AFFINITY-MATURED HUMANIZED ANTI CEA MONOCLONAL ANTIBODIES

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] 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 specific for human carcinoembryonic antigen (CEA). In addition, the present invention relates to nucleic acid molecules encoding such ABMs, and vectors and host cells comprising such nucleic acid molecules. The invention further relates to methods for producing the ABMs of the invention, and to methods of using these ABMs in treatment of disease. In addition, the present invention relates to ABMs with modified glycosylation having improved therapeutic properties, including antibodies with increased Fc receptor binding and increased effector function, such as ADCC.

Background Art

Carcinoembryonic antigen (CEA) and anti-CEA antibodies

[0002] 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).

[0003] 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.

[0004] 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).

[0005] 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).

[0006] 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.

[0007] 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).

[0008] 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).

[0009] 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).

[0010] 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.

[0011] 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

[0012] 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).

[0013] 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).

[0014] 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).

[0015] 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. IgG1-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)).

[0016] 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.

[0017] There remains a need for enhanced therapeutic approaches targeting CEA, in particular, membrane-bound CEA for the treatment of cancers in primates, including, but not limited to, humans.

BRIEF SUMMARY OF THE INVENTION

[0018] Recognizing the tremendous therapeutic potential of antigen binding molecules (ABMs) that have the binding specificity of the PR1A3 antibody and that have been affinity matured and/or glycoengineered to enhance Fc receptor binding affinity and/or effector function, the present inventors have provided such ABMs. In one aspect, the invention relates to variant ABMs and/or affinity matured ABMs that are capable of competing with the PR1A3 antibody for antigen binding. The efficacy of these ABMs is further enhanced by engineering the glycosylation profile of the antibody Fc region.

[0019] In one aspect, present invention is also directed to an antigen binding molecule (ABM) comprising a humanized, affinity-matured antigen binding domain comprising one or more complementarity determining regions (CDRs), wherein said antigen binding domain specifically binds membrane-bound human carcinoembryonic antigen (CEA), and wherein said antigen binding domain binds the same epitope as, or is capable of competing for binding with the murine monoclonal antibody PR1A3. The invention is further related to an ABM of the present invention, said ABM having modified oligosaccharides. In one embodiment the modified oligosaccharides have reduced fucosylation as compared to non-modified oligosaccharides. In other embodiments, the modified oligosaccharides are hybrid or complex. In another aspect, the invention is also directed to

polypeptides, polynucleotides, host cells, and expression vectors related to the ABMs. In a further aspect, the invention is directed methods of making the ABMs. In a further aspect, the invention is directed to methods of using the ABMs, particularly for the treatment of diseases related to abnormal expression of CEA, such as cancer.

BRIEF DESCRIPTION OF THE FIGURES

[0020] 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.

[0021] FIGURE 2 shows enhanced ADCC activity of a glycoengineered chimeric PR1A3 antibody with human PBMCs as effectors.

[0022] 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.

[0023] 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.

[0024] 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.

[0025] 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.

[0026] 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.

[0027] 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.

[0028] 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.

[0029] 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.

[0030] FIGURE 11 shows a schematic overview of CDR1 and CDR2 randomization of the humanized CH7A anti-CEA antibody heavy chain.

[0031] FIGURE 12 shows a schematic overview of CDR1 and CDR2 randomization of the humanized CL1A anti-CEA antibody light chain.

[0032] FIGURE 13 shows a schematic overview of CDR3 randomization of the humanized CH7A anti-CEA antibody heavy chain.

[0033] FIGURE 14 shows a schematic overview of CDR3 randomization of the humanized CL1A anti-CEA antibody light chain.

[0034] 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).

[0035] 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).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0036] Terms are used herein as generally used in the art, unless otherwise defined as follows.

[0037] 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 and not to the soluble CEA that is cleaved from the cell surface. By "specifically binds" is meant that the binding is selective for the antigen and can be discriminated from unwanted or nonspecific interactions.

[0038] 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)).

[0039] 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).

[0040] 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.

[0041] 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.

[0042] 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.

[0043] 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).

[0044] 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.

[0045] 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.

[0046] 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..

[0047] 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 residue*s" 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.

[0048] 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).

[0049] 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.

[0050] 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.

[0051] 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, for example, binds the same epitope as that of the parent antigen binding molecule, and preferably has properties which are superior to those of the parent antigen binding molecule. For example, the variant may have a stronger binding affinity, enhanced ability to induce antibody-mediated cellular cytotoxicity *in vitro* and *in vivo*. 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. The variant antigen binding molecule of particular interest herein is one that 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.

[0052] 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.

[0053] 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.

[0054] 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).

[0055] 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.

[0056] 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.

[0057] 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.

[0058] 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.

[0059] 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 sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence.

[0060] As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence or polypeptide sequence of the present invention can be determined conventionally using known computer programs. One method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag *et al.*, *Comp. App. Biosci. 6*:237-245 (1990). In a sequence alignment the query and subject sequences are both

DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

[0061] If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

[0062] For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

[0063] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95%

identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0064] As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a reference polypeptide can be determined conventionally using known computer programs. One method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag *et al.*, *Comp. App. Biosci. 6*:237-245 (1990). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

[0065] If the subject sequence is shorter than the query sequence due to N– or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N– and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N– and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N– and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N– and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N– and C-terminal residues of the subject sequence.

[0066] For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N– and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted

from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N– or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N– and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

[0067] Percent identity of polynucleotides and/or polypeptides can also be determined using the BLAST programs available through the National Center for Biotechnology Information (NCBI), with the default parameters indicated in the programs.

[0068] As used herein, a nucleic acid that "*hybridizes under stringent conditions*" to a nucleic acid sequence of the invention, refers to a polynucleotide that hybridizes under specified conditions, *e.g.*, in an overnight incubation at 42° C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

[0069] As used herein, the term "*polypeptide having GnTIII activity*" refers to polypeptides that are able to catalyze the addition of a N-acetylglucosamine (GlcNAc) residue in β-1-4 linkage to the β-linked mannoside of the trimannosyl core of N-linked oligosaccharides. This includes fusion polypeptides exhibiting enzymatic activity similar to, but not necessarily identical to, an activity of β(1,4)-N-acetylglucosaminyltransferase III, also known as β-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyl-transferase (EC 2.4.1.144), according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of GnTIII, but rather substantially similar to the dose-dependence in a given activity as compared to the GnTIII (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the GnTIII.).

[0070] As used herein, the term "*Golgi localization* domain" refers to the amino acid sequence of a Golgi resident polypeptide which is responsible for anchoring the polypeptide to a location within the Golgi complex. Generally, localization domains comprise amino terminal "tails" of an enzyme.

[0071] As used herein, the term "*effector function*" refers to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody.

Examples of antibody effector functions include, but are not limited to, Fc receptor binding affinity, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune-complex-mediated antigen uptake by antigen-presenting cells, down-regulation of cell surface receptors, etc.

[0072] As used herein, the terms "*engineer, engineered, engineering*" particularly with the prefix "*glyco-*," as well as the term "*glycosylation engineering*" are considered to include any manipulation of the glycosylation pattern of a naturally occurring or recombinant polypeptide or fragment thereof. Glycosylation engineering includes metabolic engineering of the glycosylation machinery of a cell, including genetic manipulations of the oligosaccharide synthesis pathways to achieve altered glycosylation of glycoproteins expressed in cells. Furthermore, glycosylation engineering includes the effects of mutations and cell environment on glycosylation. In one embodiment, the glycosylation engineering is an alteration in glycosyltransferase activity. In a particular embodiment, the engineering results in altered glucosaminyltransferase activity and/or fucosyltransferase activity.

[0073] As used herein, the term "*host cell*" covers any kind of cellular system which can be engineered to generate the polypeptides and antigen-binding molecules of the present invention. In one embodiment, the host cell is engineered to allow the production of an antigen binding molecule with modified glycoforms. In a preferred embodiment, the antigen binding molecule is an antibody, antibody fragment, or fusion protein. In certain embodiments, the host cells have been further manipulated to express increased levels of one or more polypeptides having GnTIII activity. Host cells include cultured cells, *e.g.*, mammalian cultured cells, such as CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

[0074] As used herein, the term "*Fc-mediated cellular cytotoxicity*" includes antibody-dependent cellular cytotoxicity (ADCC) and cellular cytotoxicity mediated by a soluble Fc-fusion protein containing a human Fc-region. It is an immune mechanism leading to the lysis of "targeted cells" by "human immune effector cells."

