CEA ANTIBODIES

RELATED APPLICATIONS

[0001] This application claims the benefit of European Patent Application No. 11156665.9, filed March 2, 2011, the disclosure of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to antigen binding molecules (ABMs). In particular embodiments, the present invention relates to recombinant monoclonal antibodies, including chimeric, primatized or humanized antibodies which bind to human carcinoembryonic antigen (CEA).

BACKGROUND

Carcinoembryonic antigen (CEA) and anti-CEA antibodies

[0003] Carcinoembryonic antigen (CEA, also known as CEACAM-5 or CD66e) is a glycoprotein having a molecular weight of about 180 kDa. CEA is a member of the immunoglobulin superfamily and contains seven domains that are linked to the cell membrane through a glycosylphosphatidylinositol (GPI) anchor (Thompson J.A., J Clin Lab Anal. 5:344–366, 1991) The seven domains include a single N-terminal Ig variable domain and six domains (A1-B1-A2-B2-A3-B3) homologous to the Ig constant domain (Hefta L J, et al., Cancer Res. 52:5647-5655, 1992).

[0004] The human CEA family contains 29 genes, of which 18 are expressed: 7 belonging to the CEA subgroup and 11 to the pregnancy-specific glycoprotein subgroup. Several CEA subgroup members are thought to possess cell adhesion properties. CEA is thought to have a role in innate immunity (Hammarström S., Semin Cancer Biol. 9(2):67-81 (1999)). Because of the existence of proteins closely related to CEA, it can be challenging to raise anti-CEA antibodies that are specific for CEA with minimal cross-reactivity to the other closely related proteins.

[0005] CEA has long been identified as a tumor-associated antigen (Gold and Freedman, J Exp Med., 121:439–462, 1965; Berinstein N. L., J Clin Oncol., 20:2197–2207, 2002). Originally classified as a protein expressed only in fetal tissue, CEA has now been identified in several normal adult tissues. These tissues are primarily epithelial in origin, including cells of the gastrointestinal, respiratory, and urogential tracts, and cells of colon, cervix, sweat glands, and prostate (Nap et al., Tumour Biol., 9(2-3):145-53,1988; Nap et al., Cancer Res., 52(8):2329-23339,1992).

[0006] Tumors of epithelial origin, as well as their metastases, contain CEA as a tumor associated antigen. While the presence of CEA itself does not indicate transformation to a cancerous cell, the

distribution of CEA is indicative. In normal tissue, CEA is generally expressed on the apical surface of the cell (Hammarström S., Semin Cancer Biol. 9(2):67-81 (1999)), making it inaccessible to antibody in the blood stream. In contrast to normal tissue, CEA tends to be expressed over the entire surface of cancerous cells (Hammarström S., Semin Cancer Biol. 9(2):67-81 (1999)). This change of expression pattern makes CEA accessible to antibody binding in cancerous cells. In addition, CEA expression increases in cancerous cells. Furthermore, increased CEA expression promotes increased intercellular adhesions, which may lead to metastasis (Marshall J., Semin Oncol., 30(a Suppl. 8):30-6, 2003).

[0007] CEA is readily cleaved from the cell surface and shed into the blood stream from tumors, either directly or via the lymphatics. Because of this property, the level of serum CEA has been used as a clinical marker for diagnosis of cancers and screening for recurrence of cancers, particularly colorectal cancer (Goldenberg D M., The International Journal of Biological Markers, 7:183-188, 1992; Chau I., et al., J Clin Oncol., 22:1420–1429, 2004; Flamini et al., Clin Cancer Res; 12(23):6985-6988, 2006). This property also presents one of the challenges for using CEA as a target, since serum CEA binds most of the currently available anti-CEA antibodies, hindering them from reaching their target on the cell surface and limiting potential clinical effects.

[0008] Multiple monoclonal antibodies have been raised against CEA for research purposes, as diagnostic tools, and for therapeutic purposes (e.g., Nap et al., Cancer Res., 52(8):2329-23339, 1992; Sheahan et al., Am. J. Clin. Path. 94:157-164, 1990; Sakurai et al., J. Surg. Oncol., 42:39-46, 1989; Goldenberg D M., The International Journal of Biological Markers, 7:183-188, 1992; Ledermann J A, Br. J. Cancer, 58:654, 1988; Ledermann J A, Br. J. Cancer, 68:69-73, 1993; Pedley R B, et al., Br. J. Cancer, 68:69-73, 1993; Boxer GM, et al., Br. J. Cancer, 65:825-831, 1992). Chester et al. have isolated a single chain anti-CEA antibody from a phage display library to be used in radioimmunodetection and radioimmunotherapy (U.S. Pat. No. 5,876,691), and the antibody was subsequently humanized (U.S. Pat. No. 7,232,888). Anti-CEA antibodies have also been isolated from human phage display libraries (U.S. Pat. No. 5,872,215).

