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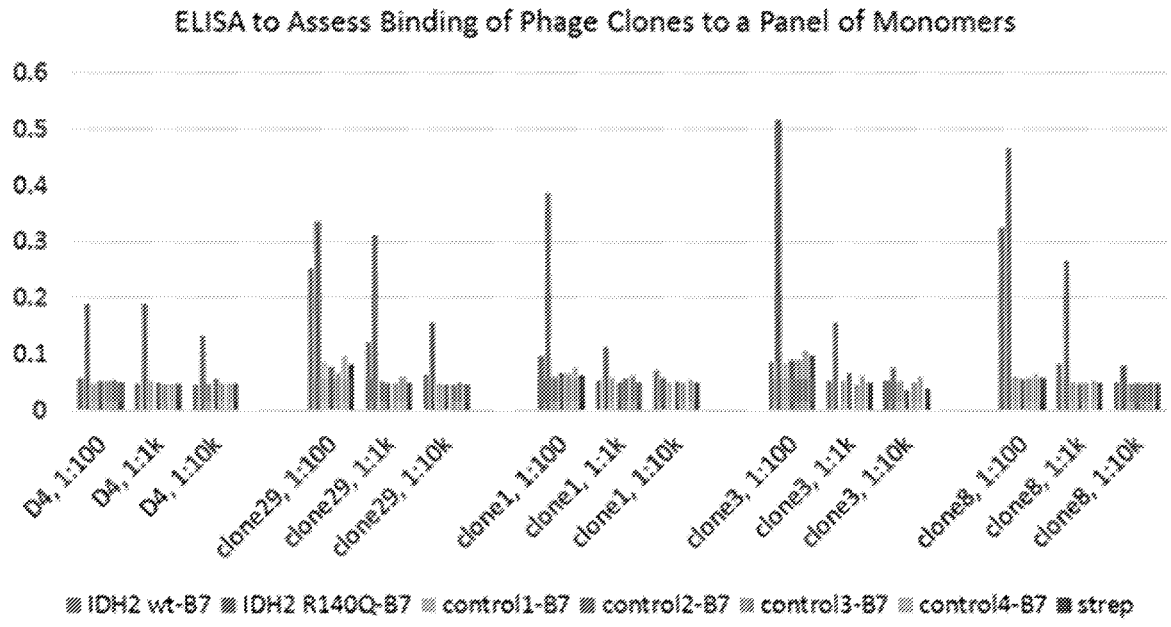

FIG. 1

(57) Abstract: This document provides methods and materials for assessing a mammal having or suspected of having cancer and/or for treating a mammal having cancer. For example, molecules including one or more antigen-binding domains (e.g., a single-chain variable fragment (scFv)) that can bind to a modified peptide (e.g., a tumor antigen), as well as method for using such molecules, are provided.

[Continued on next page]

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MANAbodies AND METHODS OF USING

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Patent Application Serial No. 62/506,674, filed on May 16, 2017. The disclosure of the prior application is considered part of (and is incorporated by reference in) the disclosure of this application.

STATEMENT REGARDING FEDERAL FUNDING

This invention was made with U.S. government support under grant No. CA62924 from the National Institutes of Health. The U.S. government has certain rights in the invention.

BACKGROUND

1. Technical Field

This document relates to methods and materials for assessing a mammal having or suspected of having cancer and/or for treating a mammal having cancer. For example, this document provides methods and materials for using a molecule including one or more antigen-binding domains (e.g., a single-chain variable fragment (scFv)) that can bind to a modified peptide (e.g., a tumor antigen) to treat a mammal having a cancer.

2. Background Information

Somatic mutations in cancer are ideal targets for cancer therapy as they are uniquely expressed only in tumor cells and not normal cells. In particular, targeting driver gene proteins (broadly subdivided into oncogene proteins and tumor suppressor proteins) have added benefits. First, the tumor's dependence on their oncogenic-endowing capacity makes resistance less likely. Second, these mutations typically occur early during the development of the tumor, thus essentially all daughter cancer cells will contain the mutation. Finally, driver gene proteins tend to have hotspot mutations shared among many patients, thus a therapy targeting a single mutation could be applied to a broad patient population.

Most mutant proteins, including most mutant driver gene proteins, are intracellular. While small molecules can target intracellular proteins, developing small molecules that can

specifically inhibit the activity of a mutant driver gene and not its wild-type (wt) counterpart has remained out of reach for the majority of such driver gene proteins. Antibodies, which can have the capacity to distinguish a single amino acid mutation, can typically only target extracellular epitopes.

The immune system samples the intracellular contents of cells through antigen processing and presentation. Following protein proteolysis, a fraction of the resulting peptides are loaded onto human leukocyte antigen (HLA) and sent to the cell surface where they serve as a way for T cells, via their T cell receptor (TCR), to distinguish self from non-self peptides. For example, a virally-infected cell will present viral peptides in its HLA, triggering T cells to kill that cell. Similarly, in cancer, mutant peptides can be presented in HLA on the cancer cell surface, referred to as MANAs, for **M**utation-**A**ssociated **N**eo-**A**ntigens. In some cases, and to varying degrees, patients may mount an anti-cancer T cell response against these mutant-peptide-HLA neoantigens, and checkpoint blockade antibodies can further augment this response. However, many patients, particularly those with a low mutational burden, cannot mount a sufficient anti-cancer T cell response. A therapy or diagnostic specifically targeting MANAs could therefore provide a truly tumor-specific method to diagnose or treat cancer.

HLA class I proteins are present on all nucleated cells. There are three classical HLA class I genes, A, B, and C, each of which are highly polymorphic. Each HLA allele has a particular peptide-binding motif, and as a result, only certain peptides will bind to certain HLA alleles.

There is a continuing need in the art to develop new methods to diagnose, monitor, and effectively treat cancers.

SUMMARY

This document provides methods and materials for treating a mammal having cancer. For example, this document provides methods and materials for using one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide (e.g., a modified peptide present in a peptide-HLA-b2M complex) to treat a mammal having a cancer (e.g., a cancer expressing the modified peptide). In some cases, one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a

modified peptide (e.g., a modified peptide present in a peptide-HLA-b2M complex) can be administered to a mammal having a cancer (e.g., a cancer expressing the modified peptide) to treat the mammal.

As demonstrated herein, scFvs were identified that target (e.g., bind to) numerous **M**utation-**A**ssociated **N**eo-**A**ntigens (MANAs) present in a peptide-HLA-b2M complex in many acute myeloid leukemia (AML) cases. Also as demonstrated herein, the scFvs were used to design both chimeric antigen receptor (CAR) T cells (CARTs; also abbreviated as CAR Ts or CAR-Ts) and bispecific antibodies capable of recognizing and killing cells expressing MANAs. The ability to specifically target MANAs provides a tumor-specific method to diagnose and/or treat cancer. For example, scFvs specifically targeting MANAs can be used in full-length antibodies or fragments thereof, antibody drug conjugates (ADCs), antibody radionuclide conjugates, CARTs, or bispecific antibodies to diagnose and/or treat a mammal having cancer.

In general, one aspect of this document a molecule comprising an antigen-binding domain that can bind to a peptide-HLA-beta-2 microglobulin complex, where the peptide includes a modified peptide, where the HLA is a class I HLA, and where the antigen-binding domain does not bind to a complex that includes a wild-type version of the modified peptide. The modified peptide can include from 7 amino acids to 15 amino acids (e.g., 10 amino acids). The modified peptide can be derived from a modified IDH2 polypeptide, a modified EGFR polypeptide, a modified p53 polypeptide, a modified KRAS polypeptide, a modified HRAS polypeptide, a modified NRAS polypeptide, or a modified CTNNB polypeptide. The modified peptide can include an amino acid sequence set forth SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, or SEQ ID NO:32. When the modified peptide includes SEQ ID NO:1, the class I HLA can be an HLA-B7, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:8. When the modified peptide includes SEQ ID NO:11, the class I HLA can be an HLA-B7, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:380, SEQ ID NO:390, SEQ ID NO:391, SEQ ID NO:392, or SEQ ID NO:393. When the modified peptide includes SEQ ID NO:13, the class I HLA

can be an HLA-A2, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:324, SEQ ID NO:325, SEQ ID NO:326, SEQ ID NO:327, SEQ ID NO:328, SEQ ID NO:329, or SEQ ID NO:330. When the modified peptide includes SEQ ID NO:15, the class I HLA can be an HLA-A2, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:336, or SEQ ID NO:337. When the modified peptide includes SEQ ID NO:16, the class I HLA can be an HLA-A2, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:332, SEQ ID NO:334, SEQ ID NO:335, SEQ ID NO:336, or SEQ ID NO:337. When the modified peptide includes SEQ ID NO:18, the class I HLA can be an HLA-A2, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:338, SEQ ID NO:339, or SEQ ID NO:340. When the modified peptide includes SEQ ID NO:20, the class I HLA can be an HLA-A3, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:341, SEQ ID NO:342, or SEQ ID NO:343. When the modified peptide includes SEQ ID NO:21, the class I HLA can be an HLA-A3, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:342, SEQ ID NO:343, SEQ ID NO:349, SEQ ID NO:350, SEQ ID NO:351, SEQ ID NO:352, SEQ ID NO:353, SEQ ID NO:354, SEQ ID NO:355, SEQ ID NO:356, or SEQ ID NO:357. When the modified peptide includes SEQ ID NO:22, the class I HLA can be an HLA-A3, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:338, SEQ ID NO:339, SEQ ID NO:340, SEQ ID NO:341, SEQ ID NO:342, SEQ ID NO:343, SEQ ID NO:344, SEQ ID NO:345, SEQ ID NO:346, SEQ ID NO:347, SEQ ID NO:348, SEQ ID NO:369, SEQ ID NO:370, SEQ ID NO:371, SEQ ID NO:372, SEQ ID NO:373, or SEQ ID NO:374. When the modified peptide includes SEQ ID NO:24, the class I HLA can be an HLA-A11, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:358, SEQ ID NO:359, SEQ ID NO:360, SEQ ID NO:361, SEQ ID NO:362, SEQ ID NO:363, SEQ ID NO:364, SEQ ID NO:365, SEQ ID NO:366, SEQ ID NO:367, or SEQ ID NO:368. When the modified peptide includes SEQ ID NO:26, the class I HLA can be an HLA-A3, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:375, SEQ ID NO:376, SEQ ID NO:377, SEQ ID NO:378, or SEQ ID NO:379. When the modified peptide includes SEQ ID NO:28, the class I HLA can be an HLA-A1, and the antigen binding fragment can include an

amino acid sequence set forth in SEQ ID NO:394. When the modified peptide includes SEQ ID NO:30, the class I HLA can be an HLA-A1, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:395. When the modified peptide includes SEQ ID NO:31, the class I HLA can be an HLA-A1, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:396. When the modified peptide includes SEQ ID NO:32, the class I HLA can be an HLA-A1, and the antigen binding fragment can include an amino acid sequence set forth in SEQ ID NO:397, SEQ ID NO:398, SEQ ID NO:399, SEQ ID NO:400, or SEQ ID NO:401. The molecule can be an antibody, an antibody fragment, a scFv, a CAR, a TCR, a TCR mimic, a tandem scFv, a bispecific T cell engager, a diabody, a single-chain diabody, an scFv-Fc, a bispecific antibody, or a dual-affinity re-targeting antibody (DART). For example, the molecule can be a single-chain diabody. The molecule also can include an antigen-binding domain that can bind to an effector cell receptor (e.g., CD3, CD28, CD4, CD8, CD16a, NKG2D, PD-1, CTLA-4, 4-1BB, OX40, ICOS, or CD27). When the antigen-binding domain that can bind to an effector cell can bind to CD3, the antigen-binding domain can include an amino acid sequence set forth in SEQ ID NO:404, SEQ ID NO:405, SEQ ID NO:406, SEQ ID NO:407, SEQ ID NO:408, SEQ ID NO:409, SEQID NO:410, SEQ ID NO:411, SEQ ID NO:412, SEQ ID NO:413, SEQ ID NO:414, SEQ ID NO:415, SEQ ID NO:416, or SEQ ID NO:417.

In another aspect, the present invention provides a molecule comprising an antigen-binding domain that can bind to a peptide-human leukocyte antigen (HLA)-beta-2 microglobulin complex, wherein said antigen-binding domain is selected from the group consisting of:

(i) an antigen-binding domain comprising a complementarity determining region (CDR)-VL1 comprising the amino acid sequence QDVNTA (SEQ ID NO:33); a CDR-VL2 comprising the amino acid sequence SAS; a CDR-VL3 comprising the amino acid sequence QQSGYAPIT (SEQ ID NO:82), a CDR-VH1 comprising the amino acid sequence GFNISYYS (SEQ ID NO:89), a CDR-VH2 comprising the amino acid sequence VDPDSDYT (SEQ ID NO:96); and a CDR-VH3 comprising the amino acid sequence SRSWIHMFSMDY (SEQ ID NO:103);

(ii) an antigen-binding domain comprising comprises a CDR-VL1 comprising the amino acid sequence QDVNTA (SEQ ID NO:33); a CDR-VL2 comprising the amino acid sequence SAS; a CDR-VL3 comprising the amino acid sequence QQSLYGPFT (SEQ ID NO:84); a CDR-VH1 comprising the amino acid sequence GFNIAYEY (SEQ ID NO:91); a CDR-VH2 comprising the amino acid sequence

IGPDSGYT (SEQ ID NO:98); and a CDR-VH3 comprising the amino acid sequence SRVWYYSTYGMDY (SEQ ID NO:105);

(iii) an antigen-binding domain comprising a CDR-VL1 comprising the amino acid sequence QDVNTA (SEQ ID NO:33); a CDR-VL2 comprising the amino acid sequence SAS; a CDR-VL3 comprising the amino acid sequence QQYLYQPWT (SEQ ID NO:87); a CDR-VH1 comprising the amino acid sequence GFNIDYYG (SEQ ID NO:94); a CDR-VH2 comprising the amino acid sequence LYGGSDST (SEQ ID NO:101); and a CDR-VH3 comprising the amino acid sequence SRQYSAYFDY (SEQ ID NO:108); and

(iv) an antigen-binding domain comprising a CDR-VL1 comprising the amino acid sequence QDVNTA (SEQ ID NO:33); a CDR-VL2 comprising the amino acid sequence SAS; a CDR-VL3 comprising the amino acid sequence QQGLYYPWT (SEQ ID NO: 88); a CDR-VH1 comprising the amino acid sequence GFNVSYSS (SEQ ID NO: 95); a CDR-VH2 comprising the amino acid sequence IWPDSGQT (SEQ ID NO: 102); and a CDR-VH3 comprising the amino acid sequence SRSSYFDAMDY (SEQ ID NO: 109);

wherein said peptide comprises a modified peptide derived from a p53 polypeptide, wherein said modified peptide comprises the amino acid sequence SEQ ID NO: 15, wherein said HLA is a class I HLA, and wherein said antigen-binding domain does not bind to a complex that includes a wild-type version of the modified peptide.

In another aspect, this document features a CAR. The CAR can include an extracellular domain that includes any antigen-binding domain described herein (e.g., an antigen-binding domain that can bind to a peptide-HLA-beta-2 microglobulin complex, where the peptide includes a modified peptide, where the HLA is a class I HLA, and where the antigen-binding domain does not bind to a complex that includes a wild-type version of the modified peptide), a transmembrane domain, and an intracellular domain. The transmembrane domain can include a transmembrane domain of CD4, CD8, or CD28. The intracellular domain can include one or more costimulatory domains from CD28, DAP10, ICOS, OX40, and/or 4-1BB. The intracellular domain can include a signaling domain from CD3-zeta.

In another aspect, this document features a T cell expressing any CAR described herein (e.g., a CAR including an extracellular domain that includes any antigen-binding domain described herein, a transmembrane domain, and an intracellular domain). The T cell

can express a CAR including an extracellular domain that includes an antigen-binding domain that can bind to a peptide-HLA-beta-2 microglobulin complex, where the peptide includes a modified peptide, where the HLA is a class I HLA, and where the antigen-binding domain does not bind to a complex that includes a wild-type version of the modified peptide), a transmembrane domain, and an intracellular domain.

In another aspect, this document features methods for treating a mammal having a cancer. The methods can include, or consist essentially of, administering to a mammal one or more molecules that include any antigen-binding domain described herein (e.g., an antigen-binding domain that can bind to a peptide-HLA-beta-2 microglobulin complex, where the peptide includes a modified peptide, where the HLA is a class I HLA, and where the antigen-binding domain does not bind to a complex that includes a wild-type version of the modified peptide), wherein the cancer includes cancer cells expressing a modified peptide. The mammal can be a human. The cancer can be Hodgkin's lymphoma, non-Hodgkin's lymphoma, AML, a lung cancer, a pancreatic cancer, a gastric cancer, a colorectal cancer, an ovarian cancer, an endometrial cancer, a biliary tract cancer, a liver cancer, myeloma, a breast cancer, a prostate cancer, an esophageal cancer, a stomach cancer, a kidney cancer, a bone cancer, a soft tissue cancer, a head and neck cancer, a glioblastoma multiforme, or an astrocytoma.

In another aspect, this document features methods for treating a mammal having a cancer. The methods can include, or consist essentially of, administering to a mammal one or more T cells expressing any one of the CARs described herein (e.g., a CAR including an extracellular domain that includes any antigen-binding domain described herein, a transmembrane domain, and an intracellular domain), where the cancer includes cancer cells expressing a modified peptide. The mammal can be a human. The cancer can be Hodgkin's lymphoma, non-Hodgkin's lymphoma, AML, a lung cancer, a pancreatic cancer, a gastric cancer, a colorectal cancer, an ovarian cancer, an endometrial cancer, a biliary tract cancer, a liver cancer, myeloma, a breast cancer, a prostate cancer, an esophageal cancer, a stomach cancer, a kidney cancer, a bone cancer, a soft tissue cancer, a head and neck cancer, a glioblastoma multiforme, or an astrocytoma.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention

pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Throughout the specification and claims, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 contains ELISA results showing specificity of IDH2 R140Q HLA-B7 scFvs. Peptide-HLA biotinylated monomers were coated on a streptavidin plate, including the wild type (wt) version of an IDH2 peptide (SPNGTIRNIL; SEQ ID NO:2), an IDH2 peptide containing the R140Q mutation (SPNGTIQNIL; SEQ ID NO:1), and four control HLA-B7 monomers containing the following control peptides: control 1 peptide is SPGAANKRPI (an artificial sequence; SEQ ID NO:418), control 2 peptide is RPIPIKYKAM (from mutant MyD88 L265P; SEQ ID NO:9), control 3 peptide is KPITIGRHAH (from a different peptide from wt IDH2; SEQ ID NO:10), and control 4 peptide is AVGVGKSAL (from mutant KRAS G12V; SEQ ID NO:11). The five clones identified in panning were incubated in the wells at the specified dilutions, followed by a rabbit anti-phage antibody, then anti-Rabbit-HRP antibody.

Figure 2 contains a graph showing flow cytometry on peptide-pulsed A2+ cells. T2 cells were peptide-pulsed overnight at 37°C in serum-free media with beta-2 microglobulin (b2M) protein only, or b2M with a EGFR T790M(789-797) peptide (IMQLMPFGC; SEQ ID NO:13). The EGFR wt(789-797) peptide (ITQLMPFGC; SEQ ID NO:14) did not bind to HLA-A2. Cells were stained with 10 µL of precipitated phage per 100 µL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 3 contains a graph showing flow cytometry on peptide-pulsed B7+ cells. RPMI-6666 cells were peptide pulsed overnight at 37°C in serum-free media with b2M protein only, b2M protein with an IDH2 mutant R140Q peptide (SPNGTIQNIL; SEQ ID NO:1), or b2M with the IDH2 wt peptide (SPNGTIRNIL; SEQ ID NO:2). Cells were stained with 10 µL of precipitated phage per 100 µL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 4 contains a graph showing flow cytometry on peptide-pulsed A2+ cells. T2 cells were peptide-pulsed overnight at 37°C in serum-free media with b2M only, b2M with a p53 mutant R248Q(245-254) peptide (GMNQRPILTI; SEQ ID NO:15), b2M with a p53 mutant R248W(245-254) peptide (GMNWRPILTI; SEQ ID NO:16), b2M with the p53 wt(245-254) peptide (GMNRRPILTI; SEQ ID NO:17), or b2M with an HLA-A2 control peptide ELA (ELAGIGILTV; SEQ ID NO:403). Cells were stained with 10 µL of precipitated phage per 100 µL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 5 contains a graph showing flow cytometry on peptide-pulsed A2+ cells. T2 cells were peptide-pulsed overnight at 37°C in serum-free media with b2M only, b2M with a KRAS mutant G12V(6-14) peptide (LVVVGAVGV; SEQ ID NO:18), or b2M with the KRAS wt(6-14) peptide (LVVVGAGGV; SEQ ID NO:19). Cells were stained with 10 µL of precipitated phage per 100 µL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 6 contains a graph showing flow cytometry on peptide-pulsed A3+ cells. T2A3 cells were peptide-pulsed overnight at 37°C in serum-free media with b2M only, b2M with a KRAS mutant G12C(8-16) peptide (VVGACGVGK; SEQ ID NO:419), b2M with a KRAS mutant G12D(8-16) peptide (VVGADGVGK; SEQ ID NO:420), b2M with a KRAS mutant G12V(8-16) peptide (VVGAVGVGK; SEQ ID NO:22), b2M with a KRAS mutant G12C(7-16) peptide (VVVGACGVGK; SEQ ID NO:20), b2M with a KRAS mutant G12D(7-16) peptide (VVVGADGVGK; SEQ ID NO:21), b2M with a KRAS mutant G12V(7-16) peptide (VVVGAVGVGK; SEQ ID NO:22), b2M with the KRAS wt(8-16)

peptide (VVGAGGVGK; SEQ ID NO:25), b2M with the KRAS wt(7-16) peptide (VVVGAGGVGK; SEQ ID NO:23), or the CTNNB S45F(41-49) peptide (TTAPFLSGK; SEQ ID NO:26). Cells were stained with 10 µL of precipitated phage per 100 µL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 7 contains a graph showing flow cytometry on peptide-pulsed A3+ cells. T2A3 cells were peptide-pulsed overnight at 37°C in serum-free media with b2M only, b2M with a KRAS mutant G12V(7-16) peptide (VVVGAVGVGK; SEQ ID NO:22), or b2M with the KRAS wt(7-16) peptide (VVVGAGGVGK; SEQ ID NO:23). Cells were stained with 10 µL of precipitated phage per 100 µL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 8 contains a graph showing flow cytometry on peptide-pulsed A3+ cells. T2A3 cells were peptide-pulsed overnight at 37°C in serum-free media with b2M only, b2M with a KRAS mutant G12C(7-16) peptide (VVVGACGVGK; SEQ ID NO:20), b2M with a KRAS mutant G12D(7-16) peptide (VVVGADGVGK; SEQ ID NO:21), b2M with a KRAS mutant G12V(7-16) peptide (VVVGAVGVGK; SEQ ID NO:22), or b2M with the KRAS wt(7-16) peptide (VVVGAGGVGK; SEQ ID NO:23). Cells were stained with 10 µL of precipitated phage per 100 µL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 9 contains a graph showing flow cytometry on peptide-pulsed A11+ cells. Hs611.T cells were peptide-pulsed overnight at 37°C in serum-free media with b2M only, b2M with a KRAS mutant G12C(7-16) peptide (VVVGACGVGK; SEQ ID NO:20), b2M with a KRAS mutant G12D(7-16) peptide (VVVGADGVGK; SEQ ID NO:21), b2M with a KRAS mutant G12V(7-16) peptide (VVVGAVGVGK; SEQ ID NO:22), or b2M with the KRAS wt(7-16) peptide (VVVGAGGVGK; SEQ ID NO:23). Cells were stained with 10 µL of precipitated phage per 100 µL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 10 contains a graph showing flow cytometry on peptide-pulsed A11+ cells. MINO cells were peptide-pulsed overnight at 37°C in serum-free media with b2M only, b2M with a KRAS mutant G12D(8-16) peptide (VVGADGVGK; SEQ ID NO:24), or b2M with the KRAS wt(8-16) peptide (VVGAGGVGK; SEQ ID NO:25). Cells were stained with 10 μL of precipitated phage per 100 μL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 11 contains a graph showing flow cytometry on peptide-pulsed A11+ cells. Hs611.T cells were peptide-pulsed overnight at 37°C in serum-free media with b2M only, b2M with a KRAS mutant G12C(7-16) peptide (VVVGACGVGK; SEQ ID NO:20), b2M with a KRAS mutant G12D(7-16) peptide (VVVGADGVGK; SEQ ID NO:21), b2M with a KRAS G12V(7-16) peptide (VVVGAVGVGK; SEQ ID NO:22), or b2M with the KRAS wt(7-16) peptide (VVVGAGGVGK; SEQ ID NO:23). Cells were stained with 10 μL of precipitated phage per 100 μL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 12 contains a graph showing flow cytometry on peptide-pulsed A3+ cells. T2A3 cells were peptide-pulsed overnight at 37°C in serum-free media with b2M only, b2M with a CTNNB mutant S45F(41-49) peptide (TTAPFLSGK; SEQ ID NO:26), or b2M with CTNNB wt(41-49) peptide (TTAPSLSGK; SEQ ID NO:27). Cells were stained with 10 μL of precipitated phage per 100 μL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 13 contains a graph showing flow cytometry on peptide-pulsed B7+ cells. RPMI-6666 cells were peptide-pulsed overnight at 37°C in serum-free media shows cells pulsed with b2M only, b2M with a KRAS mutant G12V(11-19) peptide (AVGVGKSAL; SEQ ID NO:11). The KRAS wt(11-19) peptide (AGGVGKSAL; SEQ ID NO:12) did not bind to HLA-B7. Cells were stained with 10 μL of precipitated phage per 100 μL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figure 14 contains a graph showing flow cytometry on peptide-pulsed A1+ cells. SigM5 cells were peptide-pulsed overnight at 37°C in serum-free media shows cells pulsed with b2M only, b2M with a H/K/N RAS mutant Q61H(55-64) peptide (ILDTAGHEEY; SEQ ID NO:28), b2M with a H/K/N RAS mutant Q61K(55-64) peptide (ILDTAGKEEY; SEQ ID NO:30), b2M with a H/K/N RAS mutant Q61L(55-64) peptide (ILDTAGLEEY; SEQ ID NO:31), b2M with a H/K/N RAS mutant Q61R(55-64) peptide (ILDTAGREEY; SEQ ID NO:32), or b2M with the H/K/N RAS wt(55-64) peptide (ILDTAGQEEY; SEQ ID NO:29). Cells were stained with 10 µL of precipitated phage per 100 µL of cells, washed and stained with rabbit anti-M13 antibody, then washed and stained with anti-Rabbit-PE antibody. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Figures 15A-15B show that MANAbody clones can be converted into CAR-T cells. Fig. 15A: IDH2 R140Q(134-143)-B7 cl.1 MANA-CAR-T cells were co-cultured for 4 hours at 37°C with COS-7 cells co-transfected with plasmids encoding various combinations of HLA-B7, IDH2(WT), and IDH2(R140Q). Following co-culture, conditioned media was collected and assayed for secreted IFNγ by ELISA. Fig. 15B: KRAS G12V(7-16)-A3 cl.2 MANA-CAR-T cells were co-cultured for 4 hours at 37°C with COS-7 cells transfected with plasmids encoding various combinations of HLA-A3, KRAS(WT), and KRAS(G12V). Following co-culture, conditioned media was collected and assayed for secreted IFNγ by ELISA.

Figures. 16A-16B show that MANAbody clones can be converted into single-chain diabodies (scDbs). Fig. 16A: IDH2 R140Q(134-143)-B7 cl.29, cl.1, and cl.3 scDbs, containing either the anti-CD3 clone mUCHT1 or hUCHT1v9, were incubated at the specified concentrations with T cells and COS-7 cells co-transfected with plasmids encoding various combinations of HLA-B7, IDH2(WT), IDH2(R140Q), and GFP for 24 hours at 37°C. Following co-culture, plates was snap frozen and conditioned media was collected and assayed for secreted IFNγ by ELISA. Fig. 16B: KRAS G12V(7-16)-A3 cl.2 mUCHT1 scDb was incubated at the specified concentrations with or without T cells and either 1) no target cells, 2) COS-7 cells co-transfected with plasmids encoding HLA-A3 and KRAS(WT) or HLA-A3 and KRAS(G12V), or 3) with NCI-H441 parental or HLA-A3 knockout cells for 24

hours at 37°C. Following co-culture, plates was snap frozen and conditioned media was collected and assayed for secreted IFNγ by ELISA.

Figure 17 shows that a MANAbody clone converted into a scDb can kill target cells. KRAS G12V(7-16)-A3 cl.2 mUCHT1 scDb and a pan-HLA-A3 scDb were incubated at 0 or 50 ng/mL with or without T cells and with NCI-H441 parental or HLA-A3 knockout cells for 24 hours at 37°C. Following co-culture, CellTiter-Glo was used to assay viable cells in each well. Percent target cell viability was calculated by subtracting the value from T cell only wells and normalizing to the value from target cell only wells.

DETAILED DESCRIPTION

This document provides methods and materials for assessing a mammal having cancer or suspected of having cancer and/or treating a mammal having cancer. For example, one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can target (e.g., bind to) one or more modified peptides (e.g., peptides present in a peptide-HLA complex such as a peptide-HLA-b2M complex) can be used to assess a mammal having cancer or suspected of having cancer and/or to treat a mammal having a cancer (e.g., a cancer expressing one or more modified peptides). In some cases, one or more molecules includes one or more antigen-binding domains that can bind to a modified peptide can be used to detect the presence or absence of one or more modified peptides in a sample obtained from a mammal having cancer or suspected of having cancer. In some cases, one or more molecules including one or more antigen-binding domains that can bind to a modified peptide can be administered to a mammal having a cancer (e.g., a cancer expressing the modified peptide) to treat the mammal.

As used herein, a modified peptide is a peptide derived from a modified polypeptide. A modified polypeptide can be any appropriate modified polypeptide (e.g., a polypeptide having a disease causing mutation such as an oncogenic mutation). A modified peptide can have one or more amino acid modifications (e.g., substitutions) relative to a wild type (wt) peptide (e.g., a peptide derived from a wt polypeptide from which the modified polypeptide is derived). A modified peptide also can be referred to as a mutant peptide. In some cases, a modified peptide can be a tumor antigen. Examples of tumor antigens include, without limitation, mutation-associated neo-antigens (MANAs), tumor-associated antigen, and

tumor-specific antigens. A modified peptide can be any appropriate length. In some cases, a modified peptide can be from about 7 amino acids to about 15 amino acids (e.g., from about 8 amino acids to about 15 amino acids, from about 9 amino acids to about 15 amino acids, from about 10 amino acids to about 15 amino acids, from about 11 amino acids to about 15 amino acids, from about 12 amino acids to about 15 amino acids, from about 13 amino acids to about 15 amino acids, from about 7 amino acids to about 14 amino acids, from about 7 amino acids to about 13 amino acids, from about 7 amino acids to about 12 amino acids, from about 7 amino acids to about 11 amino acids, from about 7 amino acids to about 10 amino acids, from about 7 amino acids to about 9 amino acids, or from about 9 amino acids to about 10 amino acids) in length. For example, a modified peptide can be about 9 amino acids in length. For example, a modified peptide can be about 10 amino acids in length. A modified peptide can be derived from any modified (e.g., oncogenic) polypeptide. Examples of modified polypeptides from which modified peptides described herein can be derived include, without limitation, epidermal growth factor receptor (EGFR), isocitrate dehydrogenase 2 (IDH2), p53, RAS (e.g., KRAS, HRAS, and NRAS), and CTNNB. A modified peptide can include any appropriate modification. In some cases, modified peptides described herein can include one or more modifications (e.g., mutations) shown in Table 1.

Table 1. Modified peptides.

Protein of origin	Mutation	Mutant Peptide(s)	SEQ ID NO:	WT peptide	SEQ ID NO:	Peptide Codons	HLA Allele
EGFR	T790M	IMQLMPFGC	13	ITQLMPFGC	14	789-797	A2
IDH2	R140Q	SPNGTIQNIL	1	SPNGTIRNIL	2	134-143	B7
p53	R248Q, R248W	GMNQRPILTI, GMNWRPILTI	15 16	GMNRRPILTI	17	245-254	A2
KRAS	G12V	LVVVGAVGV	18	LVVVGAGGV	19	6-14	A2
KRAS	G12C, G12D, G12V	VVVGACGVGK, VVVGADGVGK, VVVGAVGVGK	20 21 22	VVVGAGGVGK	23	7-16	A3
KRAS	G12V	VVVGAVGVGK	22	VVVGAGGVGK	23	7-16	A3
KRAS	G12D	VVVGADGVGK	21	VVVGAGGVGK	23	7-16	A3
KRAS	G12D	VVVGADGVGK	21	VVVGAGGVGK	23	7-16	A11
KRAS	G12D	VVGADGVGK	24	VVGAGGVGK	25	8-16	A11
KRAS	G12V	VVVGAVGVGK	22	VVVGAGGVGK	23	7-16	A11
CTNNB	S45F	TTAPFLSGK	26	TTAPSLSGK	27	41-49	A3

KRAS	G12V	AVGVGKSAL	11	AGGVGKSAL	12	11-19	B7
H/K/N RAS	Q61H	ILDTAGHEEY	28	ILDTAGQEEY	29	55-64	A1
H/K/N RAS	Q61K	ILDTAGKEEY	30	ILDTAGQEEY	29	55-64	A1
H/K/N RAS	Q61L	ILDTAGLEEY	31	ILDTAGQEEY	29	55-64	A1
H/K/N RAS	Q61R	ILDTAGREEY	32	ILDTAGQEEY	29	55-64	A1

A modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be in a complex with any appropriate HLA. An HLA can be any appropriate HLA allele. In some cases, an HLA can be a class I HLA (e.g., HLA-A, HLA-B, and HLA-C) allele. Examples of HLA alleles that a modified peptide described herein can complex with include, without limitation, HLA-A1, HLA-A2, HLA-A3, HLA-11, and HLA-B7. Exemplary HLA alleles for particular modified peptides are shown in Table 1. For example, a modified peptide derived from a modified EGFR polypeptide (e.g., IMQLMPFGC (SEQ ID NO:13)) can be in a complex with HLA-A2 and b2M. For example a modified peptide derived from a modified IDH2 polypeptide (e.g., SPNGTIQNIL (SEQ ID NO:1)) can be in a complex with HLA-B7 and b2M. For example a modified peptide derived from a modified p53 polypeptide (e.g., GMNQRPILTI (SEQ ID NO:15) or GMNWRPILTI 1(SEQ ID NO:16)) can be in a complex with HLA-A2 and b2M. For example a modified peptide derived from a modified KRAS polypeptide (e.g., LVVVGAVGV (SEQ ID NO:18), VVVGACGVGK (SEQ ID NO:20), VVVGADGVGK (SEQ ID NO:21), VVVGAVGVGK (SEQ ID NO:22), and VVGADGVGK (SEQ ID NO:24)) can be in a complex with HLA-A2, HLA-A3, and/or HLA-A11, and b2M. For example a modified peptide derived from a modified CTNNB polypeptide (e.g., TTAPFLSGK (SEQ ID NO:26)) can be in a complex with HLA-A3 and b2M. For example a modified peptide derived from a modified KRAS polypeptide (e.g., AVGVGKSAL (SEQ ID NO:11)) can be in a complex with HLA-B7 and b2M. For example a modified peptide derived from a modified H/K/N RAS polypeptide (e.g., ILDTAGHEEY (SEQ ID NO:28), ILDTAGKEEY (SEQ ID NO:30), ILDTAGLEEY

(SEQ ID NO:31), ILDTAGREEY (SEQ ID NO:32)) can be in a complex with HLA-A1 and b2M.

This document provides molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32). In some cases, a molecule including one or more antigen-binding domains that can bind to a modified peptide described herein does not target (e.g., does not bind to) a modified peptide described herein that is not present in a complex (e.g., a peptide-HLA-b2M complex). In some cases, a molecule including one or more antigen-binding domains that can bind to a modified peptide described herein does not target (e.g., does not bind to) a wt peptide (e.g., a peptide derived from a wt polypeptide from which the modified polypeptide is derived).

A molecule including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein can be any appropriate type of molecule. In some cases, a molecule can be a monovalent molecule (e.g., containing a single antigen-binding domain). In some cases, a molecule can be a multivalent molecule (e.g., containing two or more antigen-binding domains and simultaneously targeting two or more antigens). For example, a bispecific molecule can include two antigen-binding domains, a trispecific molecule can include three antigen-binding domains, a quadspecific molecule can include four antigen-binding domains, etc. Examples of molecules that contain antigen-binding domains include, without limitation, antibodies, antibody fragments, scFvs, CARs, T cell receptors (TCRs), TCR mimics, tandem scFvs, bispecific T cell engagers, diabodies, scDbs, scFv-Fcs, bispecific antibodies, dual-affinity re-targeting antibodies (DARTs), and any other molecule that includes at least one variable heavy chain (VH) and at least one variable light chain (VL). Any of these molecules can be used in accordance with materials and methods described herein. In some cases, an antigen-binding domain can be a scFv. For example, a molecule including one or more antigen-binding domains (e.g., one or more scFvs) that can bind to a modified peptide described herein can be a CAR. For example, a molecule

including two scFvs that can bind to a modified peptide described herein can be a single-chain diabody (scDb).

In some cases, when a molecule including one or more antigen-binding domains (e.g., one or more scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) is a CAR, the CAR can be any appropriate CAR. A CAR provided herein can include an extracellular domain having at least one antigen-binding domain that can bind to a modified peptide described herein, a transmembrane domain, and an intracellular domain (e.g., an intracellular signaling domain such as a costimulatory domain). A CAR can include any appropriate extracellular domain. For example, a CAR can include a molecule (e.g., a scFv) having an antigen binding domain that can bind to a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32. A CAR can include any appropriate transmembrane domain. A transmembrane domain can be derived from any appropriate polypeptide. Examples of transmembrane domains that can be used in CAR described herein include, without limitation, transmembrane domains of CD4, CD8 (e.g., CD8-alpha and CD8-beta), CD28, CD3 epsilon, CD5, CD6, CD9, CD16, CD22, CD33, CD37, CD45, CD64, CD80, CD86, CD134, 4-1BB, and CD154. In some cases, a CAR described herein can include a CD28 transmembrane domain. A CAR can include any appropriate intracellular domain. An intracellular domain can be derived from any appropriate polypeptide. An intracellular domain can include a costimulatory domain (e.g., a single costimulatory domain or multiple costimulatory domains). In cases where a CAR includes multiple costimulatory domains, the CAR can include multiple costimulatory domains of the same type or multiple costimulatory domains of different types. An intracellular domain can include a signaling domain. Examples of intracellular domains that can be used in CAR described herein include, without limitation, intracellular domains of CD3 (e.g., a CD3-zeta), CD28, DAP10, inducible T-cell costimulator (ICOS), OX40, 4-1BB,

CD2, CD4, CD8, CD5, CD22, DAP-12, CD22, and CD79. A CAR can be made using any appropriate method. In some cases, a CAR also can include a hinge sequence (e.g., positioned between the extracellular domain and the transmembrane domain). In some cases, a CAR can be made as described elsewhere (see, Curran et al., 2012 *J. Gene Med* 14:405-415; Kershaw et al., 2005 *Nature Reviews Immunol.* 5(12):928-940; Eshhar et al., 1993 *Proc. Natl. Acad. Sci. U.S.A.* 90(2):720-724; Sadelain et al., 2009 *Curr. Opin. Immunol.* 21(2): 215-223; WO 2015/142675; WO 2015/150526; and WO 2014/134165). Also provided here are CARTs expressing one or more CARs, which CARs can target (e.g., bind to) one or more modified peptides described herein (e.g., CARs having two or more antigen-binding domains). Also provided here are CARTs expressing one or more CARs, which CARTs can target (e.g., bind to) one or more modified peptides described herein.

In some cases, when a molecule including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) is a multivalent molecule (e.g., a bispecific molecule), a first antigen-binding domain can bind to a modified peptide described herein and a second antigen-binding domain can bind to an effector cell (e.g., an antigen present on an effector cell). Examples of effector cells include, without limitation, T cells, natural killer (NK) cells, natural killer T (NKT) cells, B cells, plasma cells, macrophages, monocytes, microglia, dendritic cells, neutrophils, fibroblasts, and mast cells. Examples of antigens present on effector cells include, without limitation, CD3, CD4, CD8, CD28, CD16a, NKG2D, PD-1, CTLA-4, 4-1BB, OX40, ICOS, CD27, and any other effector cell surface receptors. In some cases, a molecule described herein can include a first antigen-binding domain that can bind to a modified peptide described herein and a second antigen-binding domain that can bind to an antigen present on a T cell (e.g., CD3). In some cases, sequences (e.g., scFv sequences) that can bind to CD3 can be as shown in Table 4. In some cases, sequences (e.g., scFv sequences) that can bind to CD3 can be as described elsewhere (see, e.g., Rodrigues et al., 1992 *Int J Cancer Suppl.* 7:45-50; Shalaby et al., 1992 *J Exp Med.* 175:217-25; Brischwein et al., 2006 *Mol Immunol.* 43:1129-43; Li et al., 2005 *Immunology.* 116:487-98;

WO2012162067; US20070065437; US20070065437; US20070065437; US20070065437; US20070065437; and US20070065437). In some cases, a molecule described herein can include a first antigen-binding domain that can bind to a modified peptide described herein and a second antigen-binding domain that can bind to an antigen present on a NK cell (e.g., CD16a or NKG2D). By binding both the modified peptide and the effector cell, the multivalent molecule can bring the cell expressing the modified peptide (e.g., as part of the HLA complex) into proximity with the effector cell, permitting the effector cell to act on the cell expressing the modified peptide.

In some cases, when a molecule including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) is a multivalent molecule (e.g., a bispecific molecule), a molecule can be in any appropriate format which includes at least one VH and at least one VL. For example, a VH and a VL can be in any appropriate orientation. In some cases, a VH can be N-terminal to the VL. In some cases, a VH can be C-terminal to the VL. In some cases, a linker amino acid sequence can be positioned between the VH and VL.

In some cases, when a bispecific molecule is a tandem scFv, the tandem scFv can be in any appropriate orientation. Examples of tandem scFv orientations including scFv-A and scFv-B include, without limitation, VLA-LL-VHA-SL-VLB-LL-VHB, VLA-LL-VHA-SL-VHB-LL-VLB, VHA-LL-VLA-SL-VLB-LL-VHB, VHA-LL-VLA-SL-VHB-LL-VLB, VLB-LL-VHB-SL-VLA-LL-VHA, VLB-LL-VHB-SL-VHA-LL-VLA, VHB-LL-VLB-SL-VLA-LL-VHA, and VHB-LL-VLB-SL-VHA-LL-VLA, where SL is a short linker and LL is a long linker. A short linker can be from about 3 amino acids to about 10 amino acids in length. A short linker can include any appropriate amino acids (e.g., glycines and serines) in any appropriate combination. A long linker can be from about 10 amino acids to about 25 amino acids in length. A long linker can include any appropriate amino acids (e.g., glycines and serines) in any appropriate combination.

In some cases, when a bispecific molecule is a diabody, the diabody can be in any appropriate orientation. Examples of diabody orientations including scFv-A and scFv-B

include, without limitation, VLA-SL-VHB and VLB-SL-VHA, VLA-SL-VLB and VHB-SL-VHA, VHA-SL-VLB and VHB-SL-VLA, VLB-SL-VHA and VLA-SL-VHB, VLB-SL-VLA and VHA-SL-VHB, and VHB-SL-VLA and VHA-SL-VLB, where SL is a short linker. A short linker can be from about 3 amino acids to about 10 amino acids in length. A short linker can include any appropriate amino acids (e.g., glycines and serines) in any appropriate combination.

In some cases, when a bispecific molecule is a scDb, the scDb can be in any appropriate orientation. Examples of scDb orientations including scFv-A and scFv-B include, without limitation, VLA-SL-VHB-LL-VLB-SL-VHA, VHA-SL-VLB-LL-VHB-SL-VLA, VLA-SL-VLB-LL-VHB-SL-VHA, VHA-SL-VHB-LL-VLB-SL-VLA, VLB-SL-VHA-LL-VLA-SL-VHB, VHB-SL-VLA-LL-VHA-SL-VLB, VLB-SL-VLA-LL-VHA-SL-VHB, and VHB-SL-VHA-LL-VLA-SL-VLB, where SL is a short linker and LL is a long linker. A short linker can be from about 3 amino acids to about 10 amino acids in length. A short linker can include any appropriate amino acids (e.g., glycines and serines) in any appropriate combination. A long linker can be from about 10 amino acids to about 25 amino acids in length. A long linker can include any appropriate amino acids (e.g., glycines and serines) in any appropriate combination.

In some cases, when a bispecific molecule is a scFv-Fc, the scFv-Fc can be in any appropriate orientation. Examples of scFv-Fc orientations including scFv-Fc-A, scFv-Fc-B, and an Fc domain include, without limitation, VLA-LL-VHA-hinge-Fc and VLB-LL-VHB-hinge-Fc, VHA-LL-VLA-hinge-Fc and VHB-LL-VLB-hinge-Fc, VLA-LL-VHA-hinge-Fc and VHB-LL-VLB-hinge-Fc, VHA-LL-VLA-hinge-Fc and VLB-LL-VHB-hinge-Fc, where LL is a long linker. A long linker can be from about 10 amino acids to about 25 amino acids in length. A long linker can include any appropriate amino acids (e.g., glycines and serines) in any appropriate combination. In some cases, an Fc domain in a scFv-Fc can include one or more modifications to increase heterodimerization and/or to decrease homodimerization of the scFv-Fc. In some cases, an Fc domain in a scFv-Fc can exclude a hinge domain. In some cases, an Fc domain in a scFv-Fc can be at the N-terminus of the scFv.

A molecule including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID

NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can include any appropriate complementarity determining regions (CDRs). For example, a molecule including one or more antigen-binding domains that can bind to a modified peptide described herein can include a variable heavy chain (VH) having three VH complementarity determining regions (CDR-VHs) and a variable light chain (VL) having three VL CDRs (CDR-VLs). For example, a molecule that can bind to a modified peptide derived from a modified EGFR polypeptide (e.g., IMQLMPFGC (SEQ ID NO:13)) can include one of each of the CDRs set forth below:

CDR-VL1: QDVNTA (SEQ ID NO:33);

CDR-VL2: SAS;

CDR-VL3: QQYDYAPIT (SEQ ID NO:34), QQSPYYYLPIT (SEQ ID NO:35), QQYYYSPVT (SEQ ID NO:36), QQHYGNPFT (SEQ ID NO:37), QQSYYSPPT (SEQ ID NO:38), QQYYSYPPT (SEQ ID NO:39), QQYYYYPPT (SEQ ID NO:40);

CDR-VH1: GFNISWYQ (SEQ ID NO:41), GFNVSWSY (SEQ ID NO:42), GFNISWNQ (SEQ ID NO:43), GFNVGYYG (SEQ ID NO:44), GFNITSSY (SEQ ID NO:45), GFNINSSY (SEQ ID NO:46), GFNISTSY (SEQ ID NO:47);

CDR-VH2: VTPYSGYT (SEQ ID NO:48), IYGDSGYT (SEQ ID NO:49), VSPYSGYT (SEQ ID NO:50), VSGMEGYT (SEQ ID NO:51), ISPADGYN (SEQ ID NO:52), ISPTDGYY (SEQ ID NO:53), IDPNDGYS (SEQ ID NO:54); and

CDR-VH3: SRSYTDGFDY (SEQ ID NO:55), SRGQWEASYYAMDY (SEQ ID NO:56), SRSDYYAMDY (SEQ ID NO:57), SRDIYGYAMDV (SEQ ID NO:58), SRTDSTAYTAMDV (SEQ ID NO:59), SRTSDTSYAAMDV (SEQ ID NO:60), SRTNNTAADAMDV (SEQ ID NO:61).

For example, a molecule that can bind to a modified peptide derived from a modified IDH2 polypeptide (e.g., SPNGTIQNIL (SEQ ID NO:1) can include one of each of the CDRs set forth below:

CDR-VL1: QDVNTA (SEQ ID NO:33);

CDR-VL2: SAS;

CDR-VL3: QQYSYSPPT (SEQ ID NO:62), QQGKAYWPAT (SEQ ID NO:63), QQVYSSPFT (SEQ ID NO:64), QQYSLYSPMT (SEQ ID NO:65), QQSYYMPFT (SEQ ID NO:66);

CDR-VH1: GFNISDTY (SEQ ID NO:67), GFNVGHYR (SEQ ID NO:68), GFNVKYYM (SEQ ID NO:69), GFNSFLS (SEQ ID NO:70), GFNIFRGY (SEQ ID NO:71);

CDR-VH2: ISPRTGYN (SEQ ID NO:72), VSPNGYYT (SEQ ID NO:73), ISPGYDYT (SEQ ID NO:74), IFPSSDYT (SEQ ID NO:75), ISPHSDYT (SEQ ID NO:76); and

CDR-VH3: SRAYYSYAYAMDV (SEQ ID NO:77), SRGYSSYAFDY (SEQ ID NO:78), SRSYWRYSVDV (SEQ ID NO:79), SRGKHSSDSNYYMDY (SEQ ID NO:80), SRSYGWAAFDY (SEQ ID NO:81).

For example, a molecule that can bind to a modified peptide derived from a modified p53 polypeptide (e.g., GMNQRPILTI (SEQ ID NO:15) and GMNWRPILTI (SEQ ID NO:16)) can include one of each of the CDRs set forth below:

CDR-VL1: QDVNTA (SEQ ID NO:33);

CDR-VL2: SAS;

CDR-VL3: QQSGYAPIT (SEQ ID NO:82), QQYSYAPIT (SEQ ID NO:83), QQSLYGPFT (SEQ ID NO:84), QQYSYSPIT (SEQ ID NO:85), QQSGYQPDT (SEQ ID NO:86), QQYLYQPWT (SEQ ID NO:87);

CDR-VH1: GFNISYYS (SEQ ID NO:89), GFNIGYYT (SEQ ID NO:90), GFNIAYEY (SEQ ID NO:91), GFNLFGYG (SEQ ID NO:92), GFNISWYA (SEQ ID NO:93), GFNIDYYG (SEQ ID NO:94);

CDR-VH2: VDPDSDYT (SEQ ID NO:96), VSPWSYST (SEQ ID NO:97), IGPDSGYT (SEQ ID NO:98), IGPYYYYT (SEQ ID NO:99), IWPDSDWT (SEQ ID NO:100), LYGGSDST (SEQ ID NO:101); and

CDR-VH3: SRSWIHMFSMDY (SEQ ID NO:103), SRDHWDEAFDV (SEQ ID NO:104), SRVWYYSTYGMDY (SEQ ID NO:105), SRENYDMAMDY (SEQ ID NO:106), SRYYYSSAFDV (SEQ ID NO:107), SRQYSAYFDY (SEQ ID NO:108).

For example, a molecule that can bind to a modified peptide derived from a modified KRAS polypeptide (e.g., LVVVGAVGV (SEQ ID NO:18), VVVGACGVGK (SEQ ID NO:20), VVVGADGVGK (SEQ ID NO:21), VVVGAVGVGK (SEQ ID NO:22), and VVGADGVGK (SEQ ID NO:24)) can include one of each of the CDRs set forth below:

CDR-VL1: QDVNTA (SEQ ID NO:33);

CDR-VL2: SAS and SAY;

CDR-VL3: QQWYSSPVT (SEQ ID NO:110),QQYYSRPVT(SEQ ID NO:111), QQSYGSGSPWT(SEQ ID NO:121), QQTYYSPWT(SEQ ID NO:122), QQYYYPPIT(SEQ ID NO:123), QQSYYYFRPIT(SEQ ID NO:132), QQASYYYPLT(SEQ ID NO:133), QQKSEYSPWT(SEQ ID NO:134), QQSGYIPFT(SEQ ID NO:135), QQGAYYRPFT(SEQ ID NO:136), QQYMYSPVT(SEQ ID NO:152), QQSSSSPIT(SEQ ID NO:153), QQSSASPLT(SEQ ID NO:154), QQYAYSPLT(SEQ ID NO:155), QQYSYYPIT(SEQ ID NO:168), QQYSYTPVT(SEQ ID NO:169), QQYSYEPVT(SEQ ID NO:170), QQYAYYSPVT(SEQ ID NO:171), QQYEYYPMT(SEQ ID NO:172), QQYSFYPFT(SEQ ID NO:188), QQYSYSPIT(SEQ ID NO:85), QQYSAYYQPIT(SEQ ID NO:189), QQYSYYPIT(SEQ ID NO:168), QQYEYVPHT(SEQ ID NO:190), QQYSYMPIT(SEQ ID NO:191), QQYAYYPVT(SEQ ID NO:192), QQYSYMPIT(SEQ ID NO:191), QQYDYRPVT(SEQ ID NO:193), QQYDFTPMT(SEQ ID NO:194), QQYSSSSPVT(SEQ ID NO:195), QQSSYTPIT(SEQ ID NO:229), QQYAYYPIT (SEQ ID NO:230), QQYEYYPIT (SEQ ID NO:231), QQYTYYPIT (SEQ ID NO:232), QQYSYYPIT (SEQ ID NO:168), QQSSVEPWT (SEQ ID NO:233);

CDR-VH1: GFNINWAN (SEQ ID NO:112), GFNIYLHD (SEQ ID NO:113), GFNIYWSH (SEQ ID NO:114), GFNIVGGG (SEQ ID NO:124), GFNIRSYA (SEQ ID NO:125), GFNVSHTG (SEQ ID NO:126), GFNLSYSD (SEQ ID NO:137), GFNISASG (SEQ ID NO:138), GFNIYRYG (SEQ ID NO:139), GFNIYGTM (SEQ ID NO:140), GFNISYSY (SEQ ID NO:141), GFNVSAYW (SEQ ID NO:156), GFNISGYG (SEQ ID NO:157), GFNVSSVG (SEQ ID NO:158), GFNVSSYG (SEQ ID NO:159), GFNFSYGY (SEQ ID NO:173), GFNVWGPG (SEQ ID NO:174), GFNVSGSQ (SEQ ID NO:175), GFNIYGQM (SEQ ID NO:176), GFNVMYST (SEQ ID NO:177), GFNFGSY (SEQ ID NO:196), GFNISDSY (SEQ ID NO:197), GFNIFSDQ (SEQ ID NO:198), GFNLSYSY

(SEQ ID NO:199), GFNISYGY (SEQ ID NO:200), GFNISYQH (SEQ ID NO:201), GFNLSGYY (SEQ ID NO:202), GFNVSGQY (SEQ ID NO:203), GFNVSTSG (SEQ ID NO:204), GFNISYAK (SEQ ID NO:205), GFNFSSYV (SEQ ID NO:206), GFNISQGG (SEQ ID NO:234), GFNISSTG (SEQ ID NO:235), GFNFFSTV (SEQ ID NO:236), GFNLHGYL (SEQ ID NO:237), GFNLSTHV (SEQ ID NO:238), GFNVSYYS (SEQ ID NO:239);

CDR-VH2: ISPPYDYT (SEQ ID NO:115), IIPAIDYT (SEQ ID NO:116), ISSFEGYT (SEQ ID NO:117), IYPQGDYT (SEQ ID NO:127), VGPGKGYT (SEQ ID NO:128), VGPGKGYT (SEQ ID NO:128), VMPDSGHT (SEQ ID NO:142), IHPLKPYT (SEQ ID NO:143), LYPYGYST (SEQ ID NO:144), FKPDSYNT (SEQ ID NO:145), LLPYDGNT (SEQ ID NO:146), IYGGSGYT (SEQ ID NO:160), LYGGSDYT (SEQ ID NO:161), IYGTSDYT (SEQ ID NO:162), IAPRRDYT (SEQ ID NO:163), ISGYTGNT (SEQ ID NO:178), IHPFSGNT (SEQ ID NO:179), IPGWSGYT (SEQ ID NO:180), LSPFSGNT (SEQ ID NO:181), IYSWSDYT (SEQ ID NO:182), ISGYSGNT (SEQ ID NO:207), FSPYSSNT (SEQ ID NO:208), FMPYDSYYT (SEQ ID NO:209), ISGFSGNT (SEQ ID NO:210), FHYGSGNT (SEQ ID NO:211), FMPYQGST (SEQ ID NO:212), FSPYSGYT (SEQ ID NO:213), ISPVSGNT (SEQ ID NO:214), IYGAYSGT (SEQ ID NO:215), LTYWGGYT (SEQ ID NO:216), VYPDSGGT (SEQ ID NO:217), VYPGGGQT (SEQ ID NO:240), LLGGSGNT (SEQ ID NO:241), IYPWSGST (SEQ ID NO:242), IYPPNGYT (SEQ ID NO:243), FYPYVGYT (SEQ ID NO:244), IYPWNDYT (SEQ ID NO:245); and

CDR-VH3: SRSYSYYFDY (SEQ ID NO:118), SRRDGYYFDY (SEQ ID NO:119), SRSYSYYMDY (SEQ ID NO:120), SRDSSYLAFDY (SEQ ID NO:129), SRNFQSTSHAFDY (SEQ ID NO:130), SRKTYYAFDY (SEQ ID NO:131), SRATNIPVYAFDY (SEQ ID NO:147), SRYSSMYYYYFDY (SEQ ID NO:148), SRSYAYGYFAY (SEQ ID NO:149), SRGEVYHYYAFDY (SEQ ID NO:150), SRAAYSSMDV (SEQ ID NO:151), SRTHSYWSAFDY (SEQ ID NO:164), SRTVRYAFDY (SEQ ID NO:165), SRSSRYSMDY (SEQ ID NO:166), SRKSSYYFDY (SEQ ID NO:167), SRAASLSSSYYSAFDV (SEQ ID NO:183), SRGYSYSAMDY (SEQ ID NO:184), SRGYSYFAMDY (SEQ ID NO:185), SRNISYEQSSAFDY (SEQ ID NO:186), SRGYAHNSFDY (SEQ ID NO:187), SRSNQSAYSYMDY (SEQ ID NO:218),

SRSQFTFYQYFDY (SEQ ID NO:219), SRMSVRNAFDY (SEQ ID NO:220), SRSDSYYTAMDY (SEQ ID NO:221), SRSNYYYLDY (SEQ ID NO:222), SRANIYSSHSFFDY (SEQ ID NO:223), SRTHSSIYHSFDY (SEQ ID NO:224), SRPMKTSYYGAFDY (SEQ ID NO:225), SRSQSYTYWSAMDY (SEQ ID NO:226), SRGEYGTYMDY (SEQ ID NO:227), SRTSSYYAFDY (SEQ ID NO:228), SRGYDYSAFDY (SEQ ID NO:246), SRGLQYSAMDY (SEQ ID NO:247), SRSRSSNYYFDV (SEQ ID NO:248), SRGVDYAYLDY (SEQ ID NO:249), SRGYRYQYMDV (SEQ ID NO:250), SRGSYYSFDY (SEQ ID NO:251).

For example, a molecule that can bind to a modified peptide derived from a modified CTNNB polypeptide (e.g., TTAPFLSGK (SEQ ID NO:26)) can include one of each of the CDRs set forth below:

CDR-VL1: QDVNTA (SEQ ID NO:33);

CDR-VL2: SAS and SAY;

CDR-VL3: QQSYYSPPT (SEQ ID NO:38), QQIYTSPIT (SEQ ID NO:252), QQRAYFPIT (SEQ ID NO:253), QQQYAYTPIT (SEQ ID NO:254), QQIHYKPLT (SEQ ID NO:255);

CDR-VH1: GFNINNTY (SEQ ID NO:256), GFNFITTG (SEQ ID NO:257), GFNFSDYG (SEQ ID NO:258), GFNVWSYG (SEQ ID NO:259), GFNVAWYS (SEQ ID NO:260);

CDR-VH2: IYPTDGYT (SEQ ID NO:260), IGPGSDYT (SEQ ID NO:261), LIPASGYT (SEQ ID NO:262), VTPDGSYT (SEQ ID NO:263), VYGGSSYT (SEQ ID NO:264); and

CDR-VH3: SRTYYSYYSAMDV (SEQ ID NO:265), SRYYYASALDY (SEQ ID NO:266), SRGWSYYMDY (SEQ ID NO:267), SRSYGWAMDY (SEQ ID NO:268), SRDFYSSGMDY (SEQ ID NO:269).

For example, a molecule that can bind to a modified peptide derived from a modified KRAS polypeptide (e.g., AVGVGKSAL (SEQ ID NO:11)) can include one of each of the CDRs set forth below:

CDR-VL1: QDVNTA (SEQ ID NO:33);

CDR-VL2: SAS;

CDR-VL3: QQEWRLPIT (SEQ ID NO:270), QQGTSTPFT (SEQ ID NO:271), QQSWRYPMT (SEQ ID NO:272), QQSYSYPVT (SEQ ID NO:273), QQGWLYSPFT (SEQ ID NO:274);

CDR-VH1: GFNVYGNQ, (SEQ ID NO:275), GFNLSYYG (SEQ ID NO:402), GFNISRYG (SEQ ID NO:276), GFNIYSSW (SEQ ID NO:277), GFNISGYG (SEQ ID NO:157);

CDR-VH2: IYPYSGST (SEQ ID NO:278), IYPDSGYT (SEQ ID NO:279), FYPSSSYT (SEQ ID NO:280), FQPYSGYT (SEQ ID NO:281), VYGGSGYT (SEQ ID NO:282); and

CDR-VH3: SRSAYVAYSYFDY (SEQ ID NO:283), SRAYLYYYLAY (SEQ ID NO:284), SRKYYEAMDY (SEQ ID NO:285), SREYTYYFDY (SEQ ID NO:286), SRAHSSYYVDY (SEQ ID NO:287).

For example, a molecule that can bind to a modified peptide derived from a modified H/K/N RAS polypeptide (e.g., ILDTAGHEEY (SEQ ID NO:28), ILDTAGKEEY (SEQ ID NO:30), ILDTAGLEEY (SEQ ID NO:31), ILDTAGREEY (SEQ ID NO:32)) can include one of each of the CDRs set forth below:

CDR-VL1: QDVNTA (SEQ ID NO:33);

CDR-VL2: SAS;

CDR-VL3: QQHYYSPVT (SEQ ID NO:292), QQYAYAPFT (SEQ ID NO:296), QQAHMIPIT (SEQ ID NO:300), QQSVYDPIT (SEQ ID NO:301), QQSYTSPLT (SEQ ID NO:302), QQGQYSPFT (SEQ ID NO:303), QQYWYLPTT (SEQ ID NO:320);

CDR-VH1: GFNIGYYG (SEQ ID NO:289), GFNIFYQD (SEQ ID NO:293), GFNVSYSM (SEQ ID NO:297), GFNFSFPG (SEQ ID NO:305), GFNISGSW (SEQ ID NO:306), GFNIYYGV, (SEQ ID NO:307), GFNVSYEY (SEQ ID NO:308), GFNISWYD (SEQ ID NO:321);

CDR-VH2: VYPGGGYT (SEQ ID NO:290), IYPDYDYT (SEQ ID NO:294), VWGDGGVT (SEQ ID NO:298), FVGYDGYT (SEQ ID NO:310), LYPDSDYT (SEQ ID NO:311), IYPDSSWT (SEQ ID NO:312), IYGGSDNT (SEQ ID NO:313), IEPSVGYT (SEQ ID NO:322); and

CDR-VH3: SRYYYYGFDY (SEQ ID NO:291), SRTYSVYMDY (SEQ ID NO:295), SRGSYYAFDY (SEQ ID NO:299), SRDYYSFSMDY (SEQ ID NO:316), SRAHTYAFDY (SEQ ID NO:317), SRDQDFHYMNYYLSYALDY (SEQ ID NO:318), SRPLGSYFDY (SEQ ID NO:319), SRSYPYYYFDY (SEQ ID NO:323).

Examples of CDRs (e.g., particular combinations of a CDR-VL1, a CDR-VL2, a CDR-VL3, a CDR-VH1, a CDR-VH2, and a CDR-VH3) that can bind to particular modified peptides are shown in Table 2. In some cases, a molecule including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can include any appropriate set of CDR sequences (e.g., any of the CDR sequence sets described herein).

A molecule including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can include any appropriate sequence. For example, a molecule that can bind to a modified peptide derived from a modified EGFR polypeptide (e.g., IMQLMPFGC (SEQ ID NO:13)) can include, without limitation, the scFv sequence set forth in any one of SEQ ID NO:324, SEQ ID NO:325, SEQ ID NO:326, SEQ ID NO:327, SEQ ID NO:328, SEQ ID NO:329, and SEQ ID NO:330. For example, a molecule that can bind to a modified peptide derived from a modified IDH2 polypeptide (e.g., SPNGTIQNIL (SEQ ID NO:1)) can include, without limitation, the scFv sequence set forth in any one of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:8. For example, a molecule that can

bind to a modified peptide derived from a modified p53 polypeptide (e.g., GMNQRPILTI (SEQ ID NO:15) and GMNWRPILTI 1(SEQ ID NO:16)) can include, without limitation, the scFv sequence set forth in any one of SEQ ID NO:331, SEQ ID NO:332, SEQ ID NO:333, SEQ ID NO:334, SEQ ID NO:335, SEQ ID NO:336, and SEQ ID NO:337. For example, a molecule that can bind to a modified peptide derived from a modified KRAS polypeptide (e.g., LVVVGAVGV (SEQ ID NO:18), VVVGACGVGK (SEQ ID NO:20), VVVGADGVGK (SEQ ID NO:21), VVVGAVGVGK (SEQ ID NO:22), and VVGADGVGK (SEQ ID NO:24)) can include, without limitation, the scFv sequence set forth in any one of SEQ ID NO:338, SEQ ID NO:339, SEQ ID NO:340, SEQ ID NO:341, SEQ ID NO:342, SEQ ID NO:343, SEQ ID NO:344, SEQ ID NO:345, SEQ ID NO:346, SEQ ID NO:347, SEQ ID NO:348, SEQ ID NO:349, SEQ ID NO:350, SEQ ID NO:351, SEQ ID NO:352, SEQ ID NO:353, SEQ ID NO:354, SEQ ID NO:355, SEQ ID NO:356, SEQ ID NO:357, SEQ ID NO:358, SEQ ID NO:359, SEQ ID NO:360, SEQ ID NO:361, SEQ ID NO:362, SEQ ID NO:363, SEQ ID NO:364, SEQ ID NO:365, SEQ ID NO:366, SEQ ID NO:367, SEQ ID NO:368, SEQ ID NO:369, SEQ ID NO:370, SEQ ID NO:371, SEQ ID NO:372, SEQ ID NO:373, and SEQ ID NO:374. For example, a molecule that can bind to a modified peptide derived from a modified CTNNB polypeptide (e.g., TTAPFLSGK (SEQ ID NO:26)) can include, without limitation, the scFv sequence set forth in any one of SEQ ID NO:375, SEQ ID NO:376, SEQ ID NO:377, SEQ ID NO:378, SEQ ID NO:379. For example, a molecule that can bind to a modified peptide derived from a modified KRAS polypeptide (e.g., AVGVGKSAL (SEQ ID NO:11)) can include, without limitation, the scFv sequence set forth in any one of SEQ ID NO:380, SEQ ID NO:390, SEQ ID NO:391, SEQ ID NO:392, and SEQ ID NO:393. For example, a molecule that can bind to a modified peptide derived from a modified H/K/N RAS polypeptide (e.g., ILDTAGHEEY (SEQ ID NO:28), ILDTAGKEEY (SEQ ID NO:30), ILDTAGLEEY (SEQ ID NO:31), ILDTAGREEY (SEQ ID NO:32)) can include, without limitation, the scFv sequence set forth in any one of SEQ ID NO:394, SEQ ID NO:395, SEQ ID NO:396, SEQ ID NO:397, SEQ ID NO:398, SEQ ID NO:399, and SEQ ID NO:400. Examples of sequences (e.g., scFv sequences) that can bind to particular modified peptides are shown in Table 3. In some cases, a molecule including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth

in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can have a sequence that deviates from a sequence shown in Table 3, sometimes referred to as a variant sequence. For example, a molecule including one or more antigen-binding domains that can bind to a modified peptide described herein can have at least 75% sequence identity (e.g., at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or more) to any of the sequences shown in Table 3, provided the variant sequence maintains the ability to bind to a modified peptide described herein. In some cases, a molecule including one or more antigen-binding domains that can bind to a modified peptide described herein can include any appropriate set of CDR sequences described herein, and any sequence deviations from a sequence shown in Table 3 can be in the scaffold sequence(s).

A molecule including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be attached (e.g., covalently or non-covalently attached) to a label (e.g., a detectable label). A detectable label can be any appropriate label. In some cases, a label can be used to assist in detecting the presence or absence of one or more modified peptides described herein. For example, a molecule described herein that is labelled can be used *in vitro* to detect cancer cells (e.g., cancer cells expressing a modified peptide described herein) in a sample obtained from a mammal. In some cases, a label (e.g., a detectable label) can be used to assist in determining the location of one or more modified peptides described herein. For example, molecule described herein that is labelled can be used *in vivo* to monitor anti-tumor therapy and/or to detect cancer cells (e.g., cancer cells expressing a modified peptide described herein) in a mammal. Examples of labels that can be attached to a molecule described herein include, without limitation, radionuclides, chromophores, enzymes, and fluorescent molecules (e.g., green fluorescent protein).

A molecule including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be attached (e.g., covalently or non-covalently attached) to a therapeutic agent. A therapeutic agent can be any therapeutic agent. In some cases, a therapeutic agent can be an anti-cancer agent. Examples of therapeutic agents that can be attached to a molecule described herein include, without limitation, anti-cancer agents such as monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), maytansine, mertansine/emtansine (DM1), ravtansine/soravtansine (DM4), SN-38, calicheamicin, D6.5, dimeric pyrrolobenzodiazepines (PBDs), ricin, pseudomonas exotoxin A, diphtheria toxin, and gelonin.

This document also provides methods for using one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32). For example, one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can target (e.g., bind to) one or more modified peptides can be used to assess a mammal having cancer or suspected of having cancer and/or to treat a mammal having a cancer (e.g., a cancer expressing one or more modified peptides). In some cases, one or more molecules includes one or more antigen-binding domains that can bind to a modified peptide can be used to detect the presence or absence of one or more modified peptides in a sample obtained from a mammal having cancer or suspected of having cancer. In some cases, one or more molecules including one or more antigen-binding domains that can bind to a modified peptide can be administered to a mammal having a cancer (e.g., a cancer expressing the modified peptide) to treat the mammal. Administration of one or more molecules including one or more antigen-binding domains that can bind to a modified peptide described herein to a mammal (e.g., human) having a cancer can be effective to treat the mammal.

Any type of mammal can be assessed and/or treated as described herein. Examples of mammals that can be assessed and/or treated as described herein include, without limitation, primates (*e.g.*, humans and non-human primates such as chimpanzees, baboons, or monkeys), dogs, cats, pigs, sheep, rabbits, mice, and rats. In some cases, a mammal can be a human.

A mammal can be assessed and/or treated for any appropriate cancer. In some cases, a cancer can express one or more modified peptides (e.g., one or more MANAs) described herein. A cancer can be a primary cancer. A cancer can be a metastatic cancer. A cancer can include one or more solid tumors. A cancer can include one or more non-solid tumors. Examples of cancers that can be assessed as described herein (e.g., based at least in part on the presence of one or more modified peptides described herein) and/or treated as described herein (e.g, by administering one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein) include, without limitation, blood cancers (e.g., Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia such as acute myeloid leukemia (AML), and myeloma), lung cancers, pancreatic cancers, gastric cancers, colon cancers (e.g., colorectal cancers), ovarian cancers, endometrial cancers, biliary tract cancers, liver cancers, bone and soft tissue cancers, breast cancers, prostate cancers, esophageal cancers, stomach cancers, kidney cancers, head and neck cancers, and brain cancers (e.g., glioblastoma multiforme and astrocytomas).

When assessing a mammal having cancer or suspected of having cancer, one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be used to assess for the presence or absence of one or more modified peptides described herein. For example, the presence, absence, or level of one or more modified peptides described herein in a sample obtained from a human can be used to determine whether or not the human has a cancer. In some cases, the presence of one or more modified peptides described herein in a sample obtained from a mammal can be used to identify the mammal as having a cancer. For example, a mammal can be identified as having a cancer

when a sample obtained from the mammal has one or more modified peptides described herein.

Any appropriate sample obtained from a mammal can be assessed for the presence, absence, or level of one or more modified peptides described herein. For example, biological samples such as tissue samples (e.g., breast tissue), and fluid samples (e.g., blood, serum, plasma, or urine) can be obtained from a mammal and assessed for the presence, absence, or level of one or more modified peptides described herein. Any appropriate method can be used to detect the presence, absence, or level of one or more modified peptides described herein. For example, sequencing techniques including, but not limited to, Sanger sequencing, chemical sequencing, nanopore sequencing, sequencing by ligation (SOLiD sequencing), and sequencing with mass spectrometry can be used to determine the presence, absence, or level of one or more modified peptides described herein in a sample obtained from a mammal.

When treating a mammal having cancer, one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be administered to a mammal having cancer to treat the mammal. In some cases, a mammal can have a cancer expressing one or more modified peptides described herein. For example, one or more molecules including one or more antigen-binding domains that can bind to a modified peptide described herein can be administered to a mammal having a cancer expressing that modified peptide to treat the mammal. For example, one or more molecules including one or more scFvs that can bind to a modified peptide described herein (e.g., one or more CARs and/or one or more scDbs) can be administered to a mammal having a cancer expressing that modified peptide to treat the mammal.

In some cases, one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20,

SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be administered to a mammal (e.g., a mammal having a cancer) once or multiple times over a period of time ranging from days to weeks. In some cases, one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein can be formulated into a pharmaceutically acceptable composition for administration to a mammal. For example, an effective amount of one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein can be formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. A pharmaceutical composition can be formulated for administration in solid or liquid form including, without limitation, sterile solutions, suspensions, sustained-release formulations, tablets, capsules, pills, powders, and granules. Pharmaceutically acceptable carriers, fillers, and vehicles that may be used in a pharmaceutical composition described herein include, without limitation, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

A composition containing one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be designed for oral, parenteral (including subcutaneous, intramuscular, intravenous, and intradermal), or intratumoral administration. Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions that can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient. The

formulations can be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets.

A composition containing one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be administered using any appropriate technique and to any appropriate location. A composition including one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein can be administered locally (e.g., intratumorally) or systemically. For example, a composition provided herein can be administered locally by intratumoral administration (e.g., injection into tumors) or by administration into biological spaces infiltrated by tumors (e.g. intraspinal administration, intracerebellar administration, intraperitoneal administration and/or pleural administration). For example, a composition provided herein can be administered systemically by oral administration or by intravenous administration (e.g., injection or infusion) to a mammal (e.g., a human).

Effective doses can vary depending on the risk and/or the severity of the cancer, the route of administration, the age and general health condition of the subject, excipient usage, the possibility of co-usage with other therapeutic treatments such as use of other agents, and the judgment of the treating physician. An effective amount of a composition containing one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be any amount that treats a cancer present within the subject without producing significant toxicity to the subject. If a particular subject fails to respond to a

particular amount, then the amount of one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein can be increased (e.g., by two-fold, three-fold, four-fold, or more). After receiving this higher amount, the mammal can be monitored for both responsiveness to the treatment and toxicity symptoms, and adjustments made accordingly. The effective amount can remain constant or can be adjusted as a sliding scale or variable dose depending on the subject's response to treatment. Various factors can influence the actual effective amount used for a particular application. For example, the frequency of administration, duration of treatment, use of multiple treatment agents, route of administration, and severity of the condition (e.g., cancer) may require an increase or decrease in the actual effective amount administered.

The frequency of administration of one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be any frequency that effectively treats a mammal having a cancer without producing significant toxicity to the mammal. For example, the frequency of administration of one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein can be from about two to about three times a week to about two to about three times a year. In some cases, a subject having cancer can receive a single administration of one or more antibodies described herein. The frequency of administration of one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein can remain constant or can be variable during the duration of treatment. A course of treatment with a composition containing one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein can include rest periods. For example, a composition containing one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein can be administered every other month over a two-year period followed by a six-month rest period, and such a regimen can be repeated multiple times. As with the effective amount, various factors can influence the actual

frequency of administration used for a particular application. For example, the effective amount, duration of treatment, use of multiple treatment agents, route of administration, and severity of the condition (e.g., cancer) may require an increase or decrease in administration frequency.

An effective duration for administering a composition containing one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be any duration that effectively treats a cancer present within the mammal without producing significant toxicity to the mammal. In some cases, the effective duration can vary from several months to several years. In general, the effective duration for treating a mammal having a cancer can range in duration from about one or two months to five or more years. Multiple factors can influence the actual effective duration used for a particular treatment. For example, an effective duration can vary with the frequency of administration, effective amount, use of multiple treatment agents, route of administration, and severity of the condition being treated.

In certain instances, a cancer within a mammal can be monitored to evaluate the effectiveness of the cancer treatment. Any appropriate method can be used to determine whether or not a mammal having cancer is treated. For example, imaging techniques or laboratory assays can be used to assess the number of cancer cells and/or the size of a tumor present within a mammal. For example, imaging techniques or laboratory assays can be used to assess the location of cancer cells and/or a tumor present within a mammal.

In some cases, one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32) can be administered to a mammal having a cancer as a combination therapy with one or more additional cancer treatments (e.g., anti-

cancer agents). A cancer treatment can include any appropriate cancer treatments. In some cases, a cancer treatment can include surgery. In some cases, a cancer treatment can include radiation therapy. In some cases, a cancer treatment can include administration of one or more therapeutic agents (e.g., one or more anti-cancer agents). Examples of anti-cancer agents include, without limitation, platinum compounds (e.g., a cisplatin or carboplatin), taxanes (e.g., paclitaxel, docetaxel, or an albumin bound paclitaxel such as nab-paclitaxel), altretamine, capecitabine, cyclophosphamide, etoposide (vp-16), gemcitabine, ifosfamide, irinotecan (cpt-11), liposomal doxorubicin, melphalan, pemetrexed, topotecan, vinorelbine, luteinizing-hormone-releasing hormone (LHRH) agonists (e.g., goserelin and leuprolide), anti-estrogens (e.g., tamoxifen), aromatase inhibitors (e.g., letrozole, anastrozole, and exemestane), angiogenesis inhibitors (e.g., bevacizumab), poly(ADP)-ribose polymerase (PARP) inhibitors (e.g., olaparib, rucaparib, and niraparib), radioactive phosphorus, anti-CTLA-4 antibodies, anti-PD-1 antibodies, anti-PD-L1 antibodies, IL-2 and other cytokines, and any combinations thereof. In cases where one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein are used in combination with one or more additional cancer treatments, the one or more additional cancer treatments can be administered at the same time or independently. For example, a composition including one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein can be administered first, and the one or more additional cancer treatments administered second, or vice versa.

Also provided herein are kits that include one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein (e.g., a modified peptide including an amino acid sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32). For example, a kit can include a composition (e.g., a pharmaceutically acceptable composition) containing one or more molecules including one or more antigen-binding domains (e.g., scFvs) that can bind to a modified peptide described herein. In some cases, a kit can include instructions for performing any of the methods described herein. In some cases, a kit can include at least one

dose of any of the compositions (e.g., pharmaceutical compositions) described herein. In some cases, a kit can provide a means (e.g., a syringe) for administering any of the compositions (e.g., pharmaceutical compositions) described herein.

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Identification of Additional MANAbody Clones and Conversion of MANAbody Clones into T cell-based Therapeutic Formats

In this study, two phage display libraries were designed and built, both of which displayed a single chain variable fragment (scFv) on the phage surface. The scFvs present in both libraries were based on the humanized 4D5 (trastuzumab) framework with amino acid variability introduced at key positions of the scFv's complementarity determining regions (CDRs).

Phage display libraries were used to identify scFvs that specifically recognized mutation-containing peptides folded into a complex with a recombinant HLA allele alpha chain and beta-2 microglobulin (b2M). These complexes, also referred to herein as monomers, mimic the natural peptide/HLA complexes on a cancer cell surface.

Peptide-HLA targets can include mutant peptides (e.g., Mutation-Associated Neo-Antigens (MANAs)) shown in Table 1. Complementarity-determining regions (CDRs) that can specifically bind to peptide-HLA targets in Table 1 are shown in Table 2. scFvs that can specifically bind to peptide-HLA targets in Table 1 are shown in Table 3. These scFvs can also be referred to as MANAbodies for their ability to bind to **M**utation-**A**ssociated **N**eo-**A**ntigens.

Table 2. MANAbody complementarity-determining region (CDR) sequences of light (L) chains and heavy (H) chains.

Target Peptide(s)	Target HLA Allele	CDR L1	CDR L2	CDR L3	CDR H1	CDR H2	CDR H3
1) EGFR T790M(789-797)-A2							
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQYDYAPIT (SEQ ID NO:34)	GFNISWYQ (SEQ ID NO:41)	VTPYSGYT (SEQ ID NO:48)	SRSYTDGFDY (SEQ ID NO:55)
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQSPYYYLPIT (SEQ ID NO:35)	GFNVSWSY (SEQ ID NO:42)	IYGDSGYT (SEQ ID NO:49)	SRGQWEASYYAMDY (SEQ ID NO:56)
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQYYYSPVT (SEQ ID NO:36)	GFNISWNQ (SEQ ID NO:43)	VSPYSGYT (SEQ ID NO:50)	SRSDYYAMDY (SEQ ID NO:57)
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQHYGNPFT (SEQ ID NO:37)	GFNVGYYG (SEQ ID NO:44)	VSGMEGYT (SEQ ID NO:51)	SRDIYGYAMDV (SEQ ID NO:58)
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQSYYSPPT (SEQ ID NO:38)	GFNITSSY (SEQ ID NO:45)	ISPADGYN (SEQ ID NO:52)	SRTDSTAYTAMDV (SEQ ID NO:59)
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQYYSYPPT (SEQ ID NO:39)	GFNINSSY (SEQ ID NO:46)	ISPTDGYY (SEQ ID NO:53)	SRTSDTSYAAMDV (SEQ ID NO:60)
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQYYYYPPT (SEQ ID NO:40)	GFNISTSY (SEQ ID NO:47)	IDPNDGYS (SEQ ID NO:54)	SRTNNTAADAMDV (SEQ ID NO:61)
2) IDH2 R140Q(134-143)-B7							

SPNGTIQNIL (SEQ ID NO:1)	HLA-B7	QDVNTA (SEQ ID NO:33)	SAS	QQYSYSPPT (SEQ ID NO:62)	GFNISDTY (SEQ ID NO:67)	ISPRTGYN (SEQ ID NO:72)	SRAYYSYAYAMDV (SEQ ID NO:77)
SPNGTIQNIL (SEQ ID NO:1)	HLA-B7	QDVNTA (SEQ ID NO:33)	SAS	QQGKAYWPAT (SEQ ID NO:63)	GFNVGHYR (SEQ ID NO:68)	VSPNGYYT (SEQ ID NO:73)	SRGYSSYAFDY (SEQ ID NO:78)
SPNGTIQNIL (SEQ ID NO:1)	HLA-B7	QDVNTA (SEQ ID NO:33)	SAS	QQVYSSPFT (SEQ ID NO:64)	GFNVKYYM (SEQ ID NO:69)	ISPGYDYT (SEQ ID NO:74)	SRSYWRYSVDV (SEQ ID NO:79)
SPNGTIQNIL (SEQ ID NO:1)	HLA-B7	QDVNTA (SEQ ID NO:33)	SAS	QQYSLYSPMT (SEQ ID NO:65)	GFNSFLS (SEQ ID NO:70)	IFPSSDYT (SEQ ID NO:75)	SRGKHSSDSNYYMDY (SEQ ID NO:80)
SPNGTIQNIL (SEQ ID NO:1)	HLA-B7	QDVNTA (SEQ ID NO:33)	SAS	QQSYYMPFT (SEQ ID NO:66)	GFNIFRGY (SEQ ID NO:71)	ISPHSDYT (SEQ ID NO:76)	SRSYGWAAFDY (SEQ ID NO:81)
3) p53 R248Q/W(245-254)-A2							
GMNQRPILTI (SEQ ID NO:15)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQSGYAPIT (SEQ ID NO:82)	GFNISYYS (SEQ ID NO:89)	VDPDSDYT (SEQ ID NO:96)	SRSWIHMFSMDY (SEQ ID NO:103)
GMNWRPILTI (SEQ ID NO:16)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQYSYAPIT (SEQ ID NO:83)	GFNIGYYT (SEQ ID NO:90)	VSPWSYST (SEQ ID NO:97)	SRDHWDEAFDV (SEQ ID NO:104)
GMNQRPILTI (SEQ ID NO:15)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQSLYGPFT (SEQ ID NO:84)	GFNIAYEY (SEQ ID NO:91)	IGPDSGYT (SEQ ID NO:98)	SRVWYYSTYGMDY (SEQ ID NO:105)
GMNWRPILTI (SEQ ID NO:16)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQYSYSPIT (SEQ ID NO:85)	GFNLFGYG (SEQ ID NO:92)	IGPYYYYT (SEQ ID NO:99)	SRENYDMAMDY (SEQ ID NO:106)
GMNWRPILTI (SEQ ID NO:16)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQSGYQPDT (SEQ ID NO:86)	GFNISWYA (SEQ ID NO:93)	IWPDSDWT (SEQ ID NO:100)	SRYYYSSAFDV (SEQ ID NO:107)

GMNQRPILTI (SEQ ID NO:15), GMNWRPILTI (SEQ ID NO:16)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQYLYQPWT (SEQ ID NO:87)	GFNIDYYG (SEQ ID NO:94)	LYGGSDST (SEQ ID NO:101)	SRQYSAYFDY (SEQ ID NO:108)
GMNQRPILTI (SEQ ID NO:15), GMNWRPILTI (SEQ ID NO:16)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQGLYYPWT (SEQ ID NO:88)	GFNVSYSS (SEQ ID NO:95)	IWPDSGQT (SEQ ID NO:102)	SRSSYFDAMDY (SEQ ID NO:109)
4) KRAS G12V(6-14)-A2								
LVVVGAVGV (SEQ ID NO:18)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQWYSSPVT (SEQ ID NO:110)	GFNINWAN (SEQ ID NO:112)	ISPPYDYT (SEQ ID NO:115)	SRSYSYYFDY (SEQ ID NO:118)
LVVVGAVGV (SEQ ID NO:18)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQYYSRPVT (SEQ ID NO:111)	GFNIYLHD (SEQ ID NO:113)	IIPAIDYT (SEQ ID NO:116)	SRRDGYYFDY (SEQ ID NO:119)
LVVVGAVGV (SEQ ID NO:18)	HLA-A2	QDVNTA (SEQ ID NO:33)	SAS	QQWYSSPVT (SEQ ID NO:110)	GFNIYWSH (SEQ ID NO:114)	ISSFEGYT (SEQ ID NO:117)	SRSYSYYMDY (SEQ ID NO:120)
5) KRAS G12C/D/V(7-16)-A3								
VVVGACGVGK (SEQ ID NO:20), VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQSYGSGSPWT (SEQ ID NO:121)	GFNIVGGG (SEQ ID NO:124)	IYPQGDYT (SEQ ID NO:127)	SRDSSYLAFDY (SEQ ID NO:129)
VVVGACGVGK (SEQ ID NO:20), VVVGADGVGK (SEQ ID NO:21), VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQTYYSPWT (SEQ ID NO:122)	GFNIRSYA (SEQ ID NO:125)	VGPGKGYT (SEQ ID NO:128)	SRNFQSTSHAFDY (SEQ ID NO:130)

VVVGACGVGK (SEQ ID NO:20), VVVGADGVGK (SEQ ID NO:21), VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQYYYPPIT (SEQ ID NO:123)	GFNVSHTG (SEQ ID NO:126)	VGPGKGYT (SEQ ID NO:128)	SRKTYYAFDY (SEQ ID NO:131)
6) KRAS G12V(7-16)-A3							
VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQSYYYFRPIT (SEQ ID NO:132)	GFNLSYSD (SEQ ID NO:137)	VMPDSGHT (SEQ ID NO:142)	SRATNIPVYAFDY (SEQ ID NO:147)
VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQASYYYPLT (SEQ ID NO:133)	GFNISASG (SEQ ID NO:138)	IHPLKPYT (SEQ ID NO:143)	SRYSSMYYYYFD Y (SEQ ID NO:148)
VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQKSEYSPWT (SEQ ID NO:134)	GFNIYRYG (SEQ ID NO:139)	LYPYGYST (SEQ ID NO:144)	SRSYAYGYFAY (SEQ ID NO:149)
VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQSGYIPFT (SEQ ID NO:135)	GFNIYGTM (SEQ ID NO:140)	FKPDSYNT (SEQ ID NO:145)	SRGEVYHYYAFD Y (SEQ ID NO:150)
VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQGAYYRPFT (SEQ ID NO:136)	GFNISYSY (SEQ ID NO:141)	LLPYDGNT (SEQ ID NO:146)	SRAAYSSMDV (SEQ ID NO:151)
7) KRAS G12D(7-16)-A3							
VVVGADGVGK (SEQ ID NO:21)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQYMYSPVT (SEQ ID NO:152)	GFNVSAYW (SEQ ID NO:156)	IYGGSGYT (SEQ ID NO:160)	SRTHSYWSAFDY (SEQ ID NO:164)

VVVGADGVGK (SEQ ID NO:21)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQSSSSPIT (SEQ ID NO:153)	GFNISGYG (SEQ ID NO:157)	LYGGSDYT (SEQ ID NO:161)	SRTVRYAFDY (SEQ ID NO:165)
VVVGADGVGK (SEQ ID NO:21)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQSSASPLT (SEQ ID NO:154)	GFNVSSVG (SEQ ID NO:158)	IYGTSDYT (SEQ ID NO:162)	SRSSRYSMDY (SEQ ID NO:166)
VVVGADGVGK (SEQ ID NO:21)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQYAYSPLT (SEQ ID NO:155)	GFNVSSYG (SEQ ID NO:159)	IAPRRDYT (SEQ ID NO:163)	SRKSSYYFDY (SEQ ID NO:167)
8) KRAS G12D(7-16)-A11								
VVVGADGVGK (SEQ ID NO:21)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYSYYPIT (SEQ ID NO:168)	GFNFSYGY (SEQ ID NO:173)	ISGYTGNT (SEQ ID NO:178)	SRAASLSSSYYSAFDV (SEQ ID NO:183)
VVVGADGVGK (SEQ ID NO:21)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYSYTPVT (SEQ ID NO:169)	GFNVWGPG (SEQ ID NO:174)	IHPFSGNT (SEQ ID NO:179)	SRGYSYSAMDY (SEQ ID NO:184)
VVVGADGVGK (SEQ ID NO:21)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYSYEPVT (SEQ ID NO:170)	GFNVSGSQ (SEQ ID NO:175)	IPGWSGYT (SEQ ID NO:180)	SRGYSYFAMDY (SEQ ID NO:185)
VVVGADGVGK (SEQ ID NO:21)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYAYYSPVT (SEQ ID NO:171)	GFNIYGQM (SEQ ID NO:176)	LSPFSGNT (SEQ ID NO:181)	SRNISYEQSSAFDY (SEQ ID NO:186)
VVVGADGVGK (SEQ ID NO:21)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYEYYPMT (SEQ ID NO:172)	GFNVMYST (SEQ ID NO:177)	IYSWSDYT (SEQ ID NO:182)	SRGYAHNSFDY (SEQ ID NO:187)
9) KRAS G12D(8-16)-A11								
VVGADGVGK (SEQ ID NO:24)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYSFYPFT (SEQ ID NO:188)	GFNFGSY (SEQ ID NO:196)	ISGYSGNT (SEQ ID NO:207)	SRSNQSAYSYMDY (SEQ ID NO:218)

VVGADGVGK (SEQ ID NO:24)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYSYSPIT (SEQ ID NO:85)	GFNISDSY (SEQ ID NO:197)	FSPYSSNT (SEQ ID NO:208)	SRSQFTFYQYFDY (SEQ ID NO:219)
VVGADGVGK (SEQ ID NO:24)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAY	QQYSAYYQPIT (SEQ ID NO:189)	GFNIFSDQ (SEQ ID NO:198)	FMPYDSYYT (SEQ ID NO:209)	SRMSVRNAFDY (SEQ ID NO:220)
VVGADGVGK (SEQ ID NO:24)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYSYYPIT (SEQ ID NO:168)	GFNLSYSY (SEQ ID NO:199)	ISGFSGNT (SEQ ID NO:210)	SRSDSYYTAMDY (SEQ ID NO:221)
VVGADGVGK (SEQ ID NO:24)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYEYVPHT (SEQ ID NO:190)	GFNISYGY (SEQ ID NO:200)	FHYGSGNT (SEQ ID NO:211)	SRSNYYYLDY (SEQ ID NO:222)
VVGADGVGK (SEQ ID NO:24)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYSYMPIT (SEQ ID NO:191)	GFNISYQH (SEQ ID NO:201)	FMPYQGST (SEQ ID NO:212)	SRANIYSSHSFFDY (SEQ ID NO:223)
VVGADGVGK (SEQ ID NO:24)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYAYYPVT (SEQ ID NO:192)	GFNLSGYY (SEQ ID NO:202)	FSPYSGYT (SEQ ID NO:213)	SRTHSSIYHSFDY (SEQ ID NO:224)
VVGADGVGK (SEQ ID NO:24)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYSYMPIT (SEQ ID NO:191)	GFNVSGQY (SEQ ID NO:203)	ISPVSGNT (SEQ ID NO:214)	SRPMKTSYYGAFDY (SEQ ID NO:225)
VVGADGVGK (SEQ ID NO:24)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYDYRPVT (SEQ ID NO:193)	GFNVSTSG (SEQ ID NO:204)	IYGAYSGT (SEQ ID NO:215)	SRSQSYTYWSAMDY (SEQ ID NO:226)
VVGADGVGK (SEQ ID NO:24)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYDFTPMT (SEQ ID NO:194)	GFNISYAK (SEQ ID NO:205)	LTYWGGYT (SEQ ID NO:216)	SRGEYGTYMDY (SEQ ID NO:227)
VVGADGVGK (SEQ ID NO:24)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYSSSSPVT (SEQ ID NO:195)	GFNFSSYV (SEQ ID NO:206)	VYPDSGGT (SEQ ID NO:217)	SRTSSYYAFDY (SEQ ID NO:228)
10) KRAS G12V(7-16)-A11							
VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQSSYTPIT (SEQ ID NO:229)	GFNISQGG (SEQ ID NO:234)	VYPGGGQT (SEQ ID NO:240)	SRGYDYSAFDY (SEQ ID NO:246)

VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYAYYPIT (SEQ ID NO:230)	GFNISSTG (SEQ ID NO:235)	LLGGSGNT (SEQ ID NO:241)	SRGLQYSAMDY (SEQ ID NO:247)
VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	QDVNTA	SAS	QQYEYYPIT (SEQ ID NO:231)	GFNFFSTV (SEQ ID NO:236)	IYPWSGST (SEQ ID NO:242)	SRSRSSNYYFDV (SEQ ID NO:248)
VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYTYYPIT (SEQ ID NO:232)	GFNLHGYL (SEQ ID NO:237)	IYPPNGYT (SEQ ID NO:243)	SRGVDYAYLDY (SEQ ID NO:249)
VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQYSYYPIT (SEQ ID NO:168)	GFNLSTHV (SEQ ID NO:238)	FYPYVGYT (SEQ ID NO:244)	SRGYRYQYMDV (SEQ ID NO:250)
VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	QDVNTA (SEQ ID NO:33)	SAS	QQSSVEPWT (SEQ ID NO:233)	GFNVSYYS (SEQ ID NO:239)	IYPWNDYT (SEQ ID NO:245)	SRGSYYSFDY (SEQ ID NO:251)
11) CTNNB S45F(41-49)-A3							
TTAPFLSGK (SEQ ID NO:26)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQSYYSPPT (SEQ ID NO:38)	GFNINNTY (SEQ ID NO:256)	IYPTDGYT (SEQ ID NO:260)	SRTYYSYYSAMDV (SEQ ID NO:265)
TTAPFLSGK (SEQ ID NO:26)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAY	QQIYTSPIT (SEQ ID NO:252)	GFNFITTG (SEQ ID NO:257)	IGPGSDYT (SEQ ID NO:261)	SRYYYASALDY (SEQ ID NO:266)
TTAPFLSGK (SEQ ID NO:26)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQRAYFPIT (SEQ ID NO:253)	GFNFSDYG (SEQ ID NO:258)	LIPASGYT (SEQ ID NO:262)	SRGWSYYMDY (SEQ ID NO:267)
TTAPFLSGK (SEQ ID NO:26)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAS	QQQYAYTPIT (SEQ ID NO:254)	GFNVWSYG (SEQ ID NO:259)	VTPDGSYT (SEQ ID NO:263)	SRSYGWAMDY (SEQ ID NO:268)
TTAPFLSGK (SEQ ID NO:26)	HLA-A3	QDVNTA (SEQ ID NO:33)	SAY	QQIHYKPLT (SEQ ID NO:255)	GFNVAWYS (SEQ ID NO:260)	VYGGSSYT (SEQ ID NO:264)	SRDFYSSGMDY (SEQ ID NO:269)
12) KRAS G12V(11-19)-B7							

AVGVGKSAL (SEQ ID NO:11)	HLA-B7	QDVNTA (SEQ ID NO:33)	SAS	QQEWRLPIT (SEQ ID NO:270)	GFNVYGNQ (SEQ ID NO:275)	IYPYSGST (SEQ ID NO:278)	SRSAYVAYSYFDY (SEQ ID NO:283)
AVGVGKSAL (SEQ ID NO:11)	HLA-B7	QDVNTA (SEQ ID NO:33)	SAS	QQGTSTPFT (SEQ ID NO:271)	GFNLSYYG (SEQ ID NO:402)	IYPDSGYT (SEQ ID NO:279)	SRAYLYYYLAY (SEQ ID NO:284)
AVGVGKSAL (SEQ ID NO:11)	HLA-B7	QDVNTA (SEQ ID NO:33)	SAS	QQSWRYPMT (SEQ ID NO:272)	GFNISRYG (SEQ ID NO:276)	FYPSSSYT (SEQ ID NO:280)	SRKYYEAMDY (SEQ ID NO:285)
AVGVGKSAL (SEQ ID NO:11)	HLA-B7	QDVNTA (SEQ ID NO:33)	SAS	QQSYSYPVT (SEQ ID NO:273)	GFNIYSSW (SEQ ID NO:277)	FQPYSGYT (SEQ ID NO:281)	SREYTYYFDY (SEQ ID NO:286)
AVGVGKSAL (SEQ ID NO:11)	HLA-B7	QDVNTA (SEQ ID NO:33)	SAS	QQGWLYSPFT (SEQ ID NO:274)	GFNISGYG (SEQ ID NO:157)	VYGGSGYT (SEQ ID NO:282)	SRAHSSYYVDY (SEQ ID NO:287)
13) H/K/N RAS Q61H(55-64)-A1							
ILDTAGHEEY (SEQ ID NO:28)	HLA-A1	QDVNTA (SEQ ID NO:33)	SAS	QQGYFYYPNT (SEQ ID NO:288)	GFNIGYYG (SEQ ID NO:289)	VYPGGGYT (SEQ ID NO:290)	SRYYYYGFDY (SEQ ID NO:291)
14) H/K/N RAS Q61K(55-64)-A1							
ILDTAGKEEY (SEQ ID NO:30)	HLA-A1	QDVNTA (SEQ ID NO:33)	SAS	QQHYYSPVT (SEQ ID NO:292)	GFNIFYQD (SEQ ID NO:293)	IYPDYDYT (SEQ ID NO:294)	SRTYSVYMDY (SEQ ID NO:295)
15) H/K/N RAS Q61L(55-64)-A1							
ILDTAGLEEY (SEQ ID NO:31)	HLA-A1	QDVNTA (SEQ ID NO:33)	SAS	QQYAYAPFT (SEQ ID NO:296)	GFNVSYSM (SEQ ID NO:297)	VWGDGGVT (SEQ ID NO:298)	SRGSYYAFDY (SEQ ID NO:299)
16) H/K/N RAS Q61R(55-64)-A1							

ILDTAGREEY (SEQ ID NO:32)	HLA-A1	QDVNTA (SEQ ID NO:33)	SAS	QQAHMIPIT (SEQ ID NO:300)	GFNFSFPG (SEQ ID NO:305)	FVGYDGYT (SEQ ID NO:310)	SRDYYSFSMDY (SEQ ID NO:316)
ILDTAGREEY (SEQ ID NO:32)	HLA-A1	QDVNTA (SEQ ID NO:33)	SAS	QQSVYDPIT (SEQ ID NO:301)	GFNISGSW (SEQ ID NO:306)	LYPDSDYT (SEQ ID NO:311)	SRAHTYAFDY (SEQ ID NO:317)
ILDTAGREEY (SEQ ID NO:32)	HLA-A1	QDVNTA (SEQ ID NO:33)	SAS	QQSYTSPLT (SEQ ID NO:302)	GFNIYYGV (SEQ ID NO:307)	IYPDSSWT (SEQ ID NO:312)	SRDQDFHYMNYY LSYALDY (SEQ ID NO:318)
ILDTAGREEY (SEQ ID NO:32)	HLA-A1	QDVNTA (SEQ ID NO:33)	SAS	QQGQYSPFT (SEQ ID NO:303)	GFNVSYEY (SEQ ID NO:308)	IYGGSDNT (SEQ ID NO:313)	SRPLGSYFDY (SEQ ID NO:319)
ILDTAGREEY (SEQ ID NO:32)	HLA-A1	QDVNTA (SEQ ID NO:33)	SAS	QQYWYLPTT (SEQ ID NO:320)	GFNISWYD (SEQ ID NO:321)	IEPSVGYT (SEQ ID NO:322)	SRSYPYYYFDY (SEQ ID NO:323)

Table 3. MANAbody scFv sequences.

Target Peptide(s)	Target HLA Allele	scFv clone name	scFv sequence	SEQ ID NO:
1) EGFR T790M(789-797)-A2				
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	EGFR_T790M_A2_cl1	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YDYAPITFGQGTKVEIKRTGGGSGGGGSGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNISWYQMHWVRQAPGKGLEWVALVTP YSGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRSYTDGFDYWGQGTLVTVSS	324

IMQLMPFGC (SEQ ID NO:13)	HLA-A2	EGFR_T790M_A2_cl5	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS PYYYLPITFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNVSWSYMHWVRQAPGKGLEWVANIY GDSGYTHYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRGQWEASYYAMDYWGQGTLVTVSS	325
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	EGFR_T790M_A2_cl18	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YYYSPVTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNISWNQMHWVRQAPGKGLEWVALVSP YSGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRSDYYAMDYWGQGTLVTVSS	326
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	EGFR_T790M_A2_cl23	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ HYGNPFTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNVGYYGMHWVRQAPGKGLEWVAFVS GMEGYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRDIYGYAMDVWGQGTLVTVSS	327
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	EGFR_T790M_A2_D2D6	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS YYSPPTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGGLV QPGGSLRLSCAASGFNITSSYIHWVRQAPGKGLEWVAYISPADG YNRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRT DSTAYTAMDVWGQGTLVTVSS	328
IMQLMPFGC (SEQ ID NO:13)	HLA-A2	EGFR_T790M_A2_D2D8	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YYSYPPTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNINSSYIHWVRQAPGKGLEWVAYISPTD GYYRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR TSDTSYAAMDVWGQGTLVTVSS	329

IMQLMPFGC (SEQ ID NO:13)	HLA-A2	EGFR_T790M_A2_D3E6	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YYYYPPTFGQGTKVEIKRTGGGSGGGSGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNISTSYIHWVRQAPGKGLEWVATIDPN DGYSRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RTNNTAADAMDVWGQGTLVTVSS	330
2) IDH2 R140Q(134-143)-B7				
SPNGTIQNIL (SEQ ID NO:1)	HLA-B7	IDH2_R140Q_B7_D4	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSYSPPTFGQGTKVEIKRTGGGSGGGSGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNISDTYIHWVRQAPGKGLEWVASISPRTG YNRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRA YYSYAYAMDVWGQGTLVTVSS	3
SPNGTIQNIL (SEQ ID NO:1)	HLA-B7	IDH2_R140Q_B7_cl29	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ GKAYWPATFGQGTKVEIKRTGGGSGGGSGGGASEVQLVESGG GLVQPGGSLRLSCAASGFNVGHYRMHWVRQAPGKGLEWVAM VSPNGYYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV YYCSRGYSSYAFDYWGQGTLVTVSS	4
SPNGTIQNIL (SEQ ID NO:1)	HLA-B7	IDH2_R140Q_B7_cl1	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ VYSSPFTFGQGTKVEIKRTGGGSGGGSGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNVKYYMMHWVRQAPGKGLEWVAAISP GYDYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRSYWRYSVDVWGQGTLVTVSS	5

SPNGTIQNIL (SEQ ID NO:1)	HLA-B7	IDH2_R140Q_B7_cl3	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSLYSPMTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGG GLVQPGGSLRLSCAASGFNSFLSIHWVRQAPGKGLEWVAHIFPSS DYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR GKHSSDSNYYMDYWGQGTLVTVSS	6
SPNGTIQNIL (SEQ ID NO:1)	HLA-B7	IDH2_R140Q_B7_cl8	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS YYMPFTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNIFRGYMHWVRQAPGKGLEWVAMISPH SDYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR SYGWAAFDYWGQGTLVTVSS	8
3) p53 R248Q/W(245-254)-A2				
GMNQRPILTI (SEQ ID NO:15)	HLA-A2	p53_R248Q_A2_cl0	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS GYAPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGLV QPGGSLRLSCAASGFNISYYSMHWVRQAPGKGLEWVADVDPDS DYTEYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRS WIHMFSMDYWGQGTLVTVSS	331
GMNWRPILTI (SEQ ID NO:16)	HLA-A2	p53_R248W_A2_cl2	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSYAPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNIGYYTMHWVRQAPGKGLEWVAEVSP WSYSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RDHWDEAFDVWGQGTLVTVSS	332

GMNQRPILTI (SEQ ID NO:15)	HLA-A2	p53_R248Q_A2_cl4	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS LYGPFTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGGLV QPGGSLRLSCAASGFNIAYEYMHWVRQAPGKGLEWVALIGPDS GYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR VWYYSTYGMDYWGQGTLVTVSS	333
GMNWRPILTI (SEQ ID NO:16)	HLA-A2	p53_R248W_A2_cl8	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSYSPITFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNLFGYGMHWVRQAPGKGLEWVAEIGPY YYYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RENYDMAMDYWGQGTLVTVSS	334
GMNWRPILTI (SEQ ID NO:16)	HLA-A2	p53_R248W_A2_cl11	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS GYQPDTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNISWYAMHWVRQAPGKGLEWVAEIWP DSDWTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRYYYSSAFDVWGQGTLVTVSS	335
GMNQRPILTI (SEQ ID NO:15), GMNWRPILTI (SEQ ID NO:16)	HLA-A2	p53_R248QW_A2_cl14	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YLYQPWTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNIDYYGMHWVRQAPGKGLEWVASLY GGSDSTDYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRQYSAYFDYWGQGTLVTVSS	336
GMNQRPILTI (SEQ ID NO:15), GMNWRPILTI (SEQ ID NO:16)	HLA-A2	p53_R248QW_A2_cl17	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ GLYYPWTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNVSYSSIHWVRQAPGKGLEWVAEIWPD SGQTWYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RSSYFDAMDYWGQGTLVTVSS	337

4) KRAS G12V(6-14)-A2				
LVVVGAVGV (SEQ ID NO:18)	HLA-A2	KRAS_G12V_A2_A1	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ WYSSPVTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNINWANMHWVRQAPGKGLEWVAQISP PYDYTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRSYSYYFDYWGQGTLVTVSS	338
LVVVGAVGV (SEQ ID NO:18)	HLA-A2	KRAS_G12V_A2_C1	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YYSRPVTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNIYLHDMHWVRQAPGKGLEWVAQIIPAI DYTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR RDGYYFDYWGQGTLVTVSS	339
LVVVGAVGV (SEQ ID NO:18)	HLA-A2	KRAS_G12V_A2_A5	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ WYSSPVTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNIYWSHMHWVRQAPGKGLEWVAIISSF EGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RSYSYYMDYWGQGTLVTVSS	340
5) KRAS G12C/D/V(7-16)-A3				
VVVGACGVGK (SEQ ID NO:20), VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	KRAS_G12CV_A3_cl5	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS YGSGSPWTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGG GLVQPGGSLRLSCAASGFNIVGGIHWVRQAPGKGLEWVAKIYP QGDYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRDSSYLAFDYWGQGTLVTVSS	341

VVVGACGVGK (SEQ ID NO:20), VVVGADGVGK (SEQ ID NO:21), VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	KRAS_G12CDV_A3_cl9	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ TYYSPWTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNIRSYAMHWVRQAPGKGLEWVAQVGP GKGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRNFQSTSHAFDYWGQGTLVTVSS	342
VVVGACGVGK (SEQ ID NO:20), VVVGADGVGK (SEQ ID NO:21), VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	KRAS_G12CDV_A3_cl18	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YYYPPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNVSHTGMHWVRQAPGKGLEWVAVVGP GKGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRKTYYAFDYWGQGTLVTVSS	343
6) KRAS G12V(7-16)-A3				
VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	KRAS_G12V_A3_cl2	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS YYYFRPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNLSYSDIHWVRQAPGKGLEWVAVVMP DSGHTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRATNIPVYAFDYWGQGTLVTVSS	344
VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	KRAS G12V_A3_V12	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ ASYYYPLTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGG GLVQPGGSLRLSCAASGFNISASGMHWVRQAPGKGLEWVADIH PLKPYTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRYSSMYYYYFDYWGQGTLVTVSS	345

VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	KRAS G12V_A3_cl20	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ KSEYSPWTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGG GLVQPGGSLRLSCAASGFNIYRYGIHWVRQAPGKGLEWVAVLY PYGYSTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRSYAYGYFAYWGQGTLVTVSS	346
VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	KRAS G12V_A3_cl21	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS GYIPFTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGLV QPGGSLRLSCAASGFNIYGTMMHWVRQAPGKGLEWVAQFKPDS YNTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR GEVYHYYAFDYWGQGTLVTVSS	347
VVVGAVGVGK (SEQ ID NO:22)	HLA-A3	KRAS G12V_A3_cl22	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ GAYYRPFTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGG GLVQPGGSLRLSCAASGFNISYSYMHWVRQAPGKGLEWVATLL PYDGNTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRAAYSSMDVWGQGTLVTVSS	348
7) KRAS G12D(7-16)-A3				
VVVGADGVGK (SEQ ID NO:21)	HLA-A3	KRAS_G12D_A3_cl11	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YMYSPVTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNVSAYWMHWVRQAPGKGLEWVAQIY GGSGYTMYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRTHSYWSAFDYWGQGTLVTVSS	349

VVVGADGVGK (SEQ ID NO:21)	HLA-A3	KRAS _G12D _A3 _D12	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS SSSPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGLV QPGGSLRLSCAASGFNISGYGMHWVRQAPGKGLEWVAYLYGGS DYTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR TVRYAFDYWGQGTLVTVSS	350
VVVGADGVGK (SEQ ID NO:21)	HLA-A3	KRAS_G12D_A3_D15	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS SASPLTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGLV QPGGSLRLSCAASGFNVSSVGMHWVRQAPGKGLEWVAYIYGTS DYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR SSRYSMDYWGQGTLVTVSS	351
VVVGADGVGK (SEQ ID NO:21)	HLA-A3	KRAS_G12D_A3_D26	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YAYSPLTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNVSSYGMHWVRQAPGKGLEWVAFIAPR RDYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR KSSYYFDYWGQGTLVTVSS	352
8) KRAS G12D(7-16)-A11				
VVVGADGVGK (SEQ ID NO:21)	HLA-A11	KRAS_G12D_A11_D3	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSYYPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNFSYGYMHWVRQAPGKGLEWVAWISG YTGNTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRAASLSSSYYSAFDVWGQGTLVTVSS	353

VVVGADGVGK (SEQ ID NO:21)	HLA-A11	KRAS_G12D_A11_D14	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSYTPVTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNVWGPGMHWVRQAPGKGLEWVARIHP FSGNTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RGYSYSAMDYWGQGTLVTVSS	354
VVVGADGVGK (SEQ ID NO:21)	HLA-A11	KRAS_G12D_A11_D18	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSYEPVTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNVSGSQMHWVRQAPGKGLEWVARIPG WSGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRGYSYFAMDYWGQGTLVTVSS	355
VVVGADGVGK (SEQ ID NO:21)	HLA-A11	KRAS_G12D_A11_D21	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YAYYSPVTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGG GLVQPGGSLRLSCAASGFNIYGQMMHWVRQAPGKGLEWVAFL SPFSGNTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRNISYEQSSAFDYWGQGTLVTVSS	356
VVVGADGVGK (SEQ ID NO:21)	HLA-A11	KRAS_G12D_A11_D22	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YEYYPMTFGQGTKVEIKRTGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNVMYSTMHWVRQAPGKGLEWVASIYS WSDYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRGYAHNSFDYWGQGTLVTVSS	357
9) KRAS G12D(8-16)-A11				

VVGADGVGK (SEQ ID NO:24)	HLA-A11	KRAS_G12D_A11_cl4	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSFYPFTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNFGSYIHWVRQAPGKGLEWVAIISGYSG NTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRS NQSAYSYMDYWGQGTLVTVSS	358
VVGADGVGK (SEQ ID NO:24)	HLA-A11	KRAS_G12D_A11_cl6	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSYSPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNISDSYMHWVRQAPGKGLEWVATFSPY SSNTWYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RSQFTFYQYFDYWGQGTLVTVSS	359
VVGADGVGK (SEQ ID NO:24)	HLA-A11	KRAS_G12D_A11_cl7	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSAYFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSAYYQPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGG GLVQPGGSLRLSCAASGFNIFSDQMHWVRQAPGKGLEWVAGFM PYDSYYTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVY YCSRMSVRNAFDYWGQGTLVTVSS	360
VVGADGVGK (SEQ ID NO:24)	HLA-A11	KRAS_G12D_A11_cl9	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSYYPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNLSYSYMHWVRQAPGKGLEWVAVISGF SGNTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RSDSYYTAMDYWGQGTLVTVSS	361
VVGADGVGK (SEQ ID NO:24)	HLA-A11	KRAS_G12D_A11_cl10	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YEYVPHTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNISYGYMHWVRQAPGKGLEWVAKFHY GSGNTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRSNYYYLDYWGQGTLVTVSS	362

VVGADGVGK (SEQ ID NO:24)	HLA-A11	KRAS_G12D_A11_cl12	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSYMPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNISYQHIHWVRQAPGKGLEWVAVFMPY QGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RANIYSSHSFFDYWGQGTLVTVSS	363
VVGADGVGK (SEQ ID NO:24)	HLA-A11	KRAS_G12D_A11_cl14	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YAYYPVTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNLSGYYMHWVRQAPGKGLEWVAWFS PYSGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRTHSSIYHSFDYWGQGTLVTVSS	364
VVGADGVGK (SEQ ID NO:24)	HLA-A11	KRAS_G12D_A11_cl15	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSYMPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNVSGQYMHWVRQAPGKGLEWVAVISPV SGNTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RPMKTSYYGAFDYWGQGTLVTVSS	365
VVGADGVGK (SEQ ID NO:24)	HLA-A11	KRAS_G12D_A11_cl17	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YDYRPVTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNVSTSGMHWVRQAPGKGLEWVAFIYG AYSGTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRSQSYTYWSAMDYWGQGTLVTVSS	366
VVGADGVGK (SEQ ID NO:24)	HLA-A11	KRAS_G12D_A11_cl18	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YDFTPMTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNISYAKMHWVRQAPGKGLEWVAYLTY WGGYTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRGEYGTYMDYWGQGTLVTVSS	367

VVGADGVGK (SEQ ID NO:24)	HLA-A11	KRAS_G12D_A11_cl19	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSSSSPVTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNFSSYVMHWVRQAPGKGLEWVAVVY PDSGGTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRTSSYYAFDYWGQGTLVTVSS	368
10) KRAS G12V(7-16)-A11				
VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	KRAS_G12V_A11_V3	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS SYTPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGLV QPGGSLRLSCAASGFNISQGGIHWVRQAPGKGLEWVAYVYPGG GQTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR GYDYSAFDYWGQGTLVTVSS	369
VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	KRAS_G12V_A11_V9	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YAYYPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNISSTGMHWVRQAPGKGLEWVAELLGG SGNTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RGLQYSAMDYWGQGTLVTVSS	370
VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	KRAS_G12V_A11_V10	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YEYYPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNFFSTVIHWVRQAPGKGLEWVAEIYPWS GSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRS RSSNYYFDVWGQGTLVTVSS	371

VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	KRAS_G12V_A11_V21	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YTYYPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNLHGYLMHWVRQAPGKGLEWVAFIYPP NGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RGVDYAYLDYWGQGTLVTVSS	372
VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	KRAS_G12V_A11_V23	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YSYYPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNLSTHVMHWVRQAPGKGLEWVAEFYPY VGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RGYRYQYMDVWGQGTLVTVSS	373
VVVGAVGVGK (SEQ ID NO:22)	HLA-A11	KRAS_G12V_A11_V24	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS SVEPWTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNVSYYSIHWVRQAPGKGLEWVAYIYPW NDYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RGSYYSFDYWGQGTLVTVSS	374
11) CTNNB S45F(41-49)-A3				
TTAPFLSGK (SEQ ID NO:26)	HLA-A3	CTNNB_S45F_A3_E10	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS YYSPPTFGQGTKVEIKRTGGGSGGGGASEVQLVESGGGLVQPGGS LRLSCAASGFNINNTYIHWVRQAPGKGLEWVASIYPTDGYTRYA DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRTYYSYYS AMDVWGQGTLVTVSS	375

TTAPFLSGK (SEQ ID NO:26)	HLA-A3	CTNNB_S45F_A3_cl3	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSAYFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQI YTSPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGLV QPGGSLRLSCAASGFNFITTGMHWVRQAPGKGLEWVARIGPGSD YTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRY YYASALDYWGQGTLVTVSS	376
TTAPFLSGK (SEQ ID NO:26)	HLA-A3	CTNNB_S45F_A3_cl4	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ RAYFPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNFSDYGMHWVRQAPGKGLEWVAMLIPA SGYTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RGWSYYMDYWGQGTLVTVSS	377
TTAPFLSGK (SEQ ID NO:26)	HLA-A3	CTNNB_S45F_A3_cl7	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ QYAYTPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNVWSYGIHWVRQAPGKGLEWVAGVTP DGSYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRSYGWAMDYWGQGTLVTVSS	378
TTAPFLSGK (SEQ ID NO:26)	HLA-A3	CTNNB_S45F_A3_cl9	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSAYFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQI HYKPLTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNVAWYSIHWVRQAPGKGLEWVAQVYG GSSYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RDFYSSGMDYWGQGTLVTVSS	379
12) KRAS G12V(11-19)-B7				

AVGVGKSAL (SEQ ID NO:11)	HLA-B7	KRAS_G12V_B7_cl1	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ EWRLPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNVYGNQIHWVRQAPGKGLEWVARIYPY SGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR SAYVAYSYFDYWGQGTLVTVSS	380
AVGVGKSAL (SEQ ID NO:11)	HLA-B7	KRAS_G12V_B7_cl2	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ GTSTPFTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNLSYYGMHWVRQAPGKGLEWVATIYPD SGYTKYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RAYLYYYLAYWGQGTLVTVSS	390
AVGVGKSAL (SEQ ID NO:11)	HLA-B7	KRAS_G12V_B7_cl3	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS WRYPMTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNISRYGMHWVRQAPGKGLEWVAVFYPS SSYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR KYYEAMDYWGQGTLVTVSS	391
AVGVGKSAL (SEQ ID NO:11)	HLA-B7	KRAS_G12V_B7_cl5	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS YSYPVTFGHGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNIYSSWMHWVRQAPGKGLEWVAYFQPY SGYTKYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS REYTYYFDYWGQGTLVTVSS	392
AVGVGKSAL (SEQ ID NO:11)	HLA-B7	KRAS_G12V_B7_cl6	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ GWLYSPFTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGG GLVQPGGSLRLSCAASGFNISGYGMHWVRQAPGKGLEWVARV YGGSGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVY YCSRAHSSYYVDYWGQGTLVTVSS	393

13) H/K/N RAS Q61H(55-64)-A1				
ILDTAGHEEY (SEQ ID NO:28)	HLA-A1	H/K/N RAS Q61H_A1_cl0	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ GYFYYPNTFGQGTKVEIKRTGGSGGGGSGGGGSEVQLVESGG GLVQPGGSLRLSCAASGFNIGYYGMHWVRQAPGKGLEWVATV YPGGGYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVY YCSRYYYYGFDYWGQGTLVTVSS	394
14) H/K/N RAS Q61K(55-64)-A1				
ILDTAGKEEY (SEQ ID NO:30)	HLA-A1	H/K/N RAS Q61K_A1_cl6	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ HYYSPVTFGQGTKVEIKRTGGSGGGGSGGGGSEVQLVESGGG LVQPGGSLRLSCAASGFNIFYQDMHWVRQAPGKGLEWVAMIYP DYDYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRTYSVYMDYWGQGTLVTVSS	395
15) H/K/N RAS Q61L(55-64)-A1				
ILDTAGLEEY (SEQ ID NO:31)	HLA-A1	H/K/N RAS Q61K_A1_cl8	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YAYAPFTFGQGTKVEIKRTGGSGGGGSGGGGSEVQLVESGGG LVQPGGSLRLSCAASGFNVSYSMIHWVRQAPGKGLEWVARVW GDGGVTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRGSYYAFDYWGQGTLVTVSS	396
16) H/K/N RAS Q61R(55-64)-A1				

ILDTAGREEY (SEQ ID NO:32)	HLA-A1	H/R/N RAS Q61R_A1_cl16	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ AHMIPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNFSFPGMHWVRQAPGKGLEWVAWFVG YDGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRDYYSFSMDYWGQGTLVTVSS	397
ILDTAGREEY (SEQ ID NO:32)	HLA-A1	H/R/N RAS Q61R_A1_cl17	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS VYDPITFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGLV QPGGSLRLSCAASGFNISGSWIHWVRQAPGKGLEWVAWLYPDS DYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR AHTYAFDYWGQGTLVTVSS	398
ILDTAGREEY (SEQ ID NO:32)	HLA-A1	H/R/N RAS Q61R_A1_cl18	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQS YTSPLTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGLV QPGGSLRLSCAASGFNIYYGVMHWVRQAPGKGLEWVAMIYPDS SWTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR DQDFHYMNYYLSYALDYWGQGTLVTVSS	399
ILDTAGREEY (SEQ ID NO:32)	HLA-A1	H/R/N RAS Q61R_A1_cl19	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ GQYSPFTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGGL VQPGGSLRLSCAASGFNVSYEYMHWVRQAPGKGLEWVAEIYG GSDNTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC SRPLGSYFDYWGQGTLVTVSS	400
ILDTAGREEY (SEQ ID NO:32)	HLA-A1	H/R/N RAS Q61R_A1_cl22	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YWYLPTTFGQGTKVEIKRTGGGSGGGGSGGGGASEVQLVESGGG LVQPGGSLRLSCAASGFNISWYDIHWVRQAPGKGLEWVADIEPS VGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCS RSYPYYYFDYWGQGTLVTVSS	401

Representative ELISA data for a scFvs that specifically recognized an IDH2 peptide containing the R140Q mutation in complex with HLA-B7 (SPNGTIQNIL; SEQ ID NO: 1) are shown in Figure 1. The scFvs did not recognize the wt version of the peptide of interest in complex with the same HLA allele. The scFvs did not recognize other control peptides in complex with the HLA allele when tested for binding to a monomer-coated ELISA plate.

Further flow cytometry using showed that MANAbody scFv clones specifically stain the HLA allele-matched cell lines when these cells are pulsed with the mutant peptide, but not the wt peptide or other control peptides (Figures 2-14).

To demonstrate that MANAbody clones can be utilized as a therapeutic modality, selected MANAbody clones were engineered into CAR-T cells. Chimeric antigen receptor (CAR) T cells (CARTs) capable of recognizing and killing cells expressing oncogenic mutation-containing peptides in the context of HLA molecules via their endogenous processing and presentation machinery were engineered. Specifically, CARTs targeting a mutant KRAS G12V peptide presented in the context of HLA-A3 were engineered, and CARTs targeting a mutant IDH2 R140Q peptide presented in the context of HLA-B7 were engineered. MANAbody scFvs targeting either mutant peptide were grafted onto a 3rd Generation CAR construct, and CAR receptors were expressed in CD3+ T cells by mRNA electroporation. CAR-T cells were subsequently co-cultured with COS-7 cells co-transfected with plasmids encoding KRAS/IDH2 mutant and wt proteins in combination with their respective HLA. As T cells, including CAR-T cells, produce cytokines following activation by cognate antigen on target cells, the release of IFNγ in the co-culture media supernatant was measured by ELISA. Only when COS-7 cells were co-transfected with the mutant and cognate HLA plasmids was there significant release of IFNγ over background (Figure 15). CAR-T cells co-cultured with COS-7 cells co-transfected with the wt and cognate HLA released only background levels of IFNγ. Together, these findings suggest that CAR-T cells expressing MANAbody clones can target tumor cells expressing MANAs presented in the context of HLA molecules.

To demonstrate that MANAbody clones can be utilized as a therapeutic modality, selected MANAbody clones were engineered into bispecific antibodies. A bispecific antibody having one antibody-fragment binding to a target cancer cell and having one antibody-fragment binding to a CD3 protein on the T cell surface was engineered. There are

a number of different anti-CD3 scFv clones targeting human CD3 epsilon, delta, and/or gamma molecules. Examples of such clones are listed in Table 4.

Bispecific antibodies having one antibody-fragment binding to a mutant KRAS G12V peptide presented in the context of HLA-A3 and having one antibody-fragment binding to a CD3 protein on the T cell surface were engineered. Specifically, bispecific antibodies targeting a mutant KRAS G12V peptide presented in the context of HLA-A3 and CD3 were engineered, and bispecific antibodies targeting a mutant IDH2 R140Q peptide presented in the context of HLA-B7 and CD3 were engineered.

Table 4. Anti-human CD3 scFv sequences.

Clone Name	Clone scFv Sequence	SEQ ID NO:
humanized UCHT1 (hUCHT1v9)	DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLS CAASGYSFTGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAED TAVYYCARSGYYGDSDWYFDVWGQGTLVTVSS	404
murine UCHT1 (mUCHT1)	DIQMTQTTSSLSASLGDRVTISCRASQDIRNYLNWYQQKPDGTVKLLIYYTSRLHSGVPSKFSGSGSGTDYS LTISNLEQEDIATYFCQQGNTLPWTFAGGTKLEIKGGGGSGGGGSGGGGSEVQLQQSGPELVKPGASMKIS CKASGYSFTGYTMNWVKQSHGKNLEWMGLINPYKGVSTYNQKFKDKATLTVDKSSSTAYMELLSLTSED SAVYYCARSGYYGDSDWYFDVWGAGTTVTVSS	405
diL2K	DIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGTDYS LTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIKGGGGSGGGGSGGGGSDVQLVQSGAEVKKPGASVKV SCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSRGYTNYADSVKGRFTITTDKSTSTAYMELSSLRSEDT ATYYCARYYDDHYCLDYWGQGTTVTVSS	406
hXR32	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPWTPARFSGSLLG GKAALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVLGGGGSGGGGSGGGGSEVQLVESGGGLVQP GGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNSLYL QMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	407
L2K-07	DIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYS LTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKGGGGSGGGGSGGGGSDIKLQQSGAELARPGASVKM SCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSED SAVYYCARYYDDHYCLDYWGQGTTLTVSS	408
OKT3	QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYS LTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINGGGGSGGGGSGGGGSQVQLQQSGAELARPGASVKM SCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSED SAVYYCARYYDDHYCLDYWGQGTTLTVSS	409
PSMA-CD3	QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGG KAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGSGGGGSGGGGSEVQLVESGGGLVQPG GSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQ MNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSS	410

28F11	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTL TISSLEPEDFAVYYCQQRSNWPPLTFGGGTKVEIKGGGSGGGGSGGGGSQVQLVESGGGVVQPGRSLRLS CAASGFKFSGYGMHWVRQAPGKGLEWVAVIWYDGSKKYYVDSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCARQMGYWHFDLWGRGTLVTVSS	411
27H5-VL1	EIVLTQSPRTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTL TISRLDPEDFAVYYCQQYGSSPITFGQGTRLEIKGGGSGGGGSGGGGSQVQLVESGGGVVQPGRSLRLSC AASGFTFRSYGMHWVRQAPGKGLEWVAIIWYDGSKKNYADSVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARGTGYNWFDPWGQGTLVTVSS	412
27H5-VL2	DILMTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYYASSLQSGVPSRFSGSGSGTDYTL TISSLQPEDFATYYCQQYYSTLTFGGGTKVEIKGGGSGGGGSGGGGSQVQLVESGGGVVQPGRSLRLSCA ASGFTFRSYGMHWVRQAPGKGLEWVAIIWYDGSKKNYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARGTGYNWFDPWGQGTLVTVSS	413
23F10	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTL TISSLEPEDFAVYYCQQRSNWPPLTFGGGTKVEIKGGGSGGGGSGGGGSQVQLVQSGGGVVQSGRSLRLS CAASGFKFSGYGMHWVRQAPGKGLEWVAVIWYDGSKKYYVDSVKGRFTISRDNSKNTLYLQMNSLRGE DTAVYYCARQMGYWHFDLWGRGTLVTVSS	414
15C3-VL1	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTL TISSLEPEDFAVYYCQQRSNWPWTFGQGTKVEIKGGGSGGGGSGGGGSQVQLVQSGGGVVQPGRSLRLS CVASGFTFSSYGMHWVRQAPGKGLEWVAAIWYNGRKQDYADSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCTRGTGYNWFDPWGQGTLVTVSS	415
15C3-VL2	AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQFNSYPITFGQGTRLEIKGGGSGGGGSGGGGSQVQLVQSGGGVVQPGRSLRLSCV ASGFTFSSYGMHWVRQAPGKGLEWVAAIWYNGRKQDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCTRGTGYNWFDPWGQGTLVTVSS	416
hu12F6	QIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQQKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSL TISRVEAEDAATYYCQQWSSNPPTFGGGTKLETKRGGGSGGGGSGGGGSQVQLQQSGAELARPGASVK MSCKASGYTFTSYTMHWVKQRPGQGLEWIGYINPSSGYTKYNQKFKDKATLTADKSSSTAYMQLSSLTSE DSAVYYCARWQDYDVYFDYWGQGTTLTVSS	417

Representative scDb co-culture results are shown in Figure 16A for three IDH2 R140Q HLA-B7 MANAbody scFv clones combined with two different anti-CD3 scFv clones. T cells were co-cultured with COS-7 cells co-transfected with plasmids encoding HLA-B7, full-length IDH2 variants, and/or GFP in the presence of the specified concentration of scDb. As a read out of T cell activation by cognate antigen on target cells, the release of IFNγ in the co-culture media supernatant was measured by ELISA. Only when COS-7 cells were co-transfected with HLA-B7 and mutant IDH2 R140Q plasmids was there significant T cell release of IFNγ over background, with the level of IFNγ dependent on the concentration of scDb included in the well. T cells co-cultured with COS-7 cells co-transfected with HLA-B7 and wt IDH2 released only background levels of IFNγ. Representative scDb co-culture results are shown in Figure 16B for a KRAS G12V HLA-A3 MANAbody scFv clone combined with an anti-CD3 clone into a single chain diabody. In this co-culture, the single chain diabody was tested at concentrations of 0, 50, and 100 ng/mL. Only when COS-7 cells were co-transfected with HLA-A3 and mutant KRAS G12V plasmids was there significant T cell release of IFNγ over background. T cells co-cultured with COS-7 cells co-transfected with HLA-A3 and wt KRAS released only background levels of IFNγ, similar to the levels of IFNγ seen in no T cell, no target cell, and no scDb wells. An endogenous KRAS G12V HLA-A3 positive cell line NCI-H441 as a target cell line along with its isogenic HLA-A3 knockout control. IFNγ release was only seen against the parental NCI-H441 cell line but not the HLA-A3 knockout NCI-H441. Together, these findings suggest that bispecific antibodies containing MANAbody clones that target tumor cells expressing MANAs presented in the context of HLA molecules.

To evaluate the efficacy of using MANAbody clones as a therapeutic modality, target cell viability of a KRAS G12V HLA-A3 single-chain diabody was assayed using Promega's CellTiter-Glo reagent (Figure 17). CellTiter-Glo measures ATP concentration in a well, which is proportional to the number of viable cells. Percent target cell viability was measured by subtracting the CellTiter-Glo value from T cell only wells and normalizing to target cell only wells. Only when NCI-H441 parent cells were incubated with T cells in the presence of the KRAS G12V-A3 scDb or a pan-HLA-A3 scDb positive control, was there

significant target cell death. No target cell death was observed in the absence of scDb or among the NCI-H441 HLA-A3 knockout wells.

Together, these findings demonstrate that MANAbodies can be used to redirect and activate T cells to kill tumor cells expressing particular mutant protein and HLA allele pairs (e.g., IDH2 R140Q with HLA-B7 and KRAS G12V with HLA-A3).

Materials and Methods

Cells and Cell Lines.

RPMI-6666 cells (ATCC, Manassas, VA) was cultured in RPMI-1640 (ATCC) with 20% FBS (GE Hyclone, Logan, Utah, USA), and 1% penicillin streptomycin (Life Technologies). T2 cells (ATCC) and MINO cells (ATCC) were cultured in RPMI-1640 (ATCC) with 10% FBS (GE Hyclone), and 1% penicillin streptomycin (Thermo Fisher). T2A3 cells (gifted from Dr. Eric Lutz) were cultured in RPMI-1640 (ATCC) with 10% FBS (GE Hyclone), 1% penicillin streptomycin (Thermo Fisher), 0.1 mM MEM Non-Essential Amino Acids (NEAA, Thermo Fisher), and 500 μg/mL geneticin (Thermo Fisher). SigM5 cells (DSMZ, Brunswick, Germany) were cultured in Iscove's MDM (ATCC) with 20% FBS (GE Hyclone), and 1% penicillin streptomycin (Thermo Fisher). Hs611.T cells (ATCC) was cultured in Dulbecco's Modified Eagle's Medium (ATCC) with 10% FBS (GE Hyclone), and 1% penicillin streptomycin (Thermo Fisher). NCI-H441 cells (ATCC) and COS-7 cells (ATCC) was cultured in McCoy's 5A (Modified) Medium (Thermo Fisher) with 10% FBS (GE Hyclone), and 1% penicillin streptomycin (Thermo Fisher). COS-7 cells (ATCC, CRL-1651™) were cultured in DMEM (high glucose, pyruvate; Thermo Fisher) with 10% FBS (GE Hyclone), and 1% Penicillin-Streptomycin (Thermo Fisher). 293FT cells (Thermo Fisher) were cultured in high-glucose D-MEM (Thermo Fisher), with 10% FBS (GE Hyclone), 0.1 mM MEM Non-Essential Amino Acids (NEAA, Thermo Fisher), 6 mM L-glutamine (Thermo Fisher), 1 mM MEM Sodium Pyruvate (Thermo Fisher), 500 μg/ml geneticin (Thermo Fisher), and 1% Penicillin-Streptomycin (Thermo Fisher). All cell lines were maintained at 37°C under 5% CO_2.

PBMCs were obtained by Ficoll-Paque PLUS (GE Healthcare) gradient centrifugation of whole blood from healthy volunteer donors. CD3+ cells were positively selected with CD3 MicroBeads (Miltenyi Biotec) from PBMCs, and were activated and

expanded with Dynabeads® Human T-Activator CD3/CD28 (Life Technologies). Unless otherwise noted, primary CD3+ T cells were cultured in RPMI-1640 (ATCC) with 10% FBS (GE Hyclone), 1% Penicillin-Streptomycin (Life Technologies), and 100 IU/mL recombinant human inteuleukin-2 (Proleukin®) at 37°C under 5% CO_2.

Phage Display Library Construction.

For the 1st generation phage library, oligonucleotides were synthesized at DNA 2.0 (Menlo Park, CA) using mixed and split pool degenerate oligonucleotide syntheses. For the 2nd generation phage library, oligonucleotides were synthesized at GeneArt (Thermo Fisher, Halethorpe, MD) using trinucleotide mutagenesis (TRIM) technology. For both libraries, the oligonucleotides were incorporated into the pADL-10b phagemid (Antibody Design Labs, San Diego, CA). This phagemid contains an F1 origin, a transcriptional repressor to limit uninduced expression, a lac operator, and a lac repressor. The scFv was synthesized with a pelB periplasmic secretion signal and was subcloned downstream of the lac operator. For the 1st generation library, a myc epitope tag followed by a TEV protease cleavage recognition sequence was placed immediately downstream of the variable heavy chain, while in the 2nd generation library, the scFv was followed by a FLAG tag. Following the scFv, tag, and cleavage site, was the full length, in-frame M13 pIII coat protein sequence.

To transform the phagemid DNA into bacteria, 10-20 ng of the ligation product was mixed on ice with 10 µL of electrocompetent SS320 cells (Lucigen, Middleton, WI) and 14 µL of double-distilled water. This mixture was electroporated using a Gene Pulser electroporation system (Bio-Rad, Hercules, CA) and allowed to recover in Recovery Media (Lucigen) for 60 min at 37°C. Cells transformed with 60 ng of ligation product were pooled and plated on a 24-cm x 24-cm plate containing 2xYT medium supplemented with carbenicillin (100 µg/mL) and 2% glucose. Cells were grown at 37°C for 6 hours and placed at 4°C overnight. To determine the transformation efficiency for each series of electroporations, aliquots were taken and titered by serial dilution. Cells grown on plates were scraped into 850 mL of 2xYT medium with carbenicillin (100 µg/mL) plus 2% glucose for a final OD600 of 5-15. Two mL of the 850 mL culture were taken and diluted ~1:200 to reach a final OD600 of 0.05-0.07. To the remaining culture, 150 mL of sterile glycerol were added before snap freezing to produce glycerol stocks. The diluted bacteria were grown to an

OD600 of 0.2-0.4, infected with M13K07 Helper phage (Antibody Design Labs, San Diego, CA) and allowed to shake at 37°C for 1 hour. The culture was centrifuged and the cells were resuspended in 2xYT medium with carbenicillin (100 μg/mL) and kanamycin (50 μg/mL) and grown overnight at 30°C for phage production. The following morning, the bacterial culture was aliquoted into 50 mL Falcon tubes and pelleted twice at high speed to obtain clarified supernatant. The phage-laden supernatant was precipitated on ice for 40 min with a 20% PEG-8000/2.5M NaCl solution at a 4:1 ratio of PEG/NaCl to supernatant. After precipitation, phage was centrifuged at 12,000 g for 40 minutes and resuspended in a 1 mL vol 1X TBS, 2 mM EDTA. Phage from multiple tubes was pooled, re-precipitated, and resuspended to an average titer of 1×10^{13} cfu/mL. For the 1st generation library, the total number of transformants obtained was 5.5×10^{9}. For the 2nd generation library, the total number of transformants obtained was 3.6×10^{10}. Each library was aliquoted and stored in 15% glycerol at -80°C.

Next-generation sequencing of the complete phage library.

DNA from the libraries was amplified using primers that flank the CDR-H3 region. The sequences at the 5'-ends of these primers incorporated molecular barcodes to facilitate unambiguous enumeration of distinct phage sequences. The protocols for PCR-amplification and sequencing are described in Kinde et al. Sequences processed and translated using a custom SQL database and both the nucleotide sequences and amino acid translations were analyzed using Microsoft Excel.

Peptides and HLA-Monomers.

Mutant, wt, and control peptides (listed in Table 1) were predicted to bind to HLA alleles using NetMHC version 4.0. All peptides were synthesized at a purity of >90% by Peptide 2.0 (Chantilly, VA). Peptides were resuspended in DMSO or DMF at 10 mg/mL and stored at -20°C. HLA monomers were synthesized by refolding recombinant HLA with peptide and beta-2 microglobulin, purified by gel-filtration, and biotinylated (Fred Hutchinson Immune Monitoring Lab, Seattle, WA). Monomers were confirmed to be folded prior to selection by performing an ELISA using W6/32 antibody (BioLegend, San Diego, CA).

Selection for phage binding to mutant peptide-HLA monomers.

Biotinylated monomers containing HLA and beta-2-microglobulin proteins were conjugated to MyOne T1 streptavidin magnetic beads (Life Technologies, Carlsbad, CA). The biotinylated monomers were incubated with 30 µL of MyOne T1 beads (per 1ug of monomer) in blocking buffer (PBS, 0.5% BSA, 0.1% Na-azide) for 1 hour at room temperature (RT). After the initial incubation, the complexes were washed 3 times with 1ml blocking buffer and resuspended in 1 ml blocking buffer.

Enrichment phase.

In the enrichment phase of selection (round 1), phage representing 1000-fold coverage of the library was incubated with naked, washed MyOne T1 beads and heat-denatured, bead-conjugated HLA monomer overnight at 4°C on a rotator. This step was necessary to remove any phage recognizing either streptavidin or denatured monomer, present to a small extent in every preparation of biotinylated monomer. After negative selection, beads were isolated with a DynaMag-2 magnet (Life Technologies) and the supernatant containing unbound phage was transferred for positive selection against 1ug of the mutant peptide-HLA monomer conjugated to MyOne T1 streptavidin magnetic beads. Prior to elution, beads were washed 10 times with 1ml, 1X TBS containing 0.5% Tween-20 using a magnet. Phage was eluted by resuspending the beads in 1 mL of 0.2 M glycine, pH 2.2. After a 10-minute incubation, the solution was neutralized by the addition of 150 µL of 1 M Tris, pH 9.0. Neutralized phage was used to infect 10ml cultures of mid-log-phage SS320s, with the addition of M13K07 helper phage (MOI of 4) and 2% glucose. After shaking for 1 hour at 37°C, bacteria was resuspended in 2xYT medium with carbenicillin (100 µg/mL), kanamycin (50 µg/mL), and 50uM of IPTG and grown overnight at 30°C for phage production. Phage was precipitated the next morning with PEG/NaCl as previously described.

Final selection phase.

Three to five rounds of final selection were performed with phage resulting from the enrichment phase. For each round of final selection, the first negative selection was performed using 10-0.1% of the precipitated phage against HLA-allele matched cells lacking the mutated protein of interest. The unbound phage was then negatively selected against

native wt peptide-HLA monomer and unrelated HLA-allele matched monomer. After negative selection, beads were isolated with a Dynamag 2 magnet (Life Technologies) and the supernatant containing unbound phage was transferred for positive selection with 250ng to 1ug of mutant peptide-HLA monomer, as described for the enrichment phase above.

ELISA.

Streptavidin-coated, 96-well plates (R&D Systems, Minneapolis, MN) were coated with 50 ng (in 50uL) of biotinylated mutant or wt peptide-HLA monomers in blocking buffer (PBS with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide) at 4°C overnight. Plates were briefly washed with 1X TBST (TBS + 0.05% Trition-X 100). Phage was serially diluted to the specified concentrations in blocking buffer and 50uL was added to each well. Phage were incubated for 2 hrs at RT, followed by washing (6 washes with 1X TBS-0.05%Tween-20 (TBST) using an ELISA plate washer (BioTek, Winooski, VT). The bound phage were incubated with 50 µL of rabbit anti-M13 antibody (Pierce, Rockford, IL) diluted 1:3000 in 1X TBST for 1 hr at room temperature, followed by washing an additional 6X times and incubation with 50 µL of anti-Rabbit HRP (Thermo Fisher) diluted 1:10,000 in 1X TBST for 1 hour at room temperature. After a final 6 washes with 1X TBST, 50 µL of TMB substrate (BioLegend, San Diego, CA) was added to the well and the reaction was quenched with 1N sulfuric acid. Absorbance at 450 nm was measured with a Synergy H1 Multi-Mode Reader (BioTek, Winooski, VT).

Monoclonal phage ELISA was performed by selecting individual colonies of SS320 cells transformed with a limiting dilution of phage obtained from the final selection. Individual colonies were inoculated into 200 µl of 2xYT medium containing 100 µg/mL carbenicillin and 2% glucose and grown for three hours at 37°C. The cells were then infected with 1.6×10^7 M13K07 helper phage (Antibody Design Labs, San Diego, CA) and incubated for at 37°C with shaking. The cells were pelleted, resuspended in 300 µL of 2xYT medium containing carbenicillin (100 µg/mL), kanamycin (50 µg/mL), and 50uM IPTG, and grown overnight at 30°C. Cells were pelleted and the phage-laden supernatant was used for ELISA as described above.

Peptide Pulsing and Flow Cytometry.

For peptide pulsing, HLA-matched cells were washed once with PBS and once with serum-free RPMI-1640 before incubation at 10^6 cells per mL in serum-free RPMI-1640 containing 50 μg/mL peptide and 10 μg/mL human beta-2 microglobulin (ProSpec, East Brunswick, NJ) overnight at 37°C. The pulsed cells were pelleted, washed once in cold stain buffer (PBS containing 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide), and resuspended in 100 μL of stain buffer. Phage staining was performed on ice with 10uL (approximately $1x10^9$) phage for 1 hour in 100 uL total volume, followed by one 4 mL wash in cold stain buffer. Cells were then stained with 1uL of rabbit anti-M13 antibody (Pierce, Rockford, IL) in 100uL total volume on ice for 1 hour and washed once with 4 mL of cold stain buffer. Cells were stained with anti-rabbit-PE (Biolegend) on ice for 1 hour in 100 uL total volume, followed by incubation with LIVE/DEAD Fixable Near-IR Dead Cell Stain (Thermo Fisher) for 10 min at room temperature per manufacturer's instructions. Cells were washed once in 4mL of stain buffer followed by resuspension in 300uL of stain buffer before analysis. Stained cells were analyzed using an LSRII flow cytometer (Becton Dickinson, Mansfield, MA).

CAR Construction and Generation.

A third-generation Chimeric Antigen Receptor (CAR) construct, containing the MANAbody scFv, a CD28 transmembrane domain, and 4-1BB and CD3ζ intracellular domains, was synthesized (GeneArt®) and cloned into the mammalian expression vector pCI (Promega). mRNA was synthesized with the T7 mScript™ Standard mRNA Production System Kit (CellScript™) per manufacturer's instructions. CAR mRNA was electroporated into primary CD3+ T cells with the BTX ECM 2001 Electro Cell Manipulator (Harvard Apparatus) to generate CAR-T cells.

CAR-T Activation Co-Culture Assay.

COS-7 cells were transfected with various combinations of pcDNA3.1 (Life Technologies) plasmids encoding HLA-A3, HLA-B7, IDH2(WT), IDH2(R140Q), KRAS(WT), and KRAS(G12V) with Lipofectamine 3000 (Life Technologies) per manufacturer's instructions in 96-well plate format. 100,000 electroporated CAR-T cells were overlaid over the transfected COS-7 cells, and the co-culture was allowed to incubate

for 4 hours at 37°C under 5% CO_2. Following co-culture, conditioned media was collected and assayed for secreted IFNγ by ELISA (Quantikine®, R&D Systems).

Bispecific Antibody Production.

gBLOCKs encoding bispecific antibodies were ordered from IDT (Skokie, Illinois). gBLOCKs were topo-cloned into the pcDNA3.4 plasmid (Thermo Fisher) following the manufacturer's protocol. 293FT cells (Thermo Fisher) were transfected with the bispecific antibody pcDNA3.4 plasmids using Lipofectamine 3000 (Life Technologies) per manufacturer's instructions in a T75 flask. Following a 5-7 day incubation, media was harvest and centrifuged at 3,000g for 10min at 4C. Bispecific antibody protein was purified using a Clontech Capturem™ His-Tagged Purification Miniprep Kit (Takara, Mountain View, CA) per manufacturer's instructions. Bispecific antibody protein was desalted into PBS using Zeba spin 7k MWCO desalting columns per manufacturer's instructions. Bispecific antibody concentration was quantified using Mini-PROTEAN® TGX Stain-Free™ Precast Gels (Biorad, Hercules, California) using a standard curve of protein of known concentration. Stain-free gels were imaged using the ChemiDoc XRS+ Imager (Biorad).

Bispecific Antibody Co-Culture Assay.

COS-7 cells were transfected with various combinations of pcDNA3.1 (Life Technologies) plasmids encoding HLA-A3, HLA-B7, IDH2(WT), IDH2(R140Q), KRAS(WT), and KRAS(G12V) with Lipofectamine 3000 (Life Technologies) per manufacturer's instructions in a T75 flask. 50,000 T cells were combined with transfected 30,000 COS-7 cells or 10,000 NCI-H441 cells and the specified concentration of bispecific antibody in a 96-well plate, and the co-culture was allowed to incubate for 24 hours at 37°C under 5% CO_2. Following co-culture, the 96-well plate was snap frozen and conditioned media lysate was collected and assayed for secreted IFNγ by ELISA (Quantikine®, R&D Systems). Alternatively, following coculture, target cell viability was measured using CellTiter-Glo (Promega).

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A molecule comprising an antigen-binding domain that can bind to a peptide-human leukocyte antigen (HLA)-beta-2 microglobulin complex, wherein said antigen-binding domain is selected from the group consisting of:

(i) an antigen-binding domain comprising a complementarity determining region (CDR)-VL1 comprising the amino acid sequence QDVNTA (SEQ ID NO:33); a CDR-VL2 comprising the amino acid sequence SAS; a CDR-VL3 comprising the amino acid sequence QQSGYAPIT (SEQ ID NO:82), a CDR-VH1 comprising the amino acid sequence GFNISYYS (SEQ ID NO:89), a CDR-VH2 comprising the amino acid sequence VDPDSDYT (SEQ ID NO:96); and a CDR-VH3 comprising the amino acid sequence SRSWIHMFSMDY (SEQ ID NO:103);

(ii) an antigen-binding domain comprising comprises a CDR-VL1 comprising the amino acid sequence QDVNTA (SEQ ID NO:33); a CDR-VL2 comprising the amino acid sequence SAS; a CDR-VL3 comprising the amino acid sequence QQSLYGPFT (SEQ ID NO:84); a CDR-VH1 comprising the amino acid sequence GFNIAYEY (SEQ ID NO:91); a CDR-VH2 comprising the amino acid sequence IGPDSGYT (SEQ ID NO:98); and a CDR-VH3 comprising the amino acid sequence SRVWYYSTYGMDY (SEQ ID NO:105);

(iii) an antigen-binding domain comprising a CDR-VL1 comprising the amino acid sequence QDVNTA (SEQ ID NO:33); a CDR-VL2 comprising the amino acid sequence SAS; a CDR-VL3 comprising the amino acid sequence QQYLYQPWT (SEQ ID NO:87); a CDR-VH1 comprising the amino acid sequence GFNIDYYG (SEQ ID NO:94); a CDR-VH2 comprising the amino acid sequence LYGGSDST (SEQ ID NO:101); and a CDR-VH3 comprising the amino acid sequence SRQYSAYFDY (SEQ ID NO:108); and

(iv) an antigen-binding domain comprising a CDR-VL1 comprising the amino acid sequence QDVNTA (SEQ ID NO:33); a CDR-VL2 comprising the amino acid sequence SAS; a CDR-VL3 comprising the amino acid sequence QQGLYYPWT (SEQ ID NO: 88); a CDR-VH1 comprising the amino acid sequence GFNVSYSS (SEQ ID NO: 95); a CDR-VH2 comprising the amino acid sequence IWPDSGQT (SEQ ID NO: 102); and a CDR-VH3 comprising the amino acid sequence SRSSYFDAMDY (SEQ ID NO: 109);

wherein said peptide comprises a modified peptide derived from a p53 polypeptide, wherein said modified peptide comprises the amino acid sequence SEQ ID NO: 15, wherein said HLA is a class I HLA, and wherein said antigen-binding domain does not bind to a complex that includes a wild-type version of the modified peptide.

2. The molecule of claim 1, wherein said modified peptide comprises from 10 amino acids to 15 amino acids.

3. The molecule of claim 1, wherein said class I HLA is an HLA-A2, and wherein said antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:336, and SEQ ID NO:337.

4. The molecule of any one of claims 1-3, wherein said molecule further comprises a second antigen-binding domain that can bind to an effector cell receptor selected from the group consisting of CD3, CD28, CD4, CD8, CD16a, NKG2D, PD-1, CTLA-4, 4-1BB, OX40, ICOS, and CD27.

5. The molecule of claim 4, wherein said second antigen-binding domain that can bind to an effector cell can bind to CD3, wherein said second antigen-binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO:404, SEQ ID NO:405, SEQ ID NO:406, SEQ ID NO:407, SEQ ID NO:408, SEQ ID NO:409, SEQID NO:410, SEQ ID NO:411, SEQ ID NO:412, SEQ ID NO:413, SEQ ID NO:414, SEQ ID NO:415, SEQ ID NO:416, and SEQ ID NO:417.

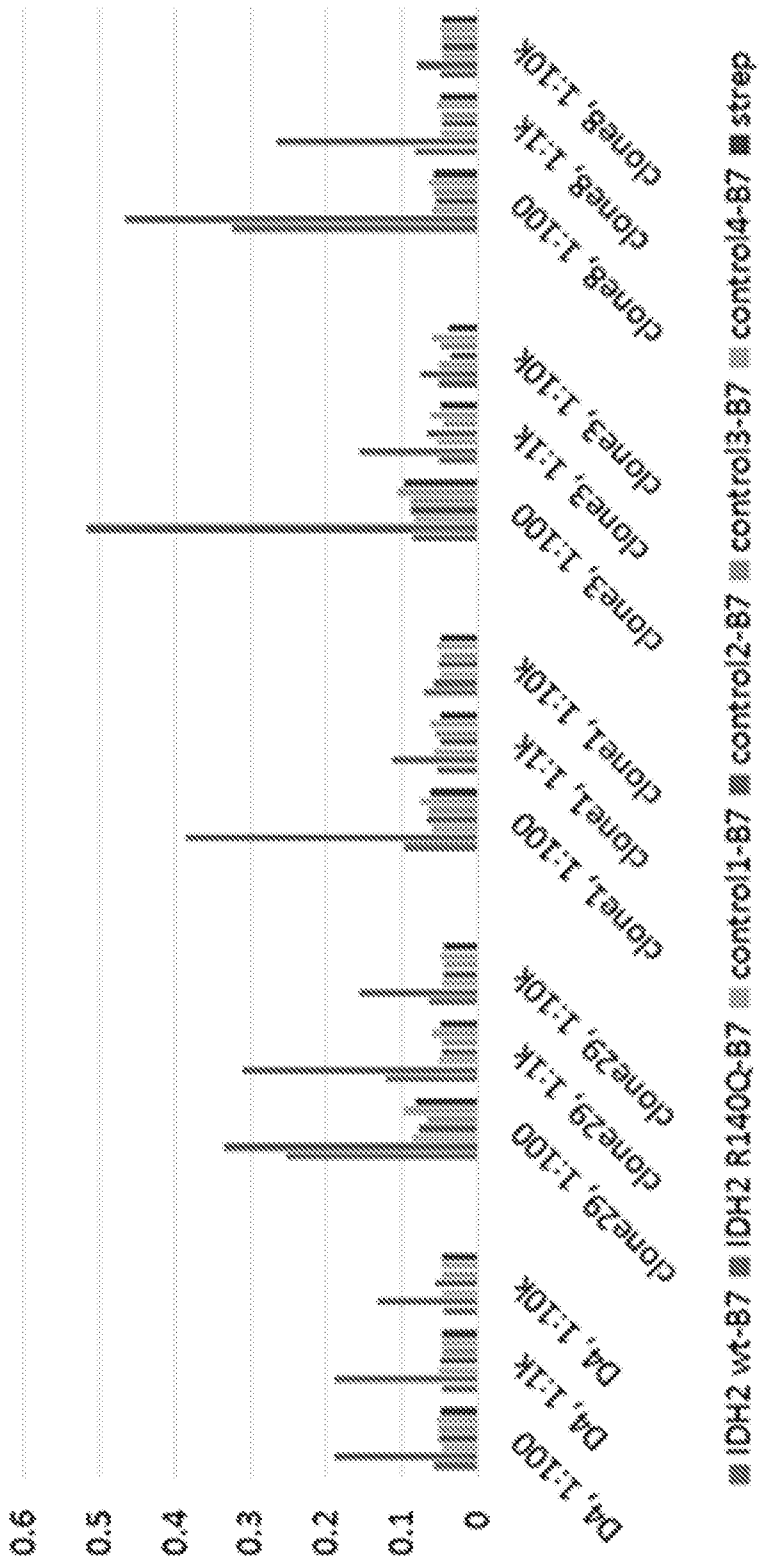
ELISA to Assess Binding of Phage Clones to a Panel of Monomers
0.6
0.5
0.4
0.3
0.2
0.1
0
DM, 1:100
DM, 1:1K
DM, 1:10K
clone29, 1:100
clone29, 1:1K
clone29, 1:10K
clone1, 1:100
clone1, 1:1K
clone1, 1:10K
clone3, 1:100
clone3, 1:1K
clone3, 1:10K
clone8, 1:100
clone8, 1:1K
clone8, 1:10K
IDH2 wt-87
IDH2 R140Q-87
control1-87
control2-87
control3-87
control4-87
strep

FIG. 1

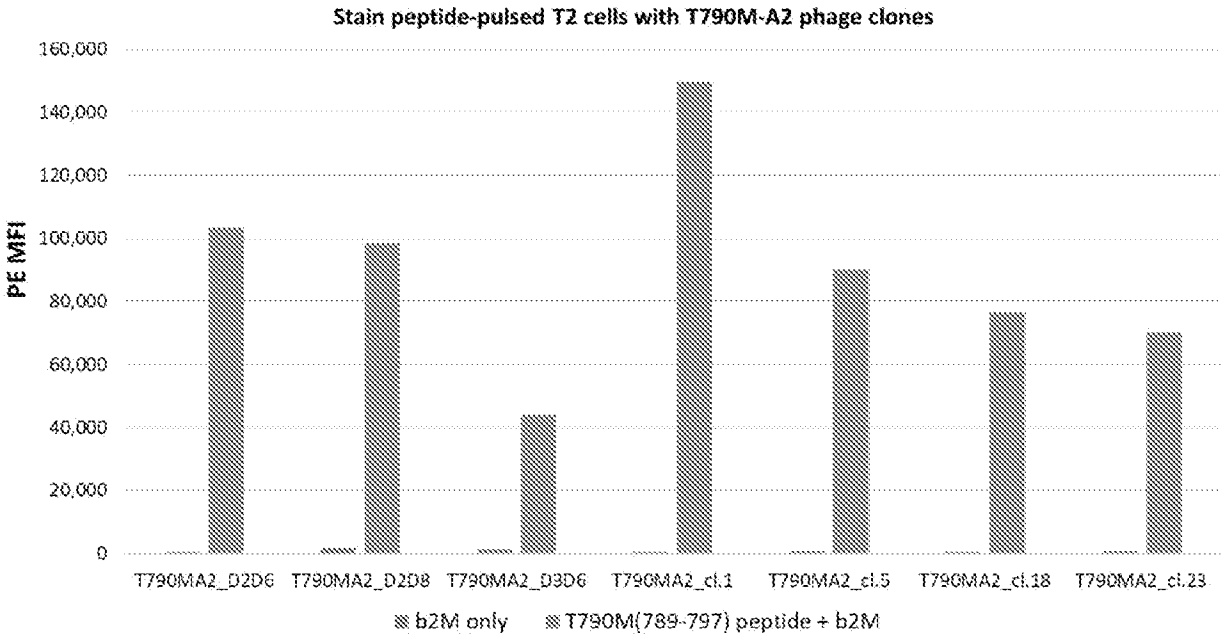
Stain peptide-pulsed T2 cells with T790M-A2 phage clones
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
PE MFI
T790MA2_D2D6
T790MA2_D2D8
T790MA2_D3D6
T790MA2_cl.1
T790MA2_cl.5
T790MA2_cl.18
T790MA2_cl.23
b2M only
T790M(789-797) peptide + b2M

FIG. 2

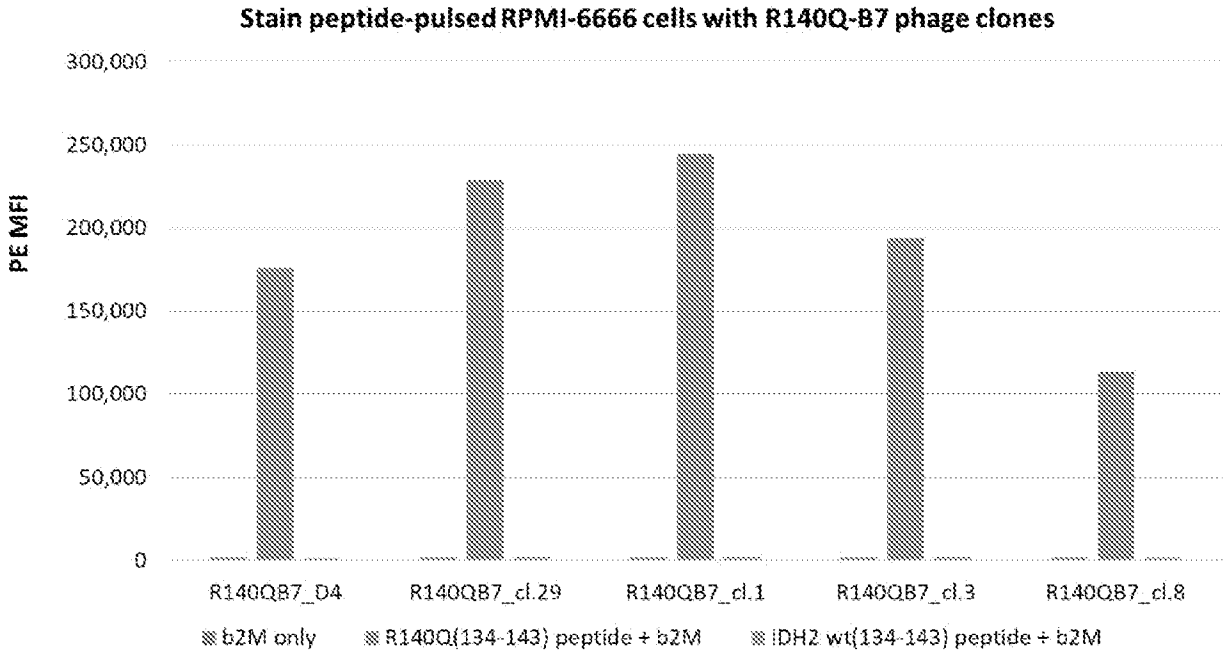
Stain peptide-pulsed RPMI-6666 cells with R140Q-B7 phage clones
PE MFI
300,000
250,000
200,000
150,000
100,000
50,000
0
R140QB7_D4
R140QB7_cl.29
R140QB7_cl.1
R140QB7_cl.3
R140QB7_cl.8
b2M only
R140Q(134-143) peptide + b2M
IDH2 wt(134-143) peptide + b2M

FIG. 3

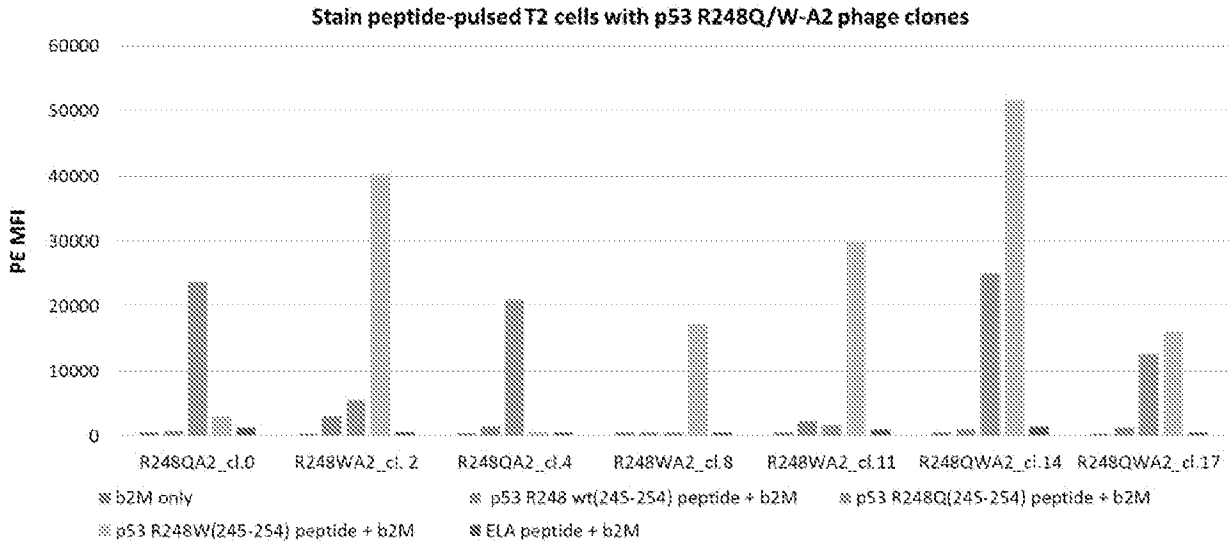
Stain peptide-pulsed T2 cells with p53 R248Q/W-A2 phage clones
PE MFI
60000
50000
40000
30000
20000
10000
0
R248QA2_cl.0
R248WA2_cl. 2
R248QA2_cl.4
R248WA2_cl.8
R248WA2_cl.11
R248QWA2_cl.14
R248QWA2_cl.17
b2M only
p53 R248 wt(245-254) peptide + b2M
p53 R248Q(245-254) peptide + b2M
p53 R248W(245-254) peptide + b2M
ELA peptide + b2M

FIG. 4

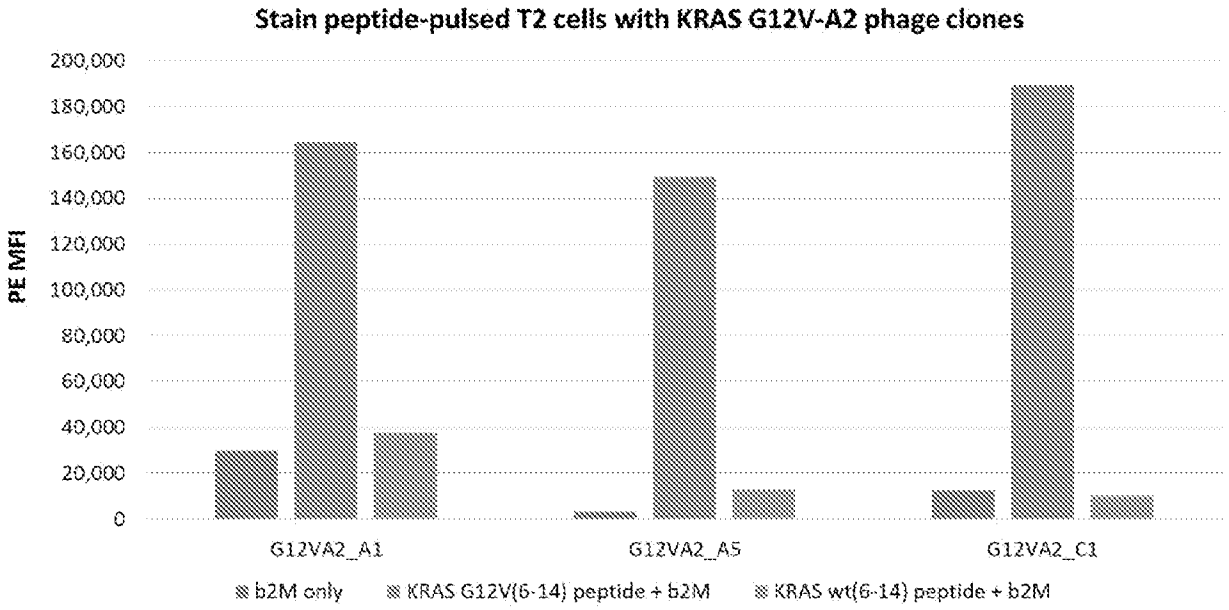
Stain peptide-pulsed T2 cells with KRAS G12V-A2 phage clones
PE MFI
200,000
180,000
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
G12VA2_A1
G12VA2_A5
G12VA2_C1
b2M only
KRAS G12V(6-14) peptide + b2M
KRAS wt(6-14) peptide + b2M

FIG. 5

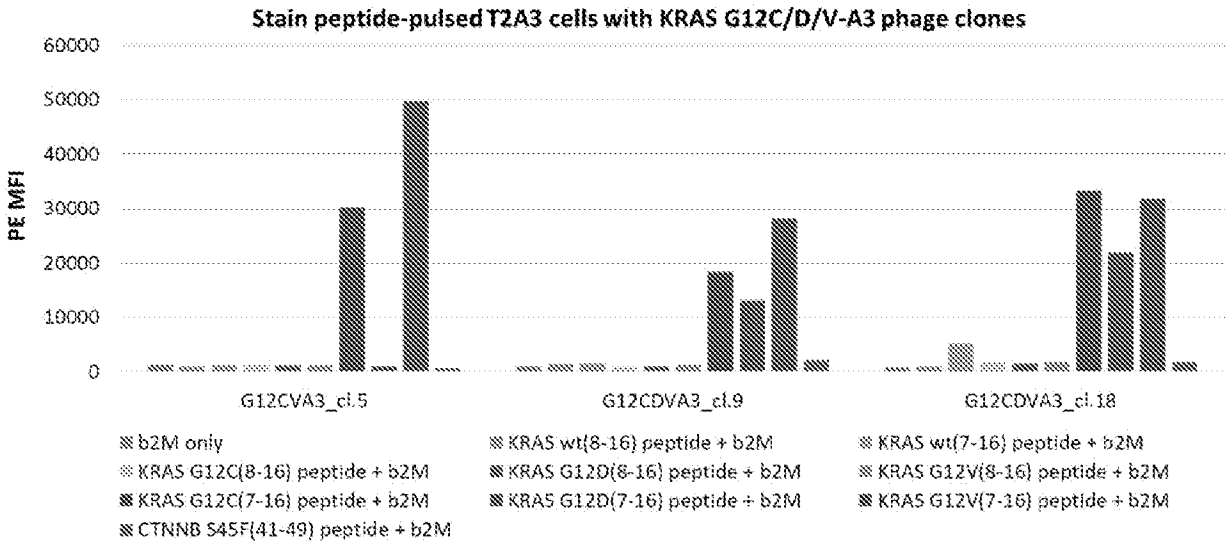
Stain peptide-pulsed T2A3 cells with KRAS G12C/D/V-A3 phage clones
PE MFI
60000
50000
40000
30000
20000
10000
0
G12CVA3_cl.5
G12CDVA3_cl.9
G12CDVA3_cl.18
b2M only
KRAS G12C(8-16) peptide + b2M
KRAS G12C(7-16) peptide + b2M
CTNNB S45F(41-49) peptide + b2M
KRAS wt(8-16) peptide + b2M
KRAS G12D(8-16) peptide + b2M
KRAS G12D(7-16) peptide + b2M
KRAS wt(7-16) peptide + b2M
KRAS G12V(8-16) peptide + b2M
KRAS G12V(7-16) peptide + b2M

FIG. 6

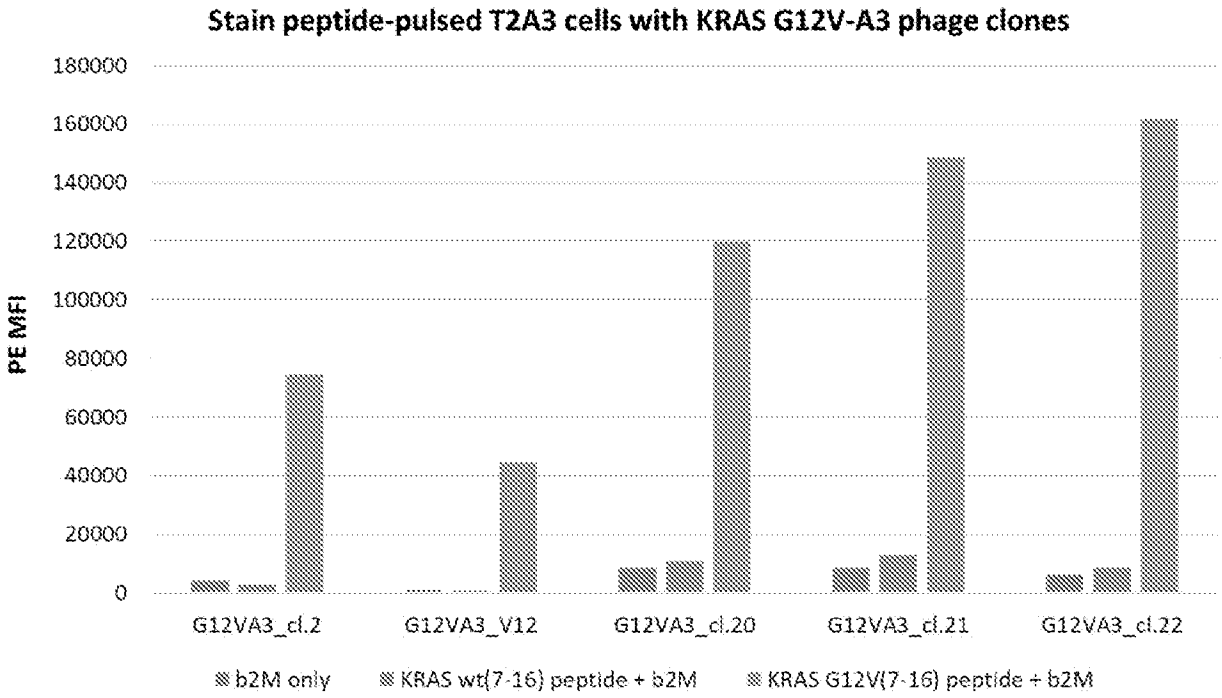
Stain peptide-pulsed T2A3 cells with KRAS G12V-A3 phage clones
PE MFI
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
G12VA3_cl.2
G12VA3_V12
G12VA3_cl.20
G12VA3_cl.21
G12VA3_cl.22
b2M only
KRAS wt(7-16) peptide + b2M
KRAS G12V(7-16) peptide + b2M

FIG. 7

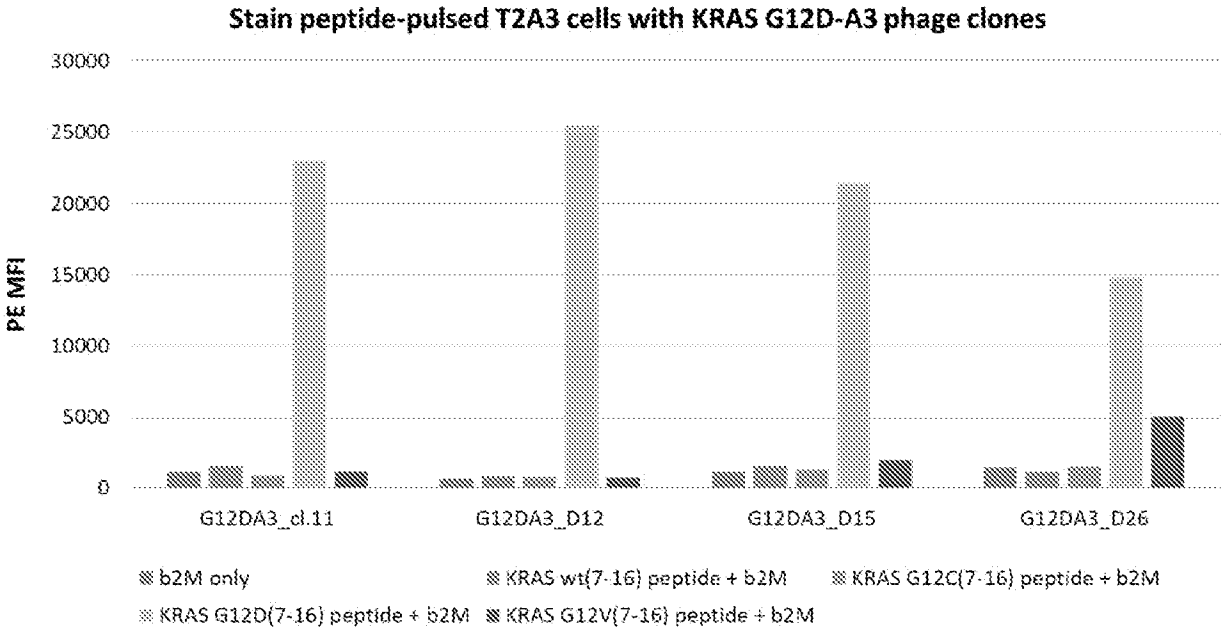
Stain peptide-pulsed T2A3 cells with KRAS G12D-A3 phage clones
PE MFI
30000
25000
20000
15000
10000
5000
0
G12DA3_cl.11
G12DA3_D12
G12DA3_D15
G12DA3_D26
b2M only
KRAS wt(7-16) peptide + b2M
KRAS G12C(7-16) peptide + b2M
KRAS G12D(7-16) peptide + b2M
KRAS G12V(7-16) peptide + b2M

FIG. 8

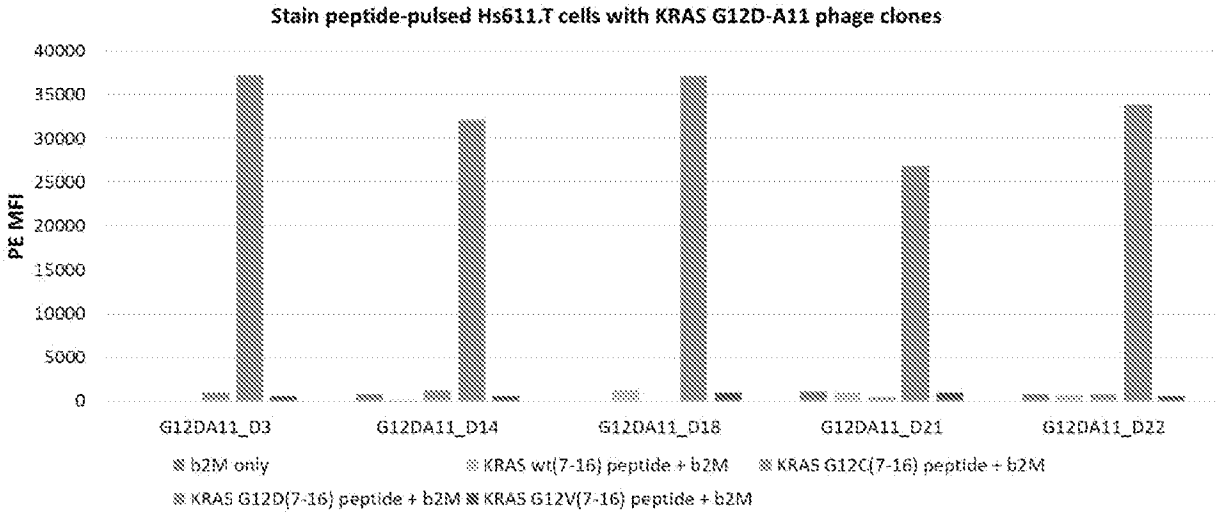
Stain peptide-pulsed Hs611.T cells with KRAS G12D-A11 phage clones
PE MFI
40000
35000
30000
25000
20000
15000
10000
5000
0
G12DA11_D3
G12DA11_D14
G12DA11_D18
G12DA11_D21
G12DA11_D22
b2M only
KRAS wt(7-16) peptide + b2M
KRAS G12C(7-16) peptide + b2M
KRAS G12D(7-16) peptide + b2M
KRAS G12V(7-16) peptide + b2M

FIG. 9

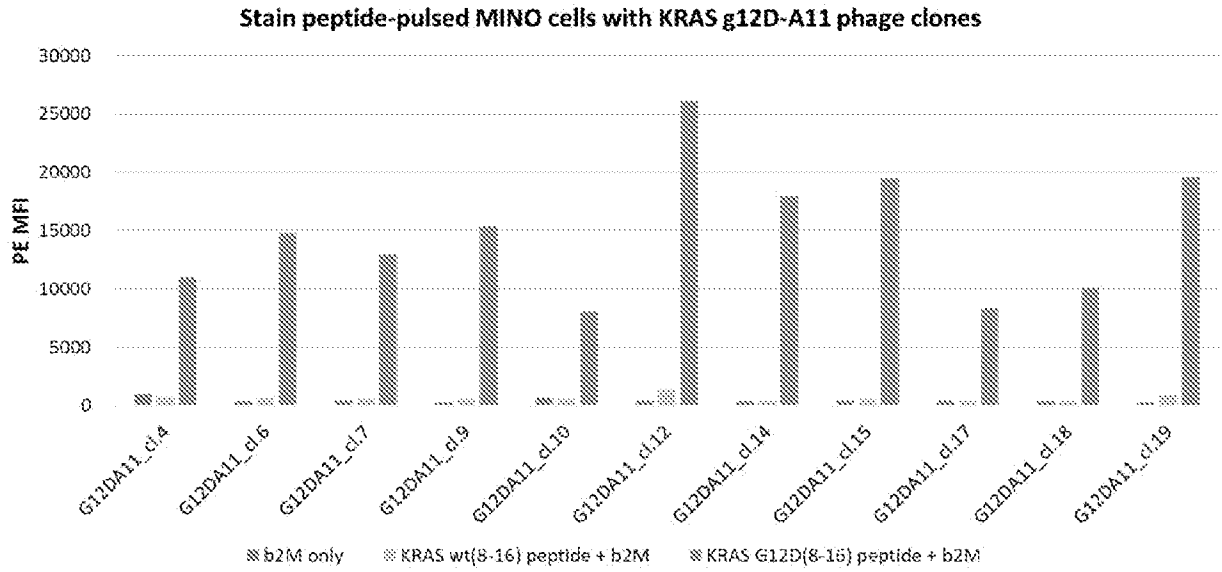
Stain peptide-pulsed MINO cells with KRAS g12D-A11 phage clones
PE MFI
30000
25000
20000
15000
10000
5000
0
G12DA11_cl.4
G12DA11_cl.6
G12DA11_cl.7
G12DA11_cl.9
G12DA11_cl.10
G12DA11_cl.12
G12DA11_cl.14
G12DA11_cl.15
G12DA11_cl.17
G12DA11_cl.18
G12DA11_cl.19
b2M only
KRAS wt(8-16) peptide + b2M
KRAS G12D(8-16) peptide + b2M

FIG. 10

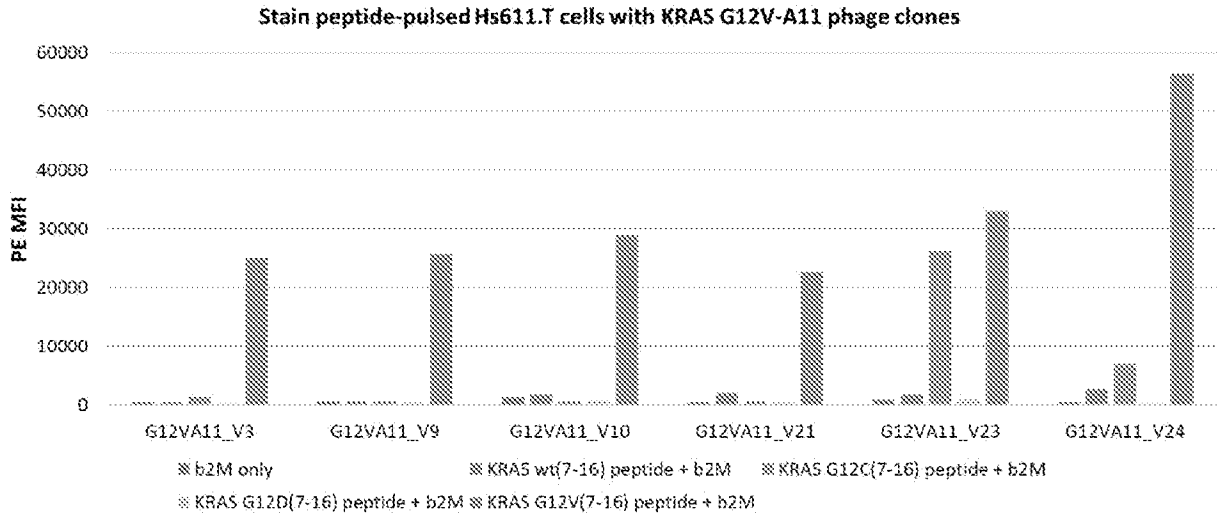
Stain peptide-pulsed Hs611.T cells with KRAS G12V-A11 phage clones
PE MFI
60000
50000
40000
30000
20000
10000
0
G12VA11_V3
G12VA11_V9
G12VA11_V10
G12VA11_V21
G12VA11_V23
G12VA11_V24
b2M only
KRAS wt(7-16) peptide + b2M
KRAS G12C(7-16) peptide + b2M
KRAS G12D(7-16) peptide + b2M
KRAS G12V(7-16) peptide + b2M

FIG. 11

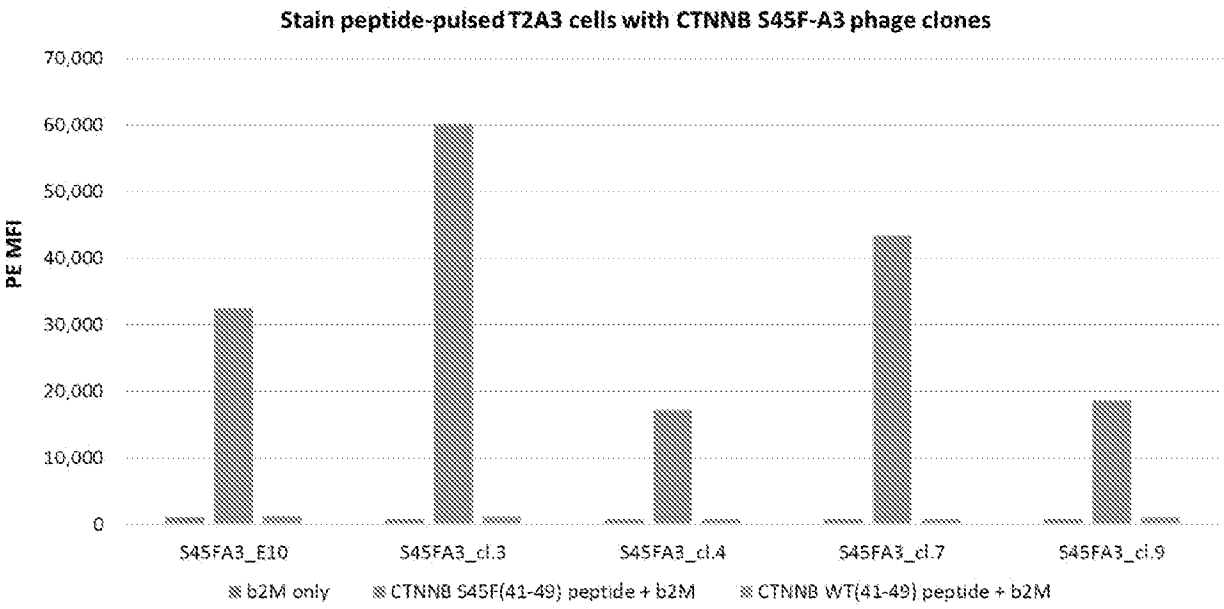
Stain peptide-pulsed T2A3 cells with CTNNB S45F-A3 phage clones
PE MFI
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
S45FA3_E10
S45FA3_cl.3
S45FA3_cl.4
S45FA3_cl.7
S45FA3_cl.9
※ b2M only
※ CTNNB S45F(41-49) peptide + b2M
※ CTNNB WT(41-49) peptide + b2M

FIG. 12

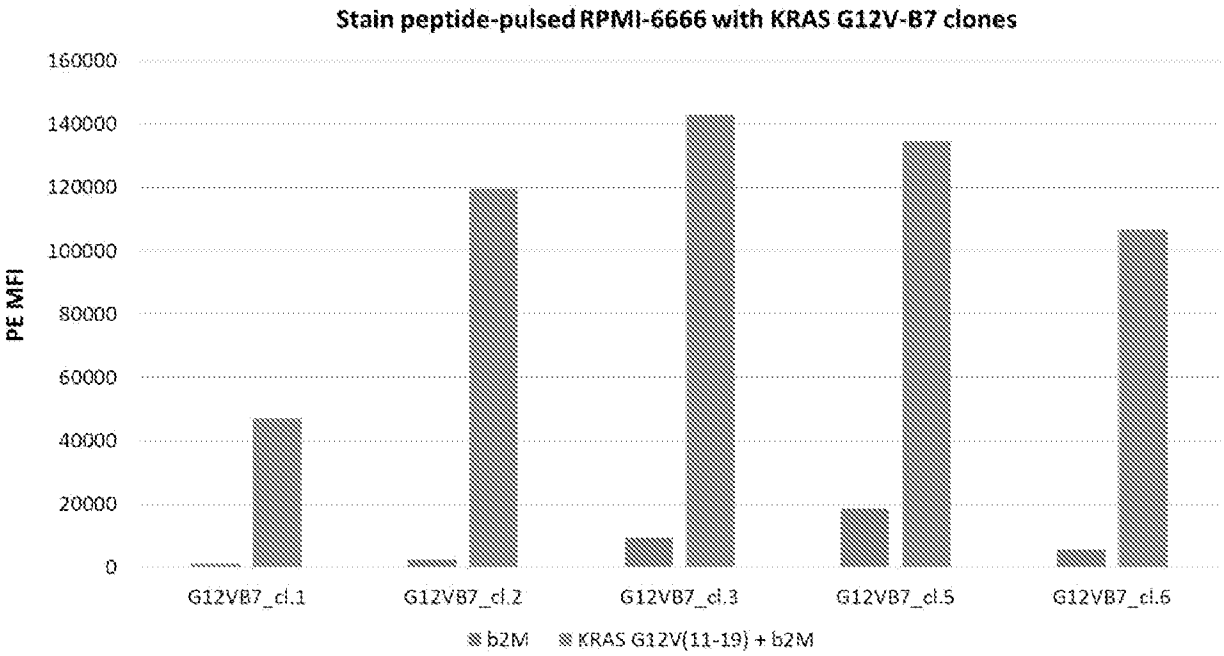
Stain peptide-pulsed RPMI-6666 with KRAS G12V-B7 clones
160000
140000
120000
100000
80000
60000
40000
20000
0
PE MFI
G12VB7_cl.1
G12VB7_cl.2
G12VB7_cl.3
G12VB7_cl.5
G12VB7_cl.6
b2M
KRAS G12V(11-19) + b2M

FIG. 13

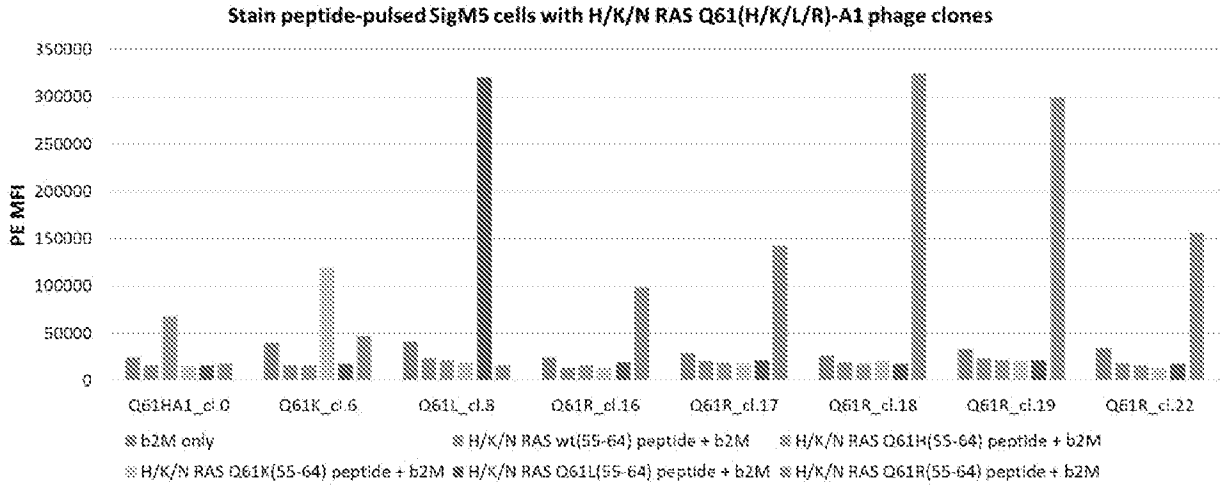
Stain peptide-pulsed SigM5 cells with H/K/N RAS Q61(H/K/L/R)-A1 phage clones
PE MFI
350000
300000
250000
200000
150000
100000
50000
0
Q61HA1_cl.0
Q61K_cl.6
Q61L_cl.8
Q61R_cl.16
Q61R_cl.17
Q61R_cl.18
Q61R_cl.19
Q61R_cl.22
b2M only
H/K/N RAS wt(55-64) peptide + b2M
H/K/N RAS Q61H(55-64) peptide + b2M
H/K/N RAS Q61K(55-64) peptide + b2M
H/K/N RAS Q61L(55-64) peptide + b2M
H/K/N RAS Q61R(55-64) peptide + b2M

FIG. 14

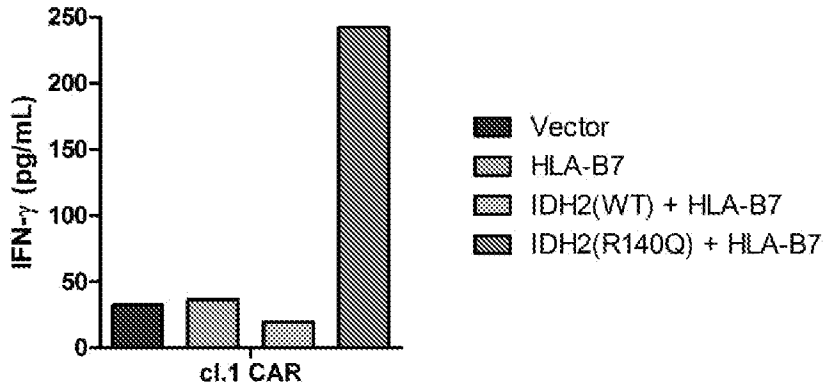

FIG. 15A

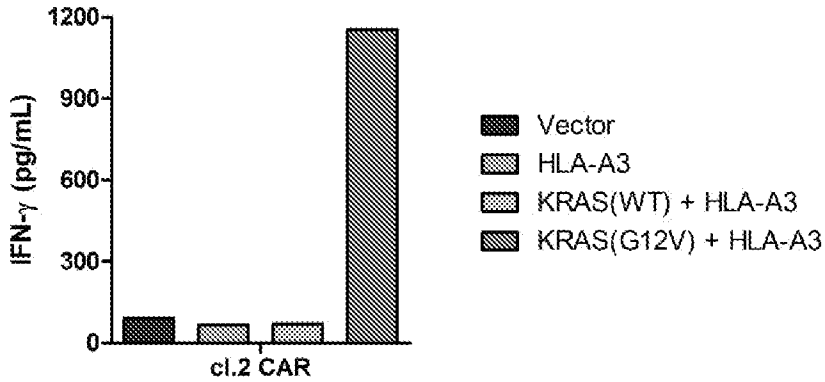

FIG. 15B

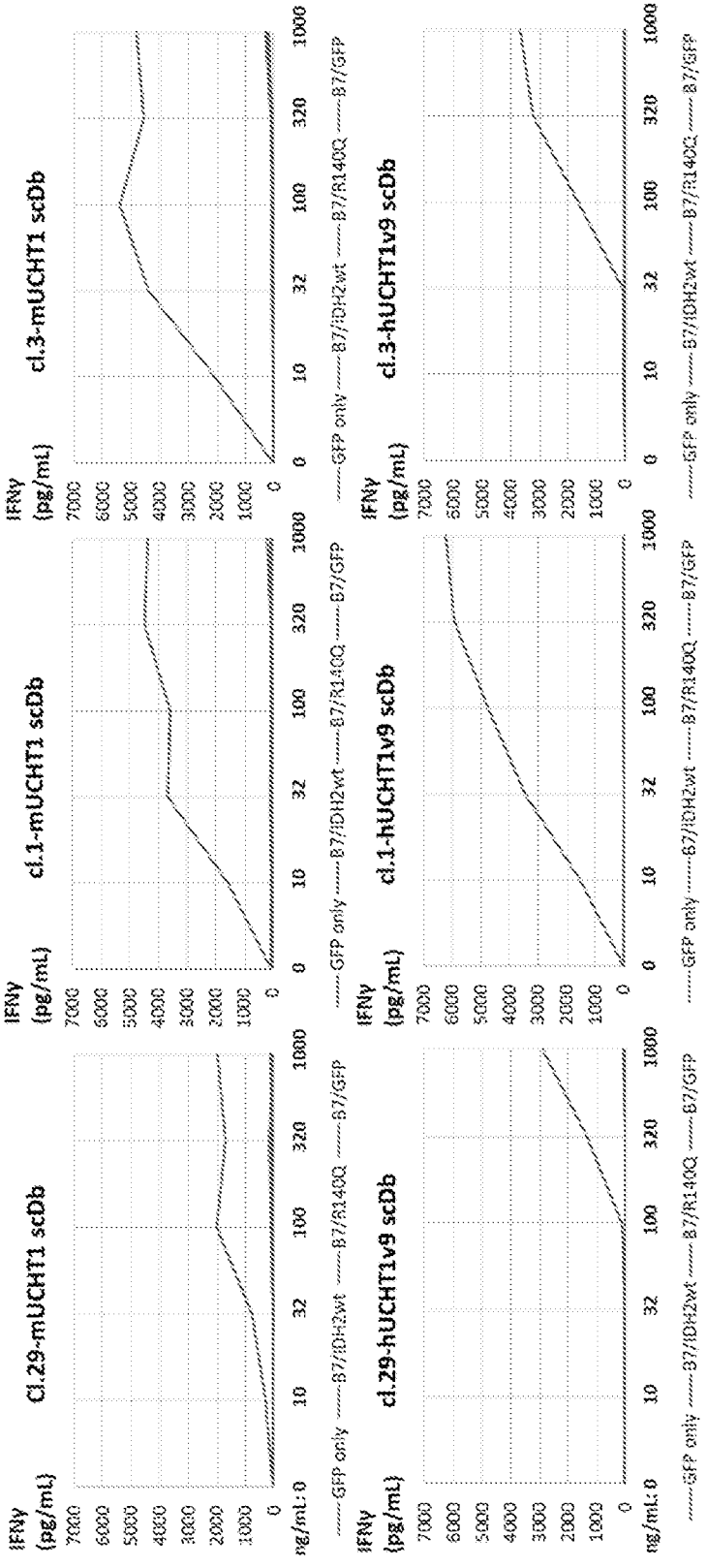
IFNγ (pg/mL)
Cl.29-mUCHT1 scDb
cl.1-mUCHT1 scDb
cl.3-mUCHT1 scDb
cl.29-hUCHT1v9 scDb
cl.1-hUCHT1v9 scDb
cl.3-hUCHT1v9 scDb
7000
6000
5000
4000
3000
2000
1000
0
ng/mL: 0 10 32 100 320 1000
GFP only
B7/IDH2wt
B7/R140Q
B7/GFP

FIG. 16A

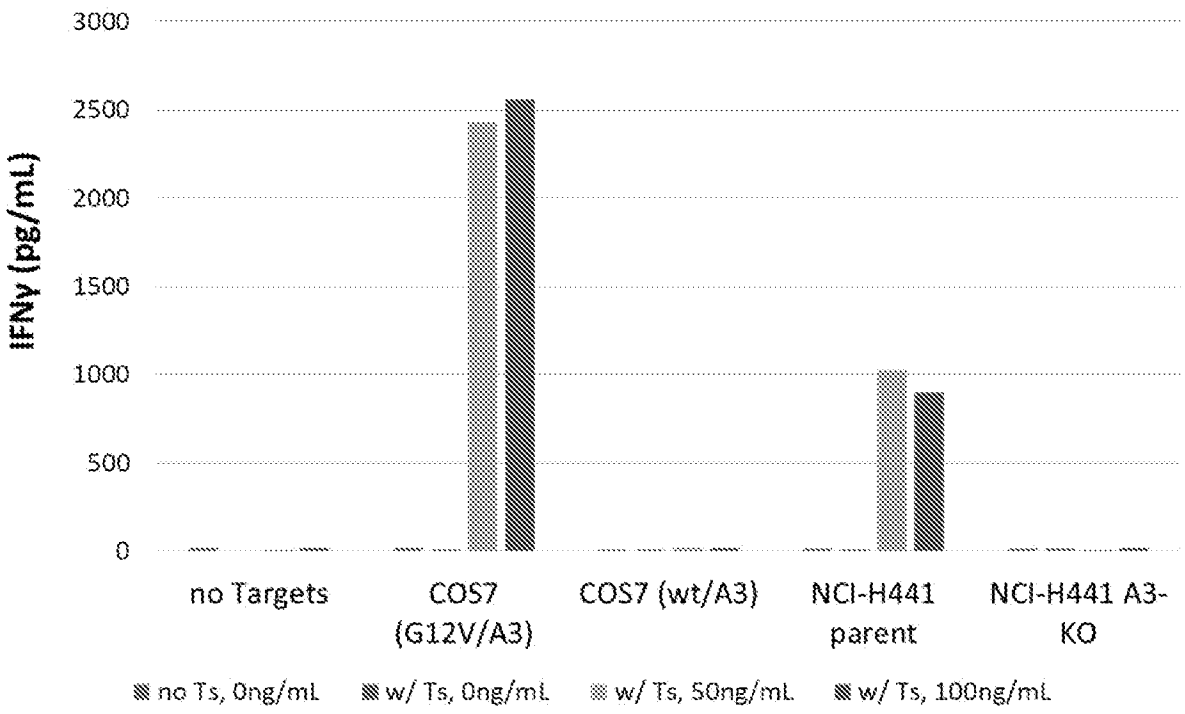
3000
2500
2000
1500
1000
500
0
IFNγ (pg/mL)
no Targets
COS7 (G12V/A3)
COS7 (wt/A3)
NCI-H441 parent
NCI-H441 A3-KO
no Ts, 0ng/mL
w/ Ts, 0ng/mL
w/ Ts, 50ng/mL
w/ Ts, 100ng/mL

FIG. 16B

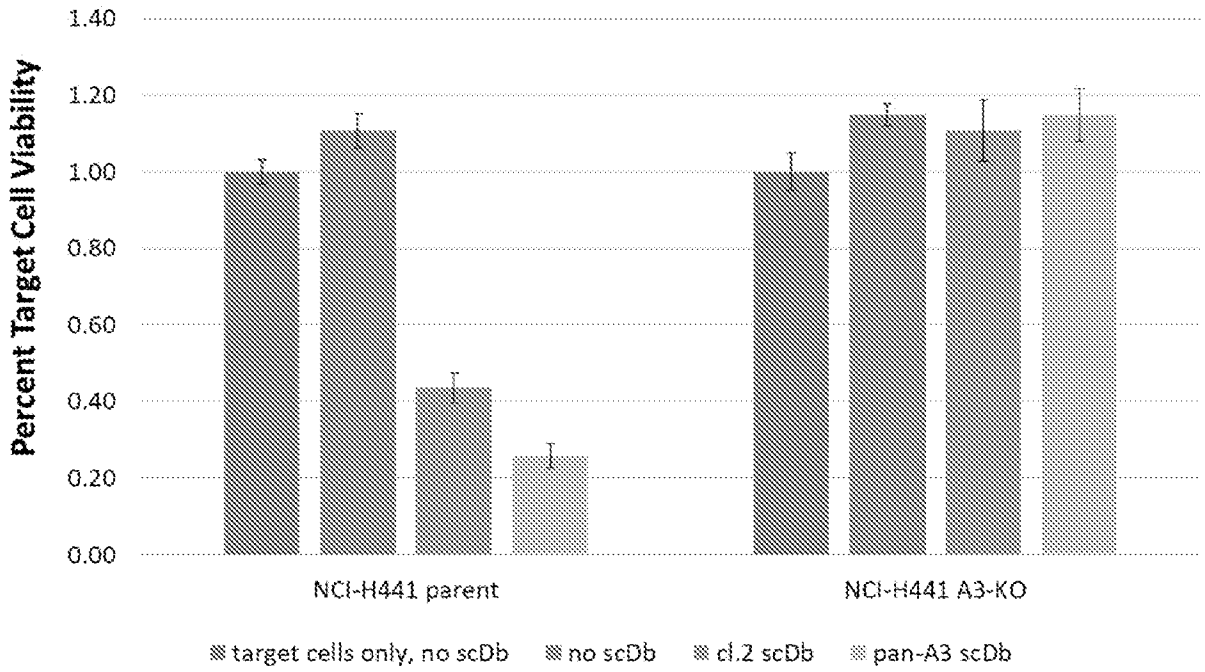
Percent Target Cell Viability
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
NCI-H441 parent
NCI-H441 A3-KO
target cells only, no scDb
no scDb
cl.2 scDb
pan-A3 scDb

FIG. 17