and d. said immunogenic peptide is inserted into CDRH1, CDRH2, CDRH3 or CDRL3 of said TMab4, CDRL1 or CDRL2 of said 3E10 or CDRL1 of said 71F12.
25. (canceled)
26. The dual function antigen binding molecule of claim 24, wherein said first antibody comprises at least one of: a. a light chain variable region of SEQ ID NO: 1022 and a heavy chain variable region selected from SEQ ID NO: 1028-1040, 1043-1045, 1047-1055, and 1058-1059; b. a heavy chain variable region of SEQ ID NO: 1021 and a light chain variable region selected from SEQ ID NO: 1041-1042, 1046, and 1056-1057; c. a heavy chain variable region of SEQ ID NO: 1023 and a light chain variable region selected from SEQ ID NO: 1060-1065; d. a heavy chain variable region

of SEQ ID NO: 1026 and a light chain variable region of SEQ ID NO: 1066; and e. a light chain variable region of SEQ ID NO: 1027 and a heavy chain variable region of SEQ ID NO: 1067; said dual function antigen binding molecule comprises two heavy chains and two light chains and wherein: a. said two heavy chains are SEQ ID NO: 1088 and 1080 and said two light chains are SEQ ID NO: 1087 and 1079; b. said two heavy chains are SEQ ID NO: 1088 and 1082 and said two light chains are SEQ ID NO: 1087 and 1081; c. said two heavy chains are SEQ ID NO: 1090 and 1080 and said two light chains are SEQ ID NO: 1089 and 1079; d. said two heavy chains are SEQ ID NO: 1090 and 1082 and said two light chains are SEQ ID NO: 1089 and 1081; or e. said two heavy chains are SEQ ID NO: 1088 and 1086 and said two light chains are SEQ ID NO: 1087 and 1085; or both.

27. (canceled)

28. An antibody or antigen binding fragment thereof comprising at least one immunogenic peptide inserted into a variable region of said antibody or antigen binding fragment thereof, and where said insertion comprises removal of antibody or antigen binding fragment sequence.

29. A pharmaceutical composition comprising a dual-function antigen binding molecule of claim 1 and a pharmaceutically acceptable carrier excipient or adjuvant.

30. A nucleic acid molecule comprising at least one open reading frame, wherein said open reading frame encodes a dual-function antigen binding molecule of claim 1, optionally wherein said nucleic acid molecule is an expression vector and comprises at least one regulatory element operatively linked to said open reading frame.

31. (canceled)

32. A method of treating EGFR positive cancer in a subject in need thereof, the method comprising administering to said subject a pharmaceutical composition of claim 29, thereby treating cancer in a subject.

33. (canceled)

34. A method of engineering an antibody or antigen binding fragment thereof, the method comprising: a. selecting an antibody or antigen binding fragment thereof of interest; b. receiving structural analysis of said selected antibody or antigen binding domain bound to its target; c. determining at least one CDR of said selected antibody or antigen binding domain that is not required for binding to said target based on said structural analysis; d. replacing said determined at least one CDR or a portion thereof with an immunogenic peptide; or a. selecting an antibody or antigen binding fragment thereof of interest; b. receiving a database of immunogenic peptides; c. performing pairwise alignment of peptides of a variable region of said selected antibody or antigen binding fragment thereof of interest with immunogenic peptides of said database; d. determining a peptide from said selected antibody or antigen binding fragment thereof and an immunogenic peptide with an alignment score above a predetermined threshold; and e. replacing said determined peptide from said selected antibody or antigen binding fragment thereof with said determined immunogenic peptide; thereby engineering an antibody or antigen binding fragment thereof.

35. (canceled)

36. The method of claim 34, where at least one of. a. said method further comprises optimizing said replacing to produce as little perturbation in the structure of said selected antibody or antigen binding fragment thereof of interest as possible, b. said engineered antibody or antigen binding fragment thereof is an immunogenic peptide delivery antibody; c. step (a) comprises selecting an antibody or antigen binding fragment thereof that binds to a surface of a target cell; d. step (a) comprises selecting an antibody or antigen binding fragment thereof that binds to a surface of a target cell and upon binding to a surface is internalized and delivered to a cytosol of said target cell; e. said method further comprises confirming at least one of: delivery of said immunogenic peptide to a cytosol of said target cell, delivery of said immunogenic peptide in complex with an HLA molecule to a surface of said target cell and specific killing of said target cell by an effector cell specific to said immunogenic peptide; and f. wherein said method further comprises selecting a

targeting antibody that binds to a protein on a surface of a target cell and producing a dual-function antigen binding molecule by combining said engineered antibody and said targeting antibody, optionally wherein combining comprises engineering a heavy chain constant region of said targeting antibody and a heavy chain constant region of said engineered antibody to promote heterodimerization and inhibit homodimerization.

37. (canceled)

38. (canceled)

39. (canceled)

40. (canceled)

41. (canceled)

42. (canceled)