[0075] As used herein, the term "*human immune effector cells*" refers to a population of leukocytes that display Fc receptors on their surfaces, through which they bind to the Fc-region of antigen binding molecules or of Fc-fusion proteins and perform effector functions. Such a population may include, but is not limited to, peripheral blood mononuclear cells (PBMC) and/or natural killer (NK) cells.

[0076] As used herein, the term "*targeted cells*" refers to cells to which antigen binding molecules comprising an Fc region (*e.g.*, antibodies or fragments thereof comprising an Fc region) or Fc-fusion proteins specifically bind. The antigen binding molecules or Fc fusion-proteins bind to target cells via the protein part that is N-terminal to the Fc region.

[0077] As used herein, the term "*increased Fc-mediated cellular cytotoxicity*" is defined as either an increase in the number of "targeted cells" that are lysed in a given time, at a given concentration of antigen binding molecule or of Fc-fusion protein in the medium surrounding the target cells, by the mechanism of Fc-mediated cellular cytotoxicity defined above, and/or a reduction in the concentration of antigen binding molecule or of Fc-fusion protein, in the medium surrounding the target cells, required to achieve the lysis of a given number of "targeted cells, " in a given time, by the mechanism of Fc-mediated cellular cytotoxicity. The increase in Fc-mediated cellular cytotoxicity is relative to the cellular cytotoxicity mediated by the same antigen binding molecule or Fc-fusion protein produced by the same type of host cells, using the same standard production, purification, formulation and storage methods, (which are known to those skilled in the art) but that has not been produced by host cells engineered to have an altered pattern of glycosylation (e.g., to express the glycosyltransferase, GnTIII, or other glycosyltransferases) by the methods described herein.

[0078] By "*antigen binding molecule having increased antibody dependent cellular cytotoxicity (ADCC)*" is meant an antigen binding molecule, as that term is defined herein, having increased ADCC as determined by any suitable method known to those of ordinary skill in the art. One accepted *in vitro* ADCC assay is as follows:

1) the assay uses target cells that are known to express the target antigen recognized by the antigen-binding region of the antibody;

2) the assay uses human peripheral blood mononuclear cells (PBMCs), isolated from blood of a randomly chosen healthy donor, as effector cells;

3) the assay is carried out according to following protocol:

i) the PBMCs are isolated using standard density centrifugation procedures and are suspended at 5 x 106 cells/ml in RPMI cell culture medium;

ii) the target cells are grown by standard tissue culture methods, harvested from the exponential growth phase with a viability higher than 90%, washed in RPMI cell culture medium, labeled with 100 micro-Curies of 51Cr, washed twice with cell culture medium, and resuspended in cell culture medium at a density of 105 cells/ml;

iii) 100 microliters of the final target cell suspension above are transferred to each well of a 96-well microtiter plate;

iv) the antibody is serially-diluted from 4000 ng/ml to 0.04 ng/ml in cell culture medium and 50 microliters of the resulting antibody solutions are added to the target cells in the 96-well microtiter plate, testing in triplicate various antibody concentrations covering the whole concentration range above;

v) for the maximum release (MR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of a 2% (V/V) aqueous solution of non-ionic detergent (Nonidet, Sigma, St. Louis), instead of the antibody solution (point iv above);

vi) for the spontaneous release (SR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of RPMI cell culture medium instead of the antibody solution (point iv above);

vii) the 96-well microtiter plate is then centrifuged at 50 x g for 1 minute and incubated for 1 hour at 4oC;

viii) 50 microliters of the PBMC suspension (point i above) are added to each well to yield an effector:target cell ratio of 25:1 and the plates are placed in an incubator under 5% CO2 atmosphere at 37oC for 4 hours;

ix) the cell-free supernatant from each well is harvested and the experimentally released radioactivity (ER) is quantified using a gamma counter;

x) the percentage of specific lysis is calculated for each antibody concentration according to the formula (ER-MR)/(MR-SR) x 100, where ER is the average radioactivity quantified (see point ix above) for that antibody concentration, MR is the average radioactivity quantified (see point ix above) for the MR controls (see point v above), and SR is the average radioactivity quantified (see point ix above) for the SR controls (see point vi above);

4) "*increased ADCC*" is defined as either an increase in the maximum percentage of specific lysis observed within the antibody concentration range tested above, and/or a reduction in the concentration of antibody required to achieve one half of the maximum percentage of specific lysis observed within the antibody concentration range tested above. The increase in ADCC is relative to the ADCC, measured with the above assay, mediated by the same antibody, produced by the same type of host cells, using the same standard production, purification, formulation and storage methods, which are known to those skilled in the art, but that has not been produced by host cells engineered to overexpress GnTIII.

Anti-CEA Antigen Binding Molecules

[0079] CEA has long been used as a cancer marker for diagnostic purposes. It is abnormally expressed (e.g., overexpressed and/or distributed in a different pattern in the cell) in many tumor tissues compared to non-tumor tissues of the same cell type. However, because CEA is generally cleaved from the tumor cell surface and most of the available anti-CEA antibodies also bind soluble CEA, unconjugated antibodies to CEA are generally not used for therapeutic purposes. For example, the anti-CEA antibodies that are currently in pilot trials are administered as radioconjugates (Wong et al., 2004; Liersch et al., 2007).

[0080] Several mechanisms are involved in the therapeutic efficacy of anti-CEA antibodies, including antibody dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Increased CEA expression promotes increased intercellular adhesion, which may lead to

metastasis of cancerous cells (Marshall J., *Semin Oncol.* 30(3) Suppl. 8:30-36). Thus, anti-CEA antigen binding molecules may also play a role in inhibiting CEA-mediated cell adhesion and metastasis of cancerous cells.

[0081] In one aspect, the invention is directed to an antigen binding molecule (e.g., an antibody or fragment thereof) comprising one or more (*e.g.*, one, two, three, four, five, or six) CDRs of the murine PR1A3 antibody, wherein at least one of the CDRs has substitution of at least one amino acid residue compared to the corresponding CDR of PR1A3, and wherein the antigen binding molecule has improved affinity for CEA, preferably membrane-bound CEA compared to a parent PR1A3 antigen binding molecule. Such one or more CDRs can be truncated CDRs and will contain, at a minimum, the specificity-determining residues (SDRs), as that term is defined herein, for a given CDR. In one embodiment, the antigen binding molecule comprises at least one (e.g., one, two, three, four, five or six) of the CDRs set forth in Table 2, below, comprising the residues of the CDRs that will retain specific binding. In another embodiment, the antigen binding molecule comprises at least one (e.g., one, two, three, four, five, or six) CDR set forth in Table 2, below, or a variant or truncated form thereof containing at least the specificity-determining residues for said CDR, and comprising a sequence derived from a heterologous polypeptide. In a specific embodiment, where the antigen binding molecule comprises a heavy chain CDR1 variant of PR1A3, the HCDR1 has a glutamate substituted for a valine at Kabat position 31. In one embodiment, the antigen binding molecule comprises three heavy chain CDRs (e.g., HCDR1, HCDR2, and HCDR3) and/or three light chain CDRs (e.g. LCDR1, LCDR2, and LCDR3) from Table 2, or variants or truncated forms thereof containing at least the specificity-determining residues for each of said three CDRs. In a more specific embodiment, the antigen binding molecule comprises three heavy chain CDRs (e.g., HCDR1, HCDR2, and HCDR3) and three light chain CDRs (e.g. LCDR1, LCDR2, and LCDR3) from Table 2. In another embodiment, the antigen binding molecule comprises the variable region(s) of an antibody light and/or heavy chain, preferably both a heavy and light chain variable region. In a more particular embodiment, the heavy chain and/or light chain variable region is selected from the heavy and/or light chain variable region of Table 4 or a combination thereof, wherein, the heavy and light chain variable region is not a combination of SEQ ID NO:99 and SEQ ID NO:103 or SEQ ID NO:100 and SEQ ID NO:104. In one embodiment, the antigen binding molecule is a chimeric antibody, more specifically, a humanized antibody. In another embodiment, the antigen binding molecule comprises an Fc region. In another embodiment, the antigen binding molecule is affinity matured. In another embodiment, the antigen binding molecule has increased ADCC activity compared to PR1A3. In one embodiment, the increased ADCC of the antigen binding molecule is due to an increase in affinity of the antigen binding molecule for membrane-bound CEA, for example by affinity maturation or other methods of improving affinity (*see* Tang *et al., J. Immunol.* 2007, 179:2815-2823, the entire contents of which is herein incorporated by reference). In another embodiment, the antigen binding molecule