[0009] The mouse monoclonal antibody PR1A3 was raised by fusion of NS1 (P3/NS1/I-Ag-4-1) myeloma cells with spleen cells from mice immunized with normal colorectal epithelium (Richman P. I. and Bodmer W. F., Int. J. Cancer, 39:317-328, 1987). PR1A3 reacts strongly to both well- and poorly-differentiated colorectal carcinomas and has advantages over other colorectal epithelium-reactive antibodies since its antigen appears to be fixed to the tumor and does not appear in the lymphatics or normal lymph nodes draining a tumor (Granowska M. et al., Eur. J. Nucl. Med., 20:690-698, 1989). For example, PR1A3 reacted with 59/60 colorectal tumors (Richman P. I. and Bodmer W. F., Int. J. Cancer, 39:317-328, 1987), whereas the CEA reactive antibody B72.3 reacted with only 75% of colorectal tumors (Mansi L., et al., Int J Rad Appl Instrum B., 16(2):127-35, 1989).

[0010] Epitope mapping of PR1A3 shows that the antibody targets the B3 domain and the GPI anchor of the CEA molecule (Durbin H. et al., Proc. Natl. Scad. Sci. USA, 91:4313-4317, 1994). Consequently, the PR1A3 antibody binds only to the membrane- bound CEA, and not the soluble CEA form that can be found in the bloodstreams of cancer patients. Because of this binding property, the PR1A3 antibody is unlikely to be sequestered by the serum CEA; instead, it can target CEA expressed on cancerous cells. The epitope bound by PR1A3 is a conformational epitope, not a linear epitope, which is thought to contribute to the loss of binding of PR1A3 to soluble CEA (Stewart et al., Cancer Immunol Immunother, 47:299-06, 1999).

[0011] The PR1A3 antibody was previously humanized by grafting the CDRs of the murine parent antibody to the heavy chain framework regions 1-3 of the human antibody RF-TS3'CL (retaining the murine framework 4 of PR1A3) and the light chain framework regions of the REI antibody. (Stewart et al., Cancer Immunol Immunother, 47:299-06, 1999). This humanized version of PR1A3 retained specificity and for surface-expressed CEA with an affinity similar to that of the murine antibody (Stewart et al., Cancer Immunol Immunother, 47:299-06, 1999; U.S. Pat. No. 5,965,710). A humanized PR1A3 (hPR1A3) antibody was shown to induce targeted killing of colorectal cancer cell lines. (Conaghhan P. J., et al., Br. J. Cancer, 98(7):1217-1225). However, the affinity of hPR1A3 for CEA is relatively low.

[0012] Radio-labeled anti-CEA antibodies have been used in clinical trials in patients with colorectal cancer. For example, an ^{123}I-labeled chimeric minibody T84.66 (cT84.66) was used in a pilot clinical study in patients with colorectal cancer. The radio-labeled minibody was able to target cancer cells. (Wong J. Y. et al., Clin Cancer Res. 10(15):5014-21, (2004)). In another example, $^{(131)}$I-labetuzumab, a radio-labeled humanized anti-CEA antibody, was tested in adjuvant radioimmunotherapy in patients with liver metastases of colorectal cancer, and was found to provide a promising survival advantage. (Liersch T., et al., Ann. Surg. Oncol. 14(9):2577-90, (2007)).

Antibody Glycosylation

[0013] The oligosaccharide component can significantly affect properties relevant to the efficacy of a therapeutic glycoprotein, including physical stability, resistance to protease attack, interactions with the immune system, pharmacokinetics, and specific biological activity. Such properties may depend not only on the presence or absence, but also on the specific structures, of oligosaccharides. Some generalizations between oligosaccharide structure and glycoprotein function can be made. For example, certain oligosaccharide structures mediate rapid clearance of the glycoprotein from the bloodstream through interactions with specific carbohydrate binding proteins, while others can be bound by antibodies and trigger undesired immune reactions. (Jenkins et al., Nature Biotechnol. 14:975-81, 1996).

