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 anti-AMC 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, wherein the anti-AMC antibody moiety binds to AMC with a K_d from 0.1 pM to 500 nM, and wherein the anti-AMC antibody moiety comprises:

(1)

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,

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)
an 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,
an 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)
an 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,
an 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)
an 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,
an 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)
an 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,
an 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.

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 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 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 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 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 an AFP/MHC class I complex on its surface, wherein the AFP peptide has the amino acid sequence of SEQ ID NO: 4, wherein the MHC class I protein is HLA-A02, and wherein the method comprises contacting the cell with an 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, the anti-AMC construct described above, or the effector cell described above in the manufacture of a medicament for treating an individual having an AFP-positive cancer, wherein the AFP-positive cancer expresses an AFP peptide complexed with HLA-A02, and wherein the AFP peptide has the amino acid sequence of SEQ ID NO: 4. 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, wherein the AFP peptide has the amino acid sequence of SEQ ID NO: 4, the method 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, wherein the AFP peptide has the amino acid sequence of SEQ ID NO: 4, 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

CLAIMS

What is claimed is:

1. An isolated anti-AMC construct comprising an anti-AMC 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, wherein the anti-AMC antibody moiety binds to AMC with a K_d from 0.1 pM to 500 nM, and wherein the anti-AMC antibody moiety comprises:

(1)
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,
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)
an 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,
an 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)

an 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,
an 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)

an 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,
an 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)

an 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,
an 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 HLA-A02.

3. The isolated anti-AMC construct of claim 1 or 2, wherein the anti-AMC 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 anti-AMC 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.

5. The isolated anti-AMC construct of any one of claims 1-3, comprising
an 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,
an 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.

6. The isolated anti-AMC construct of any one of claims 1-5, 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.

7. The isolated anti-AMC construct of any one of claims 1-6, wherein the isolated anti-AMC construct is multispecific.

8. The isolated anti-AMC construct of claim 7, 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.

9. The isolated anti-AMC construct of claim 7 or 8, wherein the isolated anti-AMC construct is a tandem scFv comprising two scFvs linked by a peptide linker.

10. The isolated anti-AMC construct of any one of claims 7-9, wherein the isolated anti-AMC construct further comprises a second antibody moiety that specifically binds to a second antigen.

11. The isolated anti-AMC construct of claim 10, wherein the second antigen is selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, CD16a, CD56, CD68, GDS2D, OX40, GITR, CD137, CD27, CD40L, and HVEM.

12. The isolated anti-AMC construct of claim 10 or 11, wherein the second antigen is an effector cell surface antigen.

13. The isolated anti-AMC construct of any one of claims 10-12, wherein the second antigen is CD3ε, and wherein the isolated anti-AMC construct is a tandem scFv comprising an N-terminal scFv specific for AMC and a C-terminal scFv specific for CD3ε.

14. The isolated anti-AMC construct of any one of claims 1-13, wherein the isolated anti-AMC construct is a chimeric antigen receptor comprising an extracellular domain comprising the anti-AMC 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 1-13, wherein the isolated anti-AMC construct is an immunoconjugate comprising the anti-AMC 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 1-13, wherein the isolated anti-AMC construct is an immunoconjugate comprising the anti-AMC antibody moiety and a label.

17. A pharmaceutical composition comprising the anti-AMC construct of any one of claims 12, 13, and 15.

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

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

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

21. The effector cell of claim 12 or 20, wherein the effector cell is a T cell.

22. An *in vitro* method of detecting a cell presenting an AFP/MHC class I complex on its surface, wherein the AFP peptide has the amino acid sequence of SEQ ID NO: 4, wherein the MHC class I protein is HLA-A02, and wherein the method comprises contacting the cell with the 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, the anti-AMC construct of any one or claims 12, 13, and 15, or the effector cell of claim 20 or 21 in the manufacture of a medicament for treating an individual having an AFP-positive cancer, wherein the AFP-positive cancer expresses an AFP peptide complexed with HLA-A02, and wherein the AFP peptide has the amino acid sequence of SEQ ID NO: 4.

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, wherein the AFP peptide has the amino acid sequence of SEQ ID NO: 4, the method 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 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, wherein the AFP peptide has the amino acid sequence of SEQ ID NO: 4, 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 any one of claims 22, 26, and 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 AFP-positive cancer is hepatocellular carcinoma, germ cell tumor, or breast cancer.

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

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

33. The use of any one of claims 23-25 and 28-31, wherein the AFP-positive cancer is hepatocellular carcinoma and metastasis is inhibited.