comprises an Fc region that is glycoengineered. In another aspect, the invention is also directed to methods of making such antigen binding molecules and their use in the treatment of disease, particularly cell proliferation disorders wherein CEA is expressed, particularly wherein CEA is abnormally expressed (e.g., overexpressed or expressed in a different pattern in the cell) compared to normal tissue of the same cell type. Such disorders include, but are not limited to colorectal cancer, NSCLC (non-small cell lung cancer), gastric cancer, pancreatic cancer and breast cancer. CEA expression levels may be determined by methods known in the art and those described herein (e.g., via immunohistochemistry assay, immunofluorescence assay, immunoenzyme assay, ELISA, flow cytometry, radioimmunoassay, Western blot, ligand binding, kinase activity, etc.).

[0082] In another aspect, the invention is also directed to an isolated polynucleotide comprising a sequence that encodes a polypeptide comprising one or more (e.g,, one, two, three, four, five, or six) complementarity determining regions of the murine PR1A3 antibody, or variants or truncated forms thereof containing at least the specificity-determining residues for said complementarity determining regions. Typically, such isolated polynucleotides encode one or more fusion polypeptides that form an antigen binding molecule. In one embodiment, the polynucleotide comprises a sequence encoding one or more (e.g., one, two, three, four, five or six) of the CDRs set forth in Table 2, below, comprising the residues of the CDRs that will retain specific binding. In one embodiment, the polynucleotide comprises a sequence that encodes at least three heavy chain CDRs (e.g., HCDR1, HCDR2, and HCDR3) and/or three light chain CDRs (e.g. LCDR1, LCDR2, and LCDR3) from Table 2, or variants or truncated forms thereof containing at least the specificity-determining residues (SDRs) for each of said three complementarity determining regions. In a more specific embodiment, the polynucleotide encodes a polypeptide comprising three heavy chain CDRs (e.g., HCDR1, HCDR2, and HCDR3) and three light chain CDRs (e.g. LCDR1, LCDR2, and LCDR3) from Table 2. In another embodiment, the polynucleotide encodes a polypeptide comprising the variable region(s) of an antibody light and/or heavy chain. The polynucleotides encoding the heavy and light chain variable region polypeptides can be expressed in one or more expression vectors. In a more particular embodiment, the polynucleotide encoding a heavy chain and/or light chain variable region is selected from the group of polynucleotides presented in Table 5 or a combination thereof, wherein, the heavy and light chain variable regions are not encoded by a combination of SEQ ID NO:11 and SEQ ID NO:115 or SEQ ID NO:112 and SEQ ID NO:116. In one embodiment, the heavy and light chain variable region polypeptides encoded by the polynucleotides combine to form a chimeric antibody, more specifically, a humanized antibody. In a specific embodiment, where the polynucleotide comprises a sequence that encodes heavy chain CDR1 of PR1A3 or a variant thereof, said polynucleotide encodes a glutamate substituted for a valine at Kabat position 31. In another embodiment, the polynucleotide comprises a sequence that encodes an Fc region. The invention is further directed to the polypeptides encoded by such polynucleotides. In one embodiment, the

polypeptide encoded by the aforementioned polynucleotides comprises an Fc region. In a more specific embodiment, the polypeptides encoded by the polynucleotides are glycoengineered to have an altered pattern of glycosylation in the Fc region. In a particular embodiment, the affinity for membrane-bound CEA of the polypeptides encoded by the polynucleotides is increased compared to the parent PR1A3 antibody. In another embodiment, the polypeptide encoded by the polynucleotide has increased ADCC activity. In one embodiment, the increased ADCC of the polypeptide encoded by the polynucleotide is due to an increase in affinity of the polypeptide for membrane-bound CEA, for example by affinity maturation or other methods of improving affinity. In another aspect, the invention is also directed to use of the polypeptides (e.g., antigen binding molecules) encoded by the polynucleotides in the treatment of disease, particularly cell proliferation disorders wherein CEA is expressed, particularly wherein CEA is abnormally expressed (e.g., overexpressed or expressed in a different pattern in the cell) compared to normal tissue of the same cell type. Such disorders include, but are not limited to colorectal cancer, NSCLC (non-small cell lung cancer), gastric cancer, pancreatic cancer and breast cancer. CEA expression levels may be determined by methods known in the art and those described herein (e.g., via immunohistochemistry assay, immunofluorescence assay, immunoenzyme assay, ELISA, flow cytometry, radioimmunoassay, Western blot, ligand binding, kinase activity, etc.).

[0083] In a particular embodiment, the invention is directed to a humanized antigen binding molecule or a portion or fragment thereof specific for membrane-bound CEA comprising a heavy chain variable region comprising the sequence of any one of SEQ ID NOs:101, 107, or 188-206. In another embodiment, the invention is directed to a humanized antigen binding molecule or a portion or fragment thereof specific for membrane-bound CEA comprising a light chain variable region comprising the sequence of any one of SEQ ID NOs:105, 108, or 207-216. In a particular embodiment, the humanized antigen binding molecule or a portion or fragment thereof specific for membrane-bound CEA comprises a heavy chain variable region comprising the sequence of any one of SEQ ID NOs:101, 107, or 188-206 and a light chain variable region comprising the sequence of any one of SEQ ID NOs:105, 108, or 207-216. In one embodiment, the humanized antigen binding molecule further comprises a human heavy chain constant region and/or a human light chain constant region. Such constant regions are described herein and are known in the art. In a more particular embodiment, the humanized antigen binding molecule comprises an Fc region, more particularly, an Fc region that has been glycoengineered.

[0084] Methods for humanizing non-human antibodies are known in the art. For example, humanized ABMs of the present invention can be prepared according to the methods of U.S. Pat. No. 5,225,539 to Winter, U.S. Pat. No. 6,180,370 to Queen *et al.,* or U.S. Pat. No. 6,632,927 to Adair *et al.,* the entire contents of each of which is hereby incorporated by reference. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which 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. 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 hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. 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. Typically, humanized antibodies are human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in a non-human (e.g., rodent) antibodies. The subject humanized anti-CEA antibodies will optionally comprise constant regions from a human immunoglobulin.

[0085] The choice of light and heavy chain human variable domains for making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a donor (e.g., rodent) antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the donor (e.g., rodent) is then accepted as the human framework region (FR) for the humanized antibody (Sims et al., *J. Immunol.*, 151:2296 (1993); Chothia et al., *J. Mol. Biol.*, 196:901 (1987)). Another method of selecting the human framework sequence is to compare the sequence of each individual subregion of the full donor (e.g., rodent) framework (i.e., FR1, FR2, FR3, and FR4) or some combination of the individual subregions (e.g., FR1 and FR2) against a library of known human variable region sequences that correspond to that framework subregion (e.g., as determined by Kabat numbering), and choose the human sequence for each subregion or combination that is the closest to that of the rodent (Leung U.S. Patent Application Publication No. 2003/0040606A1, published Feb. 27, 2003). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains (Carter et al., *Proc. Natl. Acad. Sci.* USA, 89:4285 (1992); Presta et al., *J. Immunol.*, 151:2623 (1993)). In one embodiment, the human framework regions are selected from a collection of human germline sequences. Such collections of human germline sequences can be found in databases such as IMGT or VBase. Framework regions can be selected individually (e.g., the FR1-3 selected for the acceptor for the heavy and/or light chain variable regions of the humanized anti-CEA ABMs can be encoded by different germline genes) or as part of the same germline gene. In a more specific embodiment, heavy chain FR1-3 are encoded by the IGHV7_4_1*02 human immunoglobulin germline gene sequence (Accession No. X62110, SEQ ID NO:114). In another specific embodiment, light chain FR1-3 are encoded by the IMGT_hVK_1_39 human immunoglobulin germline gene sequence (Accession No. X59315, SEQ ID NO:118). In another specific embodiment, heavy chain FR4 is encoded by the JH6 germline gene sequence (See GenBank Accession No. M63030). In another

specific embodiment, light chain FR4 is encoded by the JK2 germline gene sequence (See Genbank Accession No.X61584).