[0014] Mammalian cells have been the preferred hosts for production of therapeutic glycoproteins due to their capability to glycosylate proteins in the most compatible form for human application. (Cumming et al., Glycobiology 1:115-30, 1991; Jenkins et al., Nature Biotechnol. 14:975-981, 1996). Bacteria very rarely glycosylate proteins and, like other types of common hosts, such as yeasts, filamentous fungi, insect and plant cells, yield glycosylation patterns associated with rapid clearance from the blood stream, undesirable immune interactions, and in some specific cases, reduced biological activity. Among mammalian cells, Chinese hamster ovary (CHO) cells have been most commonly used during the last two decades. In addition to giving suitable glycosylation patterns, these cells allow consistent generation of genetically stable, highly productive clonal cell lines. They can be cultured to high densities in simple bioreactors using serum-free media, and permit the development of safe and reproducible bioprocesses. Other commonly used animal cells include baby hamster kidney (BHK) cells, NS0- and SP2/0-mouse myeloma cells. More recently, production from transgenic animals has also been tested (Jenkins et al., Nature Biotechnol. 14:975-81, 1996).

[0015] All antibodies contain carbohydrate structures at conserved positions in the heavy chain constant regions, with each isotype possessing a distinct array of N-linked carbohydrate structures, which variably affect protein assembly, secretion or functional activity. (Wright A. and Morrison S. L., Trends Biotech. 15:26-32, 1997). The structure of the attached N-linked carbohydrate varies considerably, depending on the degree of processing, and can include high-mannose, multiply-branched as well as biantennary complex oligosaccharides. (Wright, A., and Morrison, S. L., Trends Biotech. 15:26-32, 1997). Typically, there is heterogeneous processing of the core oligosaccharide structures attached at a particular glycosylation site such that even monoclonal antibodies exist as a population of multiple glycoforms. Likewise, it has been shown that major differences in antibody glycosylation occur between cell lines, and even minor differences are seen for a given cell line grown under different culture conditions. (Lifely, M. R. et al., Glycobiology 5(8):813-22, 1995).

[0016] One way to obtain large increases in potency, while maintaining a simple production process and potentially avoiding significant, undesirable side effects, is to enhance the natural, cell-mediated effector functions of monoclonal antibodies by engineering their oligosaccharide component as described in Umaña, P. et al., Nature Biotechnol. 17:176-180 (1999) and U.S. Pat. No. 6,602,684, the entire contents of which are hereby incorporated by reference in their entirety. IgGl-type antibodies, the most commonly used antibodies in cancer immunotherapy, are glycoproteins that have a conserved N-linked glycosylation site at Asn297 in each CH2 domain. The two complex biantennary oligosaccharides attached to Asn297 are buried between the CH2 domains, forming extensive contacts with the polypeptide backbone, and their presence is essential for the antibody to mediate effector functions such as antibody dependent cellular cytotoxicity (ADCC) (Lifely, M. R., et al., Glycobiology 5:813-822 (1995); Jefferis, R., et al., Immunol Rev. 163:59-76 (1998); Wright, A. and Morrison, S. L., Trends Biotechnol. 15:26-32 (1997)).

[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] In one aspect, the invention provides 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,
the heavy chain CDR3 of 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.

In another aspect, the invention provides 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: 239 and SEQ ID NO: 247 and wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 209.

In another aspect, the invention provides a composition comprising an antibody of the invention and a pharmaceutically acceptable carrier.

In a further aspect, the invention provides the use of an antibody or composition of the invention in the manufacture of medicaments for use in treating a subject having a cancer that abnormally expresses CEA, or for use in increasing survival time in such a subject.

In further aspects, the invention provides an isolated polynucleotide encoding an antibody of the invention, a vector comprising such a polynucleotide, a host cell comprising such a vector, and an *in vitro* method of inducing cell lysis of a tumour cell, the method comprising contacting the tumour cell with an antibody or composition of the invention.

The invention is as defined in the claims. However, the description which follows also includes additional antibodies and other subject matter not within the current claims. This description is retained for technical information.

[0020] Described herein is 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.

[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] Also described herein is 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.

[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] Also described herein is an isolated polynucleotide encoding the ABM or antibody of any of above described embodiments. Also described herein a vector comprising the polynucleotide encoding the ABM or antibody of any of above described embodiments. Also described herein a host cell comprising this vector.

[0029] Also described herein is a composition comprising the ABM or antibody of any of above described embodiments and a pharmaceutically acceptable carrier.

[0030] Also described herein is 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 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] Also described herein is 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] Also described herein is 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] Also described herein is the 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.

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. "PBS" refers to phosphate buffered saline, which was used as a negative control. Survival was measured according to the termination criteria defined by the Swiss regulatory authority.

