[0017] Umaña et al. showed previously that overexpression of β(1,4)-N-acetylglucosaminyltransferase III ("GnTIII"), a glycosyltransferase catalyzing the formation of bisected oligosaccharides, in Chinese hamster ovary (CHO) cells significantly increases the in vitro ADCC activity of an anti-neuroblastoma chimeric monoclonal antibody (chCE7) produced by the engineered CHO cells. (See Umaña, P. et al., Nature Biotechnol. 17:176-180 (1999); and International Publication No. WO 99/54342, the entire contents of which are hereby incorporated by reference). The antibody chCE7 belongs to a large class of unconjugated mAbs which have high tumor affinity and specificity, but have too little potency to be clinically useful when produced in standard industrial cell lines lacking the GnTIII enzyme (Umana, P., et al., Nature Biotechnol. 17:176-180 (1999)). That study was the first to show that large increases of ADCC activity could be obtained by engineering the antibody-producing cells to express GnTIII, which also led to an increase in the proportion of constant region (Fc)-associated, bisected oligosaccharides, including bisected, nonfucosylated oligosaccharides, above the levels found in naturally-occurring antibodies.

[0018] There remains a need for enhanced therapeutic approaches targeting CEA, in particular, membrane-bound CEA for the treatment of cancers.

BRIEF SUMMARY ~~OF THE INVENTION~~

[0019] The disclosure ~~One aspect of the invention~~ provides a variant antigen binding molecule (ABM), such as an antibody, which binds membrane-bound human carcinoembryonic antigen (CEA). In one embodiment, the ABM has an increase in stability as compared to its parent molecule. In one embodiment, the ABM has an increase in stability and maintains, or has an improved, binding affinity for membrane-bound CEA as compared to its parent molecule. In one embodiment, ABM is stable at a temperature that is at least 0.5, 1.0, 1.5, or 2.0 degree Celcius higher than its parent molecule. In one embodiment, the increase in stability is measured using a dynamic light scattering assay. In some embodiments, the parent comparator molecule is PR1A3 antibody or humanized version of PR1A3 antibody. In one embodiment, the parent comparator molecule is a humanized version of PR1A3 antibody which comprises the heavy chain variable region CH7A (SEQ ID NO:101) and the light chain variable region 2F1 (SEQ ID NO: 209). In one embodiment, the variant antigen binding molecule is stable at 67 degrees Celsius or higher, as measured, for example by a dynamic light scattering assay. In one embodiment, the variant antigen binding molecule binds membrane-bound CEA at a Kd of 100 nM or lower. In one embodiment, the variant antigen binding molecule binds membrane-bound CEA at a Kd of 10 nM or lower.

[0020]

[0021] In one embodiment, the ABM comprises a heavy chain variable region comprising a heavy chain CDR1 selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ

ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:12, a heavy chain CDR2 selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, and a heavy chain CDR3 selected from the group consisting of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219 SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, and SEQ ID NO:224. In one embodiment, the ABM comprises a light chain variable region comprising a light chain CDR1 selected from the group consisting of SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, and SEQ ID NO:45, and a light chain CDR2 selected from the group consisting of SEQ ID NO:46, and SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, and SEQ ID NO:55, and a light chain CDR3 of SEQ ID NO:56. In another embodiment, the heavy chain variable region of the ABM comprises the heavy chain CDR1 of SEQ ID NO:1, the heavy chain CDR2 of SEQ ID NO:13, a heavy chain CDR3 selected from the group consisting SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219 SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, and SEQ ID NO:224; and the light chain variable region of the ABM comprises the light chain CDR1 of SEQ ID NO:39, the light chain CDR2 of SEQ ID NO:49, and the light chain CDR3 of SEQ ID NO:56. In a further embodiment, the ABM comprises the framework residues of CH1A1A (SEQ ID NO: 261) or CH1A1B (SEQ ID NO: 262).

[0022] In one embodiment, the heavy chain variable region of the ABM comprises an amino acid sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, and SEQ ID NO: 247 and the light chain variable region of the ABM comprises an amino acid sequence that is at least 95% identical to the sequence of SEQ ID NO: 209. In one embodiment, the heavy chain variable region of the ABM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, and SEQ ID NO: 247 and the light chain variable region of the ABM comprises the amino acid sequence of SEQ ID NO: 209. In some embodiments, the ABM comprises an Fc region, for example, a human IgG Fc region. In certain embodiments, the ABM or is an antibody or fragment thereof , such as a whole antibody, an scFv fragment, an Fv fragment, an F(ab')2 fragment, a minibody, a diabody, a triabody, or a tetrabody.