[0086] It is generally desirable that antigen binding molecules, such as antibodies and fragments thereof, be humanized with retention of high affinity for the antigen and other favorable biological properties. Accordingly, in one embodiment, humanized antibodies are prepared by analyzing the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Analysis of these displays helps to elucidate the likely role of the residues in the functioning of the candidate immunoglobulin sequence, e.g., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[0087] In one aspect, the invention is directed to humanized, affinity matured and/or variant anti-CEA antigen binding molecules with desirable properties and characteristics that include, but are not limited to: strong binding affinity for the CEA antigen--in particular, membrane-bound CEA--while having substantially no cross-reactivity against soluble CEA; an ability to induce cell lysis of CEA-expressing cells *in vitro* and *ex vivo,* preferably in a dose-dependent manner; an ability to inhibit CEA-mediated cell adhesion *in vitro*; an ability to inhibit tumor tissue growth and/or induce tumor tissue regression (for example, as demonstrated in tumor models (*e.g.*, xenograft mouse)).

[0088] As described herein, in some embodiments, antigen binding molecules of the invention have increased binding affinity, for example, due to affinity maturation of a parent antibody comprising one or more CDRs of the PR1A3 antibody. Affinity of the antigen binding molecules of the invention can be determined by methods known in the art and as described herein. In a specific embodiment, humanized or variant anti-CEA antigen binding molecules of the invention bind to human CEA, preferably membrane-bound CEA, with a monovalent affinity constant (K_D) value of no more than about 1 μM to about 0.001 nM, more specifically no more than about 800 nM to about 1 nM, and even more specifically no more than about 550 nM to about 10 nM. In a specific embodiment, the variant anti-CEA antigen binding molecule is an affinity matured antibody or fragment thereof that binds to membrane-bound CEA with a monovalent affinity constant (K_D) value of no more than about 100 nM to about 10 nM.

[0089] In one embodiment, the antigen binding molecule of the invention typically binds the same epitope as recognized by the mouse antibody PR1A3, or are capable of competing with the PR1A

antibody for binding to membrane-bound CEA. To screen for antibodies that bind to the epitope on human CEA bound by an antibody of interest (e.g., those that block binding of the PR1A3 antibody to human CEA), a routine cross-blocking assay such as that described in ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Laboratory, Harlow and Lane eds. (1988), can be performed. Alternatively, epitope mapping, *e.g.* as described in Champe *et al.*, *J. Biol. Chem. 270*:1388-1394 (1995), can be performed to determine whether the antibody binds an epitope of interest.

[0090] In one embodiment, variant antigen bind molecules specific for human CEA are made from a parent anti-CEA antigen binding molecule comprising at least one CDR of the monoclonal antibody PR1A3, wherein the parent anti-CEA antibody binds the same epitope as the PR1A3 antibody and is capable of competing with PR1A3 for antigen binding. In one embodiment, the parent antigen binding molecule comprises at least one, two, or typically three, heavy chain CDRs of the PR1A3 antibody; in another embodiment, the parent antigen binding molecule comprises at least one, two, or typically three, light chain CDRs of the PR1A3 antibody; in another embodiment, the parent antigen binding molecule comprises the three heavy chain CDRs and the three light chain CDRs of the PR1A3 antibody. Preferably, where the antigen binding molecule comprises HCDR1 of PR1A3, said HCDR1 comprises a substitution of glutamate for valine at Kabat position 31. The variant ABMs typically have a greater affinity for CEA than the parent. In one embodiment, the variant ABM comprises an Fc region. In one embodiment, the variant ABM is glycoengineered. In one embodiment the variant ABM has increased ADCC activity compared to the parent ABM. In a particular embodiment, the increased ADCC is result of the increased affinity, achieved, for example, by affinity maturation of the parent ABM to generate the variant ABM. In a more particular embodiment, the increase in ADCC is at least about 40% to about 100% as compared to said parent antigen binding molecule. In another particular embodiment, the variant ABM increases ADCC by at least about 10% to about 100% in an in vitro cytotoxicity assay. In a more particular embodiment, the variant ABM is at least from about 10-fold to about 1000-fold more potent at inducing ADCC at a given concentration compared to the murine PR1A3 antibody. In another particular embodiment, the increased ADCC activity is a result of glycoengineering of the Fc region. In another particular embodiment, the increased ADCC activity is a result of a combination of increased affinity and glycoengineering.

[0091] In one embodiment, the variant antigen binding molecules of the invention comprise one or more amino acid substitutions in at least one CDR. The number of amino acid substitution(s) can range from one to ten (e.g, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10), preferably from two to five (e.g., 2, 3, 4, or 5). In one embodiment, at least one heavy chain CDR comprises one or more amino acid substitution(s). In another embodiment, at least one light chain CDR comprises one or more amino acid substitution(s). In another embodiment, at least one heavy chain CDR comprises one or more substitutions, and at least one light chain CDR comprises one or more substitutions. Preferably,

where the antigen binding molecule comprises HCDR1 of PR1A3, said HCDR1 comprises a substitution of glutamate for valine at Kabat position 31.

[0092] Substantial modifications in the biological properties of the antigen binding molecules are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Variant antigen binding molecules comprising amino acid substitutions may have improved biological activities, for example, improved antigen binding affinity and enhanced ADCC, compared to the parent antigen binding molecule. Amino acid substitutions can be introduced by various techniques known in the art including, but not limited to, site directed mutagenesis and/or affinity maturation of the parent antigen binding molecule e.g., by phage display.

[0093] In order to identify candidate sites, e.g., hypervariable region residues, for modification, alanine scanning mutagenesis can be performed to find residues that contribute significantly to antigen binding. Alternatively, or in addition, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and human CEA. Such contact residues and neighboring residues are candidates for substitution according by methods known in the art and/or described herein. Once such variants are generated, the panel of variants can be screened by methods known in the art and/or described herein and antibodies with superior properties in one or more relevant assays can be selected for further development.

[0094] Phage display can be used to generate a repertoire of hypervariable region sequences from a parent antigen binding molecule that containing random amino acid mutation(s). For example, several hypervariable region sites (e.g. 6-7 sites) are mutated to generate all possible amino substitutions at each site. Alternatively, random mutagenesis can be performed on heavy and/or light chain variable regions. Mutations can be generated by techniques known in the art, including but not limited to using mutagenesis primers, controlling the number of cycles and using mutagenic nucleotide analogues 8-oxo-dGTP and dPTP during PCR amplification. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activities (e.g. binding affinity) as herein disclosed and candidates that have one or more improved activities will be used for further development. Methods for making phage display libraries can be found in Huse *et al., Science*, 246:1275-1281 (1989); *Proc. Nat'l Acad. Sci., USA*, 88:4363-4366 (1991), the entire contents of each of which are hereby incorporated by reference. An alternative method for identifying affinity matured antigen binding molecules can be found in, for example, U.S. Pat. No. 7,432,063 to Balint *et al.*, the entire contents of which are hereby incorporated by reference.