[0042] FIGURE 8 shows the results of an efficacy study in SCID/bg mice that were injected intravenously with A549 lung carcinoma cells, where the tumor engrafts in the lung of the animals. 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," "SM3E," and "GA201" are as set forth for Figure 7, above. The designation "CH7ArF9 CL1A rH11" represents a CH7A antibody variant with affinity matured heavy and light chains. The designation "ge" indicates that the antibody

has been glycoengineered to have reduced numbers of fucosylated oligosaccharides in the Fc region. "Vehicle" refers to the negative control. A549 lung carcinoma cells are strongly positive for EGFR expression and weakly positive for CEA expression.

[0043] FIGURE 9 shows the results of an efficacy study in SCID/bg mice that were intrasplenically administered MKN45 gastric carcinoma cells, which generates tumor metastasis in the liver of the animals. The designations, "CH7ArF9 CL1A rH11," "SM3E," "ge," and "PBS" are as set forth for Figures 7 and 8, above.

[0044] FIGURE 10 shows kinetic analysis of affinity matured clones: (a) shows a sensorgram of anti-CEA Fabs with an affinity matured heavy chain CH7A H4E9 (SEQ ID NO: 199) together with unmatured light chain CL1A (SEQ ID NO:105); an affinity matured light chain CL1A pAC18 (SEQ ID NO:209) combined with unmatured heavy chain CH7A; and a combination thereof, CH7A H4E9 and CL1A pAC18 (SEQ ID NOs:199 and 209); (b) summary of kinetic analysis of affinity matured clones.

[0045] FIGURE 11 shows a schematic overview of the PCR strategy for the CDR1 and CDR2 randomization of the humanized CH7A anti-CEA antibody heavy chain.

[0046] FIGURE 12 shows a schematic overview of the PCR strategy for the CDR1 and CDR2 randomization of the humanized CL1A anti-CEA antibody light chain.

[0047] FIGURE 13 shows a schematic overview of the PCR strategy for the CDR3 randomization of the humanized CH7A anti-CEA antibody heavy chain.

[0048] FIGURE 14 shows a schematic overview of the PCR strategy for the CDR3 randomization of the humanized CL1A anti-CEA antibody light chain.

[0049] FIGURE 15 shows binding affinity of anti-CEA antibodies for membrane-bound CEA on MKN45 target cells. Humanized anti-CEA antibodies with either an affinity matured light chain (Panel A, CH7A,CL1ArH7 or CH7A,CL1ArH11) or affinity matured heavy and light chains (Panel B, CH7A rB9, CL1A rH11 G2(1)) that have been converted to IgG show improved binding as compared to the control antibody (CH7A,CL1A).

[0050] FIGURE 16 shows the results of an assay testing antibody-dependent cellular cytotoxicity (ADCC) by affinity matured antibodies (CH7ArB9, CL1A rH11G2(1), CH7Arf9, CL1A rH11G2(1), and CH7A, CL1A rH11 G2(1)) compared to control antibodies (CH7A, CL1A G2(R2).

[0051] FIGURE 17 shows the results of a cell binding assay for anti-CEA antibody with heavy chain CH1A as compared to the mouse-human chimeric antibody chPR1A3.

[0052] FIGURE 18 shows the results of a binding assay for anti-CEA antibodies with heavy chain CH1A1, CH1A2, CH1A3, or CH1A4 and light chain 2F1.

[0053] FIGURE 19 shows the results of a stability assay for anti-CEA antibodies with heavy chain CH1A1, CH1A2, CH1A3, or CH1A4.

[0054] FIGURE 20 shows the result of Surface Plasmon Resonance (SPR) analysis for anti-CEA antibodies generated from CH1A1.

[0055] FIGURE 21 shows the result of cell binding assays for anti-CEA antibodies generated from CH1A1.

[0056] FIGURE 22 shows the Surface Plasmon Resonance (SPR) measurements of the affinity (as measured in the bivalent form) of stability engineered anti-CEA antibodies as compared to the parent 5HFF12 heavy chain.

[0057] FIGURES 23 and 24 show the results of a stability assay for affinity-matured antibody 5HFF12 as compared to its parental heavy chain CH7A with the individual point mutations introduced that were selected in 5HFF12.

[0058] FIGURE 25 shows Surface Plasmon Resonance analysis of the combined framework and CDR-H3 variants.

[0059] FIGURE 26 shows the ADCC activity of the CHA1A-based framework variants.

