CONSTRUCTS TARGETING AFP PEPTIDE/MHC COMPLEXES AND USES THEREOF

CROSS-REREFENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/142,958, filed on April 3, 2015, U.S. Provisional Application No. 62/244,653, filed on October 21, 2015, and U.S. Provisional Application No. 62/304,915, filed on March 7, 2016, all of which are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

[0002] This invention pertains to antibody constructs that specifically bind MHC molecules complexed with AFP peptides, and uses thereof including treating and diagnosing diseases.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0003] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 750042000140SEQLIST.txt, date recorded: April 1, 2016, size: 65 KB).

BACKGROUND OF THE INVENTION

[0004] Cell surface proteins constitute only a small fraction of the cellular proteins and these proteins are often not tumor-specific. Because of the inability to easily penetrate cells, marketed therapeutic monoclonal antibodies (mAbs) recognize these cell surface proteins, most of which are lineage or differentiation antigens (Milenic, E.D., *Curr. Pharm. Des.* 8:1794-1764, 2002; Grillo-Lopez, A.J., *Expert Rev. Anticancer Ther.* 2(3):323-329, 2002; Jones, K.L. & Buzdat, A.U., *Lancet Oncol.* 10(12):1179-1187, 2009). In contrast, mutated or oncogenic tumor-associated proteins are typically nuclear, cytoplasmic or secreted, which are currently best addressed either by small molecule drugs, or in the case of secreted proteins, hardly addressed as anti-cancer drug targets (Reddy *et al.*, *Expert Opin. Ther. Targets* 3:313-324, 2012; Takeuchi, K. & Ito, F., *Biol. Pharm. Bull.* 34(12):1774-1780; Roychowdhury, S. & Talpaz, M., *Blood Rev.* 6:279-290, 2011). However, most intracellular proteins can be proteosomally degraded, processed and presented by MHC molecules on the cell surface as T cell peptide epitopes in the context of MHC molecules that are recognized by T cell receptors

(TCRs) (Morris *et al.*, *Blood Rev.* 20:61-69, 2006; Konnig, R., *Curr. Opin. Immunol.* 14(1):75-83, 2002). Therefore, generating therapeutic mAbs that recognize the secreted or intracellular tumor antigen-derived peptide/MHC complexes on the cell surface will take advantage of the enhanced specificity and therapeutic potency offered by mAbs. Recent advances in using phage display to generate mAbs have made it possible to select agents with exquisite specificity against defined epitopes from large antibody repertoires. A number of such mAbs specific for solid tumor antigens, in the context of HLA-A01 and HLA-A02, have been successfully selected from phage display libraries (Noy *et al.*, *Expert Rev. Anticancer Ther.* 5(3):523-536, 2005; Chames *et al.*, *Proc. Natl. Acad. Sci. USA* 97:7969-7974, 2000; Held *et al.*, *Eur. J. Immunol.* 34:2919-2929, 2004; Lev *et al.*, *Cancer Res.* 62:3184-3194, 2002; Klechevsky *et al.*, *Cancer Res.* 68(15):6360-6367, 2008). More recently, a human mAb specific for human WT1/HLA-A02 complex, a well-described T cell epitope, has been shown to inhibit multiple cancer cell lines and primary cancer cells via Fc-mediated effector cell function (Dao *et al.*, *Sci. Transl. Med.* 5:176ra33, 2013; Veomett *et al.*, *Clin. Cancer Res.* doi: 10.1158/1078-0432, 2014) in cellular assays and in *in vivo* models.

[0005] Alpha-fetoprotein (AFP) is a 69 kD glycoprotein produced in the yolk sac and fetal liver and secreted into circulation. The synthesis of AFP decreases dramatically after birth and only trace amounts are present in the adult liver. In normal adult human blood serum, AFP concentration is usually as low as 5-7 ng/ml (Terentiev, A.A. & Moldogazieva, N.T., *Tumour Biol.* 34(4):2075-2091, 2013). However, expression of the AFP gene is reactivated in adults during liver regeneration, hepatocarcinogensis, germ cell tumor or in some cases of viral infection (HBV/HCV). The measurement of serum AFP, therefore, plays an important role in diagnosis and in monitoring responses to the treatment of AFP-positive cancers. Currently, AFP is considered a "gold standard" among tumor-specific molecular biomarkers. However, because AFP is not a cell-surface protein, targeting AFP has not been a very active area for anti-cancer antibody drug development.

[0006] Primary liver cancer is the fifth most common form of cancer worldwide and the second most common cause of cancer-related death. In 2012, there were about 782,000 new cancer cases globally, and about 746,000 liver cancer related deaths (http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx). 90-95% of liver cancers are hepatocellular carcinoma (HCC). Chronic liver damage, such as that caused by chronic hepatitis, liver cirrhosis and fatty liver disease, is closely associated with the occurrence of

HCC. Hepatitis virus infection (*e.g.* HBV, HCV), aflatoxin B exposure, alcohol intake and other metabolic diseases (*e.g.* obesity and diabetes) are well-known risk factors for HCC. The incidence of HCC is high in East Asian and African countries due to the prevalence in HBV and HCV in these regions (Shibata, T. & Aburatani, H., *Nat. Rev. Gastroenterol. Hepatol.* 11(6):340-349, 2014). However, the number of patients infected with HCV has been rapidly increasing in Western countries, especially in the USA where viral hepatitis infection is partly mediated through drug abuse. Meanwhile, the incidences of cirrhosis owing to nonalcoholic steatohepatitis (NASH) and obesity also increased in Western countries. In the US, HCC is the 9th most common cancer (Vallanueva *et al.*, *Nat. Rev. Gastroenterol. Hepatol.* 10(1):34-42).

[0007] Mean survival of patients with HCC is 3 months from diagnosis. However this is closely related to the stage of the tumor and the extent of underlying liver disease. As only a minority of HCC patients is considered suitable for resection and transplantation at the time of diagnosis, treatment for the majority of patients with HCC is mainly palliative. Non-surgical treatments, such as trans-arterial chemoembolization (TACE/TAE), radio-frequency ablation and systemic targeted agent like sorafenib, have been shown to reduce tumor burden and improve survival rate but these treatments do not eradicate cancer cells and the patients frequently relapse. Therefore, the development of more effective therapies remains a pressing field of research (Behboudi *et al.*, *Liver Int.* 30(4):521-526, 2010).

[0008] AFP expression is reactivated in approximately 80% of HCC. Immunotherapy studies aimed at generating AFP-specific cytotoxic CD8 T cell responses that recognize peptides presented on HCC cancer cell surface by MHC class I proteins have reported many human AFP peptides as T-cell epitopes (Butterfield *et al.*, *J. Immunol.* 166:5300-5308, 2001; Pardee, A.D. & Butterfield, L.H., *OncoImmunol.* 1:48-55, 2012; Butterfield *et al.*, *J. Trans. Med.* 12:86, 2014; Liu *et al.*, *J. Immunol.* 177(1):712-721, 2006; Mizukoshi *et al.*, *Int. J. Cancer* 118(5):1194-1204, 2006). Among these peptides, FMNKFIYEI (AFP158) is an immunodominant T-cell epitope restricted by HLA-A*02:01. AFP/HLA-A*02:01 complex induced peptide-specific T cells *in vitro* from normal HLA-A*02:01 donors. These AFP158 specific T cells recognized HLA-A*02:01 positive and AFP positive tumor cells in both cytotoxicity assays and IFNγ ELISPOT assays. AFP158 was identified by mass spectrometric analysis of surface peptides from an HLA-A*02:01 positive HCC cell line, HepG2, but not from a HLA-A*02:01 negative cancer cell line, Hep3B (Butterfield *et al.*, *J. Immunol.* 166:5300-5308, 2001). These data support that AFP158 is indeed processed and presented by

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.

[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 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

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.

[0039] FIG. 8 shows SDS-PAGE analysis for molecular weight determination of purified anti-AFP158/MHC bispecific antibodies.

[0040] FIG. 9 shows SEC chromatograms of exemplary purified AFP158 bispecific antibodies to assess level of aggregation. anti-AFP158/MHC bispecific antibody monomer: ~15.8 mL.

[0041] FIG. 10 shows the T-cell killing of multiple cancer cell lines mediated by anti-AFP158/MHC bispecific antibodies (BsAb) at varying concentrations. For each concentration, the bars from left to right represent HEPG2, SK-HEP1, Hela, HEP3B, Raji, Jurkat, Daudi, and K562 cells lines.

[0042] FIG. 11 shows a schematic representation of a chimeric antigen receptor construct.

[0043] FIG. 12 shows the killing of HEPG2, SK-HEP1, and SK-HEP1-MiniG cell lines mediated by T cells expressing a panel of anti-AFP158/MHC CARs.

[0044] FIG. 13 shows the killing of a panel of cancer cell lines positive or negative for AFP158 and HLA-A*02:01, mediated by T cells expressing an exemplary anti-AFP158/MHC CAR. The tissue of origin of each cell line, the AFP/AFP158 peptide expression and whether the cells express the HLA-A02 allele are indicated in the table.

[0045] FIG. 14A shows the release of IL-2, IL-4, IL-6 , and IL-8 after co-incubation of AFP158 CAR transduced T cells or mock-transduced T cells with cancer cell lines positive or negative for AFP158 and HLA-A*02:01.

[0046] FIG. 14B shows the release of IL-10, GM-CSF, IFN-γ, and TNF-α after co-incubation of AFP158 CAR transduced T cells or mock-transduced T cells with cancer cell lines positive or negative for AFP158 and HLA-A*02:01.

[0047] FIG. 15A shows tumor growth in HepG2 subcutaneous xenograft mice treated with intravenous injection of mock-transduced T cells or AFP158 CAR-transduced T cells, or left untreated.

[0048] FIG. 15B shows tumor growth in HepG2 subcutaneous xenograft mice treated with intratumoral injection of mock-transduced T cells or AFP158 CAR-transduced T cells, or left untreated.

[0049] FIG. 16A shows the change in the photon emission from luciferase-tagged HepG2 (HepG2-Luc2) intraperitoneal xenograft mice at day 70 after treatment with intraperitoneal injection of mock-transduced T cells or AFP158 CAR-transduced T cells, or no treatment.

[0050] FIG. 16B shows photon emission images of the HepG2-Luc2 tumor-bearing mice at day 70.

[0051] FIG. 17 shows tumor growth in SK-Hep1-MiniG subcutaneous xenograft mice treated with intravenous or intratumoral injection of AFP158 CAR-transduced T cells or intravenous injection of mock-transduced T cells.

[0052] FIG. 18 shows SDS-PAGE analysis to determine the purity of full-length anti-AFP158/MHC mouse chimeric IgG1 antibodies.

[0053] FIG. 19 shows FACS analysis of full-length anti-AFP158/MHC mouse chimeric IgG1 (black line) or negative control (secondary antibody alone, gray line) binding to SK-HEP1-miniG cells presenting AFP158/HLA-A*02:01.

[0054] FIG. 20 shows the results of ELISA for full-length anti-AFP158/MHC mouse chimeric IgG1 binding to AFP158/HLA-A*02:01 complex, recombinant AFP protein or free AFP158 peptide. Left panel: dose dependence curve of full-length anti-AFP158/MHC mouse chimeric IgG1; Right: OD450 of antibody binding at 100 ng/mL for AFP158 peptide/MHC (MHC), AFP protein (AFP) and AFP158 peptide (Peptide).

[0055] FIG. 21 shows body weight measurements over time (up to 35 days post 1st dose) for mice injected intratumorally with AFP158 CAR-T cells or controls.

[0056] FIG. 22 shows tumor growth kinetics in bilateral SK-Hep1-MiniG subcutaneous xenograft mice treated with intravenous or intratumoral injection of AFP158 CAR-transduced T cells, intratumoral injection of AFP158 CAR-transduced T cells in combination with APCs, or intratumoral injection of mock-transduced T cells. Intratumoral injections were injected into right flank tumors.

[0057] FIG. 23 shows immunohistochemical staining of human CD3 in tumor sections from bilateral SK-Hep1-MiniG subcutaneous xenograft mice treated with intratumoral injection into right flank tumors of AFP158 CAR-transduced T cells in combination with APCs. L, left tumor; R, right tumor.

[0058] FIG. 24 shows flow cytometry analysis of AFP158 CAR-transduced peripheral blood lymphocytes; cells were stained with AFP158/HLA-A*02:01 tetramers and co-stained with anti-CD3, antibody, anti-CD4 antibody, or anti-CD8 antibody. Mock-transduced cells and cells without staining were included as controls.

[0059] FIG. 25 shows flow cytometry analysis of the degranulation of AFP158 CAR-transduced T cells after co-culturing with target cells (HepG2, SK-HEP-1 or SK-Hep1-

MiniG); transduced T-cells were gated for CAR and CD8 expression and stained for anti-CD107a.

[0060] FIG. 26 shows CAR cell surface distribution in cells transduced to express a representative AFP158 CAR; cells were stained with AFP158/HLA-A*02:01 tetramer-PE.

DETAILED DESCRIPTION OF THE INVENTION

[0061] The present application provides isolated constructs (referred to herein as "anti-AMC constructs") that 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 (referred to herein as an "AFP/MHC class I complex," or "AMC"). The anti-AMC constructs specifically recognize AFP/MHC class I complexes (such as MHC-presented AFP peptides on the surface of cells expressing AFP), as opposed to circulating AFP protein or free AFP peptides. When armed as anti-CD3 bispecific antibodies or present in a chimeric antigen receptor (CAR) expressed by a T cell, the anti-AMC antibody moiety specifically redirected human T cells to kill AMC-presenting target cells, such as AMC-presenting cancer cells. This strategy provides a significant technical advantage over using antibodies directed against the AFP protein, which cannot specifically target AMC-presenting cells (*i.e.*, cells presenting on their surface an AFP peptide bound to an MHC class I molecule). Furthermore, when fused to a detectable moiety, the anti-AMC antibody moiety allows for diagnosis and prognosis of AFP-positive diseases or disorders with high sensitivity to changes in the number and distribution of AMC-presenting cells, a potentially more relevant measure of disease progression than circulating AFP levels.

[0062] Using phage display technology, we generated multiple monoclonal antibodies that are specific and high affinity against human AFP158 peptide/HLA-A*02:01 complex, as well as AFP158/MHC complexes formed in the context of other subtypes of the HLA-A02 allele. Flow cytometry and T-cell mediated cytotoxicity assays demonstrated that the antibodies recognized AFP peptide-pulsed T2 cells and AMC-presenting cancer cell lines, in an AFP- and HLA-A*02:01-restricted manner. When armed as anti-CD3 bispecific antibodies or CAR T cells, the antibodies re-directed human T cells to kill AFP-positive and HLA-A*02:01-positive target cancer cells. The data presented herein demonstrate that antibodies against a secreted cancer antigen in the context of an HLA complex can be effective therapeutic agents for cancer indications, such as solid tumor indications.

[0063] The present application thus provides constructs (such as isolated constructs) comprising an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein. The construct can be, for example, a full-length anti-AMC antibody, a multi-specific anti-AMC molecule (such as a bispecific anti-AMC antibody), an anti-AMC chimeric antigen receptor ("CAR"), or an anti-AMC immunoconjugate.

[0064] In another aspect, there are provided nucleic acids encoding the anti-AMC constructs or the anti-AMC antibody moiety portion of the constructs.

[0065] In another aspect, there are provided compositions comprising an anti-AMC construct comprising an antibody moiety that specifically binds to a complex comprising an AFP-peptide and an MHC class I protein. The composition can be a pharmaceutical composition comprising an anti-AMC construct or an effector cell expressing or associated with the anti-AMC construct (for example a T cell expressing an anti-AMC CAR).

[0066] Also provided are methods of making and using the anti-AMC constructs (or cells expressing or associated with the anti-AMC constructs) for treatment or diagnostic purposes, as well as kits and articles of manufacture useful for such methods.

Definitions

[0067] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results, including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*, preventing or delaying the worsening of the disease), preventing or delaying the spread (*e.g.*, metastasis) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing or improving the quality of life, increasing weight gain, and/or prolonging survival. Also encompassed by "treatment" is a reduction of pathological consequence of cancer (such as, for example, tumor volume). The methods of the invention contemplate any one or more of these aspects of treatment.

[0068] The terms "recurrence," "relapse" or "relapsed" refers to the return of a cancer or disease after clinical assessment of the disappearance of disease. A diagnosis of distant metastasis or local recurrence can be considered a relapse.

[0069] The term "refractory" or "resistant" refers to a cancer or disease that has not responded to treatment.

[0070] "Activation", as used herein in relation to T cells, refers to the state of a T cell that has been sufficiently stimulated to induce detectable cellular proliferation. Activation can also be associated with induced cytokine production, and detectable effector functions.

[0071] The term "antibody moiety" includes full-length antibodies and antigen-binding fragments thereof. A full-length antibody comprises two heavy chains and two light chains. The variable regions of the light and heavy chains are responsible for antigen binding. The variables region in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light chain (LC) CDRs including LC-CDR1, LC-CDR2, and LC-CDR3, heavy chain (HC) CDRs including HC-CDR1 , HC-CDR2, and HC-CDR3). CDR boundaries for the antibodies and antigen-binding fragments disclosed herein may be defined or identified by the conventions of Kabat, Chothia, or Al-Lazikani (Al-Lazikani 1997; Chothia 1985; Chothia 1987; Chothia 1989; Kabat 1987; Kabat 1991). The three CDRs of the heavy or light chains are interposed between flanking stretches known as framework regions (FRs), which are more highly conserved than the CDRs and form a scaffold to support the hypervariable loops. The constant regions of the heavy and light chains are not involved in antigen binding, but exhibit various effector functions. Antibodies are assigned to classes based on the amino acid sequence of the constant region of their heavy chain. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of α, δ, ε, γ, and μ heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as lgG1 (γ1 heavy chain), lgG2 (γ2 heavy chain), lgG3 (γ3 heavy chain), lgG4 (γ4 heavy chain), lgA1 (α1 heavy chain), or lgA2 (α2 heavy chain).

[0072] The term "antigen-binding fragment" as used herein refers to an antibody fragment including, for example, a diabody, a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)2, a bispecific dsFv (dsFv- dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a

bivalent domain antibody, or any other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment (*e.g.*, a parent scFv) binds. In some embodiments, an antigen-binding fragment may comprise one or more CDRs from a particular human antibody grafted to a framework region from one or more different human antibodies.

[0073] The term "epitope" as used herein refers to the specific group of atoms or amino acids on an antigen to which an antibody or antibody moiety binds. Two antibodies or antibody moieties may bind the same epitope within an antigen if they exhibit competitive binding for the antigen.

[0074] As used herein, a first antibody moiety "competes" for binding to a target AMC with a second antibody moiety when the first antibody moiety inhibits target AMC binding of the second antibody moiety by at least about 50% (such as at least about any of 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) in the presence of an equimolar concentration of the first antibody moiety, or vice versa. A high throughput process for "binning" antibodies based upon their cross-competition is described in PCT Publication No. WO 03/48731.

[0075] As use herein, the term "specifically binds" or "is specific for" refers to measurable and reproducible interactions, such as binding between a target and an antibody or antibody moiety, that is determinative of the presence of the target in the presence of a heterogeneous population of molecules, including biological molecules. For example, an antibody or antibody moiety that specifically binds to a target (which can be an epitope) is an antibody or antibody moiety that binds this target with greater affinity, avidity, more readily, and/or with greater duration than its bindings to other targets. In some embodiments, an antibody or antibody moiety that specifically binds to an antigen reacts with one or more antigenic determinants of the antigen (for example an AFP peptide/MHC class I protein complex) with a binding affinity that is at least about 10 times its binding affinity for other targets.

[0076] An "isolated" anti-AMC construct as used herein refers to an anti-AMC construct that (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, (3) is expressed by a cell from a different species, or, (4) does not occur in nature.

[0077] The term "isolated nucleic acid" as used herein is intended to mean a nucleic acid of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated nucleic acid" (1) is not associated with all or a portion of a polynucleotide

in which the "isolated nucleic acid" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

[0078] As used herein, the term "CDR" or "complementarity determining region" is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat *et al.*, J. Biol. Chem. 252:6609-6616 (1977); Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991); by Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987); and MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison.

TABLE 1: CDR DEFINITIONS

	Kabat[1]	Chothia[2]	MacCallum[3]
V_H CDR1	31-35	26-32	30-35
V_H CDR2	50-65	53-55	47-58
V_H CDR3	95-102	96-101	93-101
V_L CDR1	24-34	26-32	30-36
V_L CDR2	50-56	50-52	46-55
V_L CDR3	89-97	91-96	89-96

[1]Residue numbering follows the nomenclature of Kabat *et al.*, *supra*
[2]Residue numbering follows the nomenclature of Chothia *et al.*, *supra*
[3]Residue numbering follows the nomenclature of MacCallum *et al.*, *supra*

[0079] The term "chimeric antibodies" refer to antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit a biological activity of this invention (see U.S. Patent No. 4,816,567; and Morrison *et al.*, Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)).

[0080] The term "semi-synthetic" in reference to an antibody or antibody moiety means that the antibody or antibody moiety has one or more naturally occurring sequences and one or more non-naturally occurring (*i.e.*, synthetic) sequences.

[0081] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the heavy and light chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0082] "Single-chain Fv," also abbreviated as "sFv" or "scFv," are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. In some embodiments, the scFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0083] The term "diabodies" refers to small antibody fragments prepared by constructing scFv fragments (see preceding paragraph) typically with short linkers (such as about 5 to about 10 residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a bivalent fragment, *i.e.*, fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" scFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

[0084] "Humanized" forms of non-human (*e.g.,* rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (HVR) of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired antibody specificity, affinity, and capability. In some

instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

[0085] "Percent (%) amino acid sequence identity" or "homology" with respect to the polypeptide and antibody sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the polypeptide being compared, after aligning the sequences considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, Megalign (DNASTAR), or MUSCLE software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program MUSCLE (Edgar, R.C., *Nucleic Acids Research* 32(5):1792-1797, 2004; Edgar, R.C., *BMC Bioinformatics* 5(1):113, 2004).

[0086] The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. In some embodiments, an FcR of this invention is one that binds an IgG antibody (a γ receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (*see*

review M. in Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). The term includes allotypes, such as FcγRIIIA allotypes: FcγRIIIA-Phe158, FcγRIIIA-Val158, FcγRIIA-R131 and/or FcγRIIA-H131. FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel *et al., Immunomethods* 4:25-34 (1994); and de Haas *et al., J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al., J. Immunol.* 117:587 (1976) and Kim *et al., J. Immunol* . 24:249 (1994)).

[0087] The term "FcRn" refers to the neonatal Fc receptor (FcRn). FcRn is structurally similar to major histocompatibility complex (MHC) and consists of an α-chain noncovalently bound to β2-microglobulin. The multiple functions of the neonatal Fc receptor FcRn are reviewed in Ghetie and Ward (2000) *Annu. Rev. Immunol.* **18**, 739-766. FcRn plays a role in the passive delivery of immunoglobulin IgGs from mother to young and the regulation of serum IgG levels. FcRn can act as a salvage receptor, binding and transporting pinocytosed IgGs in intact form both within and across cells, and rescuing them from a default degradative pathway.

[0088] The "CH1 domain" of a human IgG Fc region (also referred to as "C1" of "H1" domain) usually extends from about amino acid 118 to about amino acid 215 (EU numbering system).

[0089] "Hinge region" is generally defined as stretching from Glu216 to Pro230 of human IgG1 (Burton, *Molec. Immunol.*22:161-206 (1985)). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S bonds in the same positions.

[0090] The "CH2 domain" of a human IgG Fc region (also referred to as "C2" of "H2" domain) usually extends from about amino acid 231 to about amino acid 340. The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It has been speculated that the carbohydrate may provide a substitute for the domain-domain pairing and help stabilize the CH2 domain. Burton, *Molec Immunol.* 22:161-206 (1985).

[0091] The "CH3 domain" (also referred to as "C2" or "H3" domain) comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (*i.e.* from about amino acid residue

341 to the C-terminal end of an antibody sequence, typically at amino acid residue 446 or 447 of an IgG).

[0092] A "functional Fc fragment" possesses an "effector function" of a native sequence Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.* B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.* an antibody variable domain) and can be assessed using various assays known in the art.

[0093] An antibody with a variant IgG Fc with "altered" FcR binding affinity or ADCC activity is one which has either enhanced or diminished FcR binding activity (*e.g.*, FcγR or FcRn) and/or ADCC activity compared to a parent polypeptide or to a polypeptide comprising a native sequence Fc region. The variant Fc which "exhibits increased binding" to an FcR binds at least one FcR with higher affinity (*e.g.*, lower apparent K_d or IC_{50} value) than the parent polypeptide or a native sequence IgG Fc. According to some embodiments, the improvement in binding compared to a parent polypeptide is about 3 fold, such as about any of 5, 10, 25, 50, 60, 100, 150, 200, or up to 500 fold, or about 25% to 1000% improvement in binding. The polypeptide variant which "exhibits decreased binding" to an FcR, binds at least one FcR with lower affinity (*e.g.*, higher apparent K_d or higher IC_{50} value) than a parent polypeptide. The decrease in binding compared to a parent polypeptide may be about 40% or more decrease in binding.

[0094] "Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound to Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.* Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are absolutely required for such killing. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the

molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1998).

[0095] The polypeptide comprising a variant Fc region which "exhibits increased ADCC" or mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of human effector cells more effectively than a polypeptide having wild type IgG Fc or a parent polypeptide is one which *in vitro* or *in vivo* is substantially more effective at mediating ADCC, when the amounts of polypeptide with variant Fc region and the polypeptide with wild type Fc region (or the parent polypeptide) in the assay are essentially the same. Generally, such variants will be identified using any *in vitro* ADCC assay known in the art, such as assays or methods for determining ADCC activity, *e.g.* in an animal model etc. In some embodiments, the variant is from about 5 fold to about 100 fold, *e.g.* from about 25 to about 50 fold, more effective at mediating ADCC than the wild type Fc (or parent polypeptide) .

[0096] "Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.* as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996), may be performed. Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in US patent No. 6,194,551B1 and WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. *See*, also, Idusogie *et al. J. Immunol.* 164: 4178-4184 (2000).

[0097] Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

[0098] The term "operably linked" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the

promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

[0099] "Homologous" refers to the sequence similarity or sequence identity between two polypeptides or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared times 100. For example, if 6 of 10 of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

[0100] An "effective amount" of an anti-AMC construct or composition as disclosed herein, is an amount sufficient to carry out a specifically stated purpose. An "effective amount" can be determined empirically and by known methods relating to the stated purpose.

[0101] The term "therapeutically effective amount" refers to an amount of an anti-AMC construct or composition as disclosed herein, effective to "treat" a disease or disorder in an individual. In the case of cancer, the therapeutically effective amount of the anti-AMC construct or composition as disclosed herein can reduce the number of cancer cells; reduce the tumor size or weight; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the anti-AMC construct or composition as disclosed herein can prevent growth and/or kill existing cancer cells, it can be cytostatic and/or cytotoxic. In some embodiments, the therapeutically effective amount is a growth inhibitory amount. In some embodiments, the therapeutically effective amount is an amount that extends the survival of a patient. In some embodiments, the therapeutically effective amount is an amount that improves progression free survival of a patient.

[0102] As used herein, by "pharmaceutically acceptable" or "pharmacologically compatible" is meant a material that is not biologically or otherwise undesirable, *e.g.*, the material may be incorporated into a pharmaceutical composition administered to a patient without causing any significant undesirable biological effects or interacting in a deleterious

manner with any of the other components of the composition in which it is contained. Pharmaceutically acceptable carriers or excipients have preferably met the required standards of toxicological and manufacturing testing and/or are included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug administration.

[0103] The term "label" when used herein refers to a detectable compound or composition which can be conjugated directly or indirectly to the anti-AMC antibody moiety. The label may be detectable by itself (*e.g.*, radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

[0104] It is understood that embodiments of the invention described herein include "consisting" and/or "consisting essentially of" embodiments.

[0105] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[0106] As used herein, reference to "not" a value or parameter generally means and describes "other than" a value or parameter. For example, the method is not used to treat cancer of type X means the method is used to treat cancer of types other than X.

[0107] As used herein and in the appended claims, the singular forms "a," "or," and "the" include plural referents unless the context clearly dictates otherwise.

Anti-AMC constructs

[0108] In one aspect, the present invention provides AFP/MHC class I complex-specific constructs (anti-AMC constructs) that comprise an antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein ("AFP/MHC class I complex," or "AMC"). The specificity of the anti-AMC construct derives from an anti-AMC antibody moiety, such as a full-length antibody or antigen-binding fragment thereof, that specifically binds to the AMC. In some embodiments, reference to a moiety (such as an antibody moiety) that specifically binds to a complex comprising an AFP peptide and an MHC class I protein means that the moiety binds to the AMC with a) an affinity that is at least about 10 (including for example at least about any of 10, 20, 30, 40, 50, 75, 100, 200, 300, 400, 500, 750, 1000 or more) times its binding affinity for each of full-length AFP, free AFP peptide, MHC class I protein not bound to a peptide, and MHC class I protein bound to

a non-AFP peptide; or b) a K_d no more than about 1/10 (such as no more than about any of 1/10, 1/20, 1/30, 1/40, 1/50, 1/75, 1/100, 1/200, 1/300, 1/400, 1/500, 1/750, 1/1000 or less) times its K_d for binding to each of full-length AFP, free AFP peptide, MHC class I protein not bound to a peptide, and MHC class I protein bound to a non-AFP peptide. Binding affinity can be determined by methods known in the art, such as ELISA, fluorescence activated cell sorting (FACS) analysis, or radioimmunoprecipitation assay (RIA). K_d can be determined by methods known in the art, such as surface plasmon resonance (SPR) assay utilizing, for example, Biacore instruments, or kinetic exclusion assay (KinExA) utilizing, for example, Sapidyne instruments.

[0109] Contemplated anti-AMC constructs include, for example, full-length anti-AMC antibodies, multi-specific (such as bispecific) anti-AMC molecules, anti-AMC chimeric antigen receptors (CARs), and anti-AMC immunoconjugates.

[0110] For example, in some embodiments, there is provided an anti-AMC construct (such as an isolated anti-AMC construct) comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01 (GenBank Accession No.: AAO20853). In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the anti-AMC construct binds the AMC 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). In some embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, 5, or 6) complex comprising the MHC class I protein and a variant of the AFP peptide having one amino acid substitution (such as a conservative amino acid substitution). In some embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, or 5) complex comprising the AFP peptide and a different subtype of the MHC class I protein.

[0111] In some embodiments, there is provided an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP158 peptide

(SEQ ID NO: 4) and HLA-A*02:01. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the anti-AMC construct binds the AMC 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). In some embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, 5, or 6) complex comprising the MHC class I protein and a variant of the AFP peptide having one amino acid substitution (such as a conservative amino acid substitution). In some embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, or 5) complex comprising the AFP peptide and a different subtype of the MHC class I protein.

[0112] In some embodiments, there is provided an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the anti-AMC antibody moiety comprises: i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments,

the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the anti-AMC construct binds the AMC 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). In some embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, 5, or 6) complex comprising the MHC class I protein and a variant of the AFP peptide having one amino acid substitution (such as a conservative amino acid substitution). In some embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, or 5) complex comprising the AFP peptide and a different subtype of the MHC class I protein.

[0113] In some embodiments, there is provided an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the anti-AMC antibody moiety comprises: i) a heavy chain variable domain sequence comprising an HC-CDR1 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 57-66; or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 67-76; or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 77-86; or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a light chain variable domain sequence comprising an LC-CDR1 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 90-99; or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 100-109; or a variant thereof comprising up to about 3 (for example about any of 1, 2, or 3) amino acid substitutions; and an LC-CDR3 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 110-119; or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In

some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the anti-AMC construct binds the AMC with a Kd 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 embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, 5, or 6) complex comprising the MHC class I protein and a variant of the AFP peptide having one amino acid substitution (such as a conservative amino acid substitution). In some embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, or 5) complex comprising the AFP peptide and a different subtype of the MHC class I protein.

[0114] In some embodiments, there is provided an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the anti-AMC antibody moiety comprises: i) a heavy chain variable domain sequence comprising an HC-CDR1 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 57-66; an HC-CDR2 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 67-76; and an HC-CDR3 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 77-86; or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a light chain variable domain sequence comprising an LC-CDR1 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 90-99; an LC-CDR2 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 100-109; and an LC-CDR3 comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 110-119; or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the anti-AMC construct binds the AMC with a Kd 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 embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, 5, or 6) complex comprising the MHC class I protein and a variant of the AFP peptide having one amino acid substitution (such as a conservative amino acid substitution). In some embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, or 5) complex comprising the AFP peptide and a different subtype of the MHC class I protein.

[0115] In some embodiments, there is provided an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MCH class I protein, wherein the anti-AMC antibody moiety comprises a heavy chain variable domain comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 17-26, or a variant thereof having at least about 95% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and a light chain variable domain comprising (and in some embodiments consisting of) the amino acid sequence of any one of SEQ ID NOs: 27-36, or a variant thereof having at least about 95% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the anti-AMC construct binds the AMC with a Kd 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 embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, 5, or 6) complex comprising the MHC class I protein and a variant of the AFP peptide having one amino acid substitution (such as a conservative amino acid substitution). In some embodiments, the anti-AMC construct cross-reacts with at least one (such as at least any of 2, 3, 4, or 5) complex comprising the AFP peptide and a different subtype of the MHC class I protein.

[0116] In some embodiments, there is provided an anti-AMC construct comprising a first anti-AMC antibody moiety that competes for binding to a target AFP/MHC class I complex with a second anti-AMC antibody moiety according to any of the anti-AMC antibody moieties described herein. In some embodiments, the first anti-AMC antibody moiety binds to the same, or substantially the same, epitope as the second anti-AMC antibody moiety. In

some embodiments, binding of the first anti-AMC antibody moiety to the target AFP/MHC class I complex inhibits binding of the second anti-AMC 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 embodiments, the first anti-AMC antibody moiety and the second anti-AMC 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.

[0117] The different aspects are discussed in various sections below in further detail.

Anti-AMC antibody moiety

[0118] The anti-AMC constructs comprise an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein.

[0119] In some embodiments, the anti-AMC antibody moiety specifically binds to an AMC present on the surface of a cell. In some embodiments, the cell presents on its surface abnormally high levels of AFP. In some embodiments, the cell is a cancer cell. In some embodiments, the cancer cell is in a solid tumor. In some embodiments, the cancer cell is a metastatic cancer cell.

[0120] In some embodiments, the AFP peptide is an MHC class I-restricted peptide. In some embodiments, the AFP peptide is from about 8 to about 12 (such as about any of 8, 9, 10, 11, or 12) amino acids in length. In some embodiments, the AFP peptide is derived from human AFP (hAFP), mouse AFP (mAFP), or rat AFP (rAFP).