[0095] In some embodiments, the antigen binding molecules of the present invention comprise a Fc region, preferably a human Fc region. The sequences and structures of Fc regions are known in the art and have been characterized. In a specific embodiment, the human constant region is IgG1, as set forth in SEQ ID NOs 121 and 122, and set forth below:

IgG1 Nucleotide Sequence (SEQ ID NO:121)

ACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGC
ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTA
CTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGA
CCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGAC
TCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG
GCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC
ACCAAGGTGGACAAGAAAGCAGAGCCCAAATCTTGTGACAAAAC
TCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGAC
CGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGA
TCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGC
CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC
AACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG
GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA
AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAG
GGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGG
GATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAA
AGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATG
GGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGAC
TCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAG
AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTC
TCCGGGTAAATGA

IgG1 Amino Acid Sequence (SEQ ID NO:122)

TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
KAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
GK

[0096] However, variants and isoforms of the human Fc region are also encompassed by the present invention. For example, variant Fc regions suitable for use in the present invention can be produced according to the methods taught in U.S. Pat. No. 6,737,056 to Presta (Fc region variants with altered effector function due to one or more amino acid modifications); or in U.S. Pat. Appl. Nos. 60/439,498; 60/456,041; 60/514,549; or WO 2004/063351 (variant Fc regions with increased binding affinity due to amino acid modification); or in U.S. Pat. Appl. No. 10/672,280 or WO 2004/099249 (Fc variants with altered binding to FcγR due to amino acid modification), the contents of each of

which is herein incorporated by reference in its entirety. In a particular embodiment, the anti-CEA ABMs and variant ABMs comprise an Fc region that has been glycoengineered to alter the effector function activity of the ABM (e.g., decrease fucosylation, improve Fc receptor binding affinity, increase ADCC, etc.). Methods of glycoengineering that can be used are described in detail herein below and are known in the art.

[0097] In one embodiment, the antigen binding molecule of the present invention is conjugated to an additional moiety, such as a radiolabel or a toxin. Such conjugated antigen binding molecules can be produced by numerous methods that are well known in the art. Anti-CEA ABM conjugates of the invention are described in detail herein below in the section entitled "Anti-CEA Antigen Binding Molecule Conjugates."

Polypeptides and Polynucleotides of Anti-CEA ABMs

[0098] In one aspect, the present invention is related to antigen binding molecules and polypeptides having the same binding specificity of the murine PR1A3 antibody (e.g., binding to the same epitope of membrane-bound CEA), and having comparable or improved biological activities (e.g., improved affinity for membrane-bound CEA and/or enhanced ADCC). In one embodiment, the antigen binding molecule comprises one or more of the CDRs set forth in Table 2, below. In a more specific embodiment, the antigen binding molecule comprises 3 heavy chain CDRs in Table 2, below. In another specific embodiment, the antigen binding molecule comprises 3 light chain CDRs in Table 2, below. In another specific embodiment, the invention is directed to an antigen binding molecule specific for membrane-bound human CEA comprising: (a) a heavy chain CDR1 sequence 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, SEQ ID NO:11, and SEQ ID NO:12; (b) a heavy chain CDR2 sequence 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 (c) a heavy chain CDR3 sequence selected from the group consisting of: SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35. In another specific embodiment, the invention is directed to an antigen binding molecule specific for membrane-bound human CEA comprising: (a) a light chain CDR1 sequence 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; (b) a light chain CDR sequence 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 (c) a light chain CDR3 of SEQ ID NO:56. In a specific embodiment, the antigen binding molecule has no

substantial cross-reactivity against soluble human CEA. In another embodiment, the antigen binding molecule comprises a human heavy and/or light chain constant region. For example, in one embodiment, the antigen binding molecule comprises an Fc region. In a more specific embodiment, the antigen binding molecule comprises an Fc region that has been glycoengineered. The invention is also directed to polynucleotides encoding any one of the antigen binding molecules of the invention specific for membrane-bound human CEA.

[0100] In one aspect, the invention is directed to an antigen binding molecule specific for membrane-bound human CEA that comprises a heavy chain variable region and/or a light chain variable region. In one embodiment, the heavy chain variable region comprises a polypeptide having the sequence of SEQ ID NO:101. In another embodiment, the heavy chain variable region comprises a polypeptide having at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the sequence of SEQ ID NO:101. In one embodiment, the light chain variable region comprises a polypeptide having the sequence of SEQ ID NO:105. In another embodiment, the heavy chain variable region comprises a polypeptide having at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the sequence of SEQ ID NO:105.

[0101] In one embodiment, the antigen binding molecule specific for membrane-bound human CEA comprises a heavy chain variable region and a light chain variable region. In a specific embodiment, the heavy chain variable region comprises a polypeptide having the sequence of SEQ ID NO:4 as follows:

QVQLVQSGSELKKPGASVKVSCKASGYTFTEX^{1}X^{2}MX^{3}WVRQAPGQ
GLEWMGX^{4}INTKX^{5}GEAX^{6}YX^{7}EEFKGRFVFSLDTSVSTAYLQISSLK
AEDTAVYYCARWDX^{8}X^{9}X^{10}YX^{11}X^{12}X^{13}X^{14}DYWGQGTTVTVSS

wherein X^1 is Y or F; X^2 is S or G; X^3 is N or S; X^4 is W or Y; X^5 is N, T or S; X^6 is T or N; X^7 is V or I; X^8 is F or A; X^9 is Y, A, V, F or S; X^{10} is D, H, W, E, or Y; X^{11} is V, L or F; X^{12} is E, K or Q; X^{13} is A or T; and X^{14} is M or L.

[0102] In a specific embodiment, the light chain variable region comprises a polypeptide having the sequence of SEQ ID NO:11 as follows:

DIQMTQSPSSLSASVGDRVTITCKASX^{15}X^{16}X^{17}X^{18}X^{19}X^{20}VAWYQQKP
GKAPKX^{21}LIYX^{22}ASX^{23}X^{24}X^{25}X^{26}GVPSRFSGSGSGTDFTLTISSLQPED
FATYYCHQYYTYPLFTFGQGTKLEIK

wherein X^{15} is Q, A, K, or H; X^{16} is N, A, Y, I, K, T, or F; X^{17} is V, A, G, or M; X^{18} is G, S, T, or L; X^{19} is T, N, P, or A; X^{20} is N or Y; X^{21} is P or L; X^{22} is S, L, or W; X^{23} is Y, N, or H; X^{24} is R, L, P, or H; X^{25} is Y, S, Q, K, E, F, or P; and X^{26} is S, G, I, or R.

[0103] In another specific embodiment, the heavy chain variable region comprises a polypeptide having the sequence of SEQ ID NO:107. In another specific embodiment, the heavy chain variable region comprises a polypeptide having a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the sequence of SEQ ID NO:107. In another specific embodiment, the

antigen light chain variable region comprises a polypeptide having the sequence of SEQ ID NO:108. In another specific embodiment, the light chain variable region comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the sequence of SEQ ID NO:108. In one embodiment, the antigen binding molecule comprises a heavy chain polypeptide that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of: SEQ ID NOs: 101, 107, and 188 to 206, and a light chain polypeptide that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of: SEQ ID NOs:105, 108 and 207 to 216. An antigen binding molecule comprising the heavy and/or light chain variable region is specific for CEA, in particular membrane-bound CEA. In one embodiment, an antigen binding molecule comprising the heavy and/or light chain variable region has no substantial cross-reactivity against soluble CEA. In another embodiment, the heavy chain further comprises an Fc region. In a specific embodiment, the Fc region has been glycoengineered to have reduced fucosylation of the N-linked oligosaccharides as described in detail herein below.

[0104] In one aspect, the invention is also directed to an isolated polypeptide comprising one or more CDRs set forth in Table 2. In one embodiment, the isolated polypeptide comprises: heavy chain CDR1 sequence 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, SEQ ID NO:11, and SEQ ID NO:12; (b) a heavy chain CDR2 sequence 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 (c) a heavy chain CDR3 sequence selected from the group consisting of: SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35. In one embodiment, the isolated polypeptide comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 107. In another embodiment, the invention is directed to a polypeptide comprising: (a) a light chain CDR1 sequence 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; (b) a light chain CDR sequence 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 (c) a light chain CDR3 of SEQ ID NO:56. In one embodiment, the isolated polypeptide comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO: 108. In another aspect, the invention is also directed to polynucleotides encoding any one of these polypeptides and antigen binding molecules comprising any one of these polypeptides. An antigen binding molecule

comprising the polypeptide is specific for CEA, in particular, membrane-bound CEA. In one embodiment, the antigen binding molecule has no substantial cross-reactivity against soluble human CEA. In one embodiment, the polypeptide further comprises a human heavy and/or light chain constant region. In another embodiment, the polypeptide comprises an Fc region, more particularly a glycoengineered Fc region. In a specific embodiment, the Fc region has been glycoengineered to have reduced fucosylation of the N-linked oligosaccharides as described in detail herein.