[0060] FIGURE 27 shows the ADCC activity of the CHA1A-based framework variants.

[0061] FIGURE 28 shows the ADCC activity of the combined framework and CDR-H3 variants.

[0062] FIGURE 29 shows the ADCC activity of the combined framework and CDR-H3 variants.

[0063] FIGURE 30 shows the efficacy of glycoengineered anti-CEA antibody CH1A1A (Y98A/D99Y) x 2F1 in a colorectal carcinoma xenograft model in SCID mice transgenic for human CD16.

[0064] FIGURE 31 shows the efficacy of glycoengineered anti-CEA antibody CH1A1A (Y98A/D99Y) x 2F1 in an A549 lung carcinoma xenograft model in SCID mice transgenic for human CD16.

[0065] FIGURE 32 shows the amino acid sequences of CDRs for various anti-CEA ABMs.

[0066] FIGURE 33 shows the amino acid sequences of the light chain constructs for various anti-CEA ABMs.

[0067] FIGURE 34A-C shows amino acid sequences of affinity matured heavy and light chain CDRs and associated binding affinities.

[0068] FIGURE 35 shows affinity constants of the various affinity matured antibody sequences.

[0069] FIGURE 36 shows the amino acid sequences of CDR-H3 of various anti-CEA ABMs.

[0070] FIGURE 37A-C shows the amino acid sequences of VH regions of various anti-CEA ABMs.

[0071] FIGURE 38 shows the amino acid sequence alignments of VH regions of various stability matured anti-CEA antibodies.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0072] Terms are used herein as generally used in the art, unless otherwise defined as follows.

[0073] As used herein, the term "antigen binding molecule" refers in its broadest sense to a molecule that specifically binds an antigenic determinant. A non-limiting example of an antigen binding molecule is an antibody or fragment thereof that retains antigen-specific binding. More specifically, as used herein, an antigen binding molecule that binds membrane-bound human carcinoembryonic antigen (CEA) is a ABM that specifically binds to CEA, more particularly to cell surface or membrane-bound CEA. By "specifically binds" is meant that the binding is selective for the antigen and can be discriminated from unwanted or nonspecific interactions.

[0074] As used herein, the term "antibody" is intended to include whole antibody molecules, including monoclonal, polyclonal and multispecific (e.g., bispecific) antibodies, as well as antibody fragments having an Fc region and retaining binding specificity, and fusion proteins that include a region equivalent to the Fc region of an immunoglobulin and that retain binding specificity. Also encompassed are antibody fragments that retain binding specificity including, but not limited to, VH fragments, VL fragments, Fab fragments, $F(ab')_2$ fragments, scFv fragments, Fv fragments, minibodies, diabodies, triabodies, and tetrabodies (see, e.g., Hudson and Souriau, Nature Med. 9: 129-134 (2003)).

[0075] As used herein, the term "antigen binding domain" refers to the part of an antigen binding molecule that comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antigen binding molecule may only bind to a particular part of the antigen, which part is termed an epitope. An antigen binding domain may be provided by, for example, one or more antibody variable domains. Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

[0076] As used herein, the term "affinity matured" in the context of antigen binding molecules (e.g., antibodies) refers to an antigen binding molecule that is derived from a reference antigen binding molecule, e.g., by mutation, binds to the same antigen, preferably binds to the same epitope, as the reference antibody; and has a higher affinity for the antigen than that of the reference antigen binding molecule. Affinity maturation generally involves modification of one or more amino acid residues in one or more CDRs of the antigen binding molecule. Typically, the affinity matured antigen binding molecule binds to the same epitope as the initial reference antigen binding molecule.

[0077] As used herein "binding affinity" is generally expressed in terms of equilibrium association or dissociation constants (K_a or K_d, respectively), which are in turn reciprocal ratios of dissociation and association rate constants (k_d and k_a, respectively). Thus, equivalent affinities may comprise different rate constants, so long as the ratio of the rate constants remains the same.

[0078] As used herein, the term "Fc region" refers to a C-terminal region of an IgG heavy chain. Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region is usually defined to stretch from the amino acid residue at position Cys226 to the carboxyl-terminus.

[0079] As used herein, the term "region equivalent to the Fc region of an immunoglobulin" is intended to include naturally occurring allelic variants of the Fc region of an immunoglobulin as well as variants having alterations which produce substitutions, additions, or deletions but which do not decrease substantially the ability of the immunoglobulin to mediate effector functions (such as antibody-dependent cellular cytotoxicity). For example, one or more amino acids can be deleted from the N-terminus or C-terminus of the Fc region of an immunoglobulin without substantial loss of biological function. Such variants can be selected according to general rules known in the art so as to have minimal effect on activity. (See, e.g., Bowie, J. U. et al., Science 247:1306-10 (1990).