[0121] In some embodiments, the AFP peptide is derived from hAFP. In some embodiments, the AFP peptide comprises (and in some embodiments consists of) the sequence of amino acids 137-145 of hAFP (PLFQVPEPV, SEQ ID NO: 3), amino acids 158-166 of hAFP (FMNKFIYEI, SEQ ID NO: 4, also referred to herein as "AFP158"), amino acids 325-334 of hAFP (GLSPNLNRFL, SEQ ID NO: 5), or amino acids 542-50 of hAFP (GVALQTMKQ, SEQ ID NO: 6).

[0122] In some embodiments, the AFP peptide is derived from mAFP. In some embodiments, the AFP peptide comprises the sequence of amino acids 154-162 of mAFP (FMNRFIYEV, SEQ ID NO: 16).

[0123] In some embodiments, the MHC class I protein is HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G. In some embodiments, the MHC class I protein is HLA-A. In some

embodiments, the HLA-A is HLA-A02. In some embodiments, the HLA-A02 is HLA-A*02:01.

[0124] In some embodiments, the anti-AMC antibody moiety is a full-length antibody. In some embodiments, the anti-AMC antibody moiety is an antigen-binding fragment, for example an antigen-binding fragment selected from the group consisting of a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), and a single-chain antibody molecule (scFv). In some embodiments, the anti-AMC antibody moiety is an scFv. In some embodiments, the anti-AMC antibody moiety is human, humanized, or semi-synthetic.

[0125] In some embodiments, the anti-AMC antibody moiety specifically binds to the N-terminal portion of the AFP peptide in the complex. In some embodiments, the anti-AMC antibody moiety specifically binds to the C-terminal portion of the AFP peptide in the complex. In some embodiments, the anti-AMC antibody moiety specifically binds to the middle portion of the AFP peptide in the complex.

[0126] In some embodiments, the anti-AMC antibody moiety specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the anti-AMC antibody moiety cross-reacts with at least one complex comprising the AFP peptide and an allelic variant of the MHC class I protein. In some embodiments, the allelic variant has up to about 10 (such as about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions when compared to the MHC class I protein. In some embodiments, the allelic variant is the same serotype as the MHC class I protein. In some embodiments, the allelic variant is a different serotype than the MHC class I protein. In some embodiments, the anti-AMC antibody moiety does not cross-react with a complex comprising the AFP peptide and any allelic variant of the MHC class I protein.

[0127] In some embodiments, the anti-AMC antibody moiety specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the anti-AMC antibody moiety cross-reacts with at least one complex comprising the MHC class I protein and a variant of the AFP peptide having one amino acid substitution (such as a conservative substitution). In some embodiments, the anti-AMC antibody moiety does not cross-react with a complex comprising the MHC class I protein and any variant of the AFP peptide.

[0128] In some embodiments, the anti-AMC antibody moiety specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the anti-AMC antibody moiety cross-reacts with at least one complex comprising the MHC class I protein

and an interspecies variant of the AFP peptide. In some embodiments, for example, the AFP peptide is human AFP peptide and the interspecies variant of the AFP peptide is a mouse or rat variant thereof. In some embodiments, the anti-AMC antibody moiety does not cross-react with a complex comprising the MHC class I protein and any interspecies variant of the AFP peptide.

[0129] In some embodiments, the anti-AMC antibody moiety (or the anti-AMC construct comprising the anti-AMC antibody moiety) binds to the complex comprising the AFP peptide and the MHC class I protein with an affinity that is at least about 10 (including for example at least about any of 10, 20, 30, 40, 50, 75, 100, 200, 300, 400, 500, 750, 1000 or more) times its binding affinity for each of full-length AFP, free AFP peptide, MHC class I protein not bound to a peptide, and MHC class I protein bound to a non-AFP peptide. In some embodiments, the anti-AMC antibody moiety (or the anti-AMC construct comprising the anti-AMC antibody moiety) binds to the complex comprising the AFP peptide and the MHC class I protein with a K_d no more than about 1/10 (such as no more than about any of 1/10, 1/20, 1/30, 1/40, 1/50, 1/75, 1/100, 1/200, 1/300, 1/400, 1/500, 1/750, 1/1000 or less) times its K_d for binding to each of full-length AFP, free AFP peptide, MHC class I protein not bound to a peptide, and MHC class I protein bound to a non-AFP peptide.

[0130] In some embodiments, the anti-AMC antibody moiety (or the anti-AMC construct comprising the anti-AMC antibody moiety) binds to the complex comprising the AFP peptide and the MHC class I protein with a Kd 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 embodiments, the anti-AMC antibody moiety (or the anti-AMC construct comprising the anti-AMC antibody moiety) binds to the complex comprising the AFP peptide and the MHC class I protein with a Kd between about 1 pM to about 250 pM (such as about any of 1, 10, 25, 50, 75, 100, 150, 200, or 250 pM, including any ranges between these values). In some embodiments, the anti-AMC antibody moiety (or the anti-AMC construct comprising the anti-AMC antibody moiety) binds to the complex comprising the AFP peptide and the MHC class I protein with a Kd between about 1 nM to about 500 nM (such as about any of 1, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, or 500 nM, including any ranges between these values).

[0131] In some embodiments, the anti-AMC antibody moiety specifically binds to a complex comprising AFP158 (SEQ ID NO: 4) and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01). In some embodiments, the anti-AMC antibody moiety

further binds to at least one (including at least about any of 2, 3, 4, 5, 6, or 7) of: a complex comprising an AFP peptide of SEQ ID NO: 7 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 8 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 9 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 10 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 11 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 12 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); and a complex comprising an AFP peptide of SEQ ID NO: 13 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01).

[0132] In some embodiments, the anti-AMC antibody moiety specifically binds to: a complex comprising an AFP peptide of SEQ ID NO: 4 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 10 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 11 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 12 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); and a complex comprising an AFP peptide of SEQ ID NO: 13 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01).

[0133] In some embodiments, the anti-AMC antibody moiety specifically binds to: a complex comprising an AFP peptide of SEQ ID NO: 4 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 7 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); and a complex comprising an AFP peptide of SEQ ID NO: 8 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01).

[0134] In some embodiments, the anti-AMC antibody moiety specifically binds to: a complex comprising an AFP peptide of SEQ ID NO: 4 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 8 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 10 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID

NO: 11 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 12 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); and a complex comprising an AFP peptide of SEQ ID NO: 13 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01).

[0135] In some embodiments, the anti-AMC antibody moiety specifically binds to: a complex comprising an AFP peptide of SEQ ID NO: 4 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 7 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); and a complex comprising an AFP peptide of SEQ ID NO: 13 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01).

[0136] In some embodiments, the anti-AMC antibody moiety specifically binds to: a complex comprising an AFP peptide of SEQ ID NO: 4 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 7 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 9 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); a complex comprising an AFP peptide of SEQ ID NO: 11 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01); and a complex comprising an AFP peptide of SEQ ID NO: 13 and an MHC class I protein (such as HLA-A02, for example HLA-A*02:01).

[0137] In some embodiments, the anti-AMC antibody moiety specifically binds to a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:01. In some embodiments, the anti-antibody moiety cross-reacts with at least one (including at least about any of 2, 3, 4, 5, or 6) of: a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:02 (GenBank Accession No.: AFL91480), a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:03 (GenBank Accession No.: AAA03604), a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:05 (GenBank Accession No.: AAA03603), a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:06 (GenBank Accession No.: CCB78868), a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:07 (GenBank Accession No.: ACR55712), and a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:11 (GenBank Accession No.: CAB56609). In some embodiments, the anti-AMC antibody moiety cross-reacts with each of a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:02, a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:03, and a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:11. In some embodiments, the anti-

AMC antibody moiety cross-reacts with each of a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:02, a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:05, a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:06, a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:07, and a complex comprising AFP158 (SEQ ID NO: 4) and HLA-A*02:11.

[0138] In some embodiments, the anti-AMC antibody moiety is a semi-synthetic antibody moiety comprising fully human sequences and one or more synthetic regions. In some embodiments, the anti-AMC antibody moiety is a semi-synthetic antibody moiety comprising a fully human light chain variable domain and a semi-synthetic heavy chain variable domain comprising fully human FR1, HC-CDR1, FR2, HC-CDR2, FR3, and FR4 regions and a synthetic HC-CDR3. In some embodiments, the semi-synthetic heavy chain variable domain comprises a fully synthetic HC-CDR3 having a sequence from about 5 to about 25 (such as about any of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) amino acids in length. In some embodiments, the semi-synthetic heavy chain variable domain or the synthetic HC-CDR3 is obtained from a semi-synthetic library (such as a semi-synthetic human library) comprising fully synthetic HC-CDR3s having a sequence from about 5 to about 25 (such as about any of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) amino acids in length, wherein each amino acid in the sequence is randomly selected from the standard human amino acids, minus cysteine. In some embodiments, the synthetic HC-CDR3 is from about 10 to about 19 (such as about any of 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19) amino acids in length.

[0139] The anti-AMC antibody moieties in some embodiments comprise specific sequences or certain variants of such sequences. In some embodiments, the amino acid substitutions in the variant sequences do not substantially reduce the ability of the anti-AMC antibody moiety to bind the AMC. For example, alterations that do not substantially reduce AMC binding affinity may be made. Alterations that substantially improve AMC binding affinity or affect some other property, such as specificity and/or cross-reactivity with related variants of the AMC, are also contemplated.

[0140] In some embodiments, the anti-AMC antibody moiety comprises i) a heavy chain variable domain comprising 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid.

[0141] In some embodiments, the anti-AMC antibody moiety comprises i) a heavy chain variable domain comprising 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 an LC-CDR3 comprising the amino acid sequence of Q-S/T-Y/W-D/T-S/T-A/S (SEQ ID NO: 121); wherein X can be any amino acid.

[0142] In some embodiments, the anti-AMC antibody moiety comprises i) a heavy chain variable domain comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid.

[0143] In some embodiments, the anti-AMC antibody moiety comprises i) a heavy chain variable domain comprising an HC-CDR1 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 (for example 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 (for example 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); and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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); wherein X can be any amino acid.

[0144] In some embodiments, the anti-AMC antibody moiety comprises i) a heavy chain variable domain comprising an HC-CDR1 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); or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the HC-CDR sequences; and ii) a light chain variable domain comprising an LC-CDR1 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); or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the LC-CDR sequences; wherein X can be any amino acid.

[0145] In some embodiments, the anti-AMC antibody moiety comprises i) a heavy chain variable domain comprising an HC-CDR1 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 an LC-CDR1 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); wherein X can be any amino acid. The sequences of the CDRs noted herein are provided in Table 2 below.

TABLE 2

HC-CDR1 consensus	SEQ ID NO: 87	G-F/Y-S/T-F-D/S/T-D/N/S-Y/A-A/G/W
HC-CDR2 consensus	SEQ ID NO: 88	I/S-K/S-X-H/Y-X-G-X-T
HC-CDR3 consensus	SEQ ID NO: 89	A/G-X-W/Y-Y-X-X-X-F/Y-D
LC-CDR1 consensus	SEQ ID NO: 120	S/T-G/S-D/N-I/V-A/G-A/S/V-X-H/Y
LC-CDR3 consensus	SEQ ID NO: 121	Q-S/T-Y/W-D/T-S/T-A/S

[0146] In some embodiments, the anti-AMC antibody moiety comprises i) a heavy chain variable domain comprising an HC-CDR3 comprising 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-CDR3 comprising 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.

[0147] In some embodiments, the anti-AMC antibody moiety comprises i) a heavy chain variable domain comprising 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-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 110-119.

[0148] In some embodiments, the anti-AMC 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, 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 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 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 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 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 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.

[0149] In some embodiments, the anti-AMC 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, 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 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 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, 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 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 the amino acid sequence of any one of SEQ ID NOs: 110-119.

[0150] In some embodiments, the anti-AMC antibody moiety comprises i) a heavy chain variable domain sequence 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; 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 sequences; and ii) a light chain variable domain sequence 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; 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 sequences.

[0151] In some embodiments, the anti-AMC antibody moiety comprises i) a heavy chain variable domain sequence 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; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, wherein the amino acid substitutions are in HC-CDR1 or HC-CDR2; and ii) a light chain variable domain sequence 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; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, wherein the amino acid substitutions are in HC-CDR1 or HC-CDR2.

[0152] In some embodiments, the anti-AMC antibody moiety comprises i) a heavy chain variable domain sequence 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 sequence 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. The

sequences of the HC-CDRs noted herein are provided in Table 3 below and the LC-CDRs noted herein are provided in Table 4 below.

TABLE 3

SEQ ID NO: 57 17 HC-CDR1	GYTFTSYG	SEQ ID NO: 67 17 HC-CDR2	ISAYNGNT	SEQ ID NO: 77 17 HC-CDR3	ARYQDWWYLGQFDQ
SEQ ID NO: 58 33 HC-CDR1	VSSNSAAWN	SEQ ID NO: 68 33 HC-CDR2	YRSKWYN	SEQ ID NO: 78 33 HC-CDR3	ARGSYYSGRYDA
SEQ ID NO: 59 44 HC-CDR1	GGTFSSYA	SEQ ID NO: 69 44 HC-CDR2	IIPIFGTA	SEQ ID NO: 79 44 HC-CDR3	AREIRGYYYYYGMDV
SEQ ID NO: 60 48 HC-CDR1	GFTFDDYA	SEQ ID NO: 70 48 HC-CDR2	ISWNSGRI	SEQ ID NO: 80 48 HC-CDR3	ARADDYGAPYYYYGMDV
SEQ ID NO: 61 50 HC-CDR1	GGSISSSNW	SEQ ID NO: 71 50 HC-CDR2	IYHSGST	SEQ ID NO: 81 50 HC-CDR3	ATGYGGYFDY
SEQ ID NO: 62 52 HC-CDR1	GYTFTSYG	SEQ ID NO: 72 52 HC-CDR2	ISAYNGNT	SEQ ID NO: 82 52 HC-CDR3	ARDSYYYYYGMDV
SEQ ID NO: 63 61 HC-CDR1	GYSFPNYW	SEQ ID NO: 73 61 HC-CDR2	IDPGDSYT	SEQ ID NO: 83 61 HC-CDR3	ARYYVSLVDI
SEQ ID NO: 64 76 HC-CDR1	GFTFSNAW	SEQ ID NO: 74 76 HC-CDR2	IRSKAYGGTT	SEQ ID NO: 84 76 HC-CDR3	ARDGLYSSSWYDSDY
SEQ ID NO: 65 79 HC-CDR1	GFTFDDYA	SEQ ID NO: 75 79 HC-CDR2	ISWNSGSI	SEQ ID NO: 85 79 HC-CDR3	AKDIHSGSYYGLLYYAMDV
SEQ ID NO: 66 17-13 HC-CDR1	GYTFTSYG	SEQ ID NO: 76 17-13 HC-CDR2	ISAYNGNT	SEQ ID NO:86 17-13 HC-CDR3	ARFQDWWYLGQFDQ

TABLE 4

SEQ ID NO: 90 17 LC-CDR1	GSDVGVYYY	SEQ ID NO: 100 17 LC-CDR2	DVG	SEQ ID NO: 110 17 LC-CDR3	ASYTNRNSLGYV
SEQ ID NO: 91 33 LC-CDR1	SGSIASNY	SEQ ID NO: 101 33 LC-CDR2	EDN	SEQ ID NO: 111 33 LC-CDR3	QSYDSSTVV
SEQ ID NO: 92 44 LC-CDR1	NIGTKS	SEQ ID NO: 102 44 LC-CDR2	YDT	SEQ ID NO: 112 44 LC-CDR3	QVWDSSSDHPV
SEQ ID NO: 93 48 LC-CDR1	SSNIGAGYD	SEQ ID NO: 103 48 LC-CDR2	GNS	SEQ ID NO: 113 48 LC-CDR3	QSYDSSLSGSV
SEQ ID NO: 94 50 LC-CDR1	NIGSKS	SEQ ID NO: 104 50 LC-CDR2	YDS	SEQ ID NO: 114 50 LC-CDR3	QVWDSSSDHVV
SEQ ID NO: 95 52 LC-CDR1	TGAVTSGHY	SEQ ID NO: 105 52 LC-CDR2	DAS	SEQ ID NO: 115 52 LC-CDR3	LLSYSDALV
SEQ ID NO: 96 61 LC-CDR1	SSDVGGYNY	SEQ ID NO: 106 61 LC-CDR2	DVN	SEQ ID NO: 116 61 LC-CDR3	SSYTTGSRAV
SEQ ID NO: 97 76 LC-CDR1	SSNIGNNY	SEQ ID NO: 107 76 LC-CDR2	DNN	SEQ ID NO: 117 76 LC-CDR3	GTWDGSLYTML
SEQ ID NO: 98 79 LC-CDR1	SSNIGAGYD	SEQ ID NO: 108 79 LC-CDR2	GNS	SEQ ID NO: 118 79 LC-CDR3	QSYDSSLSGSGV
SEQ ID NO: 99 17-13 LC-CDR1	GSDVGVYYY	SEQ ID NO: 109 17-13 LC-CDR2	DVD	SEQ ID NO:119 17-13 LC-CDR3	ASYTNRNSLGYV

[0153] In some embodiments, the anti-AMC antibody moiety comprises 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% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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% (including for example at least any of 96%, 97%, 98%, or 99%) sequence identity.

[0154] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36.

[0155] The heavy and light chain variable domains can be combined in various pair-wise combinations to generate a number of anti-AMC antibody moieties.

[0156] For example, in some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 77, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 90, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 100, or a variant thereof comprising up to about 3 (for example about any of 1, 2, or 3) amino acid substitutions; and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 110, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0157] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 57, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 67, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 77, 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 sequences; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 90, an LC-CDR2 comprising the amino acid

sequence of SEQ ID NO: 100, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 110, 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 sequences.

[0158] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 57, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 67, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 77; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 90, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 100, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 110.

[0159] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 58, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 68, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 91, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 101, or a variant thereof comprising up to about 3 (for example about any of 1, 2, or 3) amino acid substitutions; and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 111, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0160] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 58, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 68, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 78, 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 sequences; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 91, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 101, and an LC-CDR3 comprising the amino acid sequence of SEQ

ID NO: 111, 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 sequences.

[0161] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 58, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 68, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 78; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 91, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 101, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 111.

[0162] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 59, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 69, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 79, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 102, or a variant thereof comprising up to about 3 (for example about any of 1, 2, or 3) amino acid substitutions; and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 112, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0163] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 59, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 79, 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 sequences; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 92, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 102, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 112, 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 sequences.

[0164] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 59, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 79; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 92, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 102, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 112.

[0165] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 60, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 70, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 80, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 93, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 103, or a variant thereof comprising up to about 3 (for example about any of 1, 2, or 3) amino acid substitutions; and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 113, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0166] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 60, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 70, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 80, 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 sequences; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 93, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 103, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 113, 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 sequences.

[0167] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID

NO: 60, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 70, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 80; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 93, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 103, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 113.

[0168] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 81, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 94, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 104, or a variant thereof comprising up to about 3 (for example about any of 1, 2, or 3) amino acid substitutions; and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 114, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0169] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 61, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 71, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 81, 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 sequences; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 94, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 104, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 114, 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 sequences.

[0170] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 61, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 71, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 81; and a light chain variable

domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 94, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 104, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 114.

[0171] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 95, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 105, or a variant thereof comprising up to about 3 (for example about any of 1, 2, or 3) amino acid substitutions; and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 115, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0172] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 62, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 82, 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 sequences; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 95, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 105, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 115, 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 sequences.

[0173] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 62, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 82; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 95, an

LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 105, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 115.

[0174] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 73, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 83, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 96, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 106, or a variant thereof comprising up to about 3 (for example about any of 1, 2, or 3) amino acid substitutions; and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 116, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0175] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 63, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 73, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 83, 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 sequences; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 96, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 106, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 116, 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 sequences.

[0176] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 63, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 73, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 83; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 96, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 106, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 116.

[0177] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 74, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 84, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 97, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 107, or a variant thereof comprising up to about 3 (for example about any of 1, 2, or 3) amino acid substitutions; and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 117, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0178] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 64, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 74, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 84, 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 sequences; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 97, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 107, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 117, 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 sequences.

[0179] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 64, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 74, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 84; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 97, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 107, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 117.

[0180] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID

NO: 65, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 98, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 108, or a variant thereof comprising up to about 3 (for example about any of 1, 2, or 3) amino acid substitutions; and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 118, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0181] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 65, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 85, 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 sequences; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 98, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 108, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 118, 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 sequences.

[0182] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 65, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 85; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 98, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 108, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 118.

[0183] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 66, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of SEQ ID

NO: 76, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 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 a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 99, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 109, or a variant thereof comprising up to about 3 (for example about any of 1, 2, or 3) amino acid substitutions; and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 119, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0184] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 66, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 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 sequences; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 99, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 109, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 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 sequences.

[0185] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 66, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 86; and a light chain variable domain comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 99, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 109, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 119.

[0186] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 17, or a variant thereof having at least about 95% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 27, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity. In some

embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 17 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 27.

[0187] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 18, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 28, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity. In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 18 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 28.

[0188] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 19, or a variant thereof having at least about 95% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 29, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity. In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 19 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 29.

[0189] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 20, or a variant thereof having at least about 95% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 30, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity. In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 20 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 30.

[0190] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 21, or a variant thereof having at least about 95% (for example at least about any of 96%, 97%, 98%, or 99%)

sequence identity, and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 31, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity. In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 21 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 31.

[0191] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 22, or a variant thereof having at least about 95% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 32, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity. In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 22 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 32.

[0192] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 23, or a variant thereof having at least about 95% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 33, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity. In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 23 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 33.

[0193] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 24, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 34, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity. In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 24 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 34.

[0194] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 25, or a variant thereof having at least about 95% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 35, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity. In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 25 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 35.

[0195] In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 26, or a variant thereof having at least about 95% (including for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 36, or a variant thereof having at least about 95% sequence identity. In some embodiments, the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 26 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 36.

[0196] In some embodiments, the anti-AMC antibody moiety competes for binding to a target AFP/MHC class I complex with a second anti-AMC antibody moiety according to any of the anti-AMC antibody moieties described herein. In some embodiments, the anti-AMC antibody moiety binds to the same, or substantially the same, epitope as the second anti-AMC antibody moiety. In some embodiments, binding of the anti-AMC antibody moiety to the target AFP/MHC class I complex inhibits binding of the second anti-AMC 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 embodiments, the anti-AMC antibody moiety and the second anti-AMC antibody moiety cross-compete for binding to the target AFP/MHC class I complex, *i.e.*, each of the antibody moieties competes with the other for binding to the target AFP/MHC class I complex.

Full-length anti-AMC antibodies

[0197] The anti-AMC constructs in some embodiments are full-length antibodies comprising an anti-AMC antibody moiety (also referred to herein as a "full-length anti-AMC antibody"). In some embodiments, the full-length antibody is a monoclonal antibody.

[0198] In some embodiments, the full-length anti-AMC antibody comprises an Fc sequence from an immunoglobulin, such as IgA, IgD, IgE, IgG, and IgM. In some embodiments, the full-length anti-AMC antibody comprises an Fc sequence of IgG, such as any of IgG1, IgG2, IgG3, or IgG4. In some embodiments, the full-length anti-AMC antibody comprises an Fc sequence of a human immunoglobulin. In some embodiments, the full-length anti-AMC antibody comprises an Fc sequence of a mouse immunoglobulin. In some embodiments, the full-length anti-AMC antibody comprises an Fc sequence that has been altered or otherwise changed so that it has enhanced antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC) effector function.

[0199] Thus, for example, in some embodiments, there is provided a full-length anti-AMC antibody comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, and b) an Fc region. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01. In some embodiments, there is provided a full-length anti-AMC antibody comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP158 peptide (SEQ ID NO: 4) and HLA-A*02:01, and b) an Fc region. In some embodiments, the Fc region comprises an IgG1 Fc sequence. In some embodiments, the Fc region comprises a human IgG1 Fc sequence. In some embodiments, the Fc region comprises a mouse IgG1 Fc sequence.

[0200] In some embodiments, there is provided a full-length anti-AMC antibody comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid, and b) an Fc region. In some embodiments, the Fc region comprises an IgG1 Fc sequence. In some embodiments, the Fc region comprises a human IgG1 Fc sequence. In some embodiments, the Fc region comprises a mouse IgG1 Fc sequence.

[0201] In some embodiments, there is provided a full-length anti-AMC antibody comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 an LC-CDR1 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); wherein X can be any amino acid, and b) an Fc region. In some embodiments, the Fc region comprises an IgG1 Fc sequence. In some embodiments, the Fc region comprises a human IgG1 Fc sequence. In some embodiments, the Fc region comprises a mouse IgG1 Fc sequence.

[0202] In some embodiments, there is provided a full-length anti-AMC antibody comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain comprising an HC-CDR1 comprising 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 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 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 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 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 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 some embodiments, the Fc region comprises an IgG1 Fc sequence. In some embodiments, the Fc region comprises a human IgG1 Fc sequence. In some embodiments, the Fc region comprises a mouse IgG1 Fc sequence.

[0203] In some embodiments, there is provided a full-length anti-AMC antibody comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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; 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 sequences; and ii) a light chain variable domain sequence 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; 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 sequences; and b) an Fc region. In some embodiments, the Fc region comprises an IgG1 Fc sequence. In some embodiments, the Fc region comprises a human IgG1 Fc sequence. In some embodiments, the Fc region comprises a mouse IgG1 Fc sequence.

[0204] In some embodiments, there is provided a full-length anti-AMC antibody comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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 sequence 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; and b) an Fc region. In some embodiments, the Fc region comprises an IgG1 Fc sequence. In some embodiments, the Fc region comprises a human IgG1 Fc sequence. In some embodiments, the Fc region comprises a mouse IgG1 Fc sequence.

[0205] In some embodiments, there is provided a full-length anti-AMC antibody comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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; and b) an Fc region. In some embodiments, the Fc region comprises an IgG1 Fc sequence. In some embodiments, the Fc region comprises a human IgG1 Fc sequence. In some embodiments, the Fc region comprises a mouse IgG1 Fc sequence.

[0206] In some embodiments, there is provided a full-length anti-AMC antibody comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36; and b) an Fc region. In some embodiments, the Fc region comprises an IgG1 Fc sequence. In some embodiments, the Fc region comprises a human IgG1 Fc sequence. In some embodiments, the Fc region comprises a mouse IgG1 Fc sequence.

[0207] In some embodiments, the full-length anti-AMC antibody binds to a complex comprising an AFP peptide and an MHC class I protein 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). In some embodiments, the full-length anti-AMC antibody binds to a complex comprising an AFP peptide and an MHC class I protein with a K_d between about 1 pM to about 250 pM (such as about any of 1, 10, 25, 50, 75, 100, 150, 200, or 250 pM, including any ranges between these values).

Multi-Specific anti-AMC molecules

[0208] The anti-AMC constructs in some embodiments comprise a multi-specific anti-AMC molecule comprising an anti-AMC antibody moiety and a second binding moiety (such as a second antigen-binding moiety). In some embodiments, the multi-specific anti-AMC molecule comprises an anti-AMC antibody moiety and a second antigen-binding moiety.

[0209] Multi-specific molecules are molecules that have binding specificities for at least two different antigens or epitopes *(e.g.,* bispecific antibodies have binding specificities for two antigens or epitopes). Multi-specific molecules with more than two valencies and/or specificities are also contemplated. For example, trispecific antibodies can be prepared. Tutt *et al. J. Immunol.* 147: 60 (1991). It is to be appreciated that one of skill in the art could select appropriate features of individual multi-specific molecules described herein to combine with one another to form a multi-specific anti-AMC molecule of the invention.

[0210] Thus, for example, in some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-AMC molecule comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, and b) a second binding moiety (such as an antigen-binding moiety). In some embodiments, the second binding moiety specifically binds to a complex comprising a different AFP peptide bound to the MHC class I protein. In some embodiments, the second scFv specifically binds to a complex comprising the AFP peptide bound to a different MHC class I protein. In some embodiments, the second binding moiety specifically binds to a different epitope on the complex comprising the AFP peptide bound to the MHC class I protein. In some embodiments, the second binding moiety specifically binds to a different antigen. In some embodiments, the second binding moiety specifically binds to an antigen on the surface of a cell, such as a cytotoxic cell. In some embodiments, the second binding moiety specifically binds to an antigen on the surface of a lymphocyte, such as a T cell, an NK cell, a neutrophil, a monocyte, a macrophage, or a dendritic cell. In some embodiments, the second binding moiety specifically binds to an effector T cell, such as a cytotoxic T cell (also known as cytotoxic T lymphocyte (CTL) or T killer cell).

[0211] In some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, and b) a second antigen-binding moiety that binds specifically to CD3. In some embodiments, the second antigen-binding moiety specifically binds to CD3ε. In some embodiments, the second antigen-binding moiety

specifically binds to an agonistic epitope of CD3ε. The term "agonistic epitope", as used herein, means (a) an epitope that, upon binding of the multi-specific molecule, optionally upon binding of several multi-specific molecules on the same cell, allows said multi-specific molecules to activate TCR signaling and induce T cell activation, and/or (b) an epitope that is solely composed of amino acid residues of the epsilon chain of CD3 and is accessible for binding by the multi-specific molecule, when presented in its natural context on T cells (*i.e.* surrounded by the TCR, the CD3γ chain, etc.), and/or (c) an epitope that, upon binding of the multi-specific molecule, does not lead to stabilization of the spatial position of CD3ε relative to CD3γ.

[0212] In some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, and b) a second antigen-binding moiety that binds specifically to an antigen on the surface of an effector cell, including for example CD3γ, CD3δ, CD3ε, CD3ζ, CD28, CD16a, CD56, CD68, and GDS2D.

[0213] In some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, and b) a second antigen-binding moiety that binds specifically to a component of the complement system, such as C1q. C1q is a subunit of the C1 enzyme complex that activates the serum complement system.

[0214] In some embodiments, the second antigen-binding moiety specifically binds to an Fc receptor. In some embodiments, the second antigen-binding moiety specifically binds to an Fcγ receptor (FcγR). The FcγR may be an FcγRIII present on the surface of natural killer (NK) cells or one of FcγRI, FcγRIIA, FcγRIIBI, FcγRIIB2, and FcγRIIIB present on the surface of macrophages, monocytes, neutrophils and/or dendritic cells. In some embodiments, the second antigen-binding moiety is an Fc region or functional fragment thereof. A "functional fragment" as used in this context refers to a fragment of an antibody Fc region that is still capable of binding to an FcR, in particular to an FcγR, with sufficient specificity and affinity to allow an FcγR bearing effector cell, in particular a macrophage, a monocyte, a neutrophil and/or a dendritic cell, to kill the target cell by cytotoxic lysis or phagocytosis. A functional Fc fragment is capable of competitively inhibiting the binding of the original, full-length Fc portion to an FcR such as the activating FcγRI. In some embodiments, a functional Fc fragment retains at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of its affinity to an activating FcγR. In some embodiments, the Fc region or functional fragment thereof is an

enhanced Fc region or functional fragment thereof. The term "enhanced Fc region", as used herein, refers to an Fc region that is modified to enhance Fc receptor-mediated effector-functions, in particular antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-mediated phagocytosis. This can be achieved as known in the art, for example by altering the Fc region in a way that leads to an increased affinity for an activating receptor (*e.g.* FcγRIIIA (CD16A) expressed on natural killer (NK) cells) and/or a decreased binding to an inhibitory receptor (*e.g.* FcγRIIB1/B2 (CD32B)). In yet other embodiments, the second antigen-binding moiety is an antibody or antigen-binding fragment thereof that specifically binds to an FcR, in particular to an FcγR, with sufficient specificity and affinity to allow an FcγR bearing effector cell, in particular a macrophage, a monocyte, a neutrophil and/or a dendritic cell, to kill the target cell by cytotoxic lysis or phagocytosis.

[0215] In some embodiments, the multi-specific anti-AMC molecule allows killing of AMC-presenting target cells and/or can effectively redirect CTLs to lyse AMC-presenting target cells. In some embodiments, the multi-specific (*e.g.*, bispecific) anti-AMC molecule of the present invention shows an *in vitro* EC_{50} ranging from 10 to 500 ng/ml, and is able to induce redirected lysis of about 50% of the target cells through CTLs at a ratio of CTLs to target cells of from about 1:1 to about 50:1 (such as from about 1:1 to about 15:1, or from about 2:1 to about 10:1).

[0216] In some embodiments, the multi-specific (*e.g.*, bispecific) anti-AMC molecule is capable of cross-linking a stimulated or unstimulated CTL and the target cell in such a way that the target cell is lysed. This offers the advantage that no generation of target-specific T cell clones or common antigen presentation by dendritic cells is required for the multi-specific anti-AMC molecule to exert its desired activity. In some embodiments, the multi-specific anti-AMC molecule of the present invention is capable of redirecting CTLs to lyse the target cells in the absence of other activating signals. In some embodiments, the second antigen-binding moiety of the multi-specific anti-AMC molecule specifically binds to CD3 (*e.g.*, specifically binds to CD3ε), and signaling through CD28 and/or IL-2 is not required for redirecting CTLs to lyse the target cells.

[0217] Methods for measuring the preference of the multi-specific anti-AMC molecule to simultaneously bind to two antigens (*e.g.*, antigens on two different cells) are within the normal capabilities of a person skilled in the art. For example, when the second binding moiety specifically binds to CD3, the multi-specific anti-AMC molecule may be contacted

with a mixture of $CD3^+/AFP^-$ cells and $CD3^-/AFP^+$ cells. The number of multi-specific anti-AMC molecule-positive single cells and the number of cells cross-linked by multi-specific anti-AMC molecules may then be assessed by microscopy or fluorescence-activated cell sorting (FACS) as known in the art.

[0218] For example, in some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, and b) a second antigen-binding moiety. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01. In some embodiments, the second antigen-binding moiety specifically binds to a complex comprising a different AFP peptide bound to the MHC class I protein. In some embodiments, the second antigen-binding moiety specifically binds to a complex comprising the AFP peptide bound to a different MHC class I protein. In some embodiments, the second antigen-binding moiety specifically binds to a different epitope on the complex comprising the AFP peptide bound to the MHC class I protein. In some embodiments, the second antigen-binding moiety specifically binds to another antigen. In some embodiments, the second antigen-binding moiety specifically binds to an antigen on the surface of a cell, such as an AMC-presenting cell. In some embodiments, the second antigen-binding moiety specifically binds to an antigen on the surface of a cell that does not express AFP. In some embodiments, the second antigen-binding moiety specifically binds to an antigen on the surface of a cytotoxic cell. In some embodiments, the second antigen-binding moiety specifically binds to an antigen on the surface of a lymphocyte, such as a T cell, an NK cell, a neutrophil, a monocyte, a macrophage, or a dendritic cell. In some embodiments, the second antigen-binding moiety specifically binds to an antigen on the surface of an effector T cell, such as a cytotoxic T cell. In some embodiments, the second antigen-binding moiety specifically binds to an antigen on the surface of an effector cell, including for example CD3γ, CD3δ, CD3ε, CD3ζ, CD28, CD16a, CD56, CD68, and GDS2D. In some embodiments, the anti-AMC antibody moiety is human, humanized, or semi-synthetic. In some embodiments, the second antigen-binding moiety is an antibody moiety. In some embodiments, the second antigen-binding moiety is a human, humanized, or semi-synthetic antibody moiety. In some embodiments, the multi-specific anti-AMC molecule further comprises at least one (such as at least about any of 2, 3, 4, 5, or more) additional antigen-binding moieties.

[0219] In some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP158 peptide (SEQ ID NO: 4) and HLA-A*02:01, and b) a second antigen-binding moiety.

[0220] In some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid, and b) a second antigen-binding moiety.

[0221] In some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 an LC-CDR1 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); wherein X can be any amino acid, and b) a second antigen-binding moiety.

[0222] In some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain comprising an HC-CDR1 comprising 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 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 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 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 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 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; and b) a second antigen-binding moiety.

[0223] In some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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; 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 sequences; and ii) a light chain variable domain sequence 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; 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 sequences; and b) a second antigen-binding moiety.

[0224] In some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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 sequence 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; and b) a second antigen-binding moiety.

[0225] In some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety comprising 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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; and b) a second scFv.

[0226] In some embodiments, there is provided a multi-specific anti-AMC molecule comprising a) an anti-AMC antibody moiety comprising a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36; and b) a second antigen-binding moiety.

[0227] In some embodiments, the multi-specific anti-AMC molecule is, for example, a diabody (Db), a single-chain diabody (scDb), a tandem scDb (Tandab), a linear dimeric scDb (LD-scDb), a circular dimeric scDb (CD-scDb), a di-diabody, a tandem scFv, a tandem di-scFv (*e.g.*, a bispecific T cell engager), a tandem tri-scFv, a tri(a)body, a bispecific Fab2, a di-miniantibody, a tetrabody, an scFv-Fc-scFv fusion, a dual-affinity retargeting (DART) antibody, a dual variable domain (DVD) antibody, an IgG-scFab, an scFab-ds-scFv, an Fv2-Fc, an IgG-scFv fusion, a dock and lock (DNL) antibody, a knob-into-hole (KiH) antibody (bispecific IgG prepared by the KiH technology), a DuoBody (bispecific IgG prepared by the Duobody technology), a heteromultimeric antibody, or a heteroconjugate antibody. In some

embodiments, the multi-specific anti-AMC molecule is a tandem scFv (*e.g.*, a tandem di-scFv, such as a bispecific T cell engager).

Tandem scFv

[0228] The multi-specific anti-AMC molecule in some embodiments is a tandem scFv comprising a first scFv comprising an anti-AMC antibody moiety and a second scFv (also referred to herein as a "tandem scFv multi-specific anti-AMC antibody"). In some embodiments, the tandem scFv multi-specific anti-AMC antibody further comprises at least one (such as at least about any of 2, 3, 4, 5, or more) additional scFv.

[0229] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, and b) a second scFv. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01. In some embodiments, the second scFv specifically binds to a complex comprising a different AFP peptide bound to the MHC class I protein. In some embodiments, the second scFv specifically binds to a complex comprising the AFP peptide bound to a different MHC class I protein. In some embodiments, the second scFv specifically binds to a different epitope on the complex comprising the AFP peptide bound to the MHC class I protein. In some embodiments, the second scFv specifically binds to another antigen. In some embodiments, the second scFv specifically binds to an antigen on the surface of a cell, such as an AMC-presenting cell. In some embodiments, the second scFv specifically binds to an antigen on the surface of a cell that does not express AFP. In some embodiments, the second scFv specifically binds to an antigen on the surface of a cytotoxic cell. In some embodiments, the second scFv specifically binds to an antigen on the surface of a lymphocyte, such as a T cell, an NK cell, a neutrophil, a monocyte, a macrophage, or a dendritic cell. In some embodiments, the second scFv specifically binds to an antigen on the surface of an effector T cell, such as a cytotoxic T cell. In some embodiments, the second scFv specifically binds to an antigen on the surface of an effector cell, including for example CD3γ, CD3δ, CD3ε, CD3ζ, CD28, CD16a, CD56, CD68, and GDS2D. In some embodiments, the first scFv is human, humanized, or semi-synthetic. In some embodiments, the second scFv is human, humanized, or semi-synthetic. In some embodiments, both the first scFv and the second scFv are human, humanized, or semi-synthetic. In some embodiments, the tandem scFv multi-

specific anti-AMC antibody further comprises at least one (such as at least about any of 2, 3, 4, 5, or more) additional scFv.

[0230] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP158 peptide (SEQ ID NO: 4) and HLA-A*02:01, and b) a second scFv.

[0231] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid, and b) a second scFv.

[0232] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 an LC-CDR1 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); wherein X can be any amino acid, and b) a second scFv.

[0233] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain comprising an HC-CDR1 comprising 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 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 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 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 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 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; and b) a second scFv.

[0234] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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; 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 sequences; and ii) a light chain variable domain sequence 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; 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 sequences; and b) a second scFv.

[0235] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable

domain sequence 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 sequence 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; and b) a second scFv.

[0236] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-AMC antibody comprising a) a first scFv comprising 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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; and b) a second scFv.

[0237] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-AMC antibody comprising a) a first scFv comprising a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36; and b) a second scFv.

[0238] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, and b) a second scFv, wherein the tandem scFv multi-specific anti-AMC antibody is a tandem di-scFv or a tandem tri-scFv. In some embodiments, the tandem scFv multi-specific anti-AMC antibody is a tandem di-scFv. In some embodiments, the tandem scFv multi-specific anti-AMC antibody is a bispecific T-cell engager.

[0239] For example, in some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, and b) a second scFv that specifically binds to an antigen on the surface of a T cell. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01. In some embodiments, the second

scFv specifically binds to an antigen on the surface of an effector T cell, such as a cytotoxic T cell. In some embodiments, the second scFv specifically binds to an antigen selected, for example, from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, and HVEM. In some embodiments, the second scFv specifically binds to an agonistic epitope on an antigen on the surface of a T cell, wherein the binding of the second scFv to the antigen enhances T cell activation. In some embodiments, the first scFv is human, humanized, or semi-synthetic. In some embodiments, the second scFv is human, humanized, or semi-synthetic. In some embodiments, both the first scFv and the second scFv are human, humanized, or semi-synthetic.

[0240] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP158 peptide (SEQ ID NO: 4) and HLA-A*02:01, and b) a second scFv that specifically binds to an antigen on the surface of a T cell.

[0241] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid, and b) a second scFv that specifically binds to an antigen on the surface of a T cell.

[0242] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence

comprising an HC-CDR1 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 an LC-CDR1 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); wherein X can be any amino acid, and b) a second scFv that specifically binds to an antigen on the surface of a T cell.

[0243] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain comprising an HC-CDR1 comprising 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 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 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 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 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 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; and b) a second scFv that specifically binds to an antigen on the surface of a T cell.

[0244] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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; 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 sequences; and ii) a light chain variable domain sequence 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; 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 sequences, and b) a second scFv that specifically binds to an antigen on the surface of a T cell.

[0245] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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 sequence 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; and b) a second scFv that specifically binds to an antigen on the surface of a T cell.

[0246] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv comprising 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and b) a second scFv that specifically binds to an antigen on the surface of a T cell.

[0247] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv comprising a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36, and b) a second scFv that specifically binds to an antigen on the surface of a T cell.

[0248] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP

peptide and an MHC class I protein, and b) a second scFv that specifically binds to CD3ε. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01. In some embodiments, the first scFv is fused to the second scFv through linkage with a peptide linker. In some embodiments, the peptide linker is between about 5 to about 20 (such as about any of 5, 10, 15, or 20, including any ranges between these values) amino acids in length. In some embodiments, the peptide linker comprises (and in some embodiments consists of) the amino acid sequence GGGGS. In some embodiments, the first scFv is human, humanized, or semi-synthetic. In some embodiments, the second scFv is human, humanized, or semi-synthetic. In some embodiments, both the first scFv and the second scFv are human, humanized, or semi-synthetic.

[0249] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP158 peptide (SEQ ID NO: 4) and HLA-A*02:01, and b) a second scFv that specifically binds to CD3ε. In some embodiments, the first scFv is fused to the second scFv through linkage with a peptide linker. In some embodiments, the peptide linker is between about 5 to about 20 (such as about any of 5, 10, 15, or 20, including any ranges between these values) amino acids in length. In some embodiments, the peptide linker comprises (and in some embodiments consists of) the amino acid sequence GGGGS. In some embodiments, the first scFv is human, humanized, or semi-synthetic. In some embodiments, the second scFv is human, humanized, or semi-synthetic. In some embodiments, both the first scFv and the second scFv are human, humanized, or semi-synthetic.

[0250] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1

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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid, and b) a second scFv that specifically binds to CD3ε. In some embodiments, the first scFv is fused to the second scFv through linkage with a peptide linker. In some embodiments, the peptide linker is between about 5 to about 20 (such as about any of 5, 10, 15, or 20, including any ranges between these values) amino acids in length. In some embodiments, the peptide linker comprises (and in some embodiments consists of) the amino acid sequence GGGGS. In some embodiments, the first scFv is human, humanized, or semi-synthetic. In some embodiments, the second scFv is human, humanized, or semi-synthetic. In some embodiments, both the first scFv and the second scFv are human, humanized, or semi-synthetic.

[0251] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 an LC-CDR1 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); wherein X can be any amino acid, and b) a second scFv that specifically binds to CD3ε. In some embodiments, the first scFv is fused to the second scFv through linkage with a peptide linker. In some embodiments, the peptide linker is between about 5 to about 20 (such as about any of 5, 10, 15, or 20, including any ranges between these values) amino acids in length. In some embodiments, the peptide linker comprises (and in some embodiments consists of) the amino acid sequence GGGGS. In some embodiments, the first scFv is human, humanized, or semi-synthetic. In some embodiments, the second scFv is human, humanized, or semi-synthetic. In some embodiments, both the first scFv and the second scFv are human, humanized, or semi-synthetic.

[0252] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain comprising an HC-CDR1 comprising 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 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 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 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 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 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, and b) a second scFv that specifically binds to CD3ε. In some embodiments, the first scFv is fused to the second scFv through linkage with a peptide linker. In some embodiments, the peptide linker is between about 5 to about 20 (such as about any of 5, 10, 15, or 20, including any ranges between these values) amino acids in length. In some embodiments, the peptide linker comprises (and in some embodiments consists of) the amino acid sequence GGGGS. In some embodiments, the first scFv is human, humanized, or semi-synthetic. In some embodiments, the second scFv is human, humanized, or semi-synthetic. In some embodiments, both the first scFv and the second scFv are human, humanized, or semi-synthetic.

[0253] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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; 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 sequences; and ii) a light chain variable domain sequence

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; 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 sequences, and b) a second scFv that specifically binds to CD3ε. In some embodiments, the first scFv is fused to the second scFv through linkage with a peptide linker. In some embodiments, the peptide linker is between about 5 to about 20 (such as about any of 5, 10, 15, or 20, including any ranges between these values) amino acids in length. In some embodiments, the peptide linker comprises (and in some embodiments consists of) the amino acid sequence GGGGS. In some embodiments, the first scFv is human, humanized, or semi-synthetic. In some embodiments, the second scFv is human, humanized, or semi-synthetic. In some embodiments, both the first scFv and the second scFv are human, humanized, or semi-synthetic.

[0254] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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 sequence 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; and b) a second scFv that specifically binds to CD3ε. In some embodiments, the first scFv is fused to the second scFv through linkage with a peptide linker. In some embodiments, the peptide linker is between about 5 to about 20 (such as about any of 5, 10, 15, or 20, including any ranges between these values) amino acids in length. In some embodiments, the peptide linker comprises (and in some embodiments consists of) the amino acid sequence GGGGS. In some embodiments, the first scFv is human, humanized, or semi-synthetic. In some embodiments, the second scFv is human, humanized, or semi-synthetic. In some embodiments, both the first scFv and the second scFv are human, humanized, or semi-synthetic.

[0255] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv comprising 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and b) a second scFv that specifically binds to CD3ε. In some embodiments, the first scFv is fused to the second scFv through linkage with a peptide linker. In some embodiments, the peptide linker is between about 5 to about 20 (such as about any of 5, 10, 15, or 20, including any ranges between these values) amino acids in length. In some embodiments, the peptide linker comprises (and in some embodiments consists of) the amino acid sequence GGGGS. In some embodiments, the first scFv is human, humanized, or semi-synthetic. In some embodiments, the second scFv is human, humanized, or semi-synthetic. In some embodiments, both the first scFv and the second scFv are human, humanized, or semi-synthetic.

[0256] In some embodiments, there is provided a tandem di-scFv bispecific anti-AMC antibody comprising a) a first scFv comprising a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36, and b) a second scFv that specifically binds to CD3ε. In some embodiments, the first scFv is fused to the second scFv through linkage with a peptide linker. In some embodiments, the peptide linker is between about 5 to about 20 (such as about any of 5, 10, 15, or 20, including any ranges between these values) amino acids in length. In some embodiments, the peptide linker comprises (and in some embodiments consists of) the amino acid sequence GGGGS. In some embodiments, the first scFv is human, humanized, or semi-synthetic. In some embodiments, the second scFv is human, humanized, or semi-synthetic. In some embodiments, both the first scFv and the second scFv are human, humanized, or semi-synthetic.

[0257] In some embodiments, the tandem di-scFv bispecific anti-AMC antibody binds to a complex comprising an AFP peptide and an MHC class I protein 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). In some embodiments, the tandem di-scFv bispecific anti-AMC antibody binds to a complex comprising an AFP peptide and an MHC class I protein with a K_d between about 1 nM to about 500 nM (such as about any of 1, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, or 500 nM, including any ranges between these values).

Chimeric Antigen Receptor (CAR) and CAR effector cells

[0258] The anti-AMC construct in some embodiments is a chimeric antigen receptor (CAR) comprising an anti-AMC antibody moiety (also referred to herein as an "anti-AMC CAR"). Also provided is a CAR effector cell (*e.g.*, T cell) comprising a CAR comprising an anti-AMC antibody moiety (also referred to herein as an "anti-AMC CAR effector cell", *e.g.*, "anti-AMC CAR T cell").

[0259] The anti-AMC CAR comprises a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein and b) an intracellular signaling domain. A transmembrane domain may be present between the extracellular domain and the intracellular domain.

[0260] Between the extracellular domain and the transmembrane domain of the anti-AMC CAR, or between the intracellular domain and the transmembrane domain of the anti-AMC CAR, there may be a spacer domain. The spacer domain can be any oligo- or polypeptide that functions to link the transmembrane domain to the extracellular domain or the intracellular domain in the polypeptide chain. A spacer domain may comprise up to about 300 amino acids, including for example about 10 to about 100, or about 25 to about 50 amino acids.

[0261] The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in this invention may be derived from (*i.e.* comprise at least the transmembrane region(s) of) the α, β, δ, or γ chain of the T-cell receptor, CD28, CD3ε, CD3ζ, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, or CD154. In some embodiments, the transmembrane domain may be synthetic, in which case it may comprise predominantly hydrophobic residues such as leucine and valine. In some embodiments, a triplet of phenylalanine, tryptophan and valine may be found at each end of a synthetic transmembrane domain. In some embodiments, a short oligo- or polypeptide linker, having a length of, for example, between about 2 and about 10 (such as about any of 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acids in length may form the linkage between the transmembrane domain and the intracellular signaling domain of the anti-AMC CAR. In some embodiments, the linker is a glycine-serine doublet.

[0262] In some embodiments, the transmembrane domain that naturally is associated with one of the sequences in the intracellular domain of the anti-AMC CAR is used (*e.g.*, if an

anti-AMC CAR intracellular domain comprises a CD28 co-stimulatory sequence, the transmembrane domain of the anti-AMC CAR is derived from the CD28 transmembrane domain). In some embodiments, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0263] The intracellular signaling domain of the anti-AMC CAR is responsible for activation of at least one of the normal effector functions of the immune cell in which the anti-AMC CAR has been placed in. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus the term "intracellular signaling domain" refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term "intracellular signaling sequence" is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

[0264] Examples of intracellular signaling domains for use in the anti-AMC CAR of the invention include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability.

[0265] It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary or co-stimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of intracellular signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (co-stimulatory signaling sequences).

[0266] Primary signaling sequences regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor

tyrosine-based activation motifs or ITAMs. The anti-AMC CAR constructs in some embodiments comprise one or more ITAMs.

[0267] Examples of ITAM containing primary signaling sequences that are of particular use in the invention include those derived from TCRζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, and CD66d.

[0268] In some embodiments, the anti-AMC CAR comprises a primary signaling sequence derived from CD3ζ. For example, the intracellular signaling domain of the CAR can comprise the CD3ζ intracellular signaling sequence by itself or combined with any other desired intracellular signaling sequence(s) useful in the context of the anti-AMC CAR of the invention. For example, the intracellular domain of the anti-AMC CAR can comprise a CD3ζ intracellular signaling sequence and a costimulatory signaling sequence. The costimulatory signaling sequence can be a portion of the intracellular domain of a costimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like.

[0269] In some embodiments, the intracellular signaling domain of the anti-AMC CAR comprises the intracellular signaling sequence of CD3ζ and the intracellular signaling sequence of CD28. In some embodiments, the intracellular signaling domain of the anti-AMC CAR comprises the intracellular signaling sequence of CD3ζ and the intracellular signaling sequence of 4-1BB. In some embodiments, the intracellular signaling domain of the anti-AMC CAR comprises the intracellular signaling sequence of CD3ζ and the intracellular signaling sequences of CD28 and 4-1BB.

[0270] Thus, for example, in some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01. In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular

signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0271] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP158 peptide (SEQ ID NO: 4) and HLA-A*02:01, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0272] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary

signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0273] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 an LC-CDR1 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); b) an intracellular signaling domain. In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0274] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain comprising an HC-CDR1 comprising 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 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 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 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 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 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; b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0275] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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; 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 sequences; and ii) a light chain variable domain sequence 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; 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 sequences; b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0276] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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 sequence 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; b) an intracellular signaling domain. In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0277] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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; b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0278] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36; b) an intracellular signaling domain. In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0279] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01.

[0280] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP158 peptide (SEQ ID NO: 4) and HLA-A*02:01, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0281] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0282] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 an LC-CDR1 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); wherein X can be any amino acid, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0283] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain comprising an HC-CDR1 comprising 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 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 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 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 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 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; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0284] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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; 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 sequences; and ii) a light chain variable domain sequence 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; 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 sequences; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0285] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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 sequence 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; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0286] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0287] In some embodiments, there is provided an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0288] Also provided herein are effector cells (such as lymphocytes, *e.g.*, T cells) expressing an anti-AMC CAR.

[0289] Also provided is a method of producing an effector cell expressing an anti-AMC CAR, the method comprising introducing a vector comprising a nucleic acid encoding the anti-AMC CAR into the effector cell. In some embodiments, introducing the vector into the effector cell comprises transducing the effector cell with the vector. In some embodiments, introducing the vector into the effector cell comprises transfecting the effector cell with the vector. Transduction or transfection of the vector into the effector cell can be carried about using any method known in the art.

Immunoconjugates

[0290] The anti-AMC constructs in some embodiments comprise an immunoconjugate comprising an anti-AMC antibody moiety attached to an effector molecule (also referred to herein as an "anti-AMC immunoconjugate"). In some embodiments the effector molecule is a therapeutic agent, such as a cancer therapeutic agent, which is either cytotoxic, cytostatic or otherwise provides some therapeutic benefit. In some embodiments, the effector molecule is a label, which can generate a detectable signal, either directly or indirectly.

[0291] In some embodiments, there is provided an anti-AMC immunoconjugate comprising an anti-AMC antibody moiety and a therapeutic agent (also referred to herein as an "antibody-drug conjugate", or "ADC"). In some embodiments, the therapeutic agent is a toxin that is either cytotoxic, cytostatic or otherwise prevents or reduces the ability of the target cells to divide. The use of ADCs for the local delivery of cytotoxic or cytostatic agents, *i.e.*, drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos, *Anticancer Research* 19:605-614 (1999); Niculescu-Duvaz and Springer, *Adv. Drg. Del. Rev.* 26:151 -172 (1997); U.S. Patent No. 4,975,278) allows targeted delivery of the drug moiety to target cells, and intracellular accumulation therein, where systemic administration of these unconjugated therapeutic agents may result in unacceptable levels of toxicity to normal cells as well as the target cells sought to be eliminated (Baldwin *et al.*, *Lancet* (Mar. 15, 1986):603-605 (1986); Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications, A. Pinchera *et al.* (eds.), pp. 475- 506). Maximal efficacy with minimal toxicity is sought thereby. Importantly, since most normal cells do not present the AMC on their surface, they cannot bind the anti-AMC immunoconjugate, and are protected from the killing effect of the toxin or other therapeutic agents.

[0292] Therapeutic agents used in anti-AMC immunoconjugates include, for example, daunomycin, doxorubicin, methotrexate, and vindesine (Rowland *et al.*, *Cancer Immunol. Immunother.* 21:183-187 (1986)). Toxins used in anti-AMC immunoconjugates include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler *et al.*, *J.Nat. Cancer Inst.* 92(19):1573-1581 (2000); Mandler *et al.*, *Bioorganic & Med. Chem. Letters* 10:1025- 1028 (2000); Mandler *et al.*, *Bioconjugate Chem.* 13:786-791 (2002)), maytansinoids (EP 1391213; Liu *et al.*, *Proc. Natl. Acad. Sci.*

USA 93:8618-8623 (1996)), and calicheamicin (Lode *et al.*, *Cancer Res.* 58:2928 (1998); Hinman *et al.*, *Cancer Res.* 53:3336-3342 (1993)). The toxins may exert their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition. Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

[0293] Enzymatically active toxins and fragments thereof that can be used include, for example, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain,α-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. *See*, *e.g.*, WO 93/21232 published October 28, 1993.

[0294] Anti-AMC immunoconjugates of an anti-AMC antibody moiety and one or more small molecule toxins, such as a calicheamicin, maytansinoids, dolastatins, aurostatins, a trichothecene, and CC1065, and the derivatives of these toxins that have toxin activity, are also contemplated herein.

[0295] In some embodiments, there is provided an anti-AMC immunoconjugate comprising a therapeutic agent that has an intracellular activity. In some embodiments, the anti-AMC immunoconjugate is internalized and the therapeutic agent is a cytotoxin that blocks the protein synthesis of the cell, therein leading to cell death. In some embodiments, the therapeutic agent is a cytotoxin comprising a polypeptide having ribosome-inactivating activity including, for example, gelonin, bouganin, saporin, ricin, ricin A chain, bryodin, diphtheria toxin, restrictocin, Pseudomonas exotoxin A and variants thereof. In some embodiments, where the therapeutic agent is a cytotoxin comprising a polypeptide having a ribosome-inactivating activity, the anti-AMC immunoconjugate must be internalized upon binding to the target cell in order for the protein to be cytotoxic to the cells.

[0296] In some embodiments, there is provided an anti-AMC immunoconjugate comprising a therapeutic agent that acts to disrupt DNA. In some embodiments, the therapeutic agent that acts to disrupt DNA is, for example, selected from the group consisting of enediyne (*e.g.*, calicheamicin and esperamicin) and non-enediyne small molecule agents (*e.g.*, bleomycin, methidiumpropyl-EDTA-Fe(II)). Other cancer therapeutic agents useful in accordance with the present application include, without limitation, daunorubicin, doxorubicin, distamycin A, cisplatin, mitomycin C, ecteinascidins, duocarmycin/CC-1065, and bleomycin/pepleomycin.

[0297] The present invention further contemplates an anti-AMC immunoconjugate formed between the anti-AMC antibody moiety and a compound with nucleolytic activity (*e.g.*, a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

[0298] In some embodiments, the anti-AMC immunoconjugate comprises an agent that acts to disrupt tubulin. Such agents may include, for example, rhizoxin/maytansine, paclitaxel, vincristine and vinblastine, colchicine, auristatin dolastatin 10 MMAE, and peloruside A.

[0299] In some embodiments, the anti-AMC immunoconjugate comprises an alkylating agent including, for example, Asaley NSC 167780, AZQ NSC 182986, BCNU NSC 409962, Busulfan NSC 750, carboxyphthalatoplatinum NSC 271674, CBDCA NSC 241240, CCNU NSC 79037, CHIP NSC 256927, chlorambucil NSC 3088, chlorozotocin NSC 178248, cis-platinum NSC 119875, clomesone NSC 338947, cyanomorpholinodoxorubicin NSC 357704, cyclodisone NSC 348948, dianhydrogalactitol NSC 132313, fluorodopan NSC 73754, hepsulfam NSC 329680, hycanthone NSC 142982, melphalan NSC 8806, methyl CCNU NSC 95441 , mitomycin C NSC 26980, mitozolamide NSC 353451 , nitrogen mustard NSC 762, PCNU NSC 95466, piperazine NSC 344007, piperazinedione NSC 135758, pipobroman NSC 25154, porfiromycin NSC 56410, spirohydantoin mustard NSC 172112, teroxirone NSC 296934, tetraplatin NSC 363812, thio-tepa NSC 6396, triethylenemelamine NSC 9706, uracil nitrogen mustard NSC 34462, and Yoshi-864 NSC 102627.

[0300] In some embodiments, the cancer therapeutic agent portion of the anti-AMC immunoconjugate of the present application may comprise an antimitotic agent including, without limitation, allocolchicine NSC 406042, Halichondrin B NSC 609395, colchicine NSC 757, colchicine derivative NSC 33410, dolastatin 10 NSC 376128 (NG - auristatin derived), maytansine NSC 153858, rhizoxin NSC 332598, taxol NSC 125973, taxol derivative NSC 608832, thiocolchicine NSC 361792, trityl cysteine NSC 83265, vinblastine sulfate NSC 49842, and vincristine sulfate NSC 67574.

[0301] In some embodiments, the anti-AMC immunoconjugate comprises a topoisomerase I inhibitor including, without limitation, camptothecin NSC 94600, camptothecin, Na salt NSC 100880, aminocamptothecin NSC 603071 , camptothecin derivative NSC 95382, camptothecin derivative NSC 107124, camptothecin derivative NSC 643833, camptothecin derivative NSC 629971 , camptothecin derivative NSC 295500, camptothecin derivative NSC 249910, camptothecin derivative NSC 606985, camptothecin derivative NSC 374028, camptothecin derivative NSC 176323, camptothecin derivative NSC 295501 , camptothecin

derivative NSC 606172, camptothecin derivative NSC 606173, camptothecin derivative NSC 610458, camptothecin derivative NSC 618939, camptothecin derivative NSC 610457, camptothecin derivative NSC 610459, camptothecin derivative NSC 606499, camptothecin derivative NSC 610456, camptothecin derivative NSC 364830, camptothecin derivative NSC 606497, and morpholinodoxorubicin NSC 354646.

[0302] In some embodiments, the anti-AMC immunoconjugate comprises a topoisomerase II inhibitor including, without limitation, doxorubicin NSC 123127, amonafide NSC 308847, m-AMSA NSC 249992, anthrapyrazole derivative NSC 355644, pyrazoloacridine NSC 366140, bisantrene HCL NSC 337766, daunorubicin NSC 82151 , deoxydoxorubicin NSC 267469, mitoxantrone NSC 301739, menogaril NSC 269148, N,N-dibenzyl daunomycin NSC 268242, oxanthrazole NSC 349174, rubidazone NSC 164011 , VM-26 NSC 122819, and VP-16 NSC 141540.

[0303] In some embodiments, the anti-AMC immunoconjugate comprises an RNA or DNA antimetabolite including, without limitation, L-alanosine NSC 153353, 5-azacytidine NSC 102816, 5-fluorouracil NSC 19893, acivicin NSC 163501 , aminopterin derivative NSC 132483, aminopterin derivative NSC 184692, aminopterin derivative NSC 134033, an antifol NSC 633713, an antifol NSC 623017, Baker's soluble antifol NSC 139105, dichlorallyl lawsone NSC 126771 , brequinar NSC 368390, ftorafur (pro-drug) NSC 148958, 5,6-dihydro-5-azacytidine NSC 264880, methotrexate NSC 740, methotrexate derivative NSC 174121 , N-(phosphonoacetyl)-L-aspartate (PALA) NSC 224131 , pyrazofurin NSC 143095, trimetrexate NSC 352122, 3-HP NSC 95678, 2'-deoxy-5-fluorouridine NSC 27640, 5-HP NSC 107392,α-TGDR NSC 71851 , aphidicolin glycinate NSC 303812, ara-C NSC 63878, 5-aza-2'- deoxycytidine NSC 127716,β-TGDR NSC 71261 , cyclocytidine NSC 145668, guanazole NSC 1895, hydroxyurea NSC 32065, inosine glycodialdehyde NSC 118994, macbecin Il NSC 330500, pyrazoloimidazole NSC 51143, thioguanine NSC 752, and thiopurine NSC 755.

[0304] In some embodiments, the anti-AMC immunoconjugate comprises a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, ^{32}P, ^{212}Pb and radioactive isotopes of Lu.

[0305] In some embodiments, the anti-AMC antibody moiety can be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate

from the circulation using a clearing agent and then administration of a "ligand" *(e.g.,* avidin) that is conjugated to a cytotoxic agent *(e.g.,* a radionucleotide).

[0306] In some embodiments, an anti-AMC immunoconjugate may comprise an anti-AMC antibody moiety conjugated to a prodrug-activating enzyme. In some such embodiments, a prodrug-activating enzyme converts a prodrug (*e.g.*, a peptidyl chemotherapeutic agent, see WO 81/01145) to an active drug, such as an anti-cancer drug. Such anti-AMC immunoconjugates are useful, in some embodiments, in antibody-dependent enzyme-mediated prodrug therapy ("ADEPT"). Enzymes that may be conjugated to an antibody include, but are not limited to, alkaline phosphatases, which are useful for converting phosphate-containing prodrugs into free drugs; arylsulfatases, which are useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase, which is useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), which are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, which are useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase, which are useful for converting glycosylated prodrugs into free drugs; β-lactamase, which is useful for converting drugs derivatized with β -lactams into free drugs; and penicillin amidases, such as penicillin V amidase and penicillin G amidase, which are useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. In some embodiments, enzymes may be covalently bound to antibody moieties by recombinant DNA techniques well known in the art. See, *e.g.*, Neuberger *et al.*, *Nature* 312:604-608 (1984).

[0307] In some embodiments, the therapeutic portion of the anti-AMC immunoconjugates may be a nucleic acid. Nucleic acids that may be used include, but are not limited to, anti-sense RNA, genes or other polynucleotides, including nucleic acid analogs such as thioguanine and thiopurine.

[0308] The present application further provides anti-AMC immunoconjugates comprising an anti-AMC antibody moiety attached to an effector molecule, wherein the effector molecule is a label, which can generate a detectable signal, indirectly or directly. These anti-AMC immunoconjugates can be used for research or diagnostic applications, such as for the *in vivo* detection of cancer. The label is preferably capable of producing, either directly or indirectly, a detectable signal. For example, the label may be radio-opaque or a radioisotope,

such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{123}I, ^{125}I, ^{131}I; a fluorescent (fluorophore) or chemiluminescent (chromophore) compound, such as fluorescein isothiocyanate, rhodamine or luciferin; an enzyme, such as alkaline phosphatase,β-galactosidase or horseradish peroxidase; an imaging agent; or a metal ion. In some embodiments, the label is a radioactive atom for scintigraphic studies, for example ^{99}Tc or ^{123}I, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, *e.g.*, for PET imaging (WO 2011/056983).

[0309] In some embodiments, the anti-AMC immunoconjugate is detectable indirectly. For example, a secondary antibody that is specific for the anti-AMC immunoconjugate and contains a detectable label can be used to detect the anti-AMC immunoconjugate.

[0310] Thus, for example, in some embodiments, there is provided an anti-AMC immunoconjugate comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, and b) an effector molecule. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01. In some embodiments, the effector molecule is covalently attached to the anti-AMC antibody moiety. In some embodiments, the effector molecule is a therapeutic agent selected, for example, from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid. In some embodiments, the effector molecular is a cancer therapeutic agent. In some embodiments, the cancer therapeutic agent is a chemotherapeutic. In some embodiments, the cancer therapeutic agent is a highly radioactive atom selected, for example, from the group consisting of ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, ^{32}P, and ^{212}Pb. In some embodiments, the effector molecule is a label that can generate a detectable signal, either directly or indirectly. In some embodiments, the label is a radioisotope selected, for example, from the group consisting of ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{123}I, ^{125}I, and ^{131}I. In some embodiments, the anti-AMC antibody moiety is an scFv. In some embodiments, the anti-AMC antibody moiety is human, humanized, or semi-synthetic.

[0311] In some embodiments, there is provided an anti-AMC immunoconjugate comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP158 peptide (SEQ ID NO: 4) and HLA-A*02:01, and b) an effector molecule. In some embodiments, the effector molecule is covalently attached to the anti-AMC antibody moiety.

In some embodiments, the effector molecule is a therapeutic agent selected, for example, from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid. In some embodiments, the effector molecular is a cancer therapeutic agent. In some embodiments, the cancer therapeutic agent is a chemotherapeutic. In some embodiments, the cancer therapeutic agent is a highly radioactive atom selected, for example, from the group consisting of ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, ^{32}P, and ^{212}Pb. In some embodiments, the effector molecule is a label that can generate a detectable signal, either directly or indirectly. In some embodiments, the label is a radioisotope selected, for example, from the group consisting of ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{123}I, ^{125}I, and ^{131}I. In some embodiments, the anti-AMC antibody moiety is an scFv. In some embodiments, the anti-AMC antibody moiety is human, humanized, or semi-synthetic.

[0312] In some embodiments, there is provided an anti-AMC immunoconjugate comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid, and b) an effector molecule.

[0313] In some embodiments, there is provided an anti-AMC immunoconjugate comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 an LC-CDR1 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); wherein X can be any amino acid, and b) an effector molecule.

[0314] In some embodiments, there is provided an anti-AMC immunoconjugate comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain comprising an HC-CDR1 comprising 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 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 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 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 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 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, and b) an effector molecule.

[0315] In some embodiments, there is provided an anti-AMC immunoconjugate comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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; 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 sequences; and ii) a light chain variable domain sequence 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; 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 sequences, and b) an effector molecule.

[0316] In some embodiments, there is provided an anti-AMC immunoconjugate comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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 sequence 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, and b) an effector molecule.

[0317] In some embodiments, there is provided an anti-AMC immunoconjugate comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and b) an effector molecule.

[0318] In some embodiments, there is provided an anti-AMC immunoconjugate comprising a) an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36, b) an effector molecule.

Nucleic Acids

[0319] Nucleic acid molecules encoding the anti-AMC constructs or anti-AMC antibody moieties are also contemplated. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding a full-length anti-AMC antibody. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding a multi-specific anti-AMC

molecule (*e.g.*, a multi-specific anti-AMC antibody, a bispecific anti-AMC antibody, or a bispecific T-cell engager anti-AMC antibody), or polypeptide portion thereof. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding an anti-AMC CAR. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding an anti-AMC immunoconjugate, or polypeptide portion thereof.

[0320] The present application also includes variants to these nucleic acid sequences. For example, the variants include nucleotide sequences that hybridize to the nucleic acid sequences encoding the anti-AMC constructs or anti-AMC antibody moieties of the present application under at least moderately stringent hybridization conditions.

[0321] The present invention also provides vectors in which a nucleic acid of the present invention is inserted.

[0322] In brief summary, the expression of an anti-AMC construct (*e.g.*, anti-AMC CAR) or polypeptide portion thereof by a natural or synthetic nucleic acid encoding the anti-AMC construct or polypeptide portion thereof can be achieved by inserting the nucleic acid into an appropriate expression vector, such that the nucleic acid is operably linked to 5' and 3' regulatory elements, including for example a promoter (*e.g.*, a lymphocyte-specific promoter) and a 3' untranslated region (UTR). The vectors can be suitable for replication and integration in eukaryotic host cells. Typical cloning and expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

[0323] The nucleic acids of the present invention may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, *e.g.*, U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties. In some embodiments, the invention provides a gene therapy vector.

[0324] The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[0325] Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook *et al.* (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses which

are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers (*see*, *e.g.*, WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[0326] A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In some embodiments, lentivirus vectors are used. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity.

[0327] Additional promoter elements, *e.g.*, enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline.

[0328] One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor-1α (EF-1α). However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as,

but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

[0329] In order to assess the expression of a polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

[0330] Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, *e.g.*, enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, β-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tel *et al.*, 2000 *FEBS Letters* 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5′ flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

[0331] Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, *e.g.*, mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the

expression vector can be transferred into a host cell by physical, chemical, or biological means.

[0332] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook *et al.* (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York). In some embodiments, the introduction of a polynucleotide into a host cell is carried out by calcium phosphate transfection.

[0333] Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method of inserting genes into mammalian, *e.g.*, human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus 1, adenoviruses and adeno-associated viruses, and the like. *See*, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[0334] Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*e.g.*, an artificial membrane vesicle).

[0335] In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (*in vitro*, *ex vivo* or *in vivo*). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape.

Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

[0336] Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; "biochemical" assays, such as detecting the presence or absence of a particular peptide, *e.g.*, by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

AFP and MHC class I proteins

[0337] Alpha-fetoprotein (AFP, α-fetoprotein; also referred to as alpha-1-fetoprotein, alpha-fetoglobulin, or alpha fetal protein) is a 591 amino acid glycoprotein that in humans is encoded by the AFP gene. The AFP gene is located on the q arm of chromosome 4 (4q25). AFP is a major plasma protein produced by the yolk sac and the liver during fetal development. It is thought to be the fetal form of serum albumin. AFP binds to copper, nickel, fatty acids and bilirubin, and is found in monomeric, dimeric and trimeric forms. AFP is the most abundant plasma protein found in the human fetus. Plasma levels decrease rapidly after birth but begin decreasing prenatally starting at the end of the first trimester. Normal adult levels are usually achieved by the age of 8 to 12 months. The function of AFP in adult humans is unknown; however, in rodents it binds estradiol to prevent the transport of this hormone across the placenta to the fetus. The main function of this is to prevent the virilization of female fetuses. As human AFP does not bind estrogen, its function in humans is less clear.

[0338] Some of the diseases in which AFP will be elevated in a person include, for example, hepatocellular carcinoma/hepatoma, germ cell tumor, metastatic liver cancer, omphalocele, neural tube defects, yolk sac tumors, and ataxia telangiectasia.

[0339] MHC class I proteins are one of two primary classes of major histocompatibility complex (MHC) molecules (the other being MHC class II) and are found on nearly every nucleated cell of the body. Their function is to display fragments of proteins from within the

cell to T cells; healthy cells will be ignored, while cells containing foreign proteins will be attacked by the immune system. Because MHC class I proteins present peptides derived from cytosolic proteins, the pathway of MHC class I presentation is often called the cytosolic or endogenous pathway. Class I MHC molecules bind peptides generated mainly from degradation of cytosolic proteins by the proteasome. The MHC I:peptide complex is then inserted into the plasma membrane of the cell. The peptide is bound to the extracellular part of the class I MHC molecule. Thus, the function of the class I MHC is to display intracellular proteins to cytotoxic T cells (CTLs). However, class I MHC can also present peptides generated from exogenous proteins, in a process known as cross-presentation.

[0340] MHC class I proteins consist of two polypeptide chains, α and β2-microglobulin (β2M). The two chains are linked noncovalently via interaction of b2m and the α3 domain. Only the α chain is polymorphic and encoded by a HLA gene, while the b2m subunit is not polymorphic and encoded by the β-2 microglobulin gene. The α3 domain is plasma membrane-spanning and interacts with the CD8 co-receptor of T-cells. The α3-CD8 interaction holds the MHC I molecule in place while the T cell receptor (TCR) on the surface of the cytotoxic T cell binds its α1-α2 heterodimer ligand, and checks the coupled peptide for antigenicity. The α1 and α2 domains fold to make up a groove for peptides to bind. MHC class I proteins bind peptides that are 8-10 amino acid in length.

[0341] The human leukocyte antigen (HLA) genes are the human versions of the MHC genes. The three major MHC class I proteins in humans are HLA-A, HLA-B, and HLA-C, while the 3 minor ones are HLA-E, HLA-F, and HLA-G. HLA-A is ranked among the genes in humans with the fastest-evolving coding sequence. As of December 2013, there were 2432 known HLA-A alleles coding for 1740 active proteins and 117 null proteins. The HLA-A gene is located on the short arm of chromosome 6 and encodes the larger, α-chain, constituent of HLA-A. Variation of HLA-A α-chain is key to HLA function. This variation promotes genetic diversity in the population. Since each HLA has a different affinity for peptides of certain structures, greater variety of HLAs means greater variety of antigens to be 'presented' on the cell surface, enhancing the likelihood that a subset of the population will be resistant to any given foreign invader. This decreases the likelihood that a single pathogen has the capability to wipe out the entire human population. Each individual can express up to two types of HLA-A, one from each of their parents. Some individuals will inherit the same HLA-A from both parents, decreasing their individual HLA diversity; however, the majority of individuals will receive two different copies of HLA-A. This same pattern follows for all

HLA groups. In other words, a person can only express either one or two of the 2432 known HLA-A alleles.

[0342] All alleles receive at least a four digit classification, *e.g.*, HLA-A*02:12. The A signifies which HLA gene the allele belongs to. There are many HLA-A alleles, so that classification by serotype simplifies categorization. The next pair of digits indicates this assignment. For example, HLA-A*02:02, HLA-A*02:04, and HLA-A*02:324 are all members of the A2 serotype (designated by the *02 prefix). This group is the primary factor responsible for HLA compatibility. All numbers after this cannot be determined by serotyping and are designated through gene sequencing. The second set of digits indicates what HLA protein is produced. These are assigned in order of discovery and as of December 2013 there are 456 different HLA-A02 proteins known (assigned names HLA-A*02:01 to HLA-A*02:456). The shortest possible HLA name includes both of these details. Each extension beyond that signifies a nucleotide change that may or may not change the protein.

[0343] In some embodiments, the anti-AMC antibody moiety specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the MHC class I protein is HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G. In some embodiments, the MHC class I protein is HLA-A, HLA-B, or HLA-C. In some embodiments, the MHC class I protein is HLA-A. In some embodiments, the MHC class I protein is HLA-B. In some embodiments, the MHC class I protein is HLA-C. In some embodiments, the MHC class I protein is HLA-A01, HLA-A02, HLA-A03, HLA-A09, HLA-A10, HLA-A11, HLA-A19, HLA-A23, HLA-A24, HLA-A25, HLA-A26, HLA-A28, HLA-A29, HLA-A30, HLA-A31, HLA-A32, HLA-A33, HLA-A34, HLA-A36, HLA-A43, HLA-A66, HLA-A68, HLA-A69, HLA-A74, or HLA-A80. In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is any one of HLA-A*02:01-555, such as HLA-A*02:01, HLA-A*02:02, HLA-A*02:03, HLA-A*02:04, HLA-A*02:05, HLA-A*02:06, HLA-A*02:07, HLA-A*02:08, HLA-A*02:09, HLA-A*02:10, HLA-A*02:11, HLA-A*02:12, HLA-A*02:13, HLA-A*02:14, HLA-A*02:15, HLA-A*02:16, HLA-A*02:17, HLA-A*02:18, HLA-A*02:19, HLA-A*02:20, HLA-A*02:21, HLA-A*02:22, or HLA-A*02:24. In some embodiments, the MHC class I protein is HLA-A*02:01. HLA-A*02:01 is expressed in 39-46% of all Caucasians, and therefore represents a suitable choice of MHC class I protein for use in the present invention.

[0344] AFP peptides suitable for use in generating anti-AMC antibody moieties can be determined, for example, based on the presence of HLA-A*02:01-binding motifs and

cleavage sites for proteasomes and immune-proteasomes using computer prediction models known to those of skill in the art. For predicting MHC binding sites, such models include, but are not limited to, IEDB (Vita *et al.*, The immune epitope database (IEDB) 3.0. *Nucleic Acids Res.* 2014 Oct 9. pii: gku938), ProPred1 (described in more detail in Singh and Raghava, *ProPred: prediction of HLA-DR binding sites. BIOINFORMATICS* 17(12):1236-1237, 2001), and SYFPEITHI (see Schuler *et al. SYFPEITHI, Database for Searching and T-Cell Epitope Prediction. in Immunoinformatics Methods in Molecular Biology*, vol 409(1): 75-93, 2007).

[0345] Once appropriate peptides have been identified, peptide synthesis may be done in accordance with protocols well known to those of skill in the art. Because of their relatively small size, the peptides of the invention may be directly synthesized in solution or on a solid support in accordance with conventional peptide synthesis techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. The synthesis of peptides in solution phase has become a well-established procedure for large-scale production of synthetic peptides and as such is a suitable alternative method of preparing the peptides of the invention (See for example, Solid Phase Peptide Synthesis by John Morrow Stewart and Martin *et al. Application of Almez-mediated Amidation Reactions to Solution Phase Peptide Synthesis*, Tetrahedron Letters Vol. 39, pages 1517-1520, 1998).

[0346] The binding activity of candidate AFP peptides can be tested using the antigen-processing-deficient T2 cell line, which increases expression of HLA-A when stabilized by a peptide in the antigen-presenting groove. T2 cells are pulsed with the candidate peptide for a time sufficient to stabilize HLA-A expression on the cell surface, which can be measured using any methods known in the art, such as by immunostaining with a fluorescently labeled monoclonal antibody specific for HLA-A (for example, BB7.2) followed by fluorescence-activated cell-sorting (FACS) analysis.

Preparation of anti-AMC antibodies and anti-AMC antibody moieties

[0347] In some embodiments, the anti-AMC antibody or anti-AMC antibody moiety is a monoclonal antibody. Monoclonal antibodies can be prepared, *e.g.,* using hybridoma methods, such as those described by Kohler and Milstein, *Nature,* 256:495 (1975) and Sergeeva *et al.*, *Blood*, 117(16):4262-4272, using the phage display methods described herein and in the Examples below, or using recombinant DNA methods (*see*, *e.g.*, US Patent No. 4,816,567).

[0348] In a hybridoma method, a hamster, mouse, or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized *in vitro.* The immunizing agent can include a polypeptide or a fusion protein of the protein of interest, or a complex comprising at least two molecules, such as a complex comprising an AFP peptide and an MHC class I protein. Generally, peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. *See, e.g.,* Goding, Monoclonal Antibodies: Principles and Practice (New York: Academic Press, 1986), pp. 59-103. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine, and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which prevents the growth of HGPRT-deficient cells.

[0349] In some embodiments, the immortalized cell lines fuse efficiently, support stable high-level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. In some embodiments, the immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies. Kozbor, *J. Immunol.,* 133:3001 (1984); Brodeur *et al.* Monoclonal Antibody Production Techniques and Applications (Marcel Dekker, Inc.: New York, 1987) pp. 51-63.

[0350] The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the polypeptide. The binding specificity of monoclonal antibodies produced by the hybridoma cells can be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in

the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, *Anal. Biochem.,* 107:220 (1980).

[0351] After the desired hybridoma cells are identified, the clones can be sub cloned by limiting dilution procedures and grown by standard methods. Goding, *supra.* Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown *in vivo* as ascites in a mammal.

[0352] The monoclonal antibodies secreted by the sub clones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[0353] The anti-AMC antibodies or antibody moieties may also be identified by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom *et al.*, *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, N.J., 2001) and further described, *e.g.*, in McCafferty *et al.*, *Nature* 348:552-554; Clackson *et al.*, *Nature* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, N.J., 2003); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0354] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.*, *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths *et al.*, *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable

CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: U.S. Pat. No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0355] The antibodies or antigen-binding fragments thereof can be prepared using phage display to screen libraries for antibodies specific to a complex comprising an AFP peptide and an MHC class I protein. The library can be a human scFv phage display library having a diversity of at least one x 10^9 (such as at least about any of 1 x 10^9, 2.5 x 10^9, 5 x 10^9, 7.5 x 10^9, 1 x 10^{10}, 2.5 x 10^{10}, 5 x 10^{10}, 7.5 x 10^{10}, or 1 x 10^{11}) unique human antibody fragments. In some embodiments, the library is a naïve human library constructed from DNA extracted from human PMBCs and spleens from healthy donors, encompassing all human heavy and light chain subfamilies. In some embodiments, the library is a naïve human library constructed from DNA extracted from PBMCs isolated from patients with various diseases, such as patients with autoimmune diseases, cancer patients, and patients with infectious diseases. In some embodiments, the library is a semi-synthetic human library, wherein heavy chain CDR3 is completely randomized, with all amino acids (with the exception of cysteine) equally likely to be present at any given position (*see, e.g.,* Hoet, R.M. *et al.*, *Nat. Biotechnol.* 23(3):344-348, 2005). In some embodiments, the heavy chain CDR3 of the semi-synthetic human library has a length from about 5 to about 24 (such as about any of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24) amino acids. In some embodiments, the library is a non-human phage display library.

[0356] Phage clones that bind to the AMC with high affinity can be selected by iterative binding of phage to the AMC, which is bound to a solid support (such as, for example, beads for solution panning or mammalian cells for cell panning), followed by removal of non-bound phage and by elution of specifically bound phage. In an example of solution panning, the AMC can be biotinylated for immobilization to a solid support. The biotinylated AMC is mixed with the phage library and a solid support, such as streptavidin-conjugated Dynabeads M-280, and then AMC-phage-bead complexes are isolated. The bound phage clones are then eluted and used to infect an appropriate host cell, such as *E. coli* XL1-Blue, for expression and purification. In an example of cell panning, T2 cells (a TAP-deficient, HLA-A*02:01$^+$ lymphoblast cell line) loaded with the AFP peptide of the AMC are mixed with the phage library, after which the cells are collected and the bound clones are eluted and used to infect

an appropriate host cell for expression and purification. The panning can be performed for multiple (such as about any of 2, 3, 4, 5, 6 or more) rounds with either solution panning, cell panning, or a combination of both, to enrich for phage clones binding specifically to the AMC. Enriched phage clones can be tested for specific binding to the AMC by any methods known in the art, including for example ELISA and FACS.

[0357] Monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures *(e.g.,* by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Hybridoma cells as described above or AMC-specific phage clones of the invention can serve as a source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains and/or framework regions in place of the homologous non-human sequences (U.S. Patent No. 4,816,567; Morrison *et al., supra)* or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a nonimmunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

[0358] The antibodies can be monovalent antibodies. Methods for preparing monovalent antibodies are known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy-chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

[0359] *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using any method known in the art.

[0360] Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant-domain sequences. The fusion

preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. In some embodiments, the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding is present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies, *see,* for example, Suresh *et al., Methods in Enzymology,* 121: 210 (1986).

Human and Humanized Antibodies

[0361] The anti-AMC antibodies or antibody moieties can be humanized antibodies or human antibodies. Humanized forms of non-human *(e.g.,* murine) antibodies are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$, scFv, or other antigen-binding subsequences of antibodies) that typically contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody can comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin, and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. In some embodiments, the humanized antibody will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. *See, e.g.*, Jones *et al.*, *Nature,* 321: 522-525 (1986); Riechmann *et al., Nature,* 332: 323-329 (1988); Presta, *Curr. Op. Struct. Biol.,* 2:593-596 (1992).

[0362] Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. According to some embodiments, humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature,* 321: 522-525 (1986); Riechmann *et al.*, *Nature,* 332: 323-327 (1988); Verhoeyen *et al.*, *Science,* 239: 1534-1536 (1988)), by

substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0363] As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals *(e.g.,* mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.,* Jakobovits *et al.*, *PNAS USA,* 90:2551 (1993); Jakobovits *et al., Nature,* 362:255-258 (1993); Bruggemann *et al.*, *Year in Immunol.,* 7:33 (1993); U.S. Patent Nos. 5,545,806, 5,569,825, 5,591,669; 5,545,807; and WO 97/17852. Alternatively, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.,* mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed that closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016, and Marks *et al., Bio/Technology,* 10: 779-783 (1992); Lonberg *et al., Nature,* 368: 856-859 (1994); Morrison, *Nature,* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnology,* 14: 845-851 (1996); Neuberger, *Nature Biotechnology,* 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.,* 13: 65-93 (1995).

[0364] Human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents 5,567,610 and 5,229,275) or by using various techniques known in the art, including phage display libraries. Hoogenboom and Winter, *J. Mol. Biol.,* 227:381 (1991); Marks *et al., J. Mol. Biol.,* 222:581 (1991). The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies. Cole *et al., Monoclonal*

Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner *et al., J. Immunol.,* 147(1): 86-95 (1991).

Multi-specific Antibodies

[0365] In some embodiments, the anti-AMC construct is a multi-specific antibody. Suitable methods for making multi-specific (*e.g.*, bispecific) antibodies are well known in the art. For example, the production of bispecific antibodies can based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two pairs each have different specificities, and upon association result in a heterodimeric antibody (*see, e.g.,* Milstein and Cuello, *Nature,* 305: 537-539 (1983); WO 93/08829, and Traunecker *et al., EMBO J.* 10: 3655 (1991)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829 and in Traunecker *et al., EMBO,* 10: 3655-3659 (1991). Alternatively, the combining of heavy and light chains can be directed by taking advantage of species-restricted pairing (*see, e.g.*, Lindhofer *et al., J. Immunol.*, 155:219-225 (1995)) and the pairing of heavy chains can be directed by use of "knob-into hole" engineering of CH3 domains (*see, e.g.,* U.S. Pat. No. 5,731,168; Ridgway *et al., Protein Eng.*, 9(7):617-621 (1996)). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (*see, e.g.,* WO 2009/089004A1). In yet another method, stable bispecific antibodies can be generated by controlled Fab-arm exchange, where two parental antibodies having distinct antigen specificity and matched point mutations in the CH3 domains are mixed in reducing condition to allow for separation, reassembly, and reoxidation to form highly pure bispecific antibodies. Labrigin *et al., Proc. Natl. Acad. Sci.*, 110(13):5145-5150 (2013). Such antibodies, comprising a mixture of heavy-chain/light-chain pairs, are also referred to herein as "heteromultimeric antibodies".

[0366] Antibodies or antigen-binding fragments thereof having different specificities can also be chemically cross-linked to generate multi-specific heteroconjugate antibodies. For example, two F(ab')2 molecules, each having specificity for a different antigen, can be chemically linked. Pullarkat *et al.*, *Trends Biotechnol.*, 48:9-21 (1999). Such antibodies have, for example, been proposed to target immune-system cells to unwanted cells (U.S. Patent No.

4,676,980), and for treatment of HIV infection. WO 91/00360; WO 92/200373; EP 03089. It is contemplated that the antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

[0367] In some embodiments, multi-specific antibodies can be prepared using recombinant DNA techniques. For example, a bispecific antibody can be engineered by fusing two scFvs, such as by fusing them through a peptide linker, resulting in a tandem scFv. One example of a tandem scFv is a bispecific T cell engager. Bispecific T cell engagers are made by linking an anti-CD3 scFv to an scFv specific for a surface antigen of a target cell, such as a tumor-associated antigen (TAA), resulting in the redirection of T cells to the target cells. Mack *et al., Proc. Natl. Acad. Sci.*, 92:7021-7025 (1995); Brischwein *et al., Mol. Immunol.*, 43(8):1129-1143 (2006). By shortening the length of a peptide linker between two variable domains, they can be prevented from self-assembling and forced to pair with domains on a second polypeptide, resulting in a compact bispecific antibody called a diabody (Db). Holliger *et al.*, *Proc. Natl. Acad. Sci.*, 90:6444-6448 (1993). The two polypeptides of a Db each comprise a VH connected to a VL by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one polypeptide are forced to pair with the complementary VL and VH domains of another polypeptide, thereby forming two antigen-binding sites. In a modification of this format, the two polypeptides are linked by another peptide linker, resulting in a single chain diabody (scDb). In yet another modification of the Db format, dual-affinity retargeting (DART) bispecific antibodies can be generated by introducing a disulfide linkage between cysteine residues at the C-terminus of each polypeptide, optionally including domains prior to the C-terminal cysteine residues that drive assembly of the desired heterodimeric structure. Veri *et al., Arthritis Rheum.*, 62(7):1933-1943 (2010). Dual-variable-domain immunoglobulins (DVD-Ig™), in which the target-binding variable domains of two monoclonal antibodies are combined via naturally occurring linkers to yield a tetravalent, bispecific antibody, are also known in the art. Gu and Ghayur, *Methods Enzymol.*, 502:25-41 (2012). In yet another format, Dock and Lock (DNL), bispecific antibodies are prepared by taking advantage of the dimerization of a peptide (DDD2) derived from the regulatory subunit of human cAMP-dependent protein kinase (PKA) with a peptide (AD2) derived from the anchoring domains of

human A kinase anchor proteins (AKAPs). Rossi *et al., Proc. Natl. Acad. Sci.*, 103:6841-6846 (2006).

[0368] Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al., J. Immunol.,* 148(5):1547-1553 (1992). This method can also be utilized for the production of antibody homodimers.

Anti-AMC variants

[0369] In some embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

[0370] In some embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

[0371] Conservative substitutions are shown in Table 5 below.

TABLE 5: CONSERVATIVE SUBSTITITIONS

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn

Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0372] Amino acids may be grouped into different classes according to common side-chain properties:

a. hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

b. neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

c. acidic: Asp, Glu;

d. basic: His, Lys, Arg;

e. residues that influence chain orientation: Gly, Pro;

f. aromatic: Trp, Tyr, Phe.

[0373] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0374] An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.,* using phage display-based affinity maturation techniques. Briefly, one or more CDR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity *(e.g.* binding affinity). Alterations *(e.g.,* substitutions) may be made in HVRs, *e.g.,* to improve antibody affinity. Such alterations may be made in HVR "hotspots," *i.e.,* residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see*, *e.g.,* Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or specificity determining residues (SDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and

reselecting from secondary libraries has been described, *e.g.,* in Hoogenboom *et al.* in *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.,* ed., Human Press, Totowa, NJ, (2001).)

[0375] In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods *(e.g.,* error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues *(e.g.,* 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.,* using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0376] In some embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In some embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0377] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex can be determined to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0378] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of

terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.* for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

Fc Region Variants

[0379] In some embodiments, one or more amino acid modifications may be introduced into the Fc region of a full-length anti-AMC antibody provided herein, thereby generating an Fc region variant. In some embodiments, the Fc region variant has enhanced antibody dependent cellular cytotoxicity (ADCC) effector function, often related to binding to Fc receptors (FcRs). In some embodiments, the Fc region variant has decreased ADCC effector function. There are many examples of changes or mutations to Fc sequences that can alter effector function. For example, WO 00/42072 and Shields *et al. J Biol. Chem.* 9(2): 6591-6604 (2001) describe antibody variants with improved or diminished binding to FcRs. The contents of those publications are specifically incorporated herein by reference.

[0380] Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) is a mechanism of action of therapeutic antibodies against tumor cells. ADCC is a cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell (*e.g.*, a cancer cell), whose membrane-surface antigens have been bound by specific antibodies (*e.g.*, an anti-AMC antibody). The typical ADCC involves activation of NK cells by antibodies. An NK cell expresses CD16 which is an Fc receptor. This receptor recognizes, and binds to, the Fc portion of an antibody bound to the surface of a target cell. The most common Fc receptor on the surface of an NK cell is called CD16 or FcγRIII. Binding of the Fc receptor to the Fc region of an antibody results in NK cell activation, release of cytolytic granules and consequent target cell apoptosis. The contribution of ADCC to tumor cell killing can be measured with a specific test that uses NK-92 cells that have been transfected with a high-affinity FcR. Results are compared to wild-type NK-92 cells that do not express the FcR.

[0381] In some embodiments, the invention contemplates an anti-AMC construct variant comprising an FC region that possesses some but not all effector functions, which makes it a desirable candidate for applications in which the half-life of the anti-AMC construct *in vivo* is important yet certain effector functions (such as CDC and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding

assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see, *e.g.* Hellstrom, I. *et al. Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I *et al.*, *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); U.S. Pat. No. 5,821,337 (see Bruggemann, M. *et al.*, *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assay methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96™ non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al. Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, *e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M. S. *et al.*, *Blood* 101:1045-1052 (2003); and Cragg, M. S. and M. J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, *e.g.*, Petkova, S. B. *et al.*, *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0382] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

[0383] Certain antibody variants with improved or diminished binding to FcRs are described. (See, *e.g.*, U.S. Pat. No. 6,737,056; WO 2004/056312, and Shields *et al.*, *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0384] In some embodiments, there is provided an anti-AMC construct (*e.g.*, a full-length anti-AMC antibody) variant comprising a variant Fc region comprising one or more amino

acid substitutions which improve ADCC. In some embodiments, the variant Fc region comprises one or more amino acid substitutions which improve ADCC, wherein the substitutions are at positions 298, 333, and/or 334 of the variant Fc region (EU numbering of residues). In some embodiments, the anti-AMC construct (*e.g.*, full-length anti-AMC antibody) variant comprises the following amino acid substitution in its variant Fc region: S298A, E333A, and K334A.

[0385] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie *et al.*, *J. Immunol.* 164: 4178-4184 (2000).

[0386] In some embodiments, there is provided an anti-AMC construct (*e.g.*, a full-length anti-AMC antibody) variant comprising a variant Fc region comprising one or more amino acid substitutions which increase half-life and/or improve binding to the neonatal Fc receptor (FcRn). Antibodies with increased half-lives and improved binding to FcRn are described in US2005/0014934A1 (Hinton *et al.*). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (U.S. Pat. No. 7,371,826).

[0387] *See* also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

[0388] Anti-AMC constructs (such as full-length anti-AMC antibodies) comprising any of the Fc variants described herein, or combinations thereof, are contemplated.

Glycosylation Variants

[0389] In some embodiments, an anti-AMC construct provided herein is altered to increase or decrease the extent to which the anti-AMC construct is glycosylated. Addition or deletion of glycosylation sites to an anti-AMC construct may be conveniently accomplished by altering the amino acid sequence of the anti-AMC construct or polypeptide portion thereof such that one or more glycosylation sites is created or removed.

[0390] Where the anti-AMC construct comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297

of the CH2 domain of the Fc region. See, *e.g.*, Wright *et al.*, *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an anti-AMC construct of the invention may be made in order to create anti-AMC construct variants with certain improved properties.

[0391] In some embodiments, anti-AMC construct (such as full-length anti-AMC antibody) variants are provided comprising an Fc region wherein a carbohydrate structure attached to the Fc region has reduced fucose or lacks fucose, which may improve ADCC function. Specifically, anti-AMC constructs are contemplated herein that have reduced fusose relative to the amount of fucose on the same anti-AMC construct produced in a wild-type CHO cell. That is, they are characterized by having a lower amount of fucose than they would otherwise have if produced by native CHO cells (*e.g.*, a CHO cell that produce a native glycosylation pattern, such as, a CHO cell containing a native FUT8 gene). In some embodiments, the anti-AMC construct is one wherein less than about 50%, 40%, 30%, 20%, 10%, or 5% of the N-linked glycans thereon comprise fucose. For example, the amount of fucose in such an anti-AMC construct may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. In some embodiments, the anti-AMC construct is one wherein none of the N-linked glycans thereon comprise fucose, *i.e.*, wherein the anti-AMC construct is completely without fucose, or has no fucose or is afucosylated. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ±3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, *e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778;

WO2005/053742; WO2002/031140; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such asα-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, *e.g.*, Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. *et al.*, *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[0392] Anti-AMC construct (such as full-length anti-AMC antibody) variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the anti-AMC construct is bisected by GlcNAc. Such anti-AMC construct (such as full-length anti-AMC antibody) variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet *et al.*); U.S. Pat. No. 6,602,684 (Umana *et al.*); US 2005/0123546 (Umana *et al.*), and Ferrara *et al.*, *Biotechnology and Bioengineering*, 93(5): 851-861 (2006). Anti-AMC construct (such as full-length anti-AMC antibody) variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such anti-AMC construct variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel *et al.*); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

[0393] In some embodiments, the anti-AMC construct (such as full-length anti-AMC antibody) variants comprising an Fc region are capable of binding to an FcγRIII. In some embodiments, the anti-AMC construct (such as full-length anti-AMC antibody) variants comprising an Fc region have ADCC activity in the presence of human effector cells or have increased ADCC activity in the presence of human effector cells compared to the otherwise same anti-AMC construct (such as full-length anti-AMC antibody) comprising a human wild-type IgG1Fc region.

Cysteine Engineered Variants

[0394] In some embodiments, it may be desirable to create cysteine engineered anti-AMC constructs (such as full-length anti-AMC antibodies) in which one or more amino acid residues are substituted with cysteine residues. In some embodiments, the substituted residues occur at accessible sites of the anti-AMC construct. By substituting those residues with

cysteine, reactive thiol groups are thereby positioned at accessible sites of the anti-AMC construct and may be used to conjugate the anti-AMC construct to other moieties, such as drug moieties or linker-drug moieties, to create an anti-AMC immunoconjugate, as described further herein. Cysteine engineered anti-AMC constructs (such as full-length anti-AMC antibodies) may be generated as described, *e.g.*, in U.S. Pat. No. 7,521,541.

Derivatives

[0395] In some embodiments, an anti-AMC construct provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the anti-AMC construct include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the anti-AMC construct may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the anti-AMC construct to be improved, whether the anti-AMC construct derivative will be used in a therapy under defined conditions, etc.

[0396] In some embodiments, conjugates of an anti-AMC construct and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In some embodiments, the nonproteinaceous moiety is a carbon nanotube (Kam *et al.*, *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the anti-AMC construct-nonproteinaceous moiety are killed.

CAR Effector Cell Preparation

[0397] The present invention in one aspect provides effector cells (such as lymphocytes, for example T cells) expressing an anti-AMC CAR. Exemplary methods of preparing effector cells (such as T cells) expressing the anti-AMC CARs (anti-AMC CAR effector cells, such as anti-AMC CAR T cells) are provided herein.

[0398] In some embodiments, an anti-AMC CAR effector cell (such as T cell) can be generated by introducing a vector (including for example a lentiviral vector) comprising an anti-AMC CAR (for example a CAR comprising an anti-AMC antibody moiety and CD28 and CD3ζ intracellular signaling sequences) into the effector cell (such as T cell). In some embodiments, the anti-AMC CAR effector cells (such as T cells) of the invention are able to replicate *in vivo*, resulting in long-term persistence that can lead to sustained control of an AFP-positive disease (such as cancer, *e.g.*, HCC).

[0399] In some embodiments, the invention relates to administering a genetically modified T cell expressing an anti-AMC CAR for the treatment of a patient having an AFP-positive disease or at risk of having an AFP-positive disease using lymphocyte infusion. In some embodiments, autologous lymphocyte infusion is used in the treatment. Autologous PBMCs are collected from a patient in need of treatment and T cells are activated and expanded using the methods described herein and known in the art and then infused back into the patient.

[0400] In some embodiments, the anti-AMC CAR T cell expresses an anti-AMC CAR comprising an anti-AMC antibody moiety (also referred to herein as an "anti-AMC CAR T cell"). In some embodiments, the anti-AMC CAR T cell expresses an anti-AMC CAR comprising an extracellular domain comprising an anti-AMC antibody moiety and an intracellular domain comprising intracellular signaling sequences of CD3ζ and CD28. The anti-AMC CAR T cells of the invention can undergo robust *in vivo* T cell expansion and can establish AMC-specific memory cells that persist at high levels for an extended amount of time in blood and bone marrow. In some embodiments, the anti-AMC CAR T cells of the invention infused into a patient can eliminate AMC-presenting cells, such as AMC-presenting cancer cells, *in vivo* in patients having an AFP-positive disease. In some embodiments, the anti-AMC CAR T cells of the invention infused into a patient can eliminate AMC-presenting cells, such as AMC-presenting cancer cells, *in vivo* in patients having an AFP-positive disease that is refractory to at least one conventional treatment.

[0401] In some embodiments, the anti-AMC CAR T cell expresses the anti-AMC CAR with even cell surface distribution. Even cell surface distribution can be characterized, for example, by staining patterns with continuous appearance and even thickness or signal intensity. For example, in some embodiments, a composition, such as a pharmaceutical composition, comprising anti-AMC CAR T cells comprises fewer than about 10 % (such as fewer than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 %) cells with aggregation of the anti-AMC CAR on the cell surface. Aggregation can be characterized, for example, by staining patterns with uneven thickness or signal intensity, or discontinuous, lumpy, punctate, and/or uneven distribution patterns. In some embodiments, the anti-AMC CAR T cell expresses the anti-AMC CAR with less than about 10 % (such as less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 %) aggregation of the anti-AMC CAR on the cell surface. In some embodiments, the anti-AMC CAR T cell has a low level of antigen-independent anti-AMC CAR activation. In some embodiments, the anti-AMC CAR T cell has a low level of T cell exhaustion. T cell exhaustion naturally occurs during conditions of extended immune activation, such as with cancer or chronic infection, where T cells become dysfunctional. T cell exhaustion may be characterized by impaired effector function, prolonged expression of inhibitory receptors, and/or an altered transcriptional state compared to functional effector or memory T cells. Optimal clearance of tumor cells and infections is prevented by T cell exhaustion. T cell exhaustion of the anti-AMC CAR T cell can be characterized by any means known in the art, for example, by determining its functional and/or phenotypic profile (Wherry, E. J., *Nature immunology* 12(6): 492-499, 2011; Jiang, Y., *et al.*, *Cell death & disease* 6(6): e1792, 2015). For example, in some embodiments, the anti-AMC CAR T cell expresses low levels of one or more markers of T cell exhaustion, including, for example, PD-1, LAG-3, TIM-3, CTLA-4, BTLA, and TIGIT. In some embodiments, the anti-AMC CAR T cell maintains levels characteristic of non-exhausted T cells for IL-2 production, TNF-α production, IFN-γ production, and granzyme B production, and/or maintains *ex vivo* killing capacity in the presence of target cells, suggesting that the anti-AMC CAR T cell is not undergoing self-activation and premature exhaustion.

[0402] Prior to expansion and genetic modification of the T cells, a source of T cells is obtained from a subject. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In some embodiments of the present invention, any number of T cell lines available in the art

may be used. In some embodiments of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In some embodiments, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In some embodiments, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as Ca^{2+}-free, Mg^{2+}-free PBS, PlasmaLyte A, or other saline solutions with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[0403] In some embodiments, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as $CD3^+$, $CD28^+$, $CD4^+$, $CD8^+$, $CD45RA^+$, and $CD45RO^+$ T cells, can be further isolated by positive or negative selection techniques. For example, in some embodiments, T cells are isolated by incubation with anti-CD3/anti-CD28 (*i.e.*, 3×28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In some embodiments, the time period is about 30 minutes. In some embodiments, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In some embodiments, the time period is at least one, 2, 3, 4, 5, or 6 hours. In some embodiments, the time period is 10 to 24 hours. In some embodiments, the incubation time period is 24 hours. For isolation of T cells from patients with leukemia, use of longer incubation times, such as 24 hours, can increase cell yield. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such as in isolating tumor infiltrating lymphocytes

(TIL) from tumor tissue or from immune-compromised individuals. Further, use of longer incubation times can increase the efficiency of capture of $CD8^+$ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention. In some embodiments, it may be desirable to perform the selection procedure and use the "unselected" cells in the activation and expansion process. "Unselected" cells can also be subjected to further rounds of selection.

[0404] Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD 14, CD20, CD11b, CD 16, HLA-DR, and CD8. In some embodiments, it may be desirable to enrich for or positively select for regulatory T cells which typically express $CD4^+$, $CD25^+$, CD62Lhi, $GITR^+$, and $FoxP3^+$. Alternatively, in some embodiments, T regulatory cells are depleted by anti-CD25 conjugated beads or other similar methods of selection.

[0405] For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (*e.g.*, particles such as beads) can be varied. In some embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (*i.e.*, increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in some embodiments, a concentration of about 2 billion cells/ml is used. In some embodiments, a concentration of about 1 billion cells/ml is used. In some embodiments, greater than about 100 million cells/ml is used. In some embodiments, a concentration of cells of about any of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In some embodiments, a concentration of cells of about any of 75, 80, 85, 90, 95, or 100 million cells/ml is used. In some embodiments, a concentration of about 125 or about 150 million cells/ml is used. Using high concentrations can result in increased cell yield, cell

activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (*i.e.*, leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of $CD8^+$ T cells that normally have weaker CD28 expression.

[0406] In some embodiments of the present invention, T cells are obtained from a patient directly following treatment. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand *ex vivo*. Likewise, following *ex vivo* manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and *in vivo* expansion. Thus, it is contemplated within the context of the present invention to collect blood cells, including T cells, dendritic cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in some embodiments, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

[0407] Whether prior to or after genetic modification of the T cells to express a desirable anti-AMC CAR, the T cells can be activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

[0408] Generally, the T cells of the invention are expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (*e.g.*, bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds

the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either $CD4^+$ T cells or $CD8^+$ T cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besançon, France) can be used as can other methods commonly known in the art (Berg *et al.*, *Transplant Proc.* 30(8):3975-3977, 1998; Haanen *et al.*, *J. Exp. Med.* 190(9):13191328, 1999; Garland *et al.*, *J. Immunol. Meth.* 227(1-2):53-63, 1999).

Immunoconjugate preparation

[0409] The anti-AMC immunoconjugates may be prepared using any methods known in the art. *See, e.g.,* WO 2009/067800, WO 2011/133886, and U.S. Patent Application Publication No. 2014322129, incorporated by reference herein in their entirety.

[0410] The anti-AMC antibody moiety of an anti-AMC immunoconjugate may be "attached to" the effector molecule by any means by which the anti-AMC antibody moiety can be associated with, or linked to, the effector molecule. For example, the anti-AMC antibody moiety of an anti-AMC immunoconjugate may be attached to the effector molecule by chemical or recombinant means. Chemical means for preparing fusions or conjugates are known in the art and can be used to prepare the anti-AMC immunoconjugate. The method used to conjugate the anti-AMC antibody moiety and effector molecule must be capable of joining the binding protein with the effector molecule without interfering with the ability of the binding protein to bind to the antigen on the target cell.

[0411] The anti-AMC antibody moiety of an anti-AMC immunoconjugate may be linked indirectly to the effector molecule. For example, the anti-AMC antibody moiety of an anti-AMC immunoconjugate may be directly linked to a liposome containing the effector molecule of one of several types. The effector molecule(s) and/or the anti-AMC antibody moiety may also be bound to a solid surface.

[0412] In some embodiments, the anti-AMC antibody moiety of an anti-AMC immunoconjugate and the effector molecule are both proteins and can be conjugated using techniques well known in the art. There are several hundred crosslinkers available that can conjugate two proteins. (See for example "Chemistry of Protein Conjugation and Crosslinking". 1991 , Shans Wong, CRC Press, Ann Arbor). The crosslinker is generally

chosen based on the reactive functional groups available or inserted on the anti-AMC antibody moiety and/or effector molecule. In addition, if there are no reactive groups, a photoactivatible crosslinker can be used. In certain instances, it may be desirable to include a spacer between the anti-AMC antibody moiety and the effector molecule. Crosslinking agents known to the art include the homobifunctional agents: glutaraldehyde, dimethyladipimidate and Bis(diazobenzidine) and the heterobifunctional agents: m Maleimidobenzoyl-N-Hydroxysuccinimide and Sulfo-m Maleimidobenzoyl-N-Hydroxysuccinimide.

[0413] In some embodiments, the anti-AMC antibody moiety of an anti-AMC immunoconjugate may be engineered with specific residues for chemical attachment of the effector molecule. Specific residues used for chemical attachment of molecule known to the art include lysine and cysteine. The crosslinker is chosen based on the reactive functional groups inserted on the anti-AMC antibody moiety, and available on the effector molecule.

[0414] An anti-AMC immunoconjugate may also be prepared using recombinant DNA techniques. In such a case a DNA sequence encoding the anti-AMC antibody moiety is fused to a DNA sequence encoding the effector molecule, resulting in a chimeric DNA molecule. The chimeric DNA sequence is transfected into a host cell that expresses the fusion protein. The fusion protein can be recovered from the cell culture and purified using techniques known in the art.

[0415] Examples of attaching an effector molecule, which is a label, to the binding protein include the methods described in Hunter, *et al.*, *Nature* 144:945 (1962); David, *et al.*, *Biochemistry* 13:1014 (1974); Pain, *et al.*, *J. Immunol. Meth.* 40:219 (1981); Nygren, J. *Histochem. and Cytochem.* 30:407 (1982); Wensel and Meares, *Radioimmunoimaging And Radioimmunotherapy*, Elsevier, N.Y. (1983); and Colcher *et al.*, "Use Of Monoclonal Antibodies As Radiopharmaceuticals For The Localization Of Human Carcinoma Xenografts In Athymic Mice", *Meth. Enzymol.*, 121:802-16 (1986).

[0416] The radio- or other labels may be incorporated in the immunoconjugate in known ways. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as ^{99}Tc or ^{123}I, ^{186}Re, ^{188}Re and ^{111}In can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker *et al.*, *Biochem. Biophys. Res. Commun.* 80:49-57 (1978)) can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

[0417] Immunoconjugates of the antibody moiety and a cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1 -carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCI), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)- ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1 ,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene tnaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, *e.g.*, WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari *et al.*, *Cancer Research* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

[0418] The anti-AMC immunoconjugates of the invention expressly contemplate, but are not limited to, ADC prepared with cross-linker reagents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (*e.g.*, from Pierce Biotechnology, Inc., Rockford, IL, U.S.A). See pages 467-498, 2003-2004 Applications Handbook and Catalog.

Pharmaceutical Compositions

[0419] Also provided herein are compositions (such as pharmaceutical compositions, also referred to herein as formulations) comprising an anti-AMC construct. In some embodiments, the composition further comprises a cell (such as an effector cell, *e.g.*, a T cell) associated with the anti-AMC construct. In some embodiments, there is provided a pharmaceutical composition comprising an anti-AMC construct and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition further comprises a cell (such as an effector cell, *e.g.*, a T cell) associated with the anti-AMC construct.

[0420] Suitable formulations of the anti-AMC constructs are obtained by mixing an anti-AMC construct having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers *(Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propylparaben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as olyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes *(e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Exemplary formulations are described in WO98/56418, expressly incorporated herein by reference. Lyophilized formulations adapted for subcutaneous administration are described in WO97/04801. Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the individual to be treated herein. Lipofectins or liposomes can be used to deliver the anti-AMC constructs of this invention into cells.

[0421] The formulation herein may also contain one or more active compounds in addition to the anti-AMC construct as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, or a chemotherapeutic agent in addition to the anti-AMC construct. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of anti-AMC construct present in the formulation, the type of disease or disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with

administration routes as described herein or about from 1 to 99% of the heretofore employed dosages.

[0422] The anti-AMC constructs may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980). Sustained-release preparations may be prepared.

[0423] Sustained-release preparations of the anti-AMC constructs can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody (or fragment thereof), which matrices are in the form of shaped articles, *e.g.,* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT TM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydro gels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they can denature or aggregate as a result of exposure to moisture at 37 °C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization of anti-AMC constructs depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization can be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[0424] In some embodiments, the anti-AMC construct is formulated in a buffer comprising a citrate, NaCl, acetate, succinate, glycine, polysorbate 80 (Tween 80), or any combination of the foregoing. In some embodiments, the anti-AMC construct is formulated in a buffer comprising about 100 mM to about 150 mM glycine. In some embodiments, the anti-AMC construct is formulated in a buffer comprising about 50mM to about 100 mM NaCl. In some

embodiments, the anti-AMC construct is formulated in a buffer comprising about 10mM to about 50 mM acetate. In some embodiments, the anti-AMC construct is formulated in a buffer comprising about 10mM to about 50 mM succinate. In some embodiments, the anti-AMC construct is formulated in a buffer comprising about 0.005% to about 0.02% polysorbate 80. In some embodiments, the anti-AMC construct is formulated in a buffer having a pH between about 5.1 and 5.6. In some embodiments, the anti-AMC construct is formulated in a buffer comprising 10 mM citrate, 100 mM NaCl, 100mM glycine, and 0.01% polysorbate 80, wherein the formulation is at pH 5.5.

[0425] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by, *e.g.*, filtration through sterile filtration membranes.

Methods for treatment using anti-AMC constructs

[0426] The anti-AMC constructs and/or compositions of the invention can be administered to individuals (*e.g.*, mammals such as humans) to treat a disease and/or disorder involving abnormally high AFP expression (also referred to herein as an "AFP-positive" disease or disorder), including, for example, cancer (such as hepatocellular carcinoma, germ cell tumor, and breast cancer). The present application thus in some embodiments provides a method of treating an AFP-positive disease (such as cancer) in an individual comprising administering to the individual an effective amount of a composition (such as a pharmaceutical composition) comprising an anti-AMC construct comprising an anti-AMC antibody moiety, such as any one of the anti-AMC constructs described herein. In some embodiments, the composition further comprises a cell (such as an effector cell) associated with the anti-AMC construct. In some embodiments, the cancer is selected, for example, from the group consisting of hepatocellular carcinoma, germ cell tumor, and breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0427] For example, in some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC

class I protein. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell) associated with the anti-AMC construct. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0428] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP158 peptide (SEQ ID NO: 4) and HLA-A*02:01. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell) associated with the anti-AMC construct. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0429] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a

composition comprising an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the anti-AMC antibody moiety comprises: i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell) associated with the anti-AMC construct. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0430] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the anti-AMC antibody moiety comprises: i) a heavy chain variable domain

sequence comprising an HC-CDR1 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 an LC-CDR1 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); wherein X can be any amino acid. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell) associated with the anti-AMC construct. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0431] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the anti-AMC antibody moiety comprises: i) a heavy chain variable domain sequence comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 57-66, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 67-76, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 77-86; or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a light chain variable domain sequence comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 90-99, or a variant thereof comprising up to about 5 (for example

about any of 1, 2, 3, 4, or 5) amino acid substitutions; an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 100-109, or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions; and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 110-119; or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell) associated with the anti-AMC construct. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0432] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the anti-AMC antibody moiety comprises: i) a heavy chain variable domain sequence 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; or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a light chain variable domain sequence 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; or a variant thereof comprising up to about 5 (for example about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC

construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell) associated with the anti-AMC construct. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0433] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, wherein the anti-AMC antibody moiety comprises: i) a heavy chain variable domain sequence 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 sequence 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. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell) associated with the anti-AMC construct. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as

preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0434] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MCH class I protein, wherein the anti-AMC antibody moiety comprises 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell) associated with the anti-AMC construct. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0435] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an anti-AMC construct comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MCH class I protein, wherein the anti-AMC antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct

is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell) associated with the anti-AMC construct. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0436] In some embodiments, there is provided a method of treating metastatic hepatocellular carcinoma in an individual comprising administering to the individual an effective amount of a composition comprising an anti-AMC construct according to any of the embodiments described above. In some embodiments, the individual is human.

[0437] In some embodiments, there is provided a method of inhibiting (such as preventing) metastasis of hepatocellular carcinoma in an individual comprising administering to the individual an effective amount of a composition comprising an anti-AMC construct according to any of the embodiments described above. In some embodiments, the individual is human.

[0438] In some embodiments of any of the methods for treating an AFP-positive disease described above, the anti-AMC construct is conjugated to a cell (such as an immune cell, *e.g.*, a T cell) prior to being administered to the individual. Thus, for example, there is provided a method of treating an AFP-positive disease in an individual comprising a) conjugating any one of the anti-AMC constructs described herein to a cell (such as an immune cell, *e.g.*, a T cell) to form an anti-AMC construct/cell conjugate, and b) administering to the individual an effective amount of a composition comprising the anti-AMC construct/cell conjugate. In some embodiments, the cell is derived from the individual. In some embodiments, the cell is not derived from the individual. In some embodiments, the anti-AMC construct is conjugated to the cell by covalent linkage to a molecule on the surface of the cell. In some embodiments, the anti-AMC construct is conjugated to the cell by non-covalent linkage to a molecule on the surface of the cell. In some embodiments, the anti-AMC construct is conjugated to the cell by insertion of a portion of the anti-AMC construct into the outer membrane of the cell. In some embodiments, the anti-AMC construct is non-naturally occurring. In some embodiments, the

anti-AMC construct is a full-length antibody. In some embodiments, the anti-AMC construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-AMC construct is a chimeric antigen receptor. In some embodiments, the anti-AMC construct is an immunoconjugate. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0439] In some embodiments, the individual is a mammal (*e.g.*, human, non-human primate, rat, mouse, cow, horse, pig, sheep, goat, dog, cat, etc.). In some embodiments, the individual is a human. In some embodiments, the individual is a clinical patient, a clinical trial volunteer, an experimental animal, etc. In some embodiments, the individual is younger than about 60 years old (including for example younger than about any of 50, 40, 30, 25, 20, 15, or 10 years old). In some embodiments, the individual is older than about 60 years old (including for example older than about any of 70, 80, 90, or 100 years old). In some embodiments, the individual is diagnosed with or genetically prone to one or more of the diseases or disorders described herein (such as hepatocellular carcinoma, germ cell tumor, and breast cancer). In some embodiments, the individual has one or more risk factors associated with one or more diseases or disorders described herein.

[0440] The present application in some embodiments provides a method of delivering an anti-AMC construct (such as any one of the anti-AMC constructs described herein) to a cell presenting on its surface a complex comprising an AFP peptide and an MHC class I protein in an individual, the method comprising administering to the individual a composition comprising the anti-AMC construct. In some embodiments, the anti-AMC construct to be delivered is associated with a cell (such as an effector cell, *e.g.*, a T cell).

[0441] Many diagnostic methods for cancer (such as hepatocellular carcinoma, germ cell tumor, and breast cancer) or any other disease exhibiting abnormal AFP expression and the clinical delineation of those diseases are known in the art. Such methods include, but are not limited to, *e.g.*, immunohistochemistry, PCR, and fluorescent in situ hybridization (FISH).

[0442] In some embodiments, the anti-AMC constructs and/or compositions of the invention are administered in combination with a second, third, or fourth agent (including,

e.g., an antineoplastic agent, a growth inhibitory agent, a cytotoxic agent, or a chemotherapeutic agent) to treat diseases or disorders involving abnormal AFP expression. In some embodiments, the anti-AMC construct is administered in combination with an agent that increases the expression of MHC class I proteins and/or enhances the surface presentation of AFP peptides by MHC class I proteins. In some embodiments, the agent includes, for example, IFN receptor agonists, Hsp90 inhibitors, enhancers of p53 expression, and chemotherapeutic agents. In some embodiments, the agent is an IFN receptor agonist including, for example, IFNγ, IFNβ, and IFNα. In some embodiments, the agent is an Hsp90 inhibitor including, for example, tanespimycin (17-AAG), alvespimycin (17-DMAG), retaspimycin (IPI-504), IPI-493, CNF2024/BIIB021, MPC-3100, Debio 0932 (CUDC-305), PU-H71, Ganetespib (STA-9090), NVP-AUY922 (VER-52269), HSP990, KW-2478, AT13387, SNX-5422, DS-2248, and XL888. In some embodiments, the agent is an enhancer of p53 expression including, for example, 5-fluorouracil and nutlin-3. In some embodiments, the agent is a chemotherapeutic agent including, for example, topotecan, etoposide, cisplatin, paclitaxel, and vinblastine.

[0443] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual, wherein the cells expressing AFP do not normally present, or present at relatively low levels, a complex comprising an AFP protein and an MHC class I protein on their surface (such as germ cell tumor cells), the method comprising administering to the individual a composition comprising an anti-AMC construct in combination with an agent that increases the expression of MHC class I proteins and/or enhances the surface presentation of AFP peptides by MHC class I proteins. In some embodiments, the agent includes, for example, IFN receptor agonists, Hsp90 inhibitors, enhancers of p53 expression, and chemotherapeutic agents. In some embodiments, the agent is an IFN receptor agonist including, for example, IFNγ, IFNβ, and IFNα. In some embodiments, the agent is an Hsp90 inhibitor including, for example, tanespimycin (17-AAG), alvespimycin (17-DMAG), retaspimycin (IPI-504), IPI-493, CNF2024/BIIB021, MPC-3100, Debio 0932 (CUDC-305), PU-H71, Ganetespib (STA-9090), NVP-AUY922 (VER-52269), HSP990, KW-2478, AT13387, SNX-5422, DS-2248, and XL888. In some embodiments, the agent is an enhancer of p53 expression including, for example, 5-fluorouracil and nutlin-3. In some embodiments, the agent is a chemotherapeutic agent including, for example, topotecan, etoposide, cisplatin, paclitaxel, and vinblastine.

[0444] Cancer treatments can be evaluated by, *e.g.,* tumor regression, tumor weight or size shrinkage, time to progression, duration of survival, progression free survival, overall response rate, duration of response, quality of life, protein expression and/or activity. Approaches to determining efficacy of the therapy can be employed, including for example, measurement of response through radiological imaging.

[0445] In some embodiments, the efficacy of treatment is measured as the percentage tumor growth inhibition (% TGI), calculated using the equation 100-(T/C x 100), where T is the mean relative tumor volume of the treated tumor, and C is the mean relative tumor volume of a non-treated tumor. In some embodiments, the %TGI is about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, or more than 95%.

Dosing and Method of Administering the anti-AMC construct Compositions

[0446] The dose of the anti-AMC construct compositions administered to an individual (such as a human) may vary with the particular composition, the mode of administration, and the type of disease being treated. In some embodiments, the amount of the composition is effective to result in an objective response (such as a partial response or a complete response). In some embodiments, the amount of the anti-AMC construct composition is sufficient to result in a complete response in the individual. In some embodiments, the amount of the anti-AMC construct composition is sufficient to result in a partial response in the individual. In some embodiments, the amount of the anti-AMC construct composition administered (for example when administered alone) is sufficient to produce an overall response rate of more than about any of 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 64%, 65%, 70%, 75%, 80%, 85%, or 90% among a population of individuals treated with the anti-AMC construct composition. Responses of an individual to the treatment of the methods described herein can be determined, for example, based on RECIST levels.

[0447] In some embodiments, the amount of the composition is sufficient to prolong progress-free survival of the individual. In some embodiments, the amount of the composition is sufficient to prolong overall survival of the individual. In some embodiments, the amount of the composition (for example when administered along) is sufficient to produce clinical benefit of more than about any of 50%, 60%, 70%, or 77% among a population of individuals treated with the anti-AMC construct composition.

[0448] In some embodiments, the amount of the composition, alone or in combination with a second, third, and/or fourth agent, is an amount sufficient to decrease the size of a tumor, decrease the number of cancer cells, or decrease the growth rate of a tumor by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100% compared to the corresponding tumor size, number of cancer cells, or tumor growth rate in the same subject prior to treatment or compared to the corresponding activity in other subjects not receiving the treatment. Standard methods can be used to measure the magnitude of this effect, such as *in vitro* assays with purified enzyme, cell-based assays, animal models, or human testing.

[0449] In some embodiments, the amount of the anti-AMC construct (*e.g.*, full-length anti-AMC antibody, multi-specific anti-AMC molecule, anti-AMC CAR, or anti-AMC immunoconjugate) in the composition is below the level that induces a toxicological effect (*i.e.*, an effect above a clinically acceptable level of toxicity) or is at a level where a potential side effect can be controlled or tolerated when the composition is administered to the individual.

[0450] In some embodiments, the amount of the composition is close to a maximum tolerated dose (MTD) of the composition following the same dosing regimen. In some embodiments, the amount of the composition is more than about any of 80%, 90%, 95%, or 98% of the MTD.

[0451] In some embodiments, the amount of an anti-AMC construct (*e.g.*, full-length anti-AMC antibody, multi-specific anti-AMC molecule, anti-AMC CAR, or anti-AMC immunoconjugate) in the composition is included in a range of about 0.001 μg to about 1000 μg.

[0452] In some embodiments of any of the above aspects, the effective amount of an anti-AMC construct (*e.g.*, full-length anti-AMC antibody, multi-specific anti-AMC molecule, anti-AMC CAR, or anti-AMC immunoconjugate) in the composition is in the range of about 0.1 μg/kg to about 100 mg/kg of total body weight.

[0453] The anti-AMC construct compositions can be administered to an individual (such as human) via various routes, including, for example, intravenous, intra-arterial, intraperitoneal, intrapulmonary, oral, inhalation, intravesicular, intramuscular, intra-tracheal, subcutaneous, intraocular, intrathecal, transmucosal, and transdermal. In some embodiments, sustained continuous release formulation of the composition may be used. In some embodiments, the composition is administered intravenously. In some embodiments, the composition is

administered intraportally. In some embodiments, the composition is administered intraarterially. In some embodiments, the composition is administered intraperitoneally. In some embodiments, the composition is administered intrahepatically. In some embodiments, the composition is administered by hepatic arterial infusion.

Anti-AMC CAR Effector Cell Therapy

[0454] The present application also provides methods of using an anti-AMC CAR to redirect the specificity of an effector cell (such as a primary T cell) to a complex comprising an AFP peptide and an MHC class I protein. Thus, the present invention also provides a method of stimulating an effector cell-mediated response (such as a T cell-mediated immune response) to a target cell population or tissue comprising AMC-presenting cells in a mammal, comprising the step of administering to the mammal an effector cell (such as a T cell) that expresses an anti-AMC CAR.

[0455] Anti-AMC CAR effector cells (such as T cells) expressing the anti-AMC CAR can be infused to a recipient in need thereof. The infused cell is able to kill AMC-presenting cells in the recipient. In some embodiments, unlike antibody therapies, anti-AMC CAR effector cells (such as T cells) are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained tumor control.

[0456] In some embodiments, the anti-AMC CAR effector cells are anti-AMC CAR T cells that can undergo robust *in vivo* T cell expansion and can persist for an extended amount of time. In some embodiments, the anti-AMC CAR T cells of the invention develop into specific memory T cells that can be reactivated to inhibit any additional tumor formation or growth.

[0457] The anti-AMC CAR T cells of the invention may also serve as a type of vaccine for *ex vivo* immunization and/or *in vivo* therapy in a mammal. In some embodiments, the mammal is a human.

[0458] With respect to *ex vivo* immunization, of least one of the following occurs *in vitro* prior to administering the cell into a mammal: i) expansion of the cells, ii) introducing a nucleic acid encoding an anti-AMC CAR to the cells, and/or iii) cryopreservation of the cells.

[0459] *Ex vivo* procedures are well known in the art and are discussed more fully below. Briefly, cells are isolated from a mammal (preferably a human) and genetically modified (*i.e.*, transduced or transfected *in vitro*) with a vector expressing an anti-AMC CAR disclosed herein. The anti-AMC CAR cell can be administered to a mammalian recipient to provide a therapeutic benefit. The mammalian recipient may be a human and the anti-AMC CAR cell

can be autologous with respect to the recipient. Alternatively, the cells can be allogeneic, syngeneic or xenogeneic with respect to the recipient.

[0460] The procedure for *ex vivo* expansion of hematopoietic stem and progenitor cells is described in U.S. Pat. No. 5,199,942, incorporated herein by reference, can be applied to the cells of the present invention. Other suitable methods are known in the art, therefore the present invention is not limited to any particular method of *ex vivo* expansion of the cells. Briefly, *ex vivo* culture and expansion of T cells comprises: (1) collecting $CD34^+$ hematopoietic stem and progenitor cells from a mammal from peripheral blood harvest or bone marrow explants; and (2) expanding such cells *ex vivo*. In addition to the cellular growth factors described in U.S. Pat. No. 5,199,942, other factors such as flt3-L, IL-1, IL-3 and c-kit ligand, can be used for culturing and expansion of the cells.

[0461] In addition to using a cell-based vaccine in terms of *ex vivo* immunization, the present invention also provides compositions and methods for *in vivo* immunization to elicit an immune response directed against an antigen in a patient.

[0462] The anti-AMC CAR effector cells (such as T cells) of the present invention may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations. Briefly, pharmaceutical compositions of the present invention may comprise anti-AMC CAR effector cells (such as T cells), in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. In some embodiments, anti-AMC CAR effector cell (such as T cell) compositions are formulated for intravenous administration.

[0463] The precise amount of the anti-AMC CAR effector cell (such as T cell) compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). In some embodiments, a pharmaceutical composition comprising the anti-AMC CAR effector cells (such as T cells) is administered at a dosage of about 10^4 to about 10^9 cells/kg body weight, such any of about 10^4 to about 10^5, about 10^5 to about 10^6, about 10^6 to about 10^7, about 10^7 to about 10^8, or about 10^8 to about 10^9 cells/kg body weight, including all integer values within those ranges. Anti-AMC CAR

effect cell (such as T cell) compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, *e.g.*, Rosenberg *et al.*, New Eng. J. of Med. 319:1676, 1988). The optimal dosage and treatment regimen for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[0464] In some embodiments, it may be desired to administer activated anti-AMC CAR T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate T cells therefrom according to the present invention, and reinfuse the patient with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In some embodiments, T cells can be activated from blood draws of from 10 cc to 400 cc. In some embodiments, T cells are activated from blood draws of 20 cc, 30 cc, 40 cc, 50 cc, 60 cc, 70 cc, 80 cc, 90 cc, or 100 cc.

[0465] The administration of the anti-AMC CAR effector cells (such as T cells) may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In some embodiments, the anti-AMC CAR effector cell (such as T cell) compositions of the present invention are administered to a patient by intradermal or subcutaneous injection. In some embodiments, the anti-AMC CAR effector cell (such as T cell) compositions of the present invention are administered by i.v. injection. The compositions of anti-AMC CAR effector cells (such as T cells) may be injected directly into a tumor, lymph node, or site of infection.

[0466] Thus, for example, in some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01. In some embodiments, the AFP-positive disease is cancer. In some embodiments,

the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the individual is human.

[0467] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP158 peptide (SEQ ID NO: 4) and HLA-A*02:01, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the individual is human.

[0468] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the individual is human.

[0469] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 an LC-CDR1 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); wherein X can be any amino acid, b) a transmembrane domain, and c) an intracellular

signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the individual is human.

[0470] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain comprising an HC-CDR1 comprising 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 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 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 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 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 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; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In

some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the individual is human.

[0471] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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; 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 sequences; and ii) a light chain variable domain sequence 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; 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 sequences; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the individual is human.

[0472] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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 sequence 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; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the individual is human.

[0473] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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; b) a transmembrane domain, and c) an

intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the individual is human.

[0474] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the individual is human.

[0475] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein, b) a

transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence, wherein the administering comprises local injection of the composition at an injection site distal to a site of the AFP-positive disease (such as an AFP-positive tumor). In some embodiments, the injection site is a first AFP-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the injection site is a first AFP-positive tumor and the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the AFP peptide is AFP158 (SEQ ID NO: 4). In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is HLA-A*02:01. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0476] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP158 peptide (SEQ ID NO: 4) and HLA-A*02:01, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence, wherein the administering comprises local injection of the composition at an injection site distal to a site of the AFP-positive disease (such as an AFP-positive tumor). In some embodiments, the injection site is a first AFP-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the injection site is a first AFP-positive tumor and the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as

preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0477] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 (for example 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; and ii) a light chain variable domain comprising an LC-CDR1 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 (for example 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 (for example about any of 1, 2, or 3) amino acid substitutions; wherein X can be any amino acid, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence, wherein the administering comprises local injection of the composition at an injection site distal to a site of the AFP-positive disease (such as an AFP-positive tumor). In some embodiments, the injection site is a first AFP-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the injection site is a first AFP-positive tumor and the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*,

inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0478] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence comprising an HC-CDR1 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 an LC-CDR1 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); wherein X can be any amino acid, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence, wherein the administering comprises local injection of the composition at an injection site distal to a site of the AFP-positive disease (such as an AFP-positive tumor). In some embodiments, the injection site is a first AFP-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the injection site is a first AFP-positive tumor and the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0479] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein

comprising i) a heavy chain variable domain comprising an HC-CDR1 comprising 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 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 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 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 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 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; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence, wherein the administering comprises local injection of the composition at an injection site distal to a site of the AFP-positive disease (such as an AFP-positive tumor). In some embodiments, the injection site is a first AFP-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the injection site is a first AFP-positive tumor and the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the individual is human.

[0480] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein

comprising i) a heavy chain variable domain sequence 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; 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 sequences; and ii) a light chain variable domain sequence 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; 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 sequences; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence, wherein the administering comprises local injection of the composition at an injection site distal to a site of the AFP-positive disease (such as an AFP-positive tumor). In some embodiments, the injection site is a first AFP-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the injection site is a first AFP-positive tumor and the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0481] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) a heavy chain variable domain sequence 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

sequence 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; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence, wherein the administering comprises local injection of the composition at an injection site distal to a site of the AFP-positive disease (such as an AFP-positive tumor). In some embodiments, the injection site is a first AFP-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the injection site is a first AFP-positive tumor and the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0482] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising i) 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% (for example at least about any of 96%, 97%, 98%, or 99%) sequence identity, and 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; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence, wherein the administering comprises local injection of the composition at an injection site distal to a site of the AFP-positive disease (such as an AFP-positive tumor). In some embodiments, the injection site is a first AFP-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the injection site is a

first AFP-positive tumor and the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0483] In some embodiments, there is provided a method of treating an AFP-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR comprising a) an extracellular domain comprising an anti-AMC antibody moiety that specifically binds to a complex comprising an AFP peptide and an MHC class I protein comprising a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36; b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence, wherein the administering comprises local injection of the composition at an injection site distal to a site of the AFP-positive disease (such as an AFP-positive tumor). In some embodiments, the injection site is a first AFP-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the injection site is a first AFP-positive tumor and the site of the AFP-positive disease is a second AFP-positive tumor. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0484] In some embodiments, there is provided a method of treating metastatic hepatocellular carcinoma in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR according to any of the embodiments described above. In some embodiments, the individual is human.

[0485] In some embodiments, there is provided a method of inhibiting (such as preventing) metastasis of hepatocellular carcinoma in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR according to any of the embodiments described above. In some embodiments, the individual is human.

[0486] In some embodiments, there is provided a method of priming T cells in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-AMC CAR according to any of the anti-AMC CARs described above. In some embodiments, the individual has an AFP-positive disease. In some embodiments, the AFP-positive disease is cancer. In some embodiments, the cancer is, for example, hepatocellular carcinoma, germ cell tumor, or breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the individual is human.

[0487] In some embodiments, according to any of the methods described above, the method further comprising administering antigen presenting cells, or APCs, (such as monocytes or monocyte-differentiated dendritic cells) to the individual. Dendritic cells can be generated ex vivo via culturing monocytes with specific cytokines (Palucka and Banchereau, *Nature Reviews Cancer 12:265-277, 2012*). In some embodiments, the APCs are administered simultaneously with the effector cell composition. In some embodiments, the APCs are administered concurrently with the effector cell composition. In some embodiments, the APCs are administered sequentially with the effector cell composition. In some embodiments, the APCs are administered via the same route as the effector cell composition. In some embodiments, the APCs are administered to the same site as the effector cell composition. In some embodiments, the effector cell composition comprises the APCs.

Cancers

[0488] The anti-AMC constructs and anti-AMC CAR cells in some embodiments can be useful for treating cancer. Cancers that may be treated using any of the methods described herein include tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. The cancers may comprise non-solid tumors (such as hematological

tumors, for example, leukemias and lymphomas) or may comprise solid tumors. Types of cancers to be treated with the anti-AMC constructs and anti-AMC CAR cells of the invention include, but are not limited to, carcinoma, blastoma, and sarcoma, and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies *e.g.*, sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

[0489] Hematologic cancers are cancers of the blood or bone marrow. Examples of hematological (or hematogenous) cancers include leukemias, including acute leukemias (such as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma (indolent and high grade forms), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia.

[0490] Solid tumors are abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumors can be benign or malignant. Different types of solid tumors are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). Examples of solid tumors, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, seminoma, bladder carcinoma, melanoma, and CNS tumors (such as a glioma (such as brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme) astrocytoma, CNS lymphoma, germinoma, medulloblastoma, Schwannoma craniopharyogioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, neuroblastoma, retinoblastoma and brain metastases).

[0491] In some embodiments, the cancer is HCC. In some embodiments, the HCC is early stage HCC, non-metastatic HCC, primary HCC, advanced HCC, locally advanced HCC, metastatic HCC, HCC in remission, or recurrent HCC. In some embodiments, the HCC is localized resectable (*i.e.*, tumors that are confined to a portion of the liver that allows for complete surgical removal), localized unresectable (*i.e.*, the localized tumors may be unresectable because crucial blood vessel structures are involved or because the liver is impaired), or unresectable (*i.e.*, the tumors involve all lobes of the liver and/or has spread to involve other organs (*e.g.*, lung, lymph nodes, bone). In some embodiments, the HCC is, according to TNM classifications, a stage I tumor (single tumor without vascular invasion), a stage II tumor (single tumor with vascular invasion, or multiple tumors, none greater than 5 cm), a stage III tumor (multiple tumors, any greater than 5 cm, or tumors involving major branch of portal or hepatic veins), a stage IV tumor (tumors with direct invasion of adjacent organs other than the gallbladder, or perforation of visceral peritoneum), N1 tumor (regional lymph node metastasis), or M1 tumor (distant metastasis). In some embodiments, the HCC is, according to AJCC (American Joint Commission on Cancer) staging criteria, stage T1, T2, T3, or T4 HCC. In some embodiments, the HCC is any one of liver cell carcinomas, fibrolamellar variants of HCC, and mixed hepatocellular cholangiocarcinomas.

[0492] In some embodiments, the cancer is a germ cell tumor.

[0493] Cancer treatments can be evaluated by, *e.g.*, tumor regression, tumor weight or size shrinkage, time to progression, duration of survival, progression free survival, overall response rate, duration of response, quality of life, protein expression and/or activity. Approaches to determining efficacy of the therapy can be employed, including for example, measurement of response through radiological imaging.

Methods for Diagnosis and Imaging Using anti-AMC constructs

[0494] Labeled anti-AMC antibody moieties and derivatives and analogs thereof, which specifically bind to an AMC on the surface of a cell, can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or disorders associated with the expression, aberrant expression and/or activity of AFP, including any of the diseases and disorders described above, such as cancer (*e.g.*, hepatocellular carcinoma, germ cell tumor, or breast cancer). For example, the anti-AMC antibody moieties of the invention can be used in *in situ, in vivo, ex vivo,* and *in vitro* diagnostic assays or imaging assays.

[0495] Additional embodiments of the invention include methods of diagnosing a disease or disorder associated with expression or aberrant expression of AFP in an individual *(e.g.,* a mammal, such as a human). The methods comprise detecting AMC-presenting cells in the individual. In some embodiments, there is provided a method of diagnosing a disease or disorder associated with expression or aberrant expression of AFP in an individual *(e.g.,* a mammal, such as a human) comprising (a) administering an effective amount of a labeled anti-AMC antibody moiety according to any of the embodiments described above to the individual; and (b) determining the level of the label in the individual, such that a level of the label above a threshold level indicates that the individual has the disease or disorder. The threshold level can be determined by various methods, including, for example, by detecting the label according to the method of diagnosing described above in a first set of individuals that have the disease or disorder and a second set of individuals that do not have the disease or disorder, and setting the threshold to a level that allows for discrimination between the first and second sets. In some embodiments, the threshold level is zero, and the method comprises determining the presence or absence of the label in the individual. In some embodiments, the method further comprises waiting for a time interval following the administering of step (a) to permit the labeled anti-AMC antibody moiety to preferentially concentrate at sites in the individual where the AMC is expressed (and for unbound labeled anti-AMC antibody moiety to be cleared). In some embodiments, the method further comprises subtracting a background level of the label. Background level can be determined by various methods, including, for example, by detecting the label in the individual prior to administration of the labeled anti-AMC antibody moiety, or by detecting the label according to the method of diagnosing described above in an individual that does not have the disease or disorder. In some embodiments, the disease or disorder is cancer. In some embodiments, the cancer is selected, for example, from the group consisting of hepatocellular carcinoma, germ cell tumor, and breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the cancer is metastatic hepatocellular carcinoma, and the method further comprises determining the level of the label in the individual's blood. In some embodiments, the individual is human.

[0496] In some embodiments, there is provided a method of diagnosing metastatic hepatocellular carcinoma in an individual *(e.g.,* a mammal, such as a human), comprising (a) administering an effective amount of a labeled anti-AMC antibody moiety according to any

of the embodiments described above to the individual; and (b) determining the level of the label in the individual's blood, such that a level of the label above a threshold level indicates that the individual has metastatic hepatocellular carcinoma. The threshold level can be determined by various methods, including, for example, by detecting the label according to the method of diagnosing described above in a first set of individuals that have metastatic hepatocellular carcinoma and a second set of individuals that do not have metastatic hepatocellular carcinoma, and setting the threshold to a level that allows for discrimination between the first and second sets. In some embodiments, the threshold level is zero, and the method comprises determining the presence or absence of the label in the individual's blood. In some embodiments, the method further comprises waiting for a time interval following the administering of step (a) to permit the labeled anti-AMC antibody moiety to preferentially concentrate at sites in the individual where the AMC is expressed (and for unbound labeled anti-AMC antibody moiety to be cleared). In some embodiments, the method further comprises subtracting a background level of the label. Background level can be determined by various methods, including, for example, by detecting the label in the individual prior to administration of the labeled anti-AMC antibody moiety, or by detecting the label according to the method of diagnosing described above in an individual that does not have metastatic hepatocellular carcinoma. In some embodiments, the individual is human.

[0497] In some embodiments, there is provided a method of diagnosing a disease or disorder associated with expression or aberrant expression of AFP in an individual *(e.g.,* a mammal, such as a human), comprising (a) contacting a labeled anti-AMC antibody moiety according to any of the embodiments described above with a sample (such as whole blood or homogenized tissue) derived from the individual; and (b) determining the number of cells bound with the labeled anti-AMC antibody moiety in the sample, such that a value for the number of cells bound with the labeled anti-AMC antibody moiety above a threshold level indicates that the individual has the disease or disorder. The threshold level can be determined by various methods, including, for example, by determining the number of cells bound with the labeled anti-AMC antibody moiety according to the method of diagnosing described above in a first set of individuals that have the disease or disorder and a second set of individuals that do not have the disease or disorder, and setting the threshold to a level that allows for discrimination between the first and second sets. In some embodiments, the threshold level is zero, and the method comprises determining the presence or absence of cells bound with the labeled anti-AMC antibody moiety in the sample. In some embodiments,

the method further comprises subtracting a background level of the number of cells bound with the labeled anti-AMC antibody moiety. Background level can be determined by various methods, including, for example, by determining the number of cells bound with the labeled anti-AMC antibody moiety in the individual prior to administration of the labeled anti-AMC antibody moiety, or by determining the number of cells bound with the labeled anti-AMC antibody moiety according to the method of diagnosing described above in an individual that does not have the disease or disorder. In some embodiments, the disease or disorder is cancer. In some embodiments, the cancer is selected, for example, from the group consisting of hepatocellular carcinoma, germ cell tumor, and breast cancer. In some embodiments, the cancer is hepatocellular carcinoma. In some embodiments, the cancer is metastatic hepatocellular carcinoma. In some embodiments, the cancer is metastatic hepatocellular carcinoma, and the sample is a blood sample (such as whole blood). In some embodiments, the individual is human.

[0498] In some embodiments, there is provided a method of diagnosing metastatic hepatocellular carcinoma in an individual *(e.g.,* a mammal, such as a human), comprising (a) contacting a labeled anti-AMC antibody moiety according to any of the embodiments described above with a sample (such as whole blood) derived from the individual; and (b) determining the number of cells bound with the labeled anti-AMC antibody moiety in the sample, such that a value for the number of cells bound with the labeled anti-AMC antibody moiety above a threshold level indicates that the individual has metastatic hepatocellular carcinoma. The threshold level can be determined by various methods, including, for example, by determining the number of cells bound with the labeled anti-AMC antibody moiety according to the method of diagnosing described above in a first set of individuals that have metastatic hepatocellular carcinoma and a second set of individuals that do not have metastatic hepatocellular carcinoma, and setting the threshold to a level that allows for discrimination between the first and second sets. In some embodiments, the threshold level is zero, and the method comprises determining the presence or absence of cells bound with the labeled anti-AMC antibody moiety in the sample. In some embodiments, the method further comprises subtracting a background level of the number of cells bound with the labeled anti-AMC antibody moiety. Background level can be determined by various methods, including, for example, by determining the number of cells bound with the labeled anti-AMC antibody moiety in the individual prior to administration of the labeled anti-AMC antibody moiety, or by determining the number of cells bound with the labeled anti-AMC antibody moiety

according to the method of diagnosing described above in an individual that does not have metastatic hepatocellular carcinoma. In some embodiments, the sample is blood (such as whole blood). In some embodiments, the individual is human.

[0499] Anti-AMC antibody moieties of the invention can be used to assay levels of AMC-presenting cell in a biological sample using methods known to those of skill in the art. Suitable antibody labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), samarium (^{153}Sm), lutetium (^{177}Lu), gadolinium (^{159}Gd), promethium (^{149}Pm), lanthanum (^{140}La), ytterbium (^{175}Yb) , holmium (^{166}Ho), yttrium (^{90}Y), scandium (^{47}Sc), rhenium (^{186}Re, ^{188}Re), praseodymium (^{142}Pr), rhodium (^{105}Rh), and ruthenium (^{97}Ru); luminol; fluorescent labels, such as fluorescein and rhodamine; and biotin.

[0500] Techniques known in the art may be applied to labeled anti-AMC antibody moieties of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see *e.g.,* U.S. Pat. Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003). Aside from the above assays, various *in vivo* and *ex vivo* assays are available to the skilled practitioner. For example, one can expose cells within the body of the subject to an anti-AMC antibody moiety which is optionally labeled with a detectable label, *e.g.,* a radioactive isotope, and binding of the anti-AMC antibody moiety to the cells can be evaluated, *e.g.,* by external scanning for radioactivity or by analyzing a sample (*e.g.*, a biopsy or other biological sample) derived from a subject previously exposed to the anti-AMC antibody moiety.

Articles of Manufacture and Kits

[0501] In some embodiments of the invention, there is provided an article of manufacture containing materials useful for the treatment of an AFP-positive disease such as cancer (for example hepatocellular carcinoma, germ cell tumor, or breast cancer), for delivering an anti-AMC construct to a cell presenting an AMC on its surface, or for isolation or detection of AMC-presenting cells in an individual. The article of manufacture can comprise a container and a label or package insert on or associated with the container. Suitable containers include,

for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. Generally, the container holds a composition which is effective for treating a disease or disorder described herein, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-AMC construct of the invention. The label or package insert indicates that the composition is used for treating the particular condition. The label or package insert will further comprise instructions for administering the anti-AMC construct composition to the patient. Articles of manufacture and kits comprising combinatorial therapies described herein are also contemplated.

[0502] Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. In some embodiments, the package insert indicates that the composition is used for treating cancer (such as hepatocellular carcinoma, germ cell tumor, or breast cancer).

[0503] Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0504] Kits are also provided that are useful for various purposes, *e.g.*, for treatment of an AFP-positive disease or disorder described herein, for delivering an anti-AMC construct to a cell presenting an AMC on its surface, or for isolation or detection of AMC-presenting cells in an individual, optionally in combination with the articles of manufacture. Kits of the invention include one or more containers comprising an anti-AMC construct composition (or unit dosage form and/or article of manufacture), and in some embodiments, further comprise another agent (such as the agents described herein) and/or instructions for use in accordance with any of the methods described herein. The kit may further comprise a description of selection of individuals suitable for treatment. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk) are also acceptable.

[0505] For example, in some embodiments, the kit comprises a composition comprising an anti-AMC construct (*e.g.*, a full-length anti-AMC antibody, a multi-specific anti-AMC molecule (such as a bispecific anti-AMC antibody), or an anti-AMC immunoconjugate). In some embodiments, the kit comprises a) a composition comprising an anti-AMC construct, and b) an effective amount of at least one other agent, wherein the other agent increases the expression of MHC class I proteins and/or enhances the surface presentation of AFP peptides by MHC class I proteins (*e.g.*, IFNγ, IFNβ, IFNα, or Hsp90 inhibitor). In some embodiments, the kit comprises a) a composition comprising an anti-AMC construct, and b) instructions for administering the anti-AMC construct composition to an individual for treatment of an AFP-positive disease, such as HCC. In some embodiments, the kit comprises a) a composition comprising an anti-AMC construct, b) an effective amount of at least one other agent, wherein the other agent increases the expression of MHC class I proteins and/or enhances the surface presentation of AFP peptides by MHC class I proteins (*e.g.*, IFNγ, IFNβ, IFNα, or Hsp90 inhibitor), and c) instructions for administering the anti-AMC construct composition and the other agent(s) to an individual for treatment of an AFP-positive disease, such as HCC. The anti-AMC construct and the other agent(s) can be present in separate containers or in a single container. For example, the kit may comprise one distinct composition or two or more compositions wherein one composition comprises an anti-AMC construct and another composition comprises another agent.

[0506] In some embodiments, the kit comprises a) a composition comprising an anti-AMC construct (*e.g.*, a full-length anti-AMC antibody, a multi-specific anti-AMC molecule (such as a bispecific anti-AMC antibody), or an anti-AMC immunoconjugate), and b) instructions for combining the anti-AMC construct with cells (such as cells, *e.g.*, immune cells, derived from an individual) to form a composition comprising anti-AMC construct/cell conjugates and administering the anti-AMC construct/cell conjugate composition to the individual for treatment of an AFP-positive disease (such as HCC). In some embodiments, the kit comprises a) a composition comprising an anti-AMC construct, and b) a cell (such as a cytotoxic cell). In some embodiments, the kit comprises a) a composition comprising an anti-AMC construct, b) a cell (such as a cytotoxic cell), and c) instructions for combining the anti-AMC construct with the cell to form a composition comprising anti-AMC construct/cell conjugates and administering the anti-AMC construct/cell conjugate composition to an individual for the treatment of an AFP-positive disease, such as HCC. In some embodiments, the kit comprises a composition comprising an anti-AMC construct in association with a cell (such as an

cytotoxic cell). In some embodiments, the kit comprises a) a composition comprising an anti-AMC construct in association with a cell (such as a cytotoxic cell), and b) instructions for administering the composition to an individual for the treatment of an AFP-positive disease, such as HCC. In some embodiments, the association is by conjugation of the anti-AMC construct to a molecule on the surface of the cell. In some embodiments, the association is by insertion of a portion of the anti-AMC construct into the outer membrane of the cell.

[0507] In some embodiments, the kit comprises a nucleic acid (or set of nucleic acids) encoding an anti-AMC construct (*e.g.*, a full-length anti-AMC antibody, a multi-specific anti-AMC molecule (such as a bispecific anti-AMC antibody), an anti-AMC CAR, or an anti-AMC immunoconjugate) or polypeptide portions thereof. In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) encoding an anti-AMC construct or polypeptide portions thereof, and b) a host cell (such as an effector cell) for expressing the nucleic acid (or set of nucleic acids). In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) encoding an anti-AMC construct or polypeptide portions thereof, and b) instructions for i) expressing the anti-AMC construct in a host cell (such as an effector cell, *e.g.*, a T cell), ii) preparing a composition comprising the anti-AMC construct or the host cell expressing the anti-AMC construct, and iii) administering the composition comprising the anti-AMC construct or the host cell expressing the anti-AMC construct to an individual for the treatment of an AFP-positive disease, such as HCC. In some embodiments, the host cell is derived from the individual. In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) encoding an anti-AMC construct or polypeptide portions thereof, b) a host cell (such as an effector cell) for expressing the nucleic acid (or set of nucleic acids), and c) instructions for i) expressing the anti-AMC construct in the host cell, ii) preparing a composition comprising the anti-AMC construct or the host cell expressing the anti-AMC construct, and iii) administering the composition comprising the anti-AMC construct or the host cell expressing the anti-AMC construct to an individual for the treatment of an AFP-positive disease, such as HCC.

[0508] In some embodiments, the kit comprises a nucleic acid encoding an anti-AMC CAR. In some embodiments, the kit comprises a vector comprising a nucleic acid encoding an anti-AMC CAR. In some embodiments, the kit comprises a) a vector comprising a nucleic acid encoding an anti-AMC CAR, and b) instructions for i) introducing the vector into effector cells, such as T cells derived from an individual, ii) preparing a composition comprising the anti-AMC CAR effector cells, and iii) administering the anti-AMC CAR

effector cell composition to the individual for treatment of an AFP-positive disease, such as HCC.

[0509] The kits of the invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information. The present application thus also provides articles of manufacture, which include vials (such as sealed vials), bottles, jars, flexible packaging, and the like.

[0510] The instructions relating to the use of the anti-AMC construct compositions generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. For example, kits may be provided that contain sufficient dosages of an anti-AMC construct (*e.g.*, a full-length anti-AMC antibody, a multi-specific anti-AMC molecule (such as a bispecific anti-AMC antibody), an anti-AMC CAR, or an anti-AMC immunoconjugate) as disclosed herein to provide effective treatment of an individual for an extended period, such as any of a week, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 3 months, 4 months, 5 months, 7 months, 8 months, 9 months, or more. Kits may also include multiple unit doses of the anti-AMC construct and pharmaceutical compositions and instructions for use and packaged in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies.

[0511] Those skilled in the art will recognize that several embodiments are possible within the scope and spirit of this invention. The invention will now be described in greater detail by reference to the following non-limiting examples. The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

Exemplary Embodiments

[0512] Embodiment 1. In some embodiments, 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).

[0513] Embodiment 2. In some further embodiments of embodiment 1, the AFP/MHC class I complex is present on a cell surface.

[0514] Embodiment 3. In some further embodiments of embodiment 1, the AFP/MHC class I complex is present on the surface of a cancer cell.

[0515] Embodiment 4. In some further embodiments of any one of embodiments 1-3, the MHC class I protein is human leukocyte antigen (HLA)-A.

[0516] Embodiment 5. In some further embodiments of embodiment 4, the MHC class I protein is HLA-A02.

[0517] Embodiment 6. In some further embodiments of embodiment 5, the MHC class I protein is the HLA-A*02:01 subtype of the HLA-A02 allele.

[0518] Embodiment 7. In some further embodiments of any one of embodiments 1-6, 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.

[0519] Embodiment 8. In some further embodiments of any one of embodiments 1-7, the AFP peptide is 8 to 12 amino acids in length.

[0520] Embodiment 9. In some further embodiments of any one of embodiments 1-8, the AFP peptide is derived from human AFP.

[0521] Embodiment 10. In some further embodiments of any one of embodiments 1-9, the AFP peptide has an amino acid sequence selected from the group consisting of SEQ ID NOs: 3-13 and 16.

[0522] Embodiment 11. In some further embodiments of embodiment 9, the AFP peptide has the amino acid sequence of FMNKFIYEI (SEQ ID NO: 4).

[0523] Embodiment 12. In some further embodiments of any one of embodiments 1-11, the isolated anti-AMC construct cross-reacts with a complex comprising an interspecies variant of the AFP peptide and the MHC class I protein.

[0524] Embodiment 13. In some further embodiments of any one of embodiments 1-12, the antibody moiety is human, humanized, or semi-synthetic.

[0525] Embodiment 14. In some further embodiments of any one of embodiments 1-13, the antibody moiety is a full-length antibody, a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv).

[0526] Embodiment 15. In some further embodiments of any one of embodiments 1-14, the antibody moiety binds to the AFP/MHC class I complex with an equilibrium dissociation constant (Kd) from about 0.1 pM to about 500 nM.

[0527] Embodiment 16. In some further embodiments of any one of embodiments 1-15, the isolated anti-AMC construct binds to the AFP/MHC class I complex with a Kd from about 0.1 pM to about 500 nM.

[0528] Embodiment 17. In some further embodiments of any one of embodiments 1-16, 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 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 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 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 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 3 amino acid substitutions, wherein

X can be any amino acid.

[0529] Embodiment 18. In some further embodiments of any one of embodiments 1-16, 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, or a variant thereof comprising up to about 5 amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 67-76, or a variant thereof comprising up to about 5 amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 77-86; or a variant thereof comprising up to about 5 amino acid substitutions; 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, or a variant thereof comprising up to about 5 amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 100-109, or a variant thereof comprising up to about 3 amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 110-119; or a variant thereof comprising up to about 5 amino acid substitutions.

[0530] Embodiment 19. In some further embodiments of any one of embodiments 1-16, the antibody moiety comprises:

i) a heavy chain (HC) 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; or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDR regions; and

ii) a light chain (LC) 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; or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDR regions.

[0531] Embodiment 20. In some further embodiments of embodiment 18 or 19, 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.

[0532] Embodiment 21. In some further embodiments of embodiment 20, the antibody moiety comprises a heavy chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 17-26 and a light chain variable domain comprising the amino acid sequence of any one of SEQ ID NOs: 27-36.

[0533] Embodiment 22. In some further embodiments of any one of embodiments 1-21, the isolated anti-AMC construct is a full-length antibody.

[0534] Embodiment 23. In some further embodiments of any one of embodiments 1-22, the isolated anti-AMC construct is monospecific.

[0535] Embodiment 24. In some further embodiments of any one of embodiments 1-22, the isolated anti-AMC construct is multispecific.

[0536] Embodiment 25. In some further embodiments of embodiment 24, the isolated anti-AMC construct is bispecific.

[0537] Embodiment 26. In some further embodiments of embodiment 24 or 25, 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.

[0538] Embodiment 27. In some further embodiments of embodiment 26, the isolated anti-AMC construct is a tandem scFv comprising two scFvs linked by a peptide linker.

[0539] Embodiment 28. In some further embodiments of embodiment 27, the peptide linker comprises the amino acid sequence GGGGS.

[0540] Embodiment 29. In some further embodiments of any one of embodiments 24-28, the isolated anti-AMC construct further comprises a second antibody moiety that specifically binds to a second antigen.

[0541] Embodiment 30. In some further embodiments of embodiment 29, the second antigen is an antigen on the surface of a T cell.

[0542] Embodiment 31. In some further embodiments of embodiment 30, the second antigen is selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, and HVEM.

[0543] Embodiment 32. In some further embodiments of embodiment 30, 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.

[0544] Embodiment 33. In some further embodiments of embodiment 30, the T cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, and a natural killer T cell.

[0545] Embodiment 34. In some further embodiments of embodiment 29, the second antigen is an antigen on the surface of a natural killer cell, a neutrophil, a monocyte, a macrophage or a dendritic cell.

[0546] Embodiment 35. In some further embodiments of any one of embodiments 1-21, the isolated anti-AMC construct is a chimeric antigen receptor.

[0547] Embodiment 36. In some further embodiments of embodiment 35, the chimeric antigen receptor comprises 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.

[0548] Embodiment 37. In some further embodiments of any one of embodiments 1-21, the isolated anti-AMC construct is an immunoconjugate comprising the antibody moiety and an effector molecule.

[0549] Embodiment 38. In some further embodiments of embodiment 36, 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.

[0550] Embodiment 39. In some further embodiments of embodiment 38, the therapeutic agent is a drug or a toxin.

[0551] Embodiment 40. In some further embodiments of embodiment 37, the effector molecule is a label.

[0552] Embodiment 41. In some embodiments there is provided a pharmaceutical composition comprising the isolated anti-AMC construct of any one of embodiments 1-39.

[0553] Embodiment 42. In some embodiments there is provided a host cell expressing the isolated anti-AMC construct of any one of embodiments 1-40.

[0554] Embodiment 43. In some embodiments there is provided a nucleic acid encoding the polypeptide components of the isolated anti-AMC construct of any one of embodiments 1-40.

[0555] Embodiment 44. In some embodiments there is provided a vector comprising the nucleic acid of embodiment 43.

[0556] Embodiment 45. In some embodiments there is provided an effector cell expressing the isolated anti-AMC construct of embodiment 35 or 36.

[0557] Embodiment 46. In some further embodiments of embodiment 45, the effector cell is a T cell.

[0558] Embodiment 47. 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 the isolated anti-AMC construct of embodiment 40 and detecting the presence of the label on the cell.

[0559] Embodiment 48. 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 the pharmaceutical composition of embodiment 41.

[0560] Embodiment 49. 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 the effector cell of embodiments 45 or 46.

[0561] Embodiment 50. In some further embodiments of embodiment 48 or 49, the administration is via intravenous route.

[0562] Embodiment 51. In some further embodiments of embodiment 48 or 49, the administration is via intratumoral route.

[0563] Embodiment 52. In some further embodiments of embodiment 48 or 49, the administration is to an injection site distal to a first disease site.

[0564] Embodiment 53. In some further embodiments of embodiment 52, the injection site is a first tumor distal to the first disease site.

[0565] Embodiment 54. In some further embodiments of embodiment 52 or 53, the first disease site is an AFP-positive tumor.

[0566] Embodiment 55.In some embodiments there is provided a method of diagnosing an individual having an AFP-positive disease, comprising:

a) administering an effective amount of the isolated anti-AMC construct of embodiment 40 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.

[0567] Embodiment 56. In some embodiments there is provided 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 embodiment 40; 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.

[0568] Embodiment 57. In some further embodiments of any one of embodiments 48-56, the AFP-positive disease is cancer.

[0569] Embodiment 58. In some further embodiments of embodiment 57, the cancer is hepatocellular carcinoma, germ cell tumor, or breast cancer.

[0570] Embodiment 59. In some further embodiments of embodiment 58, the cancer is hepatocellular carcinoma.

[0571] Embodiment 60. In some further embodiments of embodiment 59, the cancer is metastatic hepatocellular carcinoma.

[0572] Embodiment 61. In some further embodiments of any one of embodiments 48-54, the AFP-positive disease is hepatocellular carcinoma and metastasis is inhibited.

Examples

Materials

Cell Samples, Cell Lines, and Antibodies

[0573] The cell lines HepG2, SK-Hep1, MCF7, Malme-3M, CA46, THP-1, Colo205, ASPC1, OVCAR3, LnCAP, A498 and Hela were obtained from the American Type Culture Collection. SK-Hep1-MiniG (or SK-Hep1 AFP MG), MCF7-MiniG and Hela-MiniG (or Hela-MG) were generated by transducing the respective parental cell lines with an AFP158 peptide expressing minigene cassette, which results in a high level of cell surface expression of AFP158/HLA-A*02:01 complex in SK-Hep1 and MCF7 cells. Hela is HLA-A03- and HLA-A68-positive and HLA*A02:01-negative, and therefore Hela-MiniG served as AFP158 minigene expressing, but AFP158/HLA*A02:01 negative control cell line. The cell lines were cultured in RPMI 1640 supplemented with 10% FBS, 2 mM glutamine at 37°C/5% CO_2.

[0574] The following antibodies were purchased: monoclonal Ab against human HLA-A02 (clone BB7.2) conjugated to FITC or APC, and its isotype control mouse IgG2b/FITC or APC to human or mouse CD3, CD 19, CD56, CD33, CD34 (BD Biosciences, San Diego), goat $F(ab)_2$ anti-human IgG conjugated to PE or FITC and goat $F(ab)_2$ anti-mouse IgG (Invitrogen).

[0575] All peptides were synthesized by Genemed Synthesis, Inc. (San Antonio, Tex.). Peptides were >90% pure. The peptides were dissolved in DMSO and diluted in saline at 5 mg/mL and frozen at -180 °C. Biotinylated single chain AFP peptide/HLA-A*02:01 and control peptide/HLA-A*02:01 complexes were synthesized by refolding the peptides with recombinant HLA-A*02:01 and β-2 microglobulin (β2M). 19 control peptides (SEQ ID NOs: 122-140) that bind HLA-A*02:01 were generated from the following 15 genes: BCR, BTG2, CALR, CD247, CSF2RA, CTSG, DDX5, DMTN, HLA-E, IFI30, IL7, PIM1, PPP2R1B, RPS6KB1, and SSR1.

Example 1. Production of biotinylated AFP158/HLA-A*02:01 complex monomer

[0576] Biotinylated AFP158/HLA-A*02:01 complex monomers were prepared according to standard protocols (Altman, J.D. & Davis, M.M., *Current Protocols in Immunology* 17.3.1-17.3.33, 2003). In brief, DNA encoding full-length human β-2 microglobulin (β2M) was synthesized by Genewiz and cloned into vector pET-27b. The BirA substrate peptide (BSP) was added to the C-terminus of HLA-A*02:01 extracellular domain (ECD). DNA

encoding HLA-A*02:01 ECD-BSP was also synthesized by Genewiz and cloned into vector pET-27b. The vectors expressing human β2M and HLA-A*02:01 ECD-BSP were transformed into *E.coli* BL21 cells separately, and isolated as inclusion bodies from bacterial culture. Peptide ligand AFP158 refolded with human β2M and HLA-A*02:01 ECD-BSP to form AFP158/HLA-A*02:01 complex monomer. Folded peptide/HLA-A*02:01 monomers were concentrated by ultrafiltration and further purified through size-exclusion chromatography. HiPrep 26/60 Sephacryl S-300 HR was equilibrated with 1.5 column volumes of Hyclone Dulbecco's Phosphate Buffered Saline solution (Thermo Scientific, Cat No. SH3002802). The unpurified sample was loaded and eluted with 1 column volume. The first peak, corresponding to misfolded aggregates, eluted at approximately 111.3 mL, the peak corresponding to the properly folded MHC complex was observed at 212.5 mL, and the peak corresponding to free β2M was observed at 267.2 mL (FIG. 1). SDS-PAGE of the purified AFP158/MHC complex was performed to determine protein purity. In brief, 1μg of the protein complex was mixed with 2.5μL of the NuPAGE LDS Sample Buffer (Life Technologies, NP0008) and brought up to 10μL with deionized water. The sample was heated at 70 °C for 10 minutes, and then loaded onto the gel. Gel electrophoresis was performed at 180V for 1 hour. HLA-A*02:01 and β2M subunits were observed as the major bands on the gel (FIG. 2). Peptide/HLA-A*02:01 monomers were biotinylated via BirA-mediated enzymatic reaction and subsequently purified by high-resolution anion-exchange chromatography. Biotinylated peptide/HLA-A*02:01 monomers were stored in PBS at -80 °C.

Example 2. Selection and Characterization of scFv Specific for AFP158/HLA-A*02:01 Complexes.

[0577] A collection of human scFv antibody phage display libraries (diversity = $10x10^{10}$) constructed by Eureka Therapeutics was used for the selection of human mAbs specific to AFP158/HLA-A*02:01. 15 fully human phage scFv libraries were used to pan against AFP158/HLA-A*02:01 complex. In order to reduce the conformational change of MHC1 complex introduced by immobilizing the protein complex onto plastic surfaces, solution panning and cell panning were used in place of conventional plate panning. In solution panning, biotinylated antigens were first mixed with the human scFv phage library after extended washing with PBS buffer, and then antigen-scFv antibody phage complexes were pulled down by streptavidin-conjugated Dynabeads M-280 through a magnetic rack. The

bound clones were then eluted and used to infect *E.coli* XL1-Blue cells. In cell panning, T2 cells loaded with AFP158 peptide were first mixed with the human scFv phage library. T2 cells are a TAP-deficient, HLA-A*02:01^{+} lymphoblast cell line. To load peptide, T2 cells were pulsed with peptides (50ug/ml) in serum-free RPMI1640 medium in the presence of 20 μg/ml β2M overnight. After extended washing with PBS, peptide-loaded T2 cells with bound scFv antibody phage were spun down. The bound clones were then eluted and used to infect *E.coli* XL1-Blue cells. The phage clones expressed in bacteria were then purified. The panning was performed for 3-4 rounds with either solution panning, cell panning or a combination of solution and cell panning to enrich for scFv phage clones that bound AFP158/ HLA-A*02:01 specifically.

[0578] Streptavidin ELISA plates were coated with biotinylated AFP158/HLA-A*02:01 complex monomer (Bio-AFP158) or biotinylated control peptide/HLA-A*02:01 monomer (Bio-control), respectively. Individual phage clones from enriched phage display panning pools against AFP158/HLA-A*02:01 complex were incubated in the coated plates. Binding of the phage clones was detected by HRP-conjugated anti-M13 antibodies and developed using HRP substrate. The absorbance was read at 450nm. 605 positive clones were identified through ELISA screening of 1260 phage clones enriched from phage panning. FIG. 3 provides an example of phage clone binding to biotinylated AFP158/HLA-A*02:01 monomer in an ELISA assay. 82 unique clones were identified by DNA sequencing of the 605 ELISA positive phage clones. Positive clones were determined by standard ELISA against biotinylated AFP158/HLA-A*02:01 complex monomer. Then, unique antibody clones were identified through DNA sequencing of ELISA-positive clones. Specific and unique clones were further tested for their binding to HLA-A2/peptide complexes on live cell surfaces by flow cytometry (FACS analysis) using AFP158-loaded live T2 cells. T2 cells loaded with different peptides and β2M were first stained with purified scFv phage clones, followed by staining with a mouse anti-M13 mAb, and finally the R-PE conjugated horse anti-mouse IgG from Vector Labs. Each step of the staining was done between 30-60 minutes on ice and the cells were washed twice between staining. Among the 82 clones, 44 recognize AFP158-loaded T2 cells specifically. FIG. 4 provides an example of AFP158/HLA-A*02:01 specific phage clone binding to peptide-loaded T2 cells through FACS. The phage clones specifically bound to AFP158-loaded T2 cells and did not recognize T2 cells loaded with hTERT-derived

peptide ILAKFLHWL (hTERT540, SEQ ID NO: 141) in the context of HLA-A*02:01, or T2 cells with β2M but without peptide loaded.

Example 3. Characterization of FACS-positive AFP158-specific phage clones

Cross-reactivity to mouse AFP158 peptide

[0579] Clones selected from FACS binding analysis against AFP158-loaded T2 cells were characterized further for cross-reactivity towards mouse AFP158 peptide/HLA-A*02:01 complex on live cell surfaces by FACS analysis using mouse AFP158-loaded live T2 cells. Mouse AFP158 peptide differs from human AFP158 peptide by two amino acids at position 4 and position 9. The mouse AFP158 peptide sequence is FMNRFIYEV (SEQ ID NO: 16), while the human AFP158 peptide sequence is FMNKFIYEI (SEQ ID NO: 4). Antibodies cross-reacting with both human and mouse AFP158 peptide/MHC complexes are useful for assessing antibody drug toxicity in HLA-A*02:01 transgenic mice. Among the phage clones tested, 4 clones (#17, #33, #48 and #76) recognized both human AFP158- and mouse AFP158-loaded T2 cells (FIG. 5). Clones #52 and #79 also bound to mouse AFP158-loaded T2 cells, but to a lesser degree.

Epitope mapping by alanine walking

[0580] To investigate with precision the epitope for the mAb recognition, human AFP158 peptides with alanine substitutions at positions 1, 3, 4, 5, 6, 7 and 8 (SEQ ID NOs: 7-13) were pulsed onto T2 cells. Antibody phage clones were then tested for binding to these peptide-loaded T2 cells by FACS analysis. Although all the antibodies recognized the small conformational epitope formed by the AFP158 peptide and its surrounding MHCα chain residues, the key peptide residues interacting with different antibodies were quite different. For example, clone #52 is predicted to bind to the N-terminal half of the AFP158 peptide since alanine substitution at position 1 or 3 dramatically reduced binding to the peptide-loaded T2 cells, and alanine substitution at position 4 completely abrogated the binding of clone #52. In contrast, alanine substitutions at position 5, 6, 7 or 8 didn't change the binding of clone #52. Clone #17-13, on the other hand, was insensitive to alanine substitution at position 4, but was sensitive to changes at position 3, 5 and 7. FIG. 6 provides an example of FACS analysis, showing the binding of phage clone #52 to T2 cells loaded with the various AFP peptides. Table 6 summaries the binding of several AFP158/HLA-A*02:01-specific antibody clones to alanine-substituted human AFP158 peptide-loaded T2 cells.

Table 6

Peptide	Ala Position	ET1402-52	ET1402-61	ET1402-76	ET1402-79	AM1402-17-13
FMNKFIYEI		37900	30400	14400	30100	83300
AMNKFIYEI	1	468	12300	5458	21000	55600
FMAKFIYEI	3	4470	38600	38200	786	2914
FMNAFIYEI	4	20.9	15.1	38.3	79.2	50800
FMNKAIYEI	5	21800	3719	49300	34.4	1500
FMNKFAYEI	6	18800	169	40300	27.7	28800
FMNKFIAEI	7	42500	1110	29300	907	572
FMNKFIYAI	8	38500	1748	53300	16100	43200
Sensitive Position		**4, 1, 3**	**4, 6, 7,8, 5**	**4, 1**	**5, 6, 4, 3, 7**	**7, 5, 6**

Antibody binding specificity evaluation against endogenous peptides

[0581] On average, each nucleated cell in the human body expresses about half a million different peptide/MHC class I complexes. In order to develop anti-peptide/MHCI-complex antibodies into anti-cancer drugs with high specificity and therapeutic index, it is essential for the antibodies to specifically recognize the target peptide/MHCI complex, but not the MHCI molecule itself, or MHCI molecules bound to other peptides presented on cell surfaces. For the current study, the relevant MHCI molecule is HLA-A*02:01. During the early stages of our phage panning and screening, we eliminated antibodies that bound to the HLA-A*02:01 molecule alone (*see*, for example, FIGs. 3 and 4). The top phage clones were also screened against 19 endogenous HLA-A*02:01 peptides, which were derived from proteins normally expressed in multiple types of nucleated human cells, such as globin α chain, β chain, nuclear protein p68, and the like. The pool of endogenous peptides (P19, SEQ ID NOs: 122-140) was loaded into T2 cells and antibody binding was determined through FACS analysis. As shown in FIG. 7, the AFP158/HLA-A*02:01-specific antibody phage clones bound AFP158 peptide-loaded T2 cells, but not T2 cells loaded with endogenous peptides. We conclude that the identified antibodies are specific to AFP158 peptide/HLA-A*02:01 complexes, and do not recognize HLA-A*02:01 molecules bound to other HLA-A*02:01 peptides tested.

Example 4. Engineering bispecific antibodies

[0582] Bispecific antibodies (BsAbs) were generated using scFv sequences of the AFP158/HLA-A*02:01-specific phage clones. The BsAbs are single-chain bispecific

antibodies comprising the scFv sequence of an AFP158/HLA-A*02:01-specific phage clone at the N-terminal end and an anti-human CD3ε mouse monoclonal scFv at the C-terminal end (Brischwein, K. *et al.*, *Mol. Immunol.* 43:1129-1143, 2006).The DNA fragments coding for the AFP158 scFv and the anti-human CD3ε scFv were synthesized by Genewiz and subcloned into Eureka's mammalian expression vector pGSN-Hyg using standard DNA technology. A hexhistamine tag was inserted at the C-terminal end for antibody purification and detection. Chinese hamster ovary (CHO) cells were transfected with the BsAb expression vector, and then cultured for 7 days for BsAb antibody production. CHO cell supernatants containing secreted AFP158 BsAb molecules were collected. BsAb antibodies were purified using HisTrap HP column (GE healthcare) by FPLC AKTA system. Briefly, CHO cell culture was clarified and loaded onto the column with low imidazole concentration (20 mM), and then an isocratic high imidazole concentration elution buffer (500 mM) was used to elute the bound BsAb proteins. Molecular weights of the purified AFP158 BsAbs antibodies were measured under non-reducing conditions by gel electrophoresis. 4μg of the protein was mixed with 2.5μL of the NuPAGE LDS Sample Buffer (Life Technologies, NP0008) and brought up to 10μL with deionized water. The sample was heated at 70 °C for 10 minutes, and then loaded onto the gel. Gel electrophoresis was performed at 180V for 1 hour. ~50 KD bands are observed as the major bands on the gel (FIG. 8). Antibody aggregation was assessed by size-exclusion chromatography (SEC). 50μL of the sample was injected into the SEC column (Agilent, BioSEC-3,300A, 4.6x300mm) while flowing a buffer consisting of Dulbecco's Phosphate Buffered Saline (Fisher Scientific, SH30028.FS) and 0.2M arginine adjusted to pH 7.0. BsAb was observed as the major peak at the retention time ~15.8 mL. (FIG. 9). BsAbs with high molecular weight aggregation less than 10% were used for further characterization.

Example 5. Characterization of AFP158 BsAb antibodies

Binding affinity of AFP158 BsAb antibodies

[0583] The binding affinity of AFP158 BsAb antibodies to recombinant AFP158/HLA-A*02:01 complex was measured by Surface Plasma Resonance (BiaCore). The binding parameters between the AFP158 BsAb and the AFP158/HLA-A*02:01 complex were measured using a His Capture Kit (GE Healthcare, Cat# 28995056) on a Biacore X100 (GE Healthcare) according to the manufacturer's protocol for multi-cycle kinetics measurement. All of the proteins used in the assay were diluted using HBS-E buffer. In brief, 1 μg/mL of

the AFP158 BsAb was immobilized onto a Sensor Chip pre-functionalized with the anti-histidine antibody by flowing the solution through the flow cell 2 at 2 µL/min for 2 minutes. Binding towards the AFP158/A*02:01 complex was analyzed at 0.19, 0.38, 7.5, 15, and 30 µg/mL, each run consisting of a 3 minute association and 3 minute dissociation at 30 µL/min. At the end of cycle, the surface was regenerated using the regeneration buffer from the His Capture kit. Following the kinetics measurement, the surface was regenerated using the regeneration solution from the kit. The data were analyzed using 1:1 binding site mode with the BiaCore X-100 evaluation software. The binding parameters (association on rate constant k_a, dissociation constant k_d, and equilibrium dissociation constant K_d) were calculated. The binding affinities of AFP158 antibodies, in the monovalent scFv format, fall into the range of 10-500nM. (Examples provided in Table 7).

Table 7

Clone #	k_a [1/Ms]	k_d [1/s]	K_d [nM]
#52	2.63E+05	1.71E-2	65
#61	8.92E+04	3.08E-3	34
#76	9.94E+04	1.42E-2	143
#79	7.88E+04	2.51E-2	318
#17-13	7.36E+04	3.45E-3	47

T-cell Killing assay

[0584] Tumor cytotoxicity was assayed by LDH Cytotoxicity Assay (Promega). Human T cells purchased from AllCells were activated and expanded with CD3/CD28 Dynabeads (Invitrogen) according to manufacturer's protocol. Activated T cells (ATC) were cultured and maintained in RPMI1640 medium with 10% FBS plus 100 U/ml IL-2, and used at day 7-14. The T cells were > 99% $CD3^+$ by FACS analysis. Activated T cells (Effector cells) and Target cells were co-cultured at a 5:1 ratio with different concentrations of BsAb antibodies for 16 hours. Cytotoxicities were then determined by measuring LDH activities in culture supernatants.

[0585] AFP158 BsAb antibodies killed cancer cells in an AFP and HLA-A*02:01-dependent manner. Among all cell lines tested, HEPG2 (AFP and HLA-A*02:01 positive) was most effectively killed by T cells redirected through AFP158 BsAbs. Other cell lines tested that were either AFP negative or HLA-A02 negative, or negative for both were not killed as effectively under the same experimental settings (FIG. 10).

Cross-reactivity of AFP158 BsAb antibodies against multiple HLA-A02 alleles

[0586] Human MHCI molecules consist of 6 class isoforms, HLA-A, -B, -C, -E, -F and G. The HLA-A, -B and-C heavy chain genes are highly polymorphic. For each isoform, the HLA genes are further grouped according to the similarity of heavy chain sequences. For example, HLA-A is divided into different alleles such as HLA-A01, -A02, -A03, etc. For the HLA-A02 allele, there are multiple subtypes, such as HLA-A*02:01, A*02:02, etc. Between the different subtypes of HLA-A02 group, the sequence differences are limited to only several amino acids. So in many cases, peptides that bind to HLA-A*02:01 molecule can also form complexes with multiple subtypes of the HLA-A02 allele. As shown in Table 8 (http://www.allelefrequencies.net/), although HLA-A*02:01 is the dominant HLA-A02 subtype among Caucasian populations, in Asia and Africa, A*02:03, A*02:05, A*02:06, A*02:07 and A*02:11 are also common HLA-A02 subtypes. The ability of AFP158 antibodies to recognize not only AFP158 peptide in the context of HLA-A*02:01, but also other subtypes of HLA-A02, will broaden the patient population that might be able to benefit from AFP158 antibody drug treatment. We therefore generated recombinant AFP158/MHCI complexes with other subtypes of the HLA-A02 allele and tested the binding affinity of the AFP158/HLA-A*02:01-specific antibodies for these other complexes. Binding affinity was determined using a ForteBio Octet QK. 5 μg/mL biotinylated HLA-A02 MHC complex of varying subtypes was loaded onto a streptavidin biosensor. After washing off excess antigen, BsAb antibodies were tested at 10 μg/mL for association and dissociation. Binding parameters were calculated using a 1:1 binding site, partial fit model. Table 9 shows binding affinities of several AFP158 BsAbs for multiple AFP158/HLA-A02 complexes with different subtypes. All of the antibodies tested were found to recognize AFP158 bound to multiple subtypes of the HLA-A02 allele.

Table 8

	australia	china	europe	india	north africa	sub-saharan africa	taiwan	us
A*02:01	97.8%	39.5%	94.0%	53.9%	73.3%	56.3%	35.1%	79.4%
A*02:02	0.0%	0.1%	0.3%	0.9%	9.7%	24.1%	0.0%	3.6%
A*02:03	0.0%	15.3%	0.2%	4.9%	0.0%	0.4%	19.3%	2.2%
A*02:04	0.0%	0.1%	0.0%	0.3%	2.6%	0.4%	0.0%	0.2%
A*02:05	1.1%	0.9%	3.2%	5.8%	13.8%	15.9%	0.1%	4.5%
A*02:06	0.0%	16.0%	0.9%	10.6%	0.0%	0.7%	12.8%	5.5%
A*02:07	1.1%	26.1%	0.4%	0.4%	0.0%	0.0%	32.7%	2.4%
A*02:08	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
A*02:09	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

A*02:10	0.0%	1.1%	0.0%	0.0%	0.0%	0.2%	0.0%	0.1%
A*02:11	0.0%	0.1%	0.1%	22.3%	0.0%	1.5%	0.0%	1.7%
other A02 subtypes (A*02:12 - A*02:93)	0.0%	0.7%	0.8%	0.9%	0.5%	0.5%	0.0%	0.6%
	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 9

HLA-A02 subtype	#17-13 K_d(nM)	#52 K_d(nM)	#61 K_d(nM)	#76 K_d(nM)	#79 K_d(nM)
A*02:02	233	16.8	394	16.8	115
A*02:03	212	25.7	204*	25.7*	--
A*02:05	183	18.6	2000	18.6	686
A*02:06	26.4	9.4	2200*	9.4*	63.8
A*02:07	373	6.9	3200*	6.9*	387
A*02:11	11.3	10.4	433	10.4	68.5

* low fitting confidence

Example 6. Generation of AFP158/HLA-A*02:01 specific chimeric antigen receptor-presenting T cells (CAR-T)

[0587] Chimeric antigen receptor therapy (CAR-T therapy) is a new form of targeted immunotherapy. It merges the exquisite targeting specificity of monoclonal antibodies with the potent cytotoxicity and long-term persistence provided by cytotoxic T cells. This technology enables T cells to acquire long-term novel antigenic specificity independent of the endogenous TCR. Clinical trials have shown clinically significant antitumor activity of CAR-T therapy in neuroblastoma (Louis, C.U. *et al.*, *Blood* 118(23):6050-6, 2011), B-ALL (Maude, S.L. *et al.*, *N Engl J Med.* 371(16):1507-1517, 2014), CLL (Brentjens, R.J. *et al.*, *Blood.* 118(18):4817-4828, 2011), and B cell lymphoma (Kochenderfer, J.N. *et al.*, *Blood.* 116(20):4099-4102, 2010). In one study, a 90% complete remission rate in 30 patients with B-ALL treated with CD19-CAR T therapy was reported (Maude *et al.*, *supra*).

[0588] To further explore the potency of the AFP158/HLA-A*02:01 specific antibodies, we constructed anti-AFP158/HLA-A*02:01 scFv expressing CARs and transduced T cells with these CARs. AFP158/HLA-A*02:01 specific CARs were constructed using a lentiviral CAR expression vector. Anti-AFP158/HLA-A*02:01 scFvs were grafted onto a second generation CAR (Mackall, C.L. *et al.*, *Nat. Rev. Clin. Oncol.* 11(12):693-703, 2014) with CD28 signaling domain and TCRζ engineered *in cis* to provide intracellular T cell stimulation

signals and to activate T cells. FIG. 11 provides a schematic illustration of the anti-AFP158/HLA-A*02:01 CAR constructs.

Example 7. Characterization of AFP158 CAR-T cells

AFP158 CAR expressed in CD4+ and CD8+ primary T cells

[0589] Peripheral blood lymphocytes were isolated from healthy donors and transduced with an AFP158 CAR construct encoding an anti-AFP158/HLA-A*02:01 binding moiety. Five days after transduction, AFP158 CAR-T cells and mock-transduced cells were co-stained with AFP158 tetramer, and one of CD3, CD4 or CD8 antibodies and analyzed by flow cytometry. FIG. 24 shows flow cytometry results for AFP158 CAR- and mock-transfected cells indicating that the AFP158 CAR can be expressed in both $CD4^+$ and $CD8^+$ primary T cells.

Even distribution of AFP158 CAR on T cell surface

[0590] It has been demonstrated that CAR-T cells with even cell surface distribution of CARs kill tumor cells more efficiently *in vitro* and *in vivo* than CAR-T cells with uneven cell surface distribution of CARs, and they exhaust much less easily after killing. The uneven distribution, such as aggregation, of CARs can lead to antigen-independent CAR activation, premature T cell exhaustion, and no anti-tumor activity *in vivo*. Therefore, even distribution of CARs on T cell surface is desirable. To determine the distribution of anti-AFP158/HLA-A*02:01 scFv expressing CARs on T cell membrane, we used a conjugate AFP158 peptide-HLA-A2 tetramer-PE to stain the AFP158 CAR-transduced T cells as described above. Primary human CD3+ T cells were first activated by anti-CD3 and CD28 beads, and then transduced with AFP158 CAR lentivirus. On day 8 after viral transduction, AFP158 CAR-transduced T cells were stained with AFP158 peptide-HLA-A2 tetramer-PE at 2 ng/ml in the presence of protein transport inhibitor for 30 minutes at room temperature. Images were acquired using an Olympus fluorescence microscope. We found that the distribution of AFP158 CAR on T cell membrane is even and smooth. No punctate CAR distribution was observed. A representative image is shown in FIG. 26.

In vitro Cytotoxicity study of AFP158 CAR-T cells

[0591] Lentiviruses containing AFP158/HLA-A*02:01-specific chimeric antigen receptors were produced by transfection of 293T cells with CAR vectors. Human T-cells were used for transduction after 1-day stimulation with CD3/CD28 beads (Dynabeads®, Invitrogen) in the presence of interleukin-2 at 100 U/ml. Concentrated lentiviruses were applied to T-cells in

Retronectin (Takara) coated 6-well plates for 72 hours. Functional assessment of the transduced T cells (AFP158 CAR-T cells) was performed using LDH Cytotoxicity Assay. Effector-to-target ratios used were 5:1.

[0592] A panel of AFP158 CAR clones transduced into T cells was tested against the target cell lines HEPG2, SK-HEP1, and SK-HEP1-MiniG. T cells transduced with the AFP158 CAR clones specifically killed AFP158/HLA*A02:01 positive cell lines HEPG2 and SK-HEP1-MiniG (FIG. 12). The AFP158 CAR expressing T cells killed the target-positive cancer cells in a specific and highly efficient way for most of the antibody clones tested. Target-negative, wild-type SK-HEP1 cells, however, were poorly recognized by the same T cells, except for those transduced with clone #44, which showed some non-specific killing of wild-type SK-HEP1 cells.

[0593] A large panel of cancer cell lines positive or negative for AFP158 and HLA*A02:01 was tested for killing by AFP158 CAR-T cells. Primary T cells that were mock-transduced or transduced with AFP158 CAR (a CAR construct encoding an exemplar anti-AFP158/HLA*A02:01 antibody) were tested for their ability to specifically kill AFP158/HLA*A02:01 positive cancer cells, at an effector-to-target ratio at 5:1. As shown in FIG. 13, AFP158 CAR-T cells specifically killed AFP158/HLA*A02:01 positive cell lines HepG2 and SK-Hep1-MiniG, but none of the other cell lines, which are either AFP negative or HLA*A02:01 negative. Importantly, this data demonstrates that our antibody is highly specific to AFP-expressing cells and does not mediate non-specific killing of a large panel of HLA-A02 cell lines across multiple tissues types that do not express AFP.

AFP158 CAR-transduced T cells degranulate upon antigen stimulation

[0594] To further characterize the biological activities in AFP158 CAR-transduced T cells, we used a flow cytometry assay to detect CD107a surface expression as a measurement of degranulation activity. AFP158 CAR-transduced T cells were co-incubated with HepG2, SK-HEP-1 and SK-Hep1-MiniG cells for 4 hours in the presence of a 1:200 dilution of anti-CD107a antibody and protein transport inhibitor cocktail (eBioscience). After co-incubation with target cells, transduced T cells were stained with AFP158/HLA tetramers and anti-CD8. Degranulation in tetramer-positive, CD8-positive T cells is shown in FIG 25. The highest level of degranulation, as measured by CD107a expression, was observed upon co-incubation with SK-Hep1-MiniG, followed by HepG2, while no degranulation was observed with the parental antigen-negative SK-HEP-1. This is consistent with the T-cell mediated cell lysis data above.

Cytokine release

[0595] The cytokine release profile upon treatment with activated AFP158 CAR-T cells was also examined. T cells were either mock-transduced or transduced with an exemplar AFP158 CAR and co-incubated with target cells as indicated. Release of IL-2, IL-4, IL-6, IL-8, IL-10, GM-CSF, IFN-γ and TNF-α into the media was measured after 16 hours using the Magpix multiplex system (Luminex) with the Bio-plex Pro Human Cytokine 8-plex Assay (BioRad). Cytokine concentrations were determined from a standard curve, after subtracting values from media, target cells alone and AFP158 CAR transduced T cells alone. As shown in FIGs. 14A and 14B, cytokine release was detected only when AFP158 CAR-T cells were co-incubated with AFP158/HLA*A02:01 positive cells: SK-Hep1-MiniG, MCF7-MiniG and HepG2, but not with AFP158/HLA*A02:01 negative cells: SK-Hep1 and MCF7. AFP158 CAR-T cells released much higher level of cytokines when they were exposed to SK-Hep1-MiniG and MCF7-MiniG. This is consistent with AFP158 minigene-transduced cancer cells expressing much higher levels of AFP158/HLA*A02:01 on the cell surface than HepG2. Mock-transduced T cells released only trace amounts, if any, of cytokines in the presence of AFP158/HLA*A02:01 positive cancer cells. IL-6 release after co-incubation with mock-transduced (AFP negative) SK-Hep1 is due to endogenous IL-6 expression in those cells (based on data not shown).

In vivo efficacy study of AFP158 CAR T cells in human HCC xenograft model

[0596] The *in vivo* anti-tumor activity of AFP158 CAR-T cells was tested in several established human HCC xenograft models in SCID-beige immunocompromised mice. HepG2 was implanted subcutaneously (s.c.) over the right flank at $2.5x10^6$ cells per mouse. When tumor volumes reached an average of 100 mm^3, mice were randomized based on tumor volume into four groups: 1) no treatment, 2) intravenous (i.v.) treatment with mock-transduced, donor-matched T cells (mock), 3) intravenous (i.v.) treatment with an exemplar AFP158 CAR transduced T cells, and 4) intratumoral (i.t.) injection of AFP158 CAR transduced T cells. All treatments were administered at a dose of 10^7 cells per mouse and repeated every 2 weeks for a total of 3 doses.

[0597] Both control and AFP158 CAR-T cells were well-tolerated at the given dose and schedule; no treatment-related adverse responses were observed during the study. Tumors in untreated mice, as well as control T cell-treated mice, grew continuously until they reached a size that required euthanasia. As shown in FIG. 15A, intravenous administration of AFP158

CAR-T cells in HepG2 tumor-bearing mice resulted in delayed tumor growth starting 28 days after the first dose. Approximately 25% tumor growth inhibition was observed after day 35 post the first dose (FIG. 15A). In contrast to the delayed effects of i.v. administration, i.t. injections of AFP158 CAR-T cells caused rapid, profound, and lasting tumor regression in all mice, with 80% (6/8) showing complete regression (FIG. 15B).

[0598] Peritoneal dissemination of HCC occurs in a subset of patients who have very limited treatment options as a result. Therefore the anti-tumor activity of AFP158 CAR-T cells was further tested in an established intraperitoneal HCC xenograft model. In this study, luciferase-tagged HepG2 cells (HepG2-luc2) were implanted intraperitoneally (i.p.) at $2.5x10^6$ cells per mouse. Tumor burden was assessed weekly by measuring tumor-derived bioluminescence. One week post tumor implantation, animals were randomized based on total bioluminescent flux into four groups: 1) no treatment, 2) treatment with i.p. injection of 10^7 control T cells, treatment with i.p. injection of 10^6 AFP158 CAR-T cells, and 4) treatment with i.p. injection of 10^7 AFP158 CAR-T cells (n=6 mice per group). Two doses of T cells were administered to each group, two weeks apart.

[0599] As observed with i.v. and i.t. routes of administration, no clinical signs of adverse reactions were observed as a result of i.p. injections of either control or AFP158 CAR-T cells. As shown in FIGs. 16A (change of the photon emission from tumor-bearing mice at day 70 after treatment) and 16B (photon emission images of the HepG2-Luc2 tumor-bearing mice at day 70), the tumor burden in control T cell-treated animals showed no difference from that observed in the untreated control group. In contrast, mice treated with AFP158 CAR-T cells at 10^6 or 10^7 cells per mouse showed robust tumor regression. No dose-dependent anti-tumor activity was observed, indicating that both doses exceed the maximum efficacious dose in this model. Thus i.p. treatment with AFP158 CAR T cells in a model of peritoneal HCC is safe, potent and eradicated tumors effectively.

[0600] The *in vivo* activity of AFP158 CAR-T cells was also evaluated in SK-Hep1-MiniG s.c. xenograft model. SK-Hep1-MiniG was implanted subcutaneously (s.c.) over the right flank at $2.5x10^6$ cells per mouse. When tumor volume reached an average of 100 mm^3, mice were randomized based on tumor volume into three groups: 1) intravenous (i.v.) treatment with mock-transduced, donor-matched T cells (mock), 2) intravenous (i.v.) treatment with exemplar AFP158 CAR transduced T cells, and 3) intratumoral (i.t.) injection of the same AFP158 CAR transduced T cells. All treatments were administered at a dose of 10^7 cells per mouse and repeated once after 2 weeks of the first treatment. In this tumor model, i.v.

administration of AFP158 CAR-T cells resulted in immediate tumor growth inhibition, slowing tumor growth by approximately 28% by day 31 following the first dose (FIG. 17). This suggests that the delayed tumor growth inhibition activity of i.v. AFP158 CAR-T cell administration in HepG2 tumors is a model-specific phenomenon. Similar to the results obtained in HepG2 tumors, i.t. injection of AFP158 CAR-T cells in the SK-Hep1-MiniG mouse model resulted in robust and prolonged tumor regression shortly after the first dose (FIG. 17).

Example 8. Generation and Characterization of the full-length IgG1 AFP158 antibodies

[0601] Full-length human IgG1 of the selected phage clones were produced in HEK293 and Chinese hamster ovary (CHO) cell lines, as described (Tomimatsu, K. *et al.*, *Biosci. Biotechnol. Biochem.* 73(7):1465-1469, 2009) (data not shown). In brief, antibody variable regions were subcloned into mammalian expression vectors, with matching human lambda or kappa light chain constant region and human IgG1 constant region sequences. Applying the same cloning strategy, we also generated chimeric AFP158 full-length antibodies with mouse IgG1 heavy chain and light chain constant regions. Molecular weight of the purified full-length IgG antibodies was measured under both reducing and non-reducing conditions by electrophoresis. SDS-PAGE of purified AFP158 mouse chimeric IgG1 antibodies was performed to determine protein purity. In brief, 2μg of the protein was mixed with 2.5μL of the NuPAGE LDS Sample Buffer (Life Technologies, NP0008) and brought up to 10μL with deionized water. The sample was heated at 70 °C for 10 minutes, and then loaded onto the gel. Gel electrophoresis was performed at 180V for 1 hour. Examples of SDS-PAGE are shown in FIG. 18.

[0602] AFP158 chimeric IgG1 antibody was tested for the binding towards AFP158 presenting SK-HEP1 cells by flow cytometry. SK-HEP1 is an HLA-A*02:01 positive and AFP negative cell line. An AFP158 minigene cassette was transfected into SK-HEP1 cells to generate the AFP158-presenting SK-HEP1-miniG cells. 10 μg/mL of antibody was added to cells on ice for 1 hour. After washing, R-PE conjugated anti-mouse IgG(H+L) (Vector Labs#EI-2007) was added to detect antibody binding. The AFP158 antibodies were found to bind to the minigene-transfected SK-HEP1-miniG cells, while secondary control antibody alone did not bind to the same cells (FIG. 19). Binding affinity of the mouse chimeric IgG1 AFP158 antibodies was determined by ForteBio. Converting the antibodies from monovalent BsAbs into bivalent IgG antibodies dramatically increased the binding affinity of AFP158

antibodies towards target antigen. The K_d's of the full-length antibodies were determined to be in the picomolar range, a 100- to 1000-fold increase compared with the BsAb format. Table 10 shows examples of the K_d data.

Table 10

Clone #	k_a [1/Ms]	k_d [1/s]	K_d [nM]
1402-52	1.17E+06	2.35E-04	0.201
1402-61	5.13E+05	1.31E-05	0.0255
1402-76	6.32E+05	1.10E-04	0.173
1402-79	4.60E+05	5.33E-05	0.116

[0603] AFP158-specific and negative control (ET901) mouse chimeric IgG1 were tested for the binding towards AFP158/HLA-A*02:01, AFP recombinant protein and free AFP158 peptide in an ELISA assay. Antibodies were tested at 3X serial dilution, starting from 100 ng/mL, for a total of 8 concentrations. Biotinlyated AFP158/A*02:01 MHC was coated onto streptavidin plates at 2 µg/mL, AFP protein was coated at 2 µg/mL and AFP158 peptide was coated at 40 ng/mL. It was confirmed that full-length AFP158 antibodies recognize the AFP158 peptide only in the context of HLA-A02, and do not bind recombinant AFP protein or free AFP158 peptide (FIG. 20).

Example 9. Efficacy of AFP158-CART in distant SK-Hep1-MiniG s.c. xenograft model

[0604] Example 7 showed that intratumoral administration of AFP158 CAR T cells into the subcutaneous (s.c.) liver tumor model significantly inhibited the growth of treated tumors in multiple xenograft models. The goal of the study described in this Example was to assess whether intratumoral treatment would also affect the growth of distant s.c. tumors.

[0605] In one representative study, the following materials and procedures were used:

[0606] 1. Target tumor cell line SK-Hep1-MiniG (a.k.a. SK-Hep1-MG): Human HCC cell line SK-Hep1 expressing HLA-A*02:01 and AFP158 peptide minigene.

[0607] 2. Animal: SCID-beige carries no T- and B-cells. Have functional NK, monocyte/macrophage, granulocytes and dendritic cells.

[0608] 3. Animal study was carried out at a research contract lab.

[0609] 4. CART Cells: human T cells were activated on Day 0; b) activated T cells were transduced by lentivirus on Day 1; c) lentivirus and beads were removed on Day 5; d) CAR T

cells were cultured and expanded with IL-2 at 100 unit/ml; e) APC cells were isolated by depleting $CD3^+$ T cell from PBMC. The cells are mainly monocytes and B-cells.

[0610] 5. Animal study. 6-8 weeks old female SCID Beige mice were used in this study. The SK-Hep1-MiniG cell line were cultured in DMEM Medium+10% FBS and 1% L-Glutamine at 37°C in a humidified atmosphere with 5% CO_2. SK-Hep1-MiniG cells were re-suspended in 50% PBS plus 50% Matrigel and implanted subcutaneously at both right and left flanks into 40 mice at 5 x 10^6 cells/100ul/injection site.

[0611] When tumors reached 100mm^3 on average, mice were randomized based on tumor size at right flank into the 6 groups described below, at 6 mice per group, and samples were injected into the tumors on the right flank of each mouse. For the AFP158-CART groups (groups 4-6), various antibody clones as described above were used in various experiments. Results from an experiment using a representative clone are shown below. Other clones produced similar results (data not shown).

[0612] Group 1: Vehicle (PBS), 100 µL/ mouse, *i.t.* into the right site *s.c.* tumor, single dose.

[0613] Group 2: Mock* T-Cells 7 Million/100 µL/mouse, *i.t.* into the right site *s.c.* tumor, single dose. (Mock T-cells are the T-cells without CART transduction)

[0614] Group 3: Mock with APC Cells 7 Million (70% Mock + 30% APC) /100 µL/ mouse *i.t.* into the right site *s.c.* tumor, single dose.

[0615] Group 4: AFP158 CAR T Cells 7 Million /100 µL/ mouse, *i.v. via tail vein,* single dose.

[0616] Group 5: AFP158 CAR T Cells 7 Million /100 µL/ mouse, *i.t.* into the right site *s.c.* tumor, single dose.

[0617] Group 6: AFP158 CAR T Cells with APC, 7 Million (70% CART + 30% APC) / 100 µL/ mouse, *i.t.* into the right site *s.c.* tumor, single dose.

AFP158-CART is safe at the current dose/schedule

[0618] After dosing, body weight and other clinical behavior were closely monitored. As shown in Figure 21, body weight loss was not observed in any of the groups. No other clinical signs of drug related toxicity were observed.

Single i.t. dose resulted in regression of local tumors and inhibition of distant tumors

[0619] As shown in Figure 22, a single i.t. dose of AFP158-CART injected to only the right flank resulted in significant inhibition of tumors implanted on both the right flank and left

flank (the side which AFP158-CAR T cells were not directly injected). Analysis by area under curve, reflecting the overall growth kinetics of the tumor, showed that i.t. injection of AFP158-CART alone resulted in 42% tumor growth inhibition on the right flank (Dunnett's test $p<0.01$) and 33% tumor growth inhibition on the left flank ($p>0.05$).

AFP158-CART + APC enhanced anti-tumor activity in both sides

[0620] As shown in Figure 22, i.t. injection of AFP158-CART plus APC caused regression of right flank tumors with overall tumor inhibition of 68% when compared with the vehicle-treated group ($p<0.001$). A stronger inhibition of distant left flank tumor as compared to the respective vehicle-treated group was also observed (47%, $p<0.01$). This suggests the possible involvement of T-cell cross-priming in the additional inhibition of both right and left side tumors.

CD3 positive cells were observed in both right and left side tumors in AFP158-CART + APC treated animals

[0621] At the end of the study (34 days post dosing), tumors were harvested and histological staining of CD3 (T-cell marker) was performed. As shown in Figure 23, CD3 positive cells were found in both right and left side tumors from i.t. injection of AFP158-CART plus APC cells. This suggests the involvement of the human T-cells in the tumor growth inhibition.

Sequence Listing

hAFP protein (SEQ ID NO: 1)

MKWVESIFLIFLLNFTESRTLHRNEYGIASILDSYQCTAEISLADLATIFFAQFVQEATY
KEVSKMVKDALTAIEKPTGDEQSSGCLENQLPAFLEELCHEKEILEKYGHSDCCSQSE
EGRHNCFLAHKKPTPASIPLFQVPEPVTSCEAYEEDRETFMNKFIYEIARRHPFLYAPT
ILLWAARYDKIIPSCCKAENAVECFQTKAATVTKELRESSLLNQHACAVMKNFGTRT
FQAITVTKLSQKFTKVNFTEIQKLVLDVAHVHEHCCRGDVLDCLQDGEKIMSYICSQ
QDTLSNKITECCKLTTLERGQCIIHAENDEKPEGLSPNLNRFLGDRDFNQFSSGEKNIF
LASFVHEYSRRHPQLAVSVILRVAKGYQELLEKCFQTENPLECQDKGEEELQKYIQES
QALAKRSCGLFQKLGEYYLQNAFLVAYTKKAPQLTSSELMAITRKMAATAATCCQL
SEDKLLACGEGAADIIIGHLCIRHEMTPVNPGVGQCCTSSYANRRPCFSSLVVDETYV
PPAFSDDKFIFHKDLCQAQGVALQTMKQEFLINLVKQKPQITEEQLEAVIADFSGLLE
KCCQGQEQEVCFAEEGQKLISKTRAALGV

hAFP CDS (SEQ ID NO: 2)

ATGAAGTGGGTGGAATCAATTTTTTTAATTTTCCTACTAAATTTTACTGAATCCAG
AACACTGCATAGAAATGAATATGGAATAGCTTCCATATTGGATTCTTACCAATGT
ACTGCAGAGATAAGTTTAGCTGACCTGGCTACCATATTTTTTGCCCAGTTTGTTCA
AGAAGCCACTTACAAGGAAGTAAGCAAAATGGTGAAAGATGCATTGACTGCAAT
TGAGAAACCCACTGGAGATGAACAGTCTTCAGGGTGTTTAGAAAACCAGCTACC
TGCCTTTCTGGAAGAACTTTGCCATGAGAAAGAAATTTTGGAGAAGTACGGACAT
TCAGACTGCTGCAGCCAAAGTGAAGAGGGAAGACATAACTGTTTTCTTGCACAC
AAAAAGCCCACTCCAGCATCGATCCCACTTTTCCAAGTTCCAGAACCTGTCACAA
GCTGTGAAGCATATGAAGAAGACAGGGAGACATTCATGAACAAATTCATTTATG
AGATAGCAAGAAGGCATCCCTTCCTGTATGCACCTACAATTCTTCTTTGGGCTGC
TCGCTATGACAAAATAATTCCATCTTGCTGCAAAGCTGAAAATGCAGTTGAATGC
TTCCAAACAAAGGCAGCAACAGTTACAAAAGAATTAAGAGAAAGCAGCTTGTTA
AATCAACATGCATGTGCAGTAATGAAAAATTTTGGGACCCGAACTTTCCAAGCCA
TAACTGTTACTAAACTGAGTCAGAAGTTTACCAAAGTTAATTTTACTGAAATCCA
GAAACTAGTCCTGGATGTGGCCCATGTACATGAGCACTGTTGCAGAGGAGATGT
GCTGGATTGTCTGCAGGATGGGGAAAAAATCATGTCCTACATATGTTCTCAACAA
GACACTCTGTCAAACAAAATAACAGAATGCTGCAAACTGACCACGCTGGAACGT
GGTCAATGTATAATTCATGCAGAAAATGATGAAAAACCTGAAGGTCTATCTCCA
AATCTAAACAGGTTTTTAGGAGATAGAGATTTTAACCAATTTTCTTCAGGGGAAA
AAAATATCTTCTTGGCAAGTTTTGTTCATGAATATTCAAGAAGACATCCTCAGCT
TGCTGTCTCAGTAATTCTAAGAGTTGCTAAAGGATACCAGGAGTTATTGGAGAAG
TGTTTCCAGACTGAAAACCCTCTTGAATGCCAAGATAAAGGAGAAGAAGAATTA
CAGAAATACATCCAGGAGAGCCAAGCATTGGCAAAGCGAAGCTGCGGCCTCTTC
CAGAAACTAGGAGAATATTACTTACAAAATGCGTTTCTCGTTGCTTACACAAAGA
AAGCCCCCCAGCTGACCTCGTCGGAGCTGATGGCCATCACCAGAAAAATGGCAG
CCACAGCAGCCACTTGTTGCCAACTCAGTGAGGACAAACTATTGGCCTGTGGCGA
GGGAGCGGCTGACATTATTATCGGACACTTATGTATCAGACATGAAATGACTCCA
GTAAACCCTGGTGTTGGCCAGTGCTGCACTTCTTCATATGCCAACAGGAGGCCAT
GCTTCAGCAGCTTGGTGGTGGATGAAACATATGTCCCTCCTGCATTCTCTGATGA
CAAGTTCATTTTCCATAAGGATCTGTGCCAAGCTCAGGGTGTAGCGCTGCAAACA
ATGAAGCAAGAGTTTCTCATTAACCTTGTGAAGCAAAAGCCACAAATAACAGAG
GAACAACTTGAGGCTGTCATTGCAGATTTCTCAGGCCTGTTGGAGAAATGCTGCC
AAGGCCAGGAACAGGAAGTCTGCTTTGCTGAAGAGGGACAAAAACTGATTTCAA
AAACTCGTGCTGCTTTGGGAGTTTAA

hAFP137–145 (SEQ ID NO: 3)

PLFQVPEPV

hAFP158–166 (SEQ ID NO: 4)

FMNKFIYEI

hAFP325–334 (SEQ ID NO: 5)

GLSPNLNRFL

hAFP542–550 (SEQ ID NO: 6)

GVALQTMKQ

hAFP158 A1 (SEQ ID NO: 7)
AMNKFIYEI

hAFP158 A3 (SEQ ID NO: 8)
FMAKFIYEI

hAFP158 A4 (SEQ ID NO: 9)
FMNAFIYEI

hAFP158 A5 (SEQ ID NO: 10)
FMNKAIYEI

hAFP158 A6 (SEQ ID NO: 11)
FMNKFAYEI

hAFP158 A7 (SEQ ID NO: 12)
FMNKFIAEI

hAFP158 A8 (SEQ ID NO: 13)
FMNKFIYAI

mAFP protein (SEQ ID NO: 14)
MKWITPASLILLLHFAASKALHENEFGIASTLDSSQCVTEKNVLSIATITFTQFVPEATE
EEVNKMTSDVLAAMKKNSGDGCLESQLSVFLDEICHETELSNKYGLSGCCSQSGVE
RHQCLLARKKTAPASVPPFQFPEPAESCKAHEENRAVFMNRFIYEVSRRNPFMYAPA
ILSLAAQYDKVVLACCKADNKEECFQTKRASIAKELREGSMLNEHVCSVIRKFGSRN
LQATTIIKLSQKLTEANFTEIQKLALDVAHIHEECCQGNSLECLQDGEKVMTYICSQQ
NILSSKIAECCKLPMIQLGFCIIHAENGVKPEGLSLNPSQFLGDRNFAQFSSEEKIMFM
ASFLHEYSRTHPNLPVSVILRIAKTYQEILEKCSQSGNLPGCQDNLEEELQKHIEESQA
LSKQSCALYQTLGDYKLQNLFLIGYTRKAPQLTSAELIDLTGKMVSIASTCCQLSEEK
WSGCGEGMADIFIGHLCIRNEASPVNSGISHCCNSSYSNRRLCITSFLRDETYAPPPFS
EDKFIFHKDLCQAQGKALQTMKQELLINLVKQKPELTEEQLAAVTADFSGLLEKCCK
AQDQEVCFTEEGPKLISKTRDALGV

mAFP CDS (SEQ ID NO: 15)

ATGAAGTGGATCACACCCGCTTCCCTCATCCTCCTGCTACATTTCGCTGCGTCCA
AAGCATTGCACGAAAATGAGTTTGGGATAGCTTCCACGTTAGATTCCTCCCAGTG
CGTGACGGAGAAGAATGTGCTTAGCATAGCTACCATCACCTTTACCCAGTTTGTT
CCGGAAGCCACCGAGGAGGAAGTGAACAAAATGACTAGCGATGTGTTGGCTGCA
ATGAAGAAAAACTCTGGCGATGGGTGTTTAGAAAGCCAGCTATCTGTGTTTCTGG
ATGAAATTTGTCATGAGACGGAACTCTCTAACAAGTATGGACTCTCAGGCTGCTG
CAGCCAAAGTGGAGTGGAAAGACATCAGTGTCTGCTGGCACGCAAGAAGACTGC
TCCGGCCTCTGTCCCACCCTTCCAGTTTCCAGAACCTGCCGAGAGTTGCAAAGCA
CATGAAGAAAACAGGGCAGTGTTCATGAACAGGTTCATCTATGAAGTGTCAAGG
AGGAACCCCTTCATGTATGCCCCAGCCATTCTGTCCTTGGCTGCTCAGTACGACA
AGGTCGTTCTGGCATGCTGCAAAGCTGACAACAAGGAGGAGTGCTTCCAGACAA
AGAGAGCATCCATTGCAAAGGAATTAAGAGAAGGAAGCATGTTAAATGAGCATG
TATGTTCAGTGATAAGAAAATTTGGATCCCGAAACCTCCAGGCAACAACCATTAT
TAAGCTAAGTCAAAAGTTAACTGAAGCAAATTTTACTGAGATTCAGAAGCTGGC
CCTGGATGTGGCTCACATCCACGAGGAGTGTTGCCAAGGAAACTCGCTGGAGTG
TCTGCAGGATGGGGAAAAAGTCATGACATATATATGTTCTCAACAAAATATTCTG
TCAAGCAAAATAGCAGAGTGCTGCAAATTACCCATGATCCAACTAGGCTTCTGCA
TAATTCACGCAGAGAATGGCGTCAAACCTGAAGGCTTATCTCTAAATCCAAGCCA
GTTTTTGGGAGACAGAAATTTTGCCCAATTTTCTTCAGAGGAAAAAATCATGTTC
ATGGCAAGCTTTCTTCATGAATACTCAAGAACTCACCCCAACCTTCCTGTCTCAG
TCATTCTAAGAATTGCTAAAACGTACCAGGAAATATTGGAGAAGTGTTCCCAGTC
TGGAAATCTACCTGGATGTCAGGACAATCTGGAAGAAGAATTGCAGAAACACAT
CGAGGAGAGCCAGGCACTGTCCAAGCAAAGCTGCGCTCTCTACCAGACCTTAGG
AGACTACAAATTACAAAATCTGTTCCTTATTGGTTACACGAGGAAAGCCCCTCAG
CTGACCTCAGCAGAGCTGATCGACCTCACCGGGAAGATGGTGAGCATTGCCTCC
ACGTGCTGCCAGCTCAGCGAGGAGAAATGGTCCGGCTGTGGTGAGGGAATGGCC
GACATTTTCATTGGACATTTGTGTATAAGGAATGAAGCAAGCCCTGTGAACTCTG
GTATCAGCCACTGCTGCAACTCTTCGTATTCCAACAGGAGGCTATGCATCACCAG
TTTTCTGAGGGATGAAACCTATGCCCCTCCCCCATTCTCTGAGGATAAATTCATCT
TCCACAAGGATCTGTGCCAAGCTCAGGGCAAAGCCCTACAGACCATGAAACAAG
AGCTTCTCATTAACCTGGTGAAGCAAAAGCCTGAACTGACAGAGGAGCAGCTGG
CGGCTGTCACTGCAGATTTCTCGGGCCTTTTGGAGAAGTGCTGCAAAGCCCAGGA
CCAGGAAGTCTGTTTCACAGAAGAGGGTCCAAAGTTGATTTCCAAAACTCGTGAT
GCTTTGGGCGTTTAA

mAFP154-162 (SEQ ID NO: 16)

FMNRFIYEV

Clone 17 heavy chain variable domain, protein (SEQ ID NO: 17)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYN
GNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARYQDWWYLGQFD
QWGQGTLVTVSS

Clone 33 heavy chain variable domain, protein (SEQ ID NO: 18)

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRS
KWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCARGSYYSGRYDAWG
QGTLVTVSS

Clone 44 heavy chain variable domain, protein (SEQ ID NO: 19)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGT
ANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAREIRGYYYYYGMDVW
GQGTTVTVSS

Clone 48 heavy chain variable domain, protein (SEQ ID NO: 20)
EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNS
GRIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARADDYGAPYYYYG
MDVWGQGTTVTVSS

Clone 50 heavy chain variable domain, protein (SEQ ID NO: 21)
QVQLQESGPGLVKPSGTLSLTCAVSGGSISSSNWWSWVRQPPGKGLEWIGEIYHSGS
TNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCATGYGGYFDYWGQGTLV
TVSS

Clone 52 heavy chain variable domain, protein (SEQ ID NO: 22)
EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYN
GNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSYYYYYGMDV
WGQGTTVTVSS

Clone 61 heavy chain variable domain, protein (SEQ ID NO: 23)
EVQLVQSGAEVKKPGESLTISCKASGYSFPNYWITWVRQMSGGGLEWMGRIDPGDS
YTTYNPSFQGHVTISIDKSTNTAYLHWNSLKASDTAMYYCARYYVSLVDIWGQGTL
VTVSS

Clone 76 heavy chain variable domain, protein (SEQ ID NO: 24)
EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVGFIRSKA
YGGTTEYAASVKGRFTISRDDSKSIAYLQMNNLKTEDTAVYYCARDGLYSSSWYDS
DYWGQGTLVTVSS

Clone 79 heavy chain variable domain, protein (SEQ ID NO: 25)
QMQLVQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNS
GSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKDIHSGSYYGLLYYA
MDVWGQGTTVTVSS

Clone 17-13 heavy chain variable domain, protein (SEQ ID NO: 26)
EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYN
GNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARFQDWWYLGQFD
QWGQGTLVTVSS

Clone 17 light chain variable domain, protein (SEQ ID NO: 27)
QSALTQPASVSGSPGQSITISCTATGSDVGVYYYVSWYQQHPGKAPKVMIYDVGNRP
PGVSNRFSGSKSGNTASLTISGLQAEDEADYYCASYTNRNSLGYVFGTGTKVTVLG

Clone 33 light chain variable domain, protein (SEQ ID NO: 28)
NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSSPTTVIYEDNQRPSG
VPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSSTVVFGGGTKLTVLG

Clone 44 light chain variable domain, protein (SEQ ID NO: 29)
SYELTQPPSVSVAPGKTARITCGGDNIGTKSVTWYQQRPGQAPMMVIYYDTVRPSGI
PERLSGSNSGNTATLTITRVEAGDEADYYCQVWDSSSDHPVFGGGTKLTVLG

Clone 48 light chain variable domain, protein (SEQ ID NO: 30)
QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPS
GVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGSVFGTGTKVTVLG

Clone 50 light chain variable domain, protein (SEQ ID NO: 31)
QSVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIP
ERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVLG

Clone 52 light chain variable domain, protein (SEQ ID NO: 32)
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGHYPYWFQQKPGQAPRTLIYDASDKH
SWTPARFSGSLLGGKAALTLSGAQPEDEAEYYCLLSYSDALVFGGGTKLTVLG

Clone 61 light chain variable domain, protein (SEQ ID NO: 33)
QSVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVNNRP
SEVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTTGSRAVFGGGTKLTVLG

Clone 76 light chain variable domain, protein (SEQ ID NO: 34)
QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSG
IPDRFSGSKSGTSATLGITGLQTGDEADYYCGTWDGSLYTMLFGGGTKLTVLG

Clone 79 light chain variable domain, protein (SEQ ID NO: 35)
QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIFGNSNRPS
GVPDRFSGFKSGTSASLAITGLQAEDEADYFCQSYDSSLSGSGVFGTGTKVTVLG

Clone 17-13 light chain variable domain, protein (SEQ ID NO: 36)
QSALTQPASVSGSPGQSITISCTATGSDVGVYYYVSWYQQHPGKAPKVMIYDVDNRP
PGVSNRFSGSKSGNTASLTISGLQAEDEADYYCASYTNRNSLGYVFGTGTKVTVLG

Clone 17 heavy chain variable domain, nucleic acid (SEQ ID NO: 37)
GAGGTCCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTG
AAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGGG
TGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACA
ATGGTAACACAAACTATGCACAGAAGCTCCAGGGCAGAGTCACCATGACCACAG
ACACATCCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACA
CGGCCGTGTATTACTGTGCGCGCTACCAGGACTGGTGGTACCTGGGTCAGTTCGA
TCAGTGGGGTCAAGGTACTCTGGTGACCGTCTCCTCA

Clone 33 heavy chain variable domain, nucleic acid (SEQ ID NO: 38)
CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTC
TCACTCACCTGTGCCATTTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGA
ACTGGATCAGGCAGTCCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACT
ACAGGTCCAAGTGGTATAATGATTATGCAGTATCTGTGAAAAGTCGAATAACCAT
CAACCCAGACACATCCAAGAACCAGTTCTCCCTGCAGCTGAACTCTGTGACTCCC
GAGGACACGGCTGTGTATTACTGTGCGCGCGGTTCTTACTACTCTGGTCGTTACG
ATGCTTGGGGTCAAGGTACTCTGGTGACCGTCTCCTCA

Clone 44 heavy chain variable domain, nucleic acid (SEQ ID NO: 39)
CAGGTCCAGCTGGTACAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTG
AAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGG
TGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCT
TTGGTACAGCAAACTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGG
ACGAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACA
CGGCCGTGTATTACTGTGCGAGAGAAATTAGGGGCTACTACTACTACTACGGTAT
GGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

Clone 48 heavy chain variable domain, nucleic acid (SEQ ID NO: 40)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCAGGTCCCTG
AGACTCTCCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGCCATGCACTGGGT
CCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAACAG
TGGTAGAATAGGCTATGCGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGA
CAACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACAC
GGCTGTGTATTACTGTGCGAGAGCCGATGACTACGGCGCCCCCTACTACTACTAC
GGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

Clone 50 heavy chain variable domain, nucleic acid (SEQ ID NO: 41)
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGGGACCCTG
TCCCTCACCTGCGCTGTCTCTGGTGGCTCCATCAGCAGTAGTAACTGGTGGAGTT
GGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCTATCATA
GTGGGAGCACCAACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCAGTAG
ACAAGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCGGACA
CGGCCGTGTATTACTGTGCGACCGGTTATGGGGGGTACTTTGACTACTGGGGCCA
GGGAACCCTGGNCACCGTCTCCTCA

Clone 52 heavy chain variable domain, nucleic acid (SEQ ID NO: 42)
GAAGTGCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTG
AAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGGG
TGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACA
ATGGTAACACAAACTATGCACAGAAGCTCCAGGGCAGAGTCACCATGACCACAG
ACACATCCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACA
CGGCCGTGTATTACTGTGCGAGAGATTCCTACTACTACTACTACGGTATGGACGT
CTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

Clone 61 heavy chain variable domain, nucleic acid (SEQ ID NO: 43)
GAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAGCCTGGCGAGAGCCTG
ACCATCTCCTGCAAGGCCAGCGGCTACAGCTTCCCCAACTACTGGATCACCTGGG
TGCGCCAGATGTCCGGCGGAGGCCTGGAATGGATGGGCAGAATCGACCCCGGCG
ACAGCTACACAACCTACAACCCCAGCTTCCAGGGCCACGTGACCATCAGCATCG
ACAAGAGCACCAATACCGCCTACCTGCACTGGAACAGCCTGAAGGCCTCCGACA
CCGCCATGTACTACTGCGCCCGGTACTATGTGTCCCTGGTGGATATCTGGGGCCA
GGGCACACTCGTGACCGTGTCTAGC

Clone 76 heavy chain variable domain, nucleic acid (SEQ ID NO: 44)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTAAAGCCTGGGGGGTCCCTT
AGACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAACGCCTGGATGAGCTGGG
TCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTAGGTTTCATTAGAAGCAAAG
CTTATGGTGGGACAACAGAATACGCCGCCTCTGTGAAAGGCAGATTCACCATCTC
AAGAGATGATTCCAAAAGCATCGCCTATCTGCAAATGAACAACCTGAAAACCGA
GGACACAGCCGTGTATTACTGTGCTAGAGATGGGCTGTATAGCAGCAGCTGGTA
CGATTCTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

Clone 79 heavy chain variable domain, nucleic acid (SEQ ID NO: 45)
CAGATGCAGCTGGTGCAGTCTGGGGGAGGCTTGGTACAGCCTGGCAGGTCCCTG
AGACTCTCCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGCCATGCACTGGGT
CCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAATAG
TGGTAGCATAGGCTATGCGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGAC
AACGCCAAGAACTCCCTGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACACG
GCCTTGTATTACTGTGCAAAAGATATCCATAGTGGGAGCTACTACGGCCTACTCT
ACTACGCTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

Clone 17-13 heavy chain variable domain, nucleic acid (SEQ ID NO: 46)
GAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTG
AAGGTGTCCTGCAAGGCCAGCGGCTACACCTTTACCAGCTACGGCATCAGCTGG
GTGCGCCAGGCTCCTGGACAGGGCCTGGAATGGATGGGCTGGATCAGCGCCTAC
AACGGCAATACCAACTACGCCCAGAAACTGCAGGGCAGAGTGACCATGACCACC
GACACCAGCACCTCCACCGCCTACATGGAACTGCGGAGCCTGAGAAGCGACGAC
ACCGCCGTGTACTATTGCGCCCGGTTCCAGGACTGGTGGTATCTGGGCCAGTTCG
ACCAGTGGGGCCAGGGCACACTCGTGACCGTGTCTAGC

Clone 17 light chain variable domain, nucleic acid (SEQ ID NO: 47)
CAATCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCA
CCATCTCCTGCACTGCAACCGGCAGTGACGTTGGTGTTTATTACTATGTCTCCTGG
TACCAACAACACCCAGGCAAAGCCCCCAAAGTGATGATTTATGATGTCGGTAAT
CGGCCCCCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCT
CCCTGACCATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGCGCCTC
ATATACAAACAGGAACAGTCTCGGCTATGTCTTCGGAACCGGGACCAAGGTCAC
CGTCCTAGG

Clone 33 light chain variable domain, nucleic acid (SEQ ID NO: 48)
AATTTTATGCTGACTCAGCCCCACTCTGTGTCGGAGTCTCCGGGGAAGACGGTAA
CCATCTCCTGCACCCGCAGCAGTGGCAGCATTGCCAGCAACTATGTGCAGTGGTA
CCAGCAGCGCCCGGGCAGTTCCCCCACCACTGTGATCTATGAGGATAACCAAAG
ACCCTCTGGGGTCCCTGATCGGTTCTCTGGCTCCATCGACAGCTCCTCCAACTCTG
CCTCCCTCACCATCTCTGGACTGAAGACTGAGGACGAGGCTGACTACTACTGTCA
GTCTTATGATAGCAGCACCGTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTA
GGT

Clone 44 light chain variable domain, nucleic acid (SEQ ID NO: 49)
TCCTATGAGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCTGGCAAGACGGCCA
GGATTACCTGTGGGGGTGACAACATTGGAACTAAAAGTGTGACCTGGTACCAAC
AGAGGCCAGGCCAGGCCCCTATGATGGTCATCTATTATGATACCGTCCGGCCCTC
AGGGATCCCTGAGCGACTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACC
ATCACCAGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGAT
AGTAGTAGTGATCATCCGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

Clone 48 light chain variable domain, nucleic acid (SEQ ID NO: 50)
CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGGGGCCCCGGGGCAGAGGGTC
ACCATCTCCTGCACTGGGAGCAGCTCCAACATCGGGGCAGGTTATGATGTACACT
GGTACCAGCAGCTTCCAGGAACAGCCCCCAAACTCCTCATCTATGGTAACAGCA
ATCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCAGC
CTCCCTGGCCATCACTGGGCTCCAGGCTGAGGATGAGGCTGATTATTACTGCCAG
TCCTATGACAGCAGCCTGAGTGGTTCAGTCTTCGGAACTGGGACCAAGGTCACCG
TCCTAGGT

Clone 50 light chain variable domain, nucleic acid (SEQ ID NO: 51)
CAGTCTGTGTTGACTCAGCCACCCTCAGTGTCAGTGGCCCCAGGAAAGACGGCC
AGGATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAG
CAGAAGCCAGGCCAGGCCCCTGTGCTGGTCATCTATTATGATAGCGACCGGCCCT
CAGGGATCCCTGAGCGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGAC
CATCAGCAGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGA
TAGTAGTAGTGATCATGTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

Clone 52 light chain variable domain, nucleic acid (SEQ ID NO: 52)
CAGGCTGTGGTGACTCAGGAGCCCTCACTGACTGTGTCCCCAGGAGGGACAGTC
ACTCTCACCTGTGGCTCCAGCACTGGAGCTGTCACCAGTGGTCATTATCCCTACT
GGTTCCAGCAGAAGCCTGGCCAAGCCCCCAGGACACTGATTTATGATGCAAGCG
ACAAACACTCCTGGACACCTGCCCGGTTCTCAGGCTCCCTCCTTGGGGGCAAAGC
TGCCCTGACCCTTTCGGGTGCGCAGCCTGAGGATGAGGCTGAGTATTACTGCTTG
CTCTCCTATAGTGATGCTCTGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAG
GT

Clone 61 light chain variable domain, nucleic acid (SEQ ID NO: 53)
CAGAGCGTGCTGACACAGCCTGCCTCCGTGTCTGGCTCTCCTGGCCAGTCCATCA
CCATCAGCTGTACCGGCACCAGCTCCGACGTGGGCGGCTACAATTACGTGTCCTG
GTATCAGCAGCATCCCGGCAAGGCCCCCAAGCTGATGATCTACGACGTGAACAA
CCGGCCCAGCGAGGTGTCCAACAGATTCAGCGGCAGCAAGAGCGGCAACACCGC
CAGCCTGACAATCAGCGGACTGCAGGCCGAGGACGAGGCCGACTACTACTGCAG
CAGCTACACCACCGGCAGCAGAGCCGTGTTTGGCGGAGGCACCAAGCTGACAGT
GCTGGGC

Clone 76 light chain variable domain, nucleic acid (SEQ ID NO: 54)
CAGTCTGTCGTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTC
ACCATCTCCTGCTCTGGAAGCAGCTCCAACATTGGGAATAATTATGTATCCTGGT
ACCAGCAGCTCCCAGGAACAGCCCCCAAACTCCTCATTTATGACAATAATAAGC
GACCCTCAGGGATTCCTGACCGATTCTCTGGCTCCAAGTCTGGCACGTCAGCCAC
CCTGGGCATCACCGGACTCCAGACTGGGGACGAGGCCGATTACTACTGCGGAAC
ATGGGATGGCAGCCTCTATACTATGTTATTCGGCGGAGGGACCAAGCTGACCGTC
CTAGGT

Clone 79 light chain variable domain, nucleic acid (SEQ ID NO: 55)
CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGGGGCCCCAGGGCAGAGGGTC
ACCATCTCCTGCACTGGGAGCAGCTCCAACATCGGGGCAGGTTATGATGTACACT
GGTACCAGCAGCTTCCAGGAACAGCCCCCAAACTCCTCATCTTTGGTAACAGCAA
TCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTTCAAGTCTGGCACCTCAGCC
TCCCTGGCCATCACTGGGCTCCAGGCTGAGGATGAGGCTGACTATTTCTGCCAGT
CGTATGACAGTAGCCTGAGTGGTTCGGGGGTCTTCGGAACTGGGACCAAGGTCA
CCGTCCTAGGT

Clone 17-13 light chain variable domain, nucleic acid (SEQ ID NO: 56)
CAGAGCGCCCTGACACAGCCTGCCTCCGTGTCTGGATCTCCCGGCCAGAGCATCA
CCATCAGCTGCACAGCCACCGGCTCCGACGTGGGCGTGTACTACTACGTGTCCTG
GTATCAGCAGCATCCCGGCAAGGCCCCCAAAGTGATGATCTACGACGTGGACAA
CCGGCCTCCCGGCGTGTCCAATAGATTCAGCGGCAGCAAGAGCGGCAACACCGC
CAGCCTGACAATCAGCGGACTGCAGGCCGAGGACGAGGCCGATTACTACTGCGC
CAGCTACACCAACCGGAACAGCCTGGGCTACGTGTTCGGCACCGGCACCAAAGT
GACAGTGCTGGGC

Clone 17 HCDR1 (SEQ ID NO: 57)
GYTFTSYG

Clone 33 HCDR1 (SEQ ID NO: 58)
VSSNSAAWN

Clone 44 HCDR1 (SEQ ID NO: 59)
GGTFSSYA

Clone 48 HCDR1 (SEQ ID NO: 60)
GFTFDDYA

Clone 50 HCDR1 (SEQ ID NO: 61)
GGSISSSNW

Clone 52 HCDR1 (SEQ ID NO: 62)
GYTFTSYG

Clone 61 HCDR1 (SEQ ID NO: 63)
GYSFPNYW

Clone 76 HCDR1 (SEQ ID NO: 64)
GFTFSNAW

Clone 79 HCDR1 (SEQ ID NO: 65)
GFTFDDYA

Clone 17-13 HCDR1 (SEQ ID NO: 66)
GYTFTSYG

Clone 17 HCDR2 (SEQ ID NO: 67)
ISAYNGNT

Clone 33 HCDR2 (SEQ ID NO: 68)
YRSKWYN

Clone 44 HCDR2 (SEQ ID NO: 69)
IIPIFGTA

Clone 48 HCDR2 (SEQ ID NO: 70)
ISWNSGRI

Clone 50 HCDR2 (SEQ ID NO: 71)
IYHSGST

Clone 52 HCDR2 (SEQ ID NO: 72)
ISAYNGNT

Clone 61 HCDR2 (SEQ ID NO: 73)
IDPGDSYT

Clone 76 HCDR2 (SEQ ID NO: 74)
IRSKAYGGTT

Clone 79 HCDR2 (SEQ ID NO: 75)
ISWNSGSI

Clone 17-13 HCDR2 (SEQ ID NO: 76)
ISAYNGNT

Clone 17 HCDR3 (SEQ ID NO: 77)
ARYQDWWYLGQFDQ

Clone 33 HCDR3 (SEQ ID NO: 78)
ARGSYYSGRYDA

Clone 44 HCDR3 (SEQ ID NO: 79)
AREIRGYYYYYGMDV

Clone 48 HCDR3 (SEQ ID NO: 80)
ARADDYGAPYYYYGMDV

Clone 50 HCDR3 (SEQ ID NO: 81)
ATGYGGYFDY

Clone 52 HCDR3 (SEQ ID NO: 82)
ARDSYYYYYGMDV

Clone 61 HCDR3 (SEQ ID NO: 83)
ARYYVSLVDI

Clone 76 HCDR3 (SEQ ID NO: 84)
ARDGLYSSSWYDSDY

Clone 79 HCDR3 (SEQ ID NO: 85)
AKDIHSGSYYGLLYYAMDV

Clone 17-13 HCDR3 (SEQ ID NO: 86)
ARFQDWWYLGQFDQ

HCCDR1 consensus (SEQ ID NO: 87)
G-F/Y-S/T-F-D/S/T-D/N/S-Y/A-A/G/W

HCCDR2 consensus (SEQ ID NO: 88)
I/S-K/S-X-H/Y-X-G-X-T

HCCDR3 consensus (SEQ ID NO: 89)
A/G-X-W/Y-Y-X-X-X-F/Y-D

Clone 17 LCDR1 (SEQ ID NO: 90)
GSDVGVYYY

Clone 33 LCDR1 (SEQ ID NO: 91)
SGSIASNY

Clone 44 LCDR1 (SEQ ID NO: 92)
NIGTKS

Clone 48 LCDR1 (SEQ ID NO: 93)
SSNIGAGYD

Clone 50 LCDR1 (SEQ ID NO: 94)
NIGSKS

Clone 52 LCDR1 (SEQ ID NO: 95)
TGAVTSGHY

Clone 61 LCDR1 (SEQ ID NO: 96)
SSDVGGYNY

Clone 76 LCDR1 (SEQ ID NO: 97)
SSNIGNNY

Clone 79 LCDR1 (SEQ ID NO: 98)
SSNIGAGYD

Clone 17-13 LCDR1 (SEQ ID NO: 99)
GSDVGVYYY

Clone 17 LCDR2 (SEQ ID NO: 100)
DVG

Clone 33 LCDR2 (SEQ ID NO: 101)
EDN

Clone 44 LCDR2 (SEQ ID NO: 102)
YDT

Clone 48 LCDR2 (SEQ ID NO: 103)
GNS

Clone 50 LCDR2 (SEQ ID NO: 104)
YDS

Clone 52 LCDR2 (SEQ ID NO: 105)
DAS

Clone 61 LCDR2 (SEQ ID NO: 106)
DVN

Clone 76 LCDR2 (SEQ ID NO: 107)
DNN

Clone 79 LCDR2 (SEQ ID NO: 108)
GNS

Clone 17-13 LCDR2 (SEQ ID NO: 109)
DVD

Clone 17 LCDR3 (SEQ ID NO: 110)
ASYTNRNSLGYV

Clone 33 LCDR3 (SEQ ID NO: 111)
QSYDSSTVV

Clone 44 LCDR3 (SEQ ID NO: 112)
QVWDSSSDHPV

Clone 48 LCDR3 (SEQ ID NO: 113)
QSYDSSLSGSV

Clone 50 LCDR3 (SEQ ID NO: 114)
QVWDSSSDHVV

Clone 52 LCDR3 (SEQ ID NO: 115)
LLSYSDALV

Clone 61 LCDR3 (SEQ ID NO: 116)
SSYTTGSRAV

Clone 76 LCDR3 (SEQ ID NO: 117)
GTWDGSLYTML

Clone 79 LCDR3 (SEQ ID NO: 118)
QSYDSSLSGSGV

Clone 17-13 LCDR3 (SEQ ID NO: 119)
ASYTNRNSLGYV

LCCDR1 consensus (SEQ ID NO: 120)
S/T-G/S-D/N-I/V-A/G-A/S/V-X-H/Y

LCCDR3 consensus (SEQ ID NO: 121)
Q-S/T-Y/W-D/T-S/T-A/S

IFI30 control (SEQ ID NO: 122)
LLDVPTAAV

BTG2 control (SEQ ID NO: 123)
TLWVDPYEV

BCR control (SEQ ID NO: 124)
FLLDHLKRV

IFI30 control (SEQ ID NO: 125)
LLLDVPTAAV

SSR1 control (SEQ ID NO: 126)
VLFRGGPRGLLAV

PPP2R1B control (SEQ ID NO: 127)
SLLPAIVEL

DDX5 control (SEQ ID NO: 128)
YLLPAIVHI

CTSG control (SEQ ID NO: 129)
FLLPTGAEA

CD247 control (SEQ ID NO: 130)
LLDPKLCYLL

DMTN control (SEQ ID NO: 131)
SLPHFHHPET

CALR control (SEQ ID NO: 132)
MLLSVPLLLG

PIM1 control (SEQ ID NO: 133)
LLYDMVCGDIP

IFI30 control (SEQ ID NO: 134)
LLLDVPTAAVQ

IFI30 control (SEQ ID NO: 135)
LLLDVPTAAVQA

SSR1 control (SEQ ID NO: 136)
VLFRGGPRGLLAVA

HLA-E control (SEQ ID NO: 137)
MVDGTLLLL

RPS6KB1 control (SEQ ID NO: 138)
YMAPEILMRS

CSF2RA control (SEQ ID NO: 139)
FIYNADLMNC

IL7 control (SEQ ID NO: 140)
KQYESVLMVSI

hTERT540 control (SEQ ID NO: 141)
ILAKFLHWL

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.