[0023] The disclosure also~~Another aspect of the invention~~ provides an isolated antibody which binds membrane-bound CEA, wherein the antibody comprises a heavy chain variable region comprising a heavy chain CDR1 selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:12, a heavy chain CDR2 selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID

NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, and a heavy chain CDR3 selected from the group consisting of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219 SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, and SEQ ID NO:224.

[0023a] In a first aspect, there is provided an isolated antibody which binds to membrane-bound carcinoembryonic antigen (CEA), wherein the antibody comprises a heavy chain variable region comprising: the heavy chain CDR1 of SEQ ID NO:1, the heavy chain CDR2 of SEQ ID NO:13, a heavy chain CDR3 selected form the group consisting of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, and SEQ ID NO:223; and a light chain variable region comprising: the light chain CDR1 of SEQ ID NO:39, the light chian CDR2 of SEQ ID NO:49, and the light chain CDR3 of SEQ ID NO:56.

[0023b] In a second aspect, there is provided an antibody which binds membrane-bound human carcinoembryonic antigen (CEA), wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, and SEQ ID NO: 247 and wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 209.

[0024] In one embodiment, the antibody has an increase in stability as compared to its parent molecule. In one embodiment, the antibody is stable at a temperature that is at at least 0.5, 1.0, 1.5, or 2.0 degree Celcius higher its parent molecule. In one embodiment, the increase in stability is measured using a dynamic light scattering assay. In some embodiments, the parent comparator molecule is PR1A3 antibody or humanized version of PR1A3 antibody. In one embodiment, the parent comparator moleucule is a humanized version of PR1A3 antibody which comprises the heavy chain variable region CH7A (SEQ ID NO:101) and the light chain variable region 2F1 (SEQ ID NO: 209). In one embodiment, the antibody is stable at 67 degrees Celsius or higher, as measured, for example by a dynamic light scattering assay. In one embodiment, the antibody binds membrane-bound CEA at a Kd of 100 nM or lower. In one embodiment the antibody binds membrane-bound CEA at a Kd of 10 nM or lower.

[0025] In one embodiment, the antibody also comprises a light chain variable region comprising a light chain CDR1 selected from the group consisting of SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, and SEQ ID NO:45, and a light chain CDR2 selected from the group consisting of SEQ ID NO:46, and SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, and SEQ ID NO:55, and a light chain CDR3 of SEQ ID NO:56. In one embodiment, the heavy chain variable region of the antibody comprises the heavy chain CDR1 of SEQ ID NO:1 , the heavy chain CDR2 of SEQ ID NO:13, a heavy chain CDR3 selected from the group consisting SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219 SEQ ID

NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, and SEQ ID NO:224; and the light chain variable region of the antibody comprises the light chain CDR1 of SEQ ID NO:39, the light chain CDR2 of SEQ ID NO:49, and the light chain CDR3 of SEQ ID NO:56. In a further embodiment, the antibody comprises the framework residues of CH1A1A (SEQ ID NO: 261) or CH1A1B (SEQ ID NO: 262). In one embodiment, the heavy chain variable region of the antibody comprises an amino acid sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, and SEQ ID NO: 247 and the light chain variable region of the antibody comprises an amino acid sequence that is at least 95% identical to the sequence of SEQ ID NO: 209. In one embodiment, the heavy chain variable region of the antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, and SEQ ID NO:

247 and the light chain variable region of the antibody comprises the amino acid sequence of SEQ ID NO: 209.

[0026] In certain embodiments, the ABM or antibody of the above embodiments binds the same epitope as, or is capable of competing for binding with, the murine monoclonal antibody PR1A3.