[0080] As used herein, the term "membrane-bound human CEA" refers to human carcinoembryonic antigen (CEA) that is bound to a membrane-portion of a cell or to the surface of a cell, in particular, the surface of a tumor cell. The term "membrane-bound human CEA" may, in certain circumstances, refer to CEA which is not bound to the membrane of a cell, but which has been constructed so as to preserve the epitope to which the PR1A3 antibody binds. The term "soluble CEA" refers to human carcinoembryonic antigen that is not bound to or is cleaved from a cell membrane or cell surface (e.g., a tumor cell surface) and/or which, typically, does not preserve the conformation epitope that is bound by the PR1A3 antibody. Soluble CEA can, for example, be found in the blood stream or lymphatics of a subject with cancer.

[0081] As used herein, the term "no substantial cross-reactivity against soluble" CEA means that a molecule (e.g., an antigen binding molecule) does not recognize or specifically bind to soluble CEA , particularly when compared to membrane-bound CEA. For example, an antigen binding molecule may bind less than about 10% to less than about 5% soluble CEA, or may bind soluble CEA at an amount selected from the group consisting of less than about 10%, 9%, 8% 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.2%, or 0.1%, preferably less than about 2%, 1%, or 0.5% soluble CEA, and most preferably less than about 0.2% or 0.1% soluble CEA.

[0082] As used herein, the terms "fusion" and "chimeric," when used in reference to polypeptides such as ABMs, refer to polypeptides comprising amino acid sequences derived from two or more heterologous polypeptides, such as portions of antibodies from different species. For chimeric ABMs, for example, the non-antigen binding components may be derived from a wide variety of

species, including primates such as chimpanzees and humans. The constant region of the chimeric ABM is generally substantially identical to the constant region of a natural human antibody; the variable region of the chimeric antibody generally comprises a sequence that is derived from a recombinant anti-CEA antibody having the amino acid sequence of the murine PR1A3 variable region. Humanized antibodies are a particularly preferred form of fusion or chimeric antibody..

[0083] As used herein, the term "humanized" is used to refer to an antigen–binding molecule derived in part from a non-human antigen-binding molecule, for example, a murine antibody, that retains or substantially retains the antigen-binding properties of the parent molecule but which is less immunogenic in humans. This may be achieved by various methods (referred to herein as "humanization") including, but not limited to (a) grafting the entire non-human variable domains onto human constant regions to generate chimeric antibodies, (b) grafting only the non-human (e.g., donor antigen binding molecule) CDRs onto human (e.g., recipient antigen binding molecule) framework and constant regions with or without retention of critical framework residues (e.g., those that are important for retaining good antigen binding affinity or antibody functions), or (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Such methods are disclosed in Jones et al., Morrison et al., Proc. Natl. Acad. Sci., 81:6851-6855 (1984); Morrison and Oi, Adv. Immunol., 44:65-92 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988); Padlan, Molec. Immun., 28:489-498 (1991); Padlan, Molec. Immun., 31(3):169-217 (1994), all of which are incorporated by reference in their entirety herein. There are generally 3 complementarity determining regions, or CDRs, (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains of an antibody, which are flanked by four framework subregions (i.e., FR1, FR2, FR3, and FR4) in each of the heavy and light chain variable domains of an antibody: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. A discussion of humanized antibodies can be found, inter alia, in U.S. Patent No. 6,632,927, and in published U.S. Application No. 2003/0175269, both of which are incorporated herein by reference in their entirety. Humanization may also be achieved by transplanting truncated CDRs that contain only the specificity-determining amino acid residues for the given CDR onto a chosen framework. By "specificity-determining residues" is meant those residues that are directly involved in specific interaction with the antigen and/or which are necessary for antigen-specific binding. In general, only about one-fifth to one-third of the residues in a given CDR participate in binding to antigen. The specificity-determining residues in a particular CDR can be identified by, for example, computation of interatomic contacts from three-dimensional modeling and determination of the sequence variability at a given residue position in accordance with the methods described in Padlan et al., FASEB J. 9(1):133-139 (1995), the contents of which are hereby incorporated by reference in their entirety.

[0084] In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antigen binding molecules

may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antigen binding molecule performance. In general, the humanized antigen binding molecule will comprise substantially all of at least one, and typically two, variable domains, in which at least one, or substantially all, or all of the hypervariable regions 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 antigen binding molecule optionally also 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); Reichmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992).

[0085] Similarly, as used herein, the term "primatized" is used to refer to an antigen-binding molecule derived from a non-primate antigen-binding molecule, for example, a murine antibody, that retains or substantially retains the antigen-binding properties of the parent molecule but which is less immunogenic in primates.

[0086] As used herein, the term "variant" (or analog) polynucleotide or polypeptide refers to a polynucleotide or polypeptide differing from a specifically recited polynucleotide or polypeptide of the invention by insertions, deletions, and substitutions, created using, e g., recombinant DNA techniques. Specifically, recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes that produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the properties of any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate.

[0087] As used herein, the term "variant anti-CEA antigen binding molecule" refers to a molecule that differs in amino acid sequence from a "parent" anti-CEA antigen binding molecule amino acid sequence by virtue of addition, deletion and/or substitution of one or more amino acid residue(s) in the parent antibody sequence. In a specific embodiment, the variant comprises one or more amino acid substitution(s) in one or more hypervariable region(s) or CDRs of the heavy and/or light chain of the parent antigen binding molecule. For example, the variant may comprise at least one, e.g. from about one to about ten (i.e., about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10), and preferably from about two to about five, substitutions in one or more hypervariable regions or CDRs (i.e., 1, 2, 3, 4, 5, or 6 hypervariable regions or CDRs) of the parent antigen binding molecule. A variant anti-CEA antigen binding molecule may also comprise one or more additions, deletions and/or substitutions in one or more framework regions of either the heavy or the light chain. Ordinarily, the variant will have an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or

100% amino acid sequence identity with the parent antigen binding molecule heavy or light chain variable domain sequences, typically at least about 80%, 90%, 95% or 99%. Identity with respect to a sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the parent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence shall be construed as affecting sequence identity or homology. The variant antigen binding molecule retains the ability to bind membrane-bound human CEA. In one embodiment, the anti-CEA ABM binds the same epitope as that of the parent antigen binding molecule. In one embodiment, the anti-CEA ABM competes for binding to membrane-bound human CEA with the parent antigen binding molecule. In one embodiment, the anti-CEA ABM binds to membrane-bound human CEA and does not bind to soluble human CEA. The anti-CEA ABM has properties which are superior to those of the parent antigen binding molecule. For example, the variant may have a stronger binding affinity, increased stability, and/or enhanced ability to induce antibody-mediated cellular cytotoxicity in vitro and in vivo. In one embodiment, the anti-CEA ABM has increased stability and retains or has improved binding affininty for membrane-bound CEA and retains or has an enhanced ability to induce antibody-mediated cellular cytotoxicity in vitro and in vivo.

[0088] To analyze such properties, one should generally compare a variant antigen binding molecule and the parent antigen binding molecule in the same format; for example, an Fab form of the variant antigen binding molecule to an Fab form of the parent antigen binding molecule or a full length form of the variant antigen binding molecule to a full length form of the parent antigen binding molecule. In one embodiment, the variant antigen binding molecule has at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, or 20-fold enhancement in biological activity when compared to the parent antigen binding molecule. In one embodiment, the variant antigen binding molecule is a stability engineered variant that has increased stability as compared to the parent antigen binding molecule. Stability can be assayed by any method known in the art and by methods described herein, specifically in Examples 3-6. In specific embodiments, the variant antigen binding molecule has at least about a 1.5 -fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, ,20-fold, 40-fold, 50-fold, 100-fold increase in stability as compared to the parent antigen binding molecule.

[0089] In some embodiments, the variant antigen binding molecule exhibits an increase in stability that is measured as a change in stability parameter as compared to the parent antigen binding molecule. In some embodiments, the variant antigen binding molecule has at least about a 1.5 -fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 40-fold, 50-fold, 100-fold change in a

stability parameter as compared to the parent antigen binding A stability parameter is for example, temperature at which the variant antigen binding molecule unfolds or denatures, the pressure at which the variant antigen binding molecule unfolds or denature, or the time required to denature or unfold the variant antigen binding molecule under conditions designed to render the variant antigen binding molecule unstable. In one embodiment, the increase in stability is determined by a thermal denaturation assay, for example by differential scanning calorimetry (DSC). In one embodiment, the increase in stability is determined by a chemical denaturation assay. In one embodiment, the increase in stability is determined using a high pressure assay. In another embodiment, the stability of the variant antigen binding molecule is determined using a fluorescence polarization assay. In one embodiment, the stability of the variant antigen binding molecule is determined using a dynamic light scattering (DLS) assay. (See the Examples and, for example, Nobbmann, U. et al., Biotech. Genetic Eng. Rev. 24:117-128 (2007). DLS monitors the integrity of a molecule, such as an antibody, where, in general, an increase in light scattering indicates protein unfolding or denaturation. The DLS of molecules can be examined as a function of temperature or chemical denaturants to compare relative stabilities. Those molecules that remain in their native conformation (little or no increase in DLS properties) are considered to be stable under the testing conditions. In one embodiment, the variant antigen binding molecule is stable at a temperature that is at least 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0 , 9.0, or 10.0 degrees Celsius higher than the parent ABM, or other appropriate reference molecule, when analyzed using a dynamic light scattering assay. In one embodiment, the variant antigen binding molecule is stable at 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80 degrees Celsius or higher. Thermal stability can be measured, for example, using DLS, DSC, or fluorescence polarization. In one embodiment, the thermal stability of the variant antigen binding molecule is measured using DLS. In one embodiment, the DLS assay is performed using 1 mg/ml of the ABM or variant ABM in a buffer of 20 mM Histidine and 140 mM NaCl at pH 6.0. The DLS assay is conducted starting at 25°C with an incremental temperature increase of 0.05°C/min.

[0090] The term "parent" antigen binding molecule refers to an ABM that is used as the starting point or basis for the preparation of the variant. In a specific embodiment, the parent antigen binding molecule has a human framework region and, if present, has human antibody constant region(s). For example, the parent antibody may be a humanized or human antibody.

[0091] Amino acid "substitutions" can result in replacing one amino acid with another amino acid having similar structural and/or chemical properties, e.g., conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine,

threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Insertions" or "deletions" are generally in the range of about 1 to about 20 amino acids, more specifically about 1 to about 10 amino acids, and even more specifically, about 2 to about 5 amino acids. Non-conservative substitutions will entail exchanging a member of one of these classes for another class. For example, amino acid substitutions can also result in replacing one amino acid with another amino acid having different structural and/or chemical properties, for example, replacing an amino acid from one group (e.g., polar) with another amino acid from a different group (e.g., basic). The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

[0092] As used herein, the term "single-chain Fv" or "scFv" refers to an antibody fragment comprising a VH domain and a VL domain as a single polypeptide chain. Typically, the VH and VL domains are joined by a linker sequence. See, e.g., Pluckthun, in: The PHARMACOLOGY OF MONOCLONAL ANTIBODIES, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

[0093] As used herein, the term "minibody" refers to a bivalent, homodimeric scFv derivative that contains a constant region, typically the CH3 region of an immunoglobulin, preferably IgG, more preferably IgG1, as the dimerisation region. Generally, the constant region is connected to the scFv via a hinge region and/or a linker region. Examples of minibody proteins can be found in Hu et al. (1996), Cancer Res. 56: 3055-61.

[0094] As used herein, the term "diabody" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (VH -VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. 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). A triabody results from the formation of a trivalent trimer of three scFvs, yielding three binding sites, and a tetrabody is a tetravalent tetramer of four scFvs, resulting in four binding sites.

[0095] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites (also known as antigen binding regions) found within the variable region of both heavy and light chain polypeptides. CDRs are also referred to as "hypervariable regions" and that term is used interchangeably herein

with the term "CDR" in reference to the portions of the variable region that form the antigen binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia et al., J. Mol. Biol. 196:901-917 (1987), which are incorporated herein by reference, 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 variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table I as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

TABLE 1. CDR Definitions[1]

CDR	Kabat	Chothia	AbM[2]
V_H CDR1	31-35	26-32	26-35
V_H CDR2	50-65	52-58	50-58
V_H CDR3	95-102	95-102	95-102
V_L CDR1	24-34	26-32	24-34
V_L CDR2	50-56	50-52	50-56
V_L CDR3	89-97	91-96	89-97

[1]Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat et al. (see below).
[2] "AbM" with a lowercase "b" as used in Table 1 refers to the CDRs as defined by Oxford Molecular's "AbM" antibody modeling software.

[0096] Kabat et al. also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an ABM are according to the Kabat numbering system. The sequences of the sequence listing (i.e., SEQ ID NO:1 to SEQ ID NO:216) are not numbered according to the Kabat numbering system. However, one of ordinary skill in the art is familiar with how to convert the sequences in the Sequence Listing to Kabat numbering.

[0097] By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide