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 embodiments, 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 embodiments, 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). In some embodiments, the AFP/MHC class I complex is present on a cell surface. In some embodiments, the AFP/MHC class I complex is present on the surface of a cancer cell.

[0013] In some embodiments, 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 embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is the HLA-A*02:01 subtype of the HLA-A02 allele.

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 embodiments described above. In some embodiments, the effector cell is a T cell.

[0028] In some embodiments, 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 embodiments 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 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 isolated anti-AMC construct according to any of the embodiments described above, and detecting the presence of the label on the cell.

[0029] In some embodiments, 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 embodiments 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 disease. In some embodiments, the pharmaceutical composition further comprises a cell (such as an effector cell) associated with the isolated anti-AMC construct. In some embodiments, 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 embodiments, the effector cell is a T cell. In some embodiments, the administration is to an injection site distal to a first disease site in the individual. In some embodiments, the injection site is a first tumor distal to the first disease site. In some embodiments, the first disease site is an AFP-positive tumor. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is hepatocellular carcinoma or germ cell tumor. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and metastasis is inhibited. In some embodiments, the cancer is metastatic hepatocellular carcinoma.

[0030] In some embodiments, 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 embodiments 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 disease, 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 embodiments described above, wherein a level of the label above a threshold level indicates that the individual has the AFP-positive disease. In another aspect, there is provided a method of diagnosing an individual having an AFP-positive disease, comprising: a) contacting a sample derived from the individual with an isolated anti-AMC construct according to any of the embodiments described above; and b) determining

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).

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 about 0.1 pM to about 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 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), an HC-CDR2 comprising the amino acid sequence of I/S-K/S-X-H/Y-X-G-X-T (SEQ ID NO: 88), 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); 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), 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).

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

i) a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 57-66, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 67-76, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 77-86; and
ii) a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 90-99, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 100-109, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 110-119.

7. The isolated anti-AMC construct of claim 6, wherein the antibody moiety comprises a) a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26, or a variant thereof having at least about 95% sequence identify to any one of SEQ ID NOs: 17-26; and b) a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36, or a variant thereof having at least about 95% sequence identity to any one of SEQ ID NOs: 27-36.

8. 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.

9. 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, or a variant thereof having at least about 95% sequence identity to SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 33, or a variant thereof having at least about 95% sequence identity to SEQ ID NO: 33.

10. The isolated anti-AMC construct of any one of claims 1-9, wherein the isolated anti-AMC construct is multispecific.

11. The isolated anti-AMC construct of claim 10, 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.

12. The isolated anti-AMC construct of claim 11, wherein the isolated anti-AMC construct is a tandem scFv comprising two scFvs linked by a peptide linker.

13. The isolated anti-AMC construct of any one of claims 10-12, wherein the isolated anti-AMC construct further comprises a second antibody moiety that specifically binds to a second antigen.

14. The isolated anti-AMC construct of claim 13, wherein the second antigen is selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, and HVEM.

15. The isolated anti-AMC construct of claim 13, 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.

16. The isolated anti-AMC construct of any one of claims 1-9, 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.

17. The isolated anti-AMC construct of any one of claims 1-9, 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.

18. The isolated anti-AMC construct of any one of claims 1-9, wherein the isolated anti-AMC construct is an immunoconjugate comprising the antibody moiety and a label.

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

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

21. A nucleic acid encoding the polypeptide components of the isolated anti-AMC construct of any one of claims 1-18.

22. An effector cell expressing the isolated anti-AMC construct of claim 16.

23. The effector cell of claim 22, wherein the effector cell is a T cell.

24. 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 the isolated anti-AMC construct of claim 18 and detecting the presence of the label on the cell.

25. Use of the pharmaceutical composition of claim 19 or the effector cell of claim 22 or 23 in the manufacture of a medicament for treating an individual having an AFP-positive disease.

26. The use of claim 25, wherein the medicament is to be administered via intravenous or intratumoral route.

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

28. An *in vitro* method of diagnosing an individual having an AFP-positive disease, 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 18, wherein a level of the label above a threshold level indicates that the individual has the AFP-positive disease.

29. A method of diagnosing an individual having an AFP-positive disease, comprising:

a) contacting a sample derived from the individual with the isolated anti-AMC construct of claim 18; 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.

30. The use of any one of claims 25-27, or the method of any one of claims 28-29, wherein the AFP-positive disease is cancer.

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

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

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

34. The use of any one of claims 25-27, wherein the AFP-positive disease is hepatocellular carcinoma and metastasis is inhibited.