[0027] In one embodiment, the ABM or the antibody comprises an Fc region that has been glycoengineered. In one embodiment, at least about 20% to about 100% of the N-linked oligosaccharides in the Fc region of the glycoengineered antibody are nonfucosylated. In one embodiment, at least about 20% to about 100% of the N-linked oligosaccharides in the glycoengineered Fc region are bisected. In one embodiment, wherein at least about 20% to about 50% of the N-linked oligosaccharides in the glycoengineered Fc region are bisected, nonfucosylated. In one embodiment, the glycoengineered ABM or antibody has at least one increased effector function. The increased effector function is, for example, increased Fc receptor binding affinity, increased antibody-mediated cellular cytotoxicity (ADCC), increased binding to NK cells, increased binding to macrophages, increased binding to monocytes, increased binding to polymorphonuclear cells, direct signaling inducing apoptosis, increased dendritic cell maturation, and increased T cell priming. In one embodiment, the glycoengineered ABM or antibody has an increase in ADCC of at least about 40% to about 100% as compared to the non-glycoengineered parent antigen binding molecule.

[0028] The disclosure also~~Another aspect of the invention~~ provides for an isolated polynucleotide encoding the ABM or antibody of any of above described embodiments. In a third aspect, there is provided an isolated polynucleotide encoding the antibody as defined above. The disclosure also~~Another aspect of the invention~~ provides for a vector comprising the polynucleotide encoding the ABM or antibody of any of above described embodiments. In a fourth aspect, there is provided a vector comprising the polynucleotide as defined above. In a fifth aspect, there is~~Another aspect of the invention~~ providesd for host cell comprising this vector.

[0029] The disclosure also~~Another aspect of the invention~~ provides a composition comprising the ABM or antibody of any of above described embodiments and a pharmaceutically acceptable carrier. In a sixth aspect, there is provided a composition comprising the antibody as defined above and a pharmaceutically acceptable carrier.

[0030] The disclosure also~~Another aspect of the invention~~ provides a method of inducing cell lysis of a tumor cell comprising contacting the tumor cell with the ABM or antibody of any of above

described embodiments. In a seventh aspect, there is provided an in vitro method of inducing cell lysis of a tumor cell, the method comprising contacting the tumor cell with the antibody as defined above or the composition as defined above. In some embodiments, the tumor cell is a colorectal cancer cell, NSCLC (non-small cell lung cancer), gastric cancer cell, pancreatic cancer cell or breast cancer cell. In one embodiment, the cell lysis is induced by antibody dependent cell cytotoxicity of the ABM or antibody.

[0031] The disclosure also~~Another aspect of the invention~~ provides a method of treating a subject having a cancer that abnormally expresses CEA, the method comprising administering to the subject a therapeutically effective amount of the ABM or antibody of any of above described embodiments

[0032] The disclosure also~~Another aspect of the invention~~ provides a method of increasing survival time in a subject having a cancer that abnormally expresses CEA, said method comprising administering to said

subject a therapeutically effective amount of the ABM or antibody of any of above described embodiments. In one embodiment, the cancer is colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, pancreatic cancer or breast cancer.

[0033] In certain embodiments of these methods, the ABM, antibody, or composition is administered in combination with chemotherapy or radiation therapy. In one embodiment, the subject is a human.

[0034] The disclosure also~~Another aspect of the invention~~ provides for use of the ABM or antibody of any of above described embodiments in the manufacture of a medicament for treating a subject having a cancer that abnormally expresses CEA. In one embodiment, the cancer is selected from the group consisting of colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, pancreatic cancer and breast cancer.

[0034a] In an eighth aspect, there is provided the antibody as defined above or the composition as defined above for use in treating a subject having a cancer that abnormally expresses CEA.

[0034b] In a ninth aspect, there is provided the antibody as defined above or the composition as defined above for use in increasing survival time in a subject having a cancer that abnormally expresses CEA.

[0034c] In a tenth aspect, there is provided the use of the antibody as defined above or the composition as defined above in the manufacture of a medicament for treating a subject having a cancer that abnormally expresses CEA.

BRIEF DESCRIPTION OF THE FIGURES

[0035] FIGURE 1 shows a schematic diagram of the CEA (CEACAM-5, CD66e) antigen. The PR1A3 antibody binds specifically to the B3 domain of the antigen when it is bound to the cell membrane.

[0036] FIGURE 2 shows enhanced ADCC activity of a glycoengineered chimeric PR1A3 antibody in comparison to non-glycoengineered chimeric PR1A3 antibody with human PBMCs as effectors.

[0037] FIGURE 3 shows antigen binding activity of a humanized PR1A3 antibody comprising a heavy chain variable region construct, CH7A, and a light chain variable region construct, CL1A, in comparison to chimeric PR1A3 antibody.

[0038] FIGURE 4 shows randomization sites for generating an antibody library for affinity maturation of the humanized PR1A3 antibody light chain. Positions marked with an X were randomized.

[0039] FIGURE 5 shows randomization sites for generating an antibody library for affinity maturation of the humanized PR1A3 antibody heavy chain. Positions marked with an X were randomized.

[0040] FIGURE 6 shows binding activity of affinity matured anti-CEA antibodies derived from a humanized PR1A3 antibody comprising a heavy chain variable region construct CH7ArF9 and a light chain variable region construct CL1ArH11.

[0041] FIGURE 7 shows the results of an efficacy study in SCID/bg mice that were intrasplenically administered LS174T human colorectal carcinoma cells in order to have an orthotopic tumor model. Antibody therapy was started at seven days later by injection of the antibodies at a dose of 25 mg/kg body weight, followed by two additional weekly injections. "CH7A" represents a humanized antibody comprising the CDRs of PR1A3 as described herein. "SM3E" refers to a previously generated anti-CEA antibody. "GA201" represents a humanized anti-EGF antibody used as a positive control.

What Is Claimed Is:

1. An isolated antibody which binds to membrane-bound carcinoembryonic antigen (CEA), wherein the antibody comprises a heavy chain variable region comprising:

the heavy chain CDR1 of SEQ ID NO:1 ,

the heavy chain CDR2 of SEQ ID NO:13,

a heavy chain CDR3 selected from the group consisting of SEQ ID NO:217, SEQ ID NO:218, ~~SEQ ID NO:219~~ SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, and SEQ ID NO:223; and

a light chain variable region comprising:

the light chain CDR1 of SEQ ID NO:39,

the light chain CDR2 of SEQ ID NO:49, and

the light chain CDR3 of SEQ ID NO:56.

2. The antibody of claim 1, wherein the antibody comprises the framework residues of CH1A1A (SEQ ID NO: 261) or CH1A1B (SEQ ID NO: 262).

3. The antibody of claim 1 or 2, wherein the heavy chain variable region comprises an amino acid sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NO: 233, SEQ ID NO: 234~~, SEQ ID NO: 235~~, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, and SEQ ID NO: 247 and wherein the light chain variable region comprises an amino acid sequence that is at least 95% identical to the sequence of SEQ ID NO: 209.

4. The antibody of any one of claims 1-3, wherein the antibody comprises a heavy chain variable region comprising the heavy chain CDR1 of SEQ ID NO: 1, the heavy chain CDR2 of SEQ ID NO: 13, the heavy chain CDR3 of SEQ ID NO: 233; and a light chain variable region comprising the light chain CDR1 of SEQ ID NO: 39, the light chain CDR2 of SEQ ID NO: 49, and the light chain CDR 3 of SEQ ID NO: 56.

5. The antibody of any one of claims 1-4, wherein the antibody embodiments binds the same epitope as, or is capable of competing for binding with, the murine monoclonal antibody PR1A3.

6. The antibody of any one of claims 1-5, wherein the antibody binds to membrane-bound CEA with a Kd of 100 nM, 10 nM, 1 nM or lower.

7. An antibody which binds membrane-bound human carcinoembryonic antigen (CEA), wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, and SEQ ID NO: 247 and wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 209.

8. The antibody of claim 7, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 239~~ or SEQ ID NO: 247~~ and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 209.

9. The antibody of any one of claims 1-8, wherein the antibody comprises an Fc region that has been glycoengineered.

10. The antibody of claim 9, wherein at least 20% to 100% of the N-linked oligosaccharides in the Fc region are nonfucosylated.

11. The antibody of claim 9, wherein at least 20% to 100% of the N-linked oligosaccharides in the Fc region are bisected.

12. The antibody of claim 9, wherein at least 20% to 50% of the N-linked oligosaccharides in the Fc region are bisected, nonfucosylated.

13. The antibody of any of claims 9-12, wherein the antibody has at least one increased effector function.