[0105] In another aspect, the invention is further directed to isolated polynucleotides encoding antigen binding molecules specific for membrane-bound human CEA. In one embodiment, the isolated polynucleotide comprises one or more of the CDR sequences shown in Table 3, below, or a combination thereof, wherein the polynucleotide encodes a polypeptide that, as part of an antigen binding molecule, specifically binds CEA, particularly membrane-bound CEA. In one embodiment, the isolated polynucleotide comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to one or more of the variable region sequences shown in Table 5. In one embodiment, the invention is directed to a composition that comprises a first isolated polynucleotide comprising a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of SEQ ID NOs:113, 119, and 159-177. and a second isolated polynucleotide comprising a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of SEQ ID NOs:117, 120, and 178-187. In one embodiment, the polynucleotide encodes a heavy chain comprising a variable region and a constant region (e.g., an IgG constant region or fragment thereof, in particular an IgG constant region comprising an Fc region). In one embodiment, the polynucleotide encodes a light chain comprising a variable region and a constant region (e.g., a kappa or lambda constant region). In one embodiment, antigen binding molecules encoded by these isolated polynucleotides have no substantial cross-reactivity against soluble human CEA.

[0106] In another embodiment, the invention also encompasses an isolated polynucleotide comprising a sequence that encodes a polypeptide having a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98% , 99% or 100% identical to a sequence selected from the group consisting of: SEQ ID NOs:101, 105, 107, 108, and 188-216 with conservative amino acid substitutions, wherein an antigen binding molecule comprising the polypeptide (for example, an antigen binding molecule comprising a polypeptide that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98% , 99% or 100% identical to a sequence selected from the group consisting of: SEQ ID NOs:101, 107, and 188-206 and a polypeptide that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98% , 99% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs:105, 108, and 207-216) specifically binds to CEA, particularly membrane-bound CEA. In one embodiment, the polypeptide further comprises a human constant region. In a specific embodiment, the polypeptide

comprises a human Fc region. In a more specific embodiment, the Fc region is a glycoengineered IgG Fc region.

[0107] In one embodiment, the invention is directed to a polynucleotide comprising: (a) a sequence encoding a heavy chain CDR1 selected from the group consisting of SEQ ID NOs:57-59 and 61, (b) a heavy chain CDR2 sequence selected from the group consisting of SEQ ID NOs:62-66, and (c) a heavy chain CDR3 sequence selected from the group consisting of SEQ ID NOs:67-77. In another embodiment, the invention is directed to a polynucleotide comprising: (a) a light chain CDR1 sequence selected from the group consisting of SEQ ID NOs:78-88, (b) a light chain CDR2 sequence selected from the group consisting of SEQ ID NOs:89-97, and (c) a light chain CDR3 sequence of SEQ ID NO:98.

TABLE 2

CDR		Amino Acid Sequence	SEQ ID NO
Heavy Chain CDR1	**Kabat**	EFGMN	1
		E**Y**GMN	2
		E**YS**MN	3
		EFGM**S**	5
	Chothia	GYTFTEF	6
		GYTFTE**Y**	7
	AbM	GYTFTEFGMN	8
		GYTFTE**Y**GMN	9
		GYTFTE**YS**MN	10
		GYTFTEFGM**S**	12
Heavy Chain CDR2	**Kabat**	WINTKTGEATYVEEFKG	13
		WINTKTGEATY**I**EEFKG	14
		WINTK**S**GEATYVEEFKG	15
		YINTK**N**GEA**N**YVEEFKG	16
		WINTK**N**GEATY**I**EEFKG	17
	Chothia	NTKTGEAT	18
		NTK**S**GEAT	19
		NTK**N**GEA**N**	20
	AbM	WINTKTGEAT	21
		WINTK**S**GEAT	22
		YINTK**N**GEA**N**	23
		WINTK**N**GEAT	24
Heavy Chain CDR3	**Kabat Chothia and AbM**	WDFYDYVEAMDY	25
		WDFY**H**YVEAMDY	26
		WDF**V**DYVEAMDY	27
		WDFY**W**YVEAMDY	28
		WD**AFE**YV**K**A**L**DY	29
		WDF**FE**Y**FKT**MDY	30
		WDF**FY**YV**QT**MDY	31
		WDF**SY**YVEAMDY	32
		WDF**AHY FQT**MDY	33
		WDF**AYY FQT**MDY	34
		WDF**AYYL**EAMDY	35
Light Chain		KASQNVGTNVA	36

CDR	Amino Acid Sequence	SEQ ID NO
CDR1	KAS**A**NVG**N**NVA	37
	KAS**K**NVGTNVA	38
	KAS**AA**VGT**Y**VA	39
	KASQ**YAS**TNVA	40
	KAS**H**NVGTNVA	41
	KASQ**IMG**P**N**VA	42
	KASQ**I**VGTNVA	43
	KASQ**K**V**L**TNVA	44
	KASQ**T**V**SA**NVA	45
Light Chain CDR2	SASYRYS	46
	Y**L**ASNLSG	47
	Y**L**ASY**PQI**	48
	YSASYR**KR**	49
	Y**W**ASYRYS	50
	YSAS**H**RYS	51
	YLASY**HE**S	52
	YSAS**H**R**P**S	53
	Y**L**ASYRYS	54
	Y**L**ASYRY**R**	55
Light Chain CDR3	HQYYTYPLFT	56

TABLE 3

CDR	NUCLEOTIDE SEQUENCE	SEQ ID NO
Heavy Chain CDR1	GGATACACCTTCACTGAGTTTGGAATGAAC	57
	GGATACACCTTCACTGAG**TAT**GGTATGAAC	58
	GGATACACCTTCACTGAG**TATTCT**ATGAAC	59
	GGATACACCTTCACTGAGTTTGGAATG**AGC**	61
Heavy Chain CDR2	TGGATAAACACCAAAACTGGAGAGGCAACATATGT TGAAGAGTTTAAGGGA	62
	TGGATAAACACCAAAACTGGAGAGGCAACATAT**AT** **T**GAAGAGTTTAAGGGA	63
	TGGATAAACACCAAA**AGT**GGAGAGGCAACATATG TTGAAGAGTTTAAGGGA	64
	TATATAAACACCAAA**AAT**GGAGAGGCA**AAC**TATG TTGAAGAGTTTAAGGGA	65
	TGGATAAACACCAAA**AAT**GGAGAGGCAACATAT**A** **TT**GAAGAGTTTAAGGGA	66
Heavy Chain CDR3	TGGGACTTCTATGATTACGTGGAGGCTATGGACTA C	67
	TGGGACTTCTAT**CAT**TACGTGGAGGCTATGGACTA C	68
	TGGGACTTC**GTG**GATTACGTGGAGGCTATGGACTA C	69
	TGGGACTTCTAT**TGG**TACGTGGAGGCTATGGACTA C	70
	TGGGAC**GCCTTTGAG**TACGTG**AAGGCGCTG**GACT AC	71

CDR	NUCLEOTIDE SEQUENCE	SEQ ID NO
	TGG**GAT**TTC**TTTGAGTATTTTAAGACT**ATGGACTAC	72
	TGGGAC**TTTTTTTAT**TACGTG**CAGACT**ATGGACTAC	73
	TGG**GAT**TTT**TCTTAT**TAC**GTT**GAG**GCG**ATGGACTAC	74
	TGGGAC**TTTGCTCAT**TAC**TTTCAGACT**ATGGACTAC	75
	TGGGACTTC**GCTTAT**TAC**TTTCAGACT**ATGGACTAC	76
	TGG**GAT**TTC**GCGTAT**TAC**CTT**GAGGCTATGGACTAC	77
Light Chain CDR1	AAGGCCAGTCAGAATGTGGGTACTAATGTTGCC	78
	AAGGCCAGT**GCC**AATGTGGGT**AAT**AATGTTGCC	79
	AAGGCCAGT**AAG**AATGTGGGGACTAATGTTGCG	80
	AAGGCCAGT**GCGGCT**GTGGGT**ACGTAT**GTTGCG	81
	AAGGCCAGTCAG**ATAGCGAGT**ACTAATGTTGCC	82
	AAGGCCAGT**CAC**AATGTGGGT**ACCAAC**GTT**GCG**	83
	AAGGCCAGTCAG**ATTATG**GGT**CCT**AATGTTGCG	84
	AAGGCCAGTCAA**ATT**GTGGGTACTAATGTTGCG	85
	AAGGCCAGTCAG**AAG**GTG**CTT**ACTAATGTTGCG	86
	AAGGCCAGTCAG**ACT**GTG**AGTGCT**AATGTTGCG	87
Light Chain CDR2	TATTCGGCATCCTACCGCTACAGT	88
	TAT**TTGGCC**TCC**AACCTCTCCGGT**	89
	TACCTGGCATCCTAC**CCCCAGATT**	90
	TATTCGGCATCCTACCGC**AAAAGG**	91
	TAT**TGG**GCATCCTACCGCTATAGT	92
	TATTCGGCATCC**CACCGG**TACAGT	93
	TAT**TTG**GCATCCTAC**CACGAA**AGT	94
	TATTCGGCATCC**CACCGTCCC**AGT	95
	TAT**TTG**GCATCCTACCGCTACAGT	96
	TAT**TTG**GCATCCTACCGCTAC**AGA**	97
Light Chain CDR3	CACCAATATTACACCTATCCTCTATTCACG	98

TABLE 4

CONSTRUCT	PEPTIDE SEQUENCE	SEQ ID NO
PR1A3 VH	QVKLQQSGPELKKPGETVKISCKASGYTFTEFGMNW VKQAPGKGLKWMGWINTKTGEATYVEEFKGRFAFS LETSATTAYLQINNLKNEDTAKYFCARWDFYDYVE AMDYWGQGTTVTVSS	99
pEM1496 huPR1A3 VH	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKADDTAVYYCARWDFYDYVE AMDYWGQGTTVTVSS	100
CH7A	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFYDYVE AMDYWGQGTTVTVSS	101

CONSTRUCT	PEPTIDE SEQUENCE	SEQ ID NO
IGHV7-4-1*02	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMN WVRQAPGQGLEWMGWINTNTGNPTYAQGFTGRFV FSLDTSVSTAYLQISSLKAEDTAVYYCAR	102
PR1A3 VL	DIVMTQSQRFMSTSVGDRVSVTCKASQNVGTNVAW YQQKPGQSPKALIYSASYRYSGVPDRFTGSGSGTDFT LTISNVQSEDLAEYFCHQYYTYPLFTFGSGTKLEMKR T	103
pEM1495 huPR1A3 VL	DIQMTQSPSSLSASVGDRVTITCKASQNVGTNVAWY QQKPGKAPKLLIYSASYRYSGVPSRFSGSGSGTDFTF TISSLQPEDIATYYCHQYYTYPLFSFGQGTKVEIKR	104
CL1A	DIQMTQSPSSLSASVGDRVTITCKASQNVGTNVAWY QQKPGKAPKLLIYSASYRYSGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIK	105
IMGT_hVK_1_39	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQ QKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQSYSTP	106
CH7 rF9	QVQLVQSGSELKKPGASVKVSCKASGYTFTEYGMN WVRQAPGQGLEWMGWINTKSGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFYDYVE AMDYWGQGTTVTVSS	107
CLA1 rH11	DIQMTQSPSSLSASVGDRVTITCKASQTVSANVAWY QQKPGKAPKLLIYLASYRYRGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRT	108
PMS22	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFYDYVE AMDYWGQGTTVTVSS	188
1C8	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFYHYVE AMDYWGQGTTVTVSS	189
3E1	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFVDYVE AMDYWGQGTTVTVSS	190
2D7	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFYWYVE AMDYWGQGTTVTVSS	191
Affinity Matured Heavy Chain	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFAHYFQ TMDYWGQGTTVTVSS	192
Affinity Matured Heavy Chain	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFAYYFQ TMDYWGQGTTVTVSS	193
Affinity Matured Heavy Chain	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFAYYLE AMDYWGQGTTITVSS	194

CONSTRUCT	PEPTIDE SEQUENCE	SEQ ID NO
H3 Full (5) 19	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMS WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDAFEYVK ALDYWGQGTTVTVSS	195
H3 Full (5) 8	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFFEYFK TMDYWGQGTTVTVSS	196
H3 Full (5) 28	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFFYYVQ TMDYWGQGTTVTVSS	197
H3 Full (5) 27	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFSYYVE AMDYWGQGTTVTVSS	198
H4E9 Heavy Chain	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKTGEATYIEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFYDYVE AMDYWGQGTTVTVSS	199
pAC14 (B9)	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGWINTKSGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFYDYVE AMDYWGQGTTVTVSS	200
pAC15 (F9)	QVQLVQSGSELKKPGASVKVSCKASGYTFTEYGMN WVRQAPGQGLEWMGWINTKSGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFYDYVE AMDYWGQGTTVTVSS	201
H1/H2 (5) 2	QVQLVQSGSELKKPGASVKVSCKASGYTFTEYSMN WVRQAPGQGLEWMGYINTKNGEANYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFYDYVE AMDYWGQGTTVTVSS	202
H1/H2 (5) 11	QVQLVQSGSELKKPGASVKVSCKASGYTFTEYGMN WVRQAPGQGLEWMGWINTKNGEATYIEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFYDYVE AMDYWGQGTTVTVSS	203
H1/H2 (5) 13	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMN WVRQAPGQGLEWMGYINTKNGEANYVEEFKGRFVF SLDASVSTAYLQISSLKAEDTAVYYCARWDFYDYVE AMDYWGQGTTVTVSS	204
H1/H2 (5) 14	QVQLVQSGSELKKPGASVKVSCKASGYTFTEYGMN WVRQAPGQGLEWMGYINTKNGEANYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDFYDYVE AMDYWGQGTTVTVSS	205
H3 Full (5) 19	QVQLVQSGSELKKPGASVKVSCKASGYTFTEFGMS WVRQAPGQGLEWMGWINTKTGEATYVEEFKGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCARWDAFEYVK ALDYWGQGTTVTVSS	206
pAC21 (3A1)	DIQMTQSPSSLSASVGDRVTITCKASANVGNNVAWY QQKPGKAPKLLIYLASNRSGGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRT	207

CONSTRUCT	PEPTIDE SEQUENCE	SEQ ID NO
pAC19 (2C6)	DIQMTQSPSSLSASVGDRVTITCKASKNVGTNVAWY QQKPGKAPKPLIYLASYPQIGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRT	208
pAC18 (2F1)	DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWY QQKPGKAPKLLIYSASYRKRGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRT	209
pAC23 (2F11)	DIQMTQSPSSLSASVGDRVTITCKASQIASTNVAWYQ QKPGKAPKLLIYWASYRYSGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRT	210
H4E9 light chain	DIQMTQSPSSLSASVGDRVTITCKASQNVGTNVAWY QQKPGKAPKPLIYSASYRYSGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRT	211
L2D2	DIQMTQSPSSLSASVGDRVTITCKASHNVGTNVAWY QQKPGKAPKLLIYSASHRYSGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRT	212
pAC6 (C1)	DIQMTQSPSSLSASVGDRVTITCKASQIMGPNVAWY QQKPGKAPKLLIYLASYHESGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRT	213
pAC7 (E10)	DIQMTQSPSSLSASVGDRVTITCKASQIVGTNVAWY QQKPGKAPKLLIYSASHRPSGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRT	214
pAC12 (H7)	DIQMTQSPSSLSASVGDRVTITCKASQKVLTNVAWY QQKPGKAPKLLIYLASYRYSGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRT	215
pAC13 (H11)	DIQMTQSPSSLSASVGDRVTITCKASQTVSANVAWY QQKPGKAPKLLIYLASYRYRGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRT	216

TABLE 5

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
PR1A3 VH	CAGGTGAAGCTGCAGCAGTCAGGACCTGAGTTGA AGAAGCCTGGAGAGACAGTCAAGATCTCCTGCAA GGCTTCTGGATATACCTTCACAGAATTCGGAATGA ACTGGGTGAAGCAGGCTCCTGGAAAGGGTTTAAA GTGGATGGGCTGGATAAACACCAAAACTGGAGAG GCAACATATGTTGAAGAGTTTAAGGGACGGTTTGC CTTCTCTTTGGAGACCTCTGCCACCACTGCCTATTT GCAGATCAACAACCTCAAAAATGAGGACACGGCT AAATATTTCTGTGCTCGATGGGATTTCTATGACTAT GTTGAAGCTATGGACTACTGGGGCCAAGGGACCA CCGTGACCGTCTCCTCA	111

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
pEM1496	CAGGTGCAGCTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTGGAATGAACT GGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTG GATGGGATGGATAAACACCAAAACTGGAGAGGCA ACATATGTTGAAGAGTTTAAGGGACGGTTTGTCTT CTCCTTGGACACCTCTGTCAGCACGGCATATCTGC AGATCAGCAGCCTAAAGGCTGACGACACTGCCGT GTATTACTGTGCGAGATGGGACTTCTATGATTACG TGGAGGCTATGGACTACTGGGGCCAAGGGACCAC GGTCACCGTCTCCTCA	112
CH7A	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCTATGATTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	113
IGHV7-4-1*02	CAGGTGCAGCTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTAGCTATGCTATGAATT GGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTG GATGGGATGGATCAACACCAACACTGGGAACCCA ACGTATGCCCAGGGCTTCACAGGACGGTTTGTCTT CTCCTTGGACACCTCTGTCAGCACGGCATATCTGC AGATCAGCAGCCTAAAGGCTGAGGACACTGCCGT GTATTACTGTGCGAGA	114
PR1A3 VL	GATATCGTGATGACCCAGTCTCAAAGATTCATGTC CACATCAGTAGGAGACAGGGTCAGCGTCACCTGC AAGGCCAGTCAGAATGTGGGTACTAATGTTGCCTG GTATCAACAGAAACCAGGACAATCCCCTAAAGCA CTGATTTACTCGGCATCCTACCGGTACAGTGGAGT CCCTGATCGCTTCACAGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAATGTACAGTCTGAA GACTTGGCGGAGTATTTCTGTCACCAATATTACAC CTATCCTCTATTCACGTTCGGCTCGGGGACAAAGT TGGAAATGAAACGTACG	115
pEM1495	GACATCCAGATGACTCAGAGCCCAAGCAGCCTGA GCGCCAGCGTGGGTGACAGAGTGACCATCACCTGT AAGGCCAGTCAGAATGTGGGTACTAATGTTGCCTG GTACCAGCAGAAGCCAGGTAAGGCTCCAAAGCTG CTGATCTACTCGGCATCCTACCGGTACAGTGGTGT GCCAAGCAGATTCAGCGGTAGCGGTAGCGGTACC GACTTCACCTTCACCATCAGCAGCCTCCAGCCAGA GGACATCGCCACCTACTACTGCCACCAATATTACA CCTATCCTCTATTCAGCTTCGGCCAAGGGACCAAG GTGGAAATCAAACGT	116

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
CL1A	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTCAGAATGTGGGTACTAATGTTGCCTGG TATCAGCAGAAACCAGGGAAAGCACCTAAGCTCC TGATCTATTCGGCATCCTACCGCTACAGTGGAGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCTCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAG	117
IMGT_hVK_1_39	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTAGGAGACAGAGTCACCATCACTTGCC GGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCC TGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTTGCAACTTACTACTGTCAACAGAGTTACAG TACCCCT	118
CH7 rF9	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTATGGTATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACGAAATCTGGAGAGGC AACCTATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCTATGATTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCAGCTAGC	119
CLA1 rH11	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTCAGACTGTGAGTGCTAATGTTGCGTGG TATCAGCAGAAACCAGGGAAAGCACCTAAGCTCC TGATCTACTTGGCATCCTACCGCTACAGAGGAGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCTCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAGCGTACG	120
PMS22	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCTATGATTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	159

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
1C8	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCTATCATTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	160
3E1	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCGTGGATTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	161
2D7	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCTATTGGTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	162
Affinity Matured Heavy Chain	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTTGCTCATTAC TTTCAGACTATGGACTACTGGGGCCAAGGGACCAC GGTCACCGTCTCCTCA	163

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
Affinity Matured Heavy Chain	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCGCTTATTAC TTTCAGACTATGGACTACTGGGGCCAAGGGACCAC GGTCACCGTCTCCTCA	164
Affinity Matured Heavy Chain	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGATTTCGCGTATTAC CTTGAGGCTATGGACTACTGGGGCCAAGGGACCA CGATCACCGTCTCCTCA	165
H3 Full (5) 19	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAGC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCTG TGTATTACTGTGCGAGATGGGACGCCTTTGAGTAC GTGAAGGCGCTGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	166
H3 Full (5) 8	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGATTTCTTTGAGTATT TTAAGACTATGGACTACTGGGGCCAAGGGACCAC GGTCACCGTCTCCTCA	167

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
H3 Full (5) 28	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTTTTTTATTACG TGCAGACTATGGACTACTGGGGCCAAGGGACCAC GGTCACCGTCTCCTCA	168
H3 Full (5) 27	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGATTTTTCTTATTACG TTGAGGCGATGGACTACTGGGGCCAAGGGACCAC AGTCACCGTCTCCTCA	169
H4E9 Heavy Chain	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGTATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAATACCAAAACTGGAGAGGC AACTTATATTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCTATGATTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	170
pAC14 (B9)	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGTATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAAGTGGAGAGGC AACCTATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCTATGATTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	171

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
pAC15 (F9)	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTATGGTATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACGAAATCTGGAGAGGC AACCTATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCTATGATTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	172
H1/H2 (5) 2	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTATTCTATGAACT GGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTG GATGGGATACATAAACACCAAAAATGGAGAGGCA AACTATGTTGAAGAGTTTAAGGGACGGTTTGTCTT CTCCTTGGACACCTCTGTCAGCACGGCATATCTGC AGATCAGCAGCCTAAAGGCTGAAGACACTGCCGT GTATTACTGTGCGAGATGGGACTTCTATGATTACG TGGAGGCTATGGACTACTGGGGCCAAGGGACCAC GGTCACCGTCTCCTCA	173
H1/H2 (5) 11	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTATGGTATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAAATGGAGAGGC AACCTATATTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCTATGATTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	174
H1/H2 (5) 13	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGTATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATATATAAACACCAAAAATGGAGAGGC AAACTATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACGCCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCTATGATTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	175

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
H1/H2 (5) 14	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTATGGTATGAAC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATATATAAACACCAAAAATGGAGAGGC AAACTATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCCG TGTATTACTGTGCGAGATGGGACTTCTATGATTAC GTGGAGGCTATGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	176
H3 Full (5) 19	CAGGTGCAATTGGTGCAATCTGGGTCTGAGTTGAA GAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGG CTTCTGGATACACCTTCACTGAGTTTGGAATGAGC TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT GGATGGGATGGATAAACACCAAAACTGGAGAGGC AACATATGTTGAAGAGTTTAAGGGACGGTTTGTCT TCTCCTTGGACACCTCTGTCAGCACGGCATATCTG CAGATCAGCAGCCTAAAGGCTGAAGACACTGCTG TGTATTACTGTGCGAGATGGGACGCCTTTGAGTAC GTGAAGGCGCTGGACTACTGGGGCCAAGGGACCA CGGTCACCGTCTCCTCA	177
pAC21 (3A1)	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTGCCAATGTGGGTAATAATGTTGCCTGG TATCAGCAGAAACCAGGGAAAGCACCTAAGCTCC TGATCTATTTGGCCTCCAACCGCTCCGGTGGAGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCTCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAGCGTACG	178
pAC19 (2C6)	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTAAGAATGTGGGGACTAATGTTGCGTG GTATCAGCAGAAACCAGGGAAAGCACCTAAGCCC CTGATCTACCTGGCATCCTACCCCCAGATTGGAGT CCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCCCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAGCGTACG	179
pAC18 (2F1)	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTGCGGCTGTGGGTACGTATGTTGCGTGG TATCAGCAGAAