HLA-A*02:01 molecules in AFP-positive cancer cells. Therefore, AFP158/HLA-A*02:01 is a good target candidate for mAb cancer drug development.

[0009] Traditional approaches to using AFP as a therapeutic target for treating cancers that overexpress AFP have relied on using antibodies that target the AFP protein or vaccination with various AFP peptides. Antibodies directed against the AFP protein have little therapeutic efficacy since AFP is not a cell-surface protein, and may be present at high circulating levels, thus reducing any specific targeting of the antibody to the cells expressing AFP. While vaccination with various MHC-restricted AFP peptides and variants thereof has been observed to lead to activation of AFP-specific T cells and increased cytotoxicity of AFP-presenting cells *in vitro*, a clinically significant therapeutic benefit has yet to be observed.

[0010] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

SUMMARY

[0011] The present application provides constructs (such as isolated constructs) that bind to a complex comprising an AFP peptide and an MHC class I protein (referred to herein as an "AFP/MHC class I complex," or "AMC"). In some examples, the constructs ("anti-AMC constructs") comprise an antibody moiety (referred to herein as an "anti-AMC antibody moiety") that specifically binds to a complex comprising an AFP peptide and an MHC class I protein.

[0012] Thus, in some examples, there is provided an anti-AMC construct (such as an isolated anti-AMC construct) comprising an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein. In one aspect, there is provided an isolated anti-AMC construct comprising an antibody moiety that specifically binds to a complex comprising an alpha-fetoprotein (AFP) peptide and a major histocompatibility (MHC) class I protein (an AFP/MHC class I complex, or AMC), wherein the AFP peptide has the amino acid sequence of FMNKFIYEI (SEQ ID NO: 4), wherein the MHC class I protein is HLA-A02, and wherein the antibody moiety comprises:

(1)

i. a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 62, an HC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 72, an HC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 82; and

ii. a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 95, an LC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 105, and an LC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 115;

(2)
i. a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 63, an HC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 73, an HC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 83; and
ii. a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 96, an LC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 106, and an LC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 116;
(3)
i. a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 64, an HC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 74, an HC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 84; and
ii. a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 97, an LC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 107, and an LC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 117;
(4)
i. a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 65, an HC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 75, an HC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 85; and
ii. a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 98, an LC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 108, and an LC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 118; or
(5)
i. a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 66, an HC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 76, an HC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 86; and
ii. a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 99, an LC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 109, and an LC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 119. In some examples, the AFP/MHC class I complex is present on a cell surface. In some examples, the AFP/MHC class I complex is present on the surface of a cancer cell.

[0013] In some examples, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the MHC class I protein is HLA-A. In some examples, the MHC class I protein is HLA-A02. In some examples, the MHC class I protein is the HLA-A*02:01 subtype of the HLA-A02 allele.

[0014] In some examples, according to any of the anti-AMC constructs (such as isolated anti-AMC constructs) described above, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the antibody moiety cross-reacts with a complex comprising the AFP peptide and a second MHC class I protein having a different HLA allele than the MHC class I protein. In some examples, the antibody moiety cross-reacts with a complex comprising an interspecies variant of the AFP peptide and the MHC class I protein. In some examples, the antibody moiety cross-reacts with a complex comprising a variant of the AFP peptide comprising one amino acid substitution (such as a conservative amino acid substitution) and the MHC class I protein.

[0015] In some examples, according to any of the anti-AMC constructs (such as isolated anti-AMC constructs) described above, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the AFP peptide is about 8 to about 12 (such as about any of 8, 9, 10, 11, or 12) amino acids in length. In some examples, the AFP peptide is derived from human AFP. In some examples, the AFP peptide has an amino acid sequence selected from the group consisting of SEQ ID NOs: 3-13 and 16**.** In some examples, the AFP peptide has the amino acid sequence FMNKFIYEI (SEQ ID NO: 4).

[0016] In some examples, according to any of the anti-AMC constructs (such as isolated anti-AMC constructs) described above, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the antibody moiety is a full-length antibody, a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some examples, the antibody moiety is fully human, semi-synthetic with human antibody framework regions, or humanized.

[0017] In some examples, according to any of the anti-AMC constructs (such as isolated anti-AMC constructs) described above, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the antibody moiety binds to the AFP/MHC class I complex with an equilibrium dissociation constant (K_d) between about 0.1 pM to about 500 nM (such as about any of 0.1 pM, 1.0 pM, 10 pM, 50 pM, 100 pM, 500 pM, 1 nM, 10 nM, 50 nM, 100 nM, or 500 nM, including any ranges between these values). In some examples, the isolated anti-AMC construct binds to the AFP/MHC class I complex with a K_d between about 0.1 pM to

about 500 nM (such as about any of 0.1 pM, 1.0 pM, 10 pM, 50 pM, 100 pM, 500 pM, 1 nM, 10 nM, 50 nM, 100 nM, or 500 nM, including any ranges between these values).

[0018] In some examples, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the antibody moiety comprises: i) a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of G-F/Y-S/T-F-D/S/T-D/N/S-Y/A-A/G/W (SEQ ID NO: 87), or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of I/S-K/S-X-H/Y-X-G-X-T (SEQ ID NO: 88), or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of A/G-X-W/Y-Y-X-X-X-F/Y-D (SEQ ID NO: 89), or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of S/T-G/S-D/N-I/V-A/G-A/S/V-X-H/Y (SEQ ID NO: 120), or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of Q-S/T-Y/W-D/T-S/T-A/S (SEQ ID NO: 121), or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, wherein X can be any amino acid.

[0019] In some examples, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the antibody moiety comprises: i) a heavy chain variable domain comprising an HC-CDR1 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 57-66, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 67-76, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 77-86, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 90-99, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an

LC-CDR2 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 100-109, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 110-119, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0020] In some examples, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the antibody moiety comprises: i) a heavy chain variable domain comprising (and in some examples consisting of) an HC-CDR1 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 57-66, an HC-CDR2 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 67-76, and an HC-CDR3 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 77-86; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR regions; and ii) a light chain variable domain comprising an LC-CDR1 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 90-99, an LC-CDR2 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 100-109, and an LC-CDR3 comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 110-119; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR regions.

[0021] In some examples, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the antibody moiety comprises a) a heavy chain variable domain comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 17-26 or a variant thereof having at least about 95% (such as at least about any of 95%, 96%, 97%, 98%, or 99%) sequence identify to any one of SEQ ID NOs: 17-26; and b) a light chain variable domain comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 27-36 or a variant thereof having at least about 95% (such as at least about any of 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 27-36. In some examples, the antibody moiety comprises a heavy chain variable domain comprising (and in some examples consisting of) the amino acid

sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising (and in some examples consisting of) the amino acid sequence of any one of SEQ ID NOs: 27-36.

[0022] In some examples, the anti-AMC construct comprises a first antibody moiety that competes for binding to a target AFP/MHC class I complex with a second antibody moiety according to any of the antibody moieties described above. In some examples, the first antibody moiety binds to the same, or substantially the same, epitope as the second antibody moiety. In some examples, binding of the first antibody moiety to the target AFP/MHC class I complex inhibits binding of the second antibody moiety to the target AFP/MHC class I complex by at least about 70% (such as by at least about any of 75%, 80%, 85%, 90%, 95%, 98% or 99%), or vice versa. In some examples, the first antibody moiety and the second antibody moiety cross-compete for binding to the target AFP/MHC class I complex, *i.e.*, each of the first and second antibody moieties competes with the other for binding to the target AFP/MHC class I complex.

[0023] In some examples, according to any of the anti-AMC constructs (such as isolated anti-AMC constructs) described above, the isolated anti-AMC construct is a full-length antibody. In some examples, the isolated anti-AMC construct is monospecific. In some examples, the isolated anti-AMC construct is multi-specific. In some examples, the isolated anti-AMC construct is bispecific. In some examples, the isolated anti-AMC molecule is a tandem scFv, a diabody (Db), a single chain diabody (scDb), a dual-affinity retargeting (DART) antibody, a dual variable domain (DVD) antibody, a knob-into-hole (KiH) antibody, a dock and lock (DNL) antibody, a chemically cross-linked antibody, a heteromultimeric antibody, or a heteroconjugate antibody. In some examples, the isolated anti-AMC construct is a tandem scFv comprising two scFvs linked by a peptide linker. In some examples, the peptide linker comprises (and in some examples consists of) the amino acid sequence GGGGS.

[0024] In some examples, according to any of the anti-AMC constructs (such as isolated anti-AMC constructs) described above, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the isolated anti-AMC construct further comprises a second antigen-binding moiety that specifically binds to a second antigen. In some examples, the second antigen-binding moiety is an antibody moiety. In some examples, the second antigen is an antigen on the surface of a T cell. In some examples, the T cell is selected from the group

consisting of a cytotoxic T cell, a helper T cell, and a natural killer T cell. In some examples, the second antigen is selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, and HVEM. In some examples, the second antigen is CD3ε, and the isolated anti-AMC construct is a tandem scFv comprising an N-terminal scFv specific for the AFP/MHC class I complex and a C-terminal scFv specific for CD3ε. In some examples, the second antigen is an antigen on the surface of a natural killer cell, a neutrophil, a monocyte, a macrophage, or a dendritic cell.

[0025] In some examples, according to any of the anti-AMC constructs (such as isolated anti-AMC constructs) described above, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the isolated anti-AMC construct is a chimeric antigen receptor. In some examples, the chimeric antigen receptor comprises an extracellular domain comprising the antibody moiety, a transmembrane domain, and an intracellular signaling domain. In some examples, the intracellular signaling domain comprises a CD3ζ intracellular signaling sequence and a co-stimulatory signaling sequence. In some examples, the co-stimulatory signaling sequence is a CD28 intracellular signaling sequence.

[0026] In some examples, according to any of the anti-AMC constructs (such as isolated anti-AMC constructs) described above, the anti-AMC construct comprises an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the isolated anti-AMC construct is an immunoconjugate comprising the antibody moiety and an effector molecule. In some examples, the effector molecule is a therapeutic agent selected from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid. In some examples, the therapeutic agent is a drug or a toxin. In some examples, the effector molecule is a label.

[0027] In yet other examples, there is provided a pharmaceutical composition comprising an anti-AMC construct (such as an isolated anti-AMC construct) according to any of the examples described above. In one aspect, there is provided a pharmaceutical composition comprising the isolated anti-AMC construct according to any of the examples described above. In some examples, the pharmaceutical composition further comprises a cell (such as an effector cell) associated with the anti-AMC construct. In some examples, there is provided a host cell expressing or associated with an anti-AMC construct or polypeptide component thereof. In one aspect, there is provided a host cell expressing the isolated anti-AMC construct according to any of the examples described above. In some examples, there is provided a nucleic acid encoding an anti-AMC construct or polypeptide component thereof. In one aspect, there is provided a nucleic acid encoding the polypeptide components of the isolated anti-AMC construct according to any of the examples described above. In some examples, there is provided a vector comprising the nucleic acid. In some examples,

there is provided an effector cell expressing or associated with an anti-AMC construct. In one aspect, there is provided an effector cell expressing the isolated anti-AMC construct according to any of the examples described above. In some examples, the effector cell is a T cell.

[0028] In some examples, there is provided a method of detecting a cell presenting a complex comprising an AFP peptide and an MHC class I protein on its surface, comprising contacting the cell with an anti-AMC construct (such as an isolated anti-AMC construct) according to any of the examples described above comprising a) an antibody moiety that specifically binds to a complex comprising the AFP peptide and the MHC class I protein and b) a label, and detecting the presence of the label on the cell. In one aspect, there is provided an *in vitro* method of detecting a cell presenting a complex comprising an AFP peptide and an MHC class I protein on its surface, comprising contacting the cell with an isolated anti-AMC construct according to any of the examples described above, and detecting the presence of the label on the cell.

[0029] In some examples, there is provided a method of treating an individual having an AFP-positive disease, comprising administering to the individual an effective amount of a pharmaceutical composition comprising an anti-AMC construct (such as an isolated anti-AMC construct) according to any of the examples described above. In one aspect, there is provided use of the pharmaceutical composition described above or the effector cell described above in the manufacture of a medicament for treating an individual having an AFP-positive cancer, and wherein the cancer expresses an AFP peptide complexed with HLA-A02. In some examples, the pharmaceutical composition further comprises a cell (such as an effector cell) associated with the isolated anti-AMC construct. In some examples, there is provided a method of treating an individual having an AFP-positive disease, comprising administering to the individual an effective amount of an effector cell expressing any of the anti-AMC CARs described above. In some examples, the effector cell is a T cell. In some examples, the administration is to an injection site distal to a first disease site in the individual. In some examples, the injection site is a first tumor distal to the first disease site. In some examples, the first disease site is an AFP-positive tumor. In some examples, the AFP-positive disease is cancer. In some examples, the cancer is hepatocellular carcinoma or germ cell tumor. In some examples, the cancer is hepatocellular carcinoma. In some examples, the cancer is hepatocellular carcinoma and metastasis is inhibited. In some examples, the cancer is metastatic hepatocellular carcinoma.

[0030] In some examples, there is provided a method of diagnosing an individual having an AFP-positive disease, comprising: a) administering an effective amount of an isolated anti-AMC construct according to any of the examples described above to the individual; and b) determining the level of the label in the individual, wherein a level of the label above a threshold level indicates that the individual has the AFP-positive disease. In one aspect, there is provided an *in vitro* method of diagnosing an individual having an AFP-positive cancer, wherein the cancer expresses an AFP peptide complexed with HLA-A02, comprising determining the level of a label in a sample obtained from the individual who has been administered an effective amount of the isolated anti-AMC construct according to any of the examples described above, wherein a level of the label above a threshold level indicates that the individual has the AFP-positive cancer. In another aspect, there is provided a method of diagnosing an individual having an AFP-positive cancer, wherein the cancer expresses an AFP peptide complexed with HLA-A02, the method comprising: a) contacting a sample derived from the individual with an isolated anti-AMC construct according to any of the examples described above; and b) determining

the number of cells bound with the isolated anti-AMC construct in the sample, wherein a value for the number of cells bound with the isolated anti-AMC construct above a threshold level indicates that the individual has the AFP-positive disease. In some examples, the AFP-positive disease is cancer. In some examples, the cancer is hepatocellular carcinoma or germ cell tumor. In some examples, the cancer is hepatocellular carcinoma. In some examples, the cancer is hepatocellular carcinoma and metastasis is inhibited. In some examples, the cancer is metastatic hepatocellular carcinoma.

[0031] Also provided are methods of making any of the constructs described herein, articles of manufacture, and kits that are suitable for the methods described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] FIG. 1 shows the size exclusion chromatography (SEC) chromatogram of AFP158 peptide/HLA-A*02:01 complex following concentration by ultrafiltration. Properly folded peptide/MHC complex monomers: 212.49 mL; misfolded aggregates: 111.27 mL; free β2M: 267.21 mL.

[0033] FIG. 2 shows reducing SDS-PAGE analysis to determine the purity of AFP158/HLA-A*02:01 complex isolated following SEC. Major bands correspond to HLA-A*02:01 and β2M subunits.

[0034] FIG. 3 shows the results of phage clone ELISA for specific binding of biotinylated AFP158 peptide/HLA-A*02:01 (Bio-AFP158) versus biotinylated control peptide/HLA-A*02:01 (Bio-control).

[0035] FIG. 4 shows the results of phage clone FACS binding assays for binding of β2M-loaded, β2M/AFP158 peptide-loaded and β2M/hTERT peptide-loaded T2 cells.

[0036] FIG. 5 shows the results of phage clone FACS binding assays for cross-reactivity with β2M/human AFP158 peptide-loaded T2 cells and β2M/mouse AFP158 peptide-loaded T2 cells versus β2M-loaded T2 cells.

[0037] FIG. 6 shows the results of phage clone #52 FACS binding assays for T2 cells loaded with AFP158 peptide having single alanine substitutions at varying positions.

[0038] FIG. 7 shows the results of phage clone FACS binding assays for binding of β2M AFP158 peptide-loaded T2 cells, T2 cells loaded with β2M and a mixture of peptides derived from normally expressed endogenous proteins or β2M-loaded T2 cells.

CLAIMS

What is claimed is:

1. An isolated anti-AMC construct comprising an antibody moiety that specifically binds to a complex comprising an alpha-fetoprotein (AFP) peptide and a major histocompatibility (MHC) class I protein (an AFP/MHC class I complex, or AMC), wherein the AFP peptide has the amino acid sequence of FMNKFIYEI (SEQ ID NO: 4), wherein the MHC class I protein is HLA-A02, and wherein the antibody moiety comprises:

(1)

i. a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 62, an HC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 72, an HC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 82; and

ii. a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 95, an LC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 105, and an LC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 115;

(2)

i. a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 63, an HC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 73, an HC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 83; and

ii. a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 96, an LC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 106, and an LC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 116;

(3)

i. a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 64, an HC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 74, an HC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 84; and

ii. a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 97, an LC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 107, and an LC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 117;

(4)

i. a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 65, an HC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 75, an HC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 85; and

ii. a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 98, an LC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 108, and an LC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 118; or

(5)

i. a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 66, an HC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 76, an HC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 86; and

ii. a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 99, an LC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 109, and an LC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 119.

2. The isolated anti-AMC construct of claim 1, wherein the MHC class I protein is the HLA-A*02:01 subtype of the HLA-A02 allele.

3. The isolated anti-AMC construct of claim 1 or 2, wherein the antibody moiety is a full-length antibody, a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv).

4. The isolated anti-AMC construct of any one of claims 1-3, wherein the isolated anti-AMC construct binds to the AFP/MHC class I complex with a K_d from 0.1 pM to 500 nM**.**

5. The isolated anti-AMC construct of any one of claims 1-4, wherein the antibody moiety comprises:

(i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 22, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 32;

(ii) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 24, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 34;

(iii) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 25, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 35;

(iv) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 26, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 36; or
(v) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 33.

6. The isolated anti-AMC construct of any one of claims 1-4, comprising:
i) a heavy chain variable domain comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 63, an HC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 73, an HC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 83; and
ii) a light chain variable domain comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 96, an LC-CDR 2 comprising the amino acid sequence of SEQ ID NO: 106, and an LC-CDR 3 comprising the amino acid sequence of SEQ ID NO: 116.

7. The isolated anti-AMC construct of any one of claims 1-4, wherein the anti-AMC antibody moiety comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 33.

8. The isolated anti-AMC construct of any one of claims 1-7, wherein the isolated anti-AMC construct is multispecific.

9. The isolated anti-AMC construct of claim 8, wherein the isolated anti-AMC construct is a tandem scFv, a diabody (Db), a single chain diabody (scDb), a dual-affinity retargeting (DART) antibody, a dual variable domain (DVD) antibody, a knob-into-hole (KiH) antibody, a dock and lock (DNL) antibody, a chemically cross-linked antibody, a heteromultimeric antibody, or a heteroconjugate antibody.

10. The isolated anti-AMC construct of claim 9, wherein the isolated anti-AMC construct is a tandem scFv comprising two scFvs linked by a peptide linker.

11. The isolated anti-AMC construct of any one of claims 8-10, wherein the isolated anti-AMC construct further comprises a second antibody moiety that specifically binds to a second antigen.

12. The isolated anti-AMC construct of claim 11, wherein the second antigen is selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, and HVEM.

13. The isolated anti-AMC construct of claim 11, wherein the second antigen is CD3ε, and wherein the isolated anti-AMC construct is a tandem scFv comprising an N-terminal scFv specific for the AFP/MHC class I complex and a C-terminal scFv specific for CD3ε.

14. The isolated anti-AMC construct of any one of claims 1-7, wherein the isolated anti-AMC construct is a chimeric antigen receptor comprising an extracellular domain comprising the antibody moiety, a transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

15. The isolated anti-AMC construct of any one of claims 1-7, wherein the isolated anti-AMC construct is an immunoconjugate comprising the antibody moiety and an effector

molecule, wherein the effector molecule is a therapeutic agent selected from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid.

16. The isolated anti-AMC construct of any one of claims 1-7, wherein the isolated anti-AMC construct is an immunoconjugate comprising the antibody moiety and a label.

17. A pharmaceutical composition comprising the isolated anti-AMC construct of any one of claims 1-15.

18. A host cell expressing the isolated anti-AMC construct of any one of claims 1-16.

19. A nucleic acid encoding the polypeptide components of the isolated anti-AMC construct of any one of claims 1-16.

20. An effector cell expressing the isolated anti-AMC construct of claim 14.

21. The effector cell of claim 20, wherein the effector cell is a T cell.

22. An *in vitro* method of detecting a cell presenting a complex comprising an AFP peptide and an MHC class I protein on its surface, comprising contacting the cell with the isolated anti-AMC construct of claim 16 and detecting the presence of the label on the cell.

23. Use of the pharmaceutical composition of claim 17 or the effector cell of claim 20 or 21 in the manufacture of a medicament for treating an individual having an AFP-positive cancer, and wherein the cancer expresses an AFP peptide complexed with HLA-A02.

24. The use of claim 23, wherein the medicament is to be administered via intravenous or intratumoral route.

25. The use of claim 23, wherein the medicament is to be administered to an injection site distal to a first disease site.

26. An *in vitro* method of diagnosing an individual having an AFP-positive cancer, wherein the cancer expresses an AFP peptide complexed with HLA-A02, comprising determining the level of the label in a sample obtained from the individual who has been administered an effective amount of the isolated anti-AMC construct of claim 16, wherein a level of the label above a threshold level indicates that the individual has the AFP-positive cancer.

27. A method of diagnosing an individual having an AFP-positive cancer, wherein the cancer expresses an AFP peptide complexed with HLA-A02, the method comprising:

a) contacting a sample derived from the individual with the isolated anti-AMC construct of claim 16; and

b) determining the number of cells bound with the isolated anti-AMC construct in the sample, wherein a value for the number of cells bound with the isolated anti-AMC construct above a threshold level indicates that the individual has the AFP-positive cancer.

28. The use of any one of claims 23-25, or the method of claim 26 or 27, wherein the HLA-A02 is selected from the group consisting of HLA-A*02*01, HLA-A02*02, HLA*02:03, HLA-A*02:05, HLA-A*02:06, HLA-A*02:07, and HLA-A*02:11.

29. The use or method of claim 28, wherein the HLA-A02 is HLA-A*02:01.

30. The use or method of any one of claims 23-29, wherein the cancer is hepatocellular carcinoma, germ cell tumor, or breast cancer.

31. The use or method of claim 30, wherein the cancer is hepatocellular carcinoma.

32. The use or method of claim 31, wherein the cancer is metastatic hepatocellular carcinoma.

33. The use of any one of claims 23-25, wherein the AFP-positive cancer is hepatocellular carcinoma and metastasis is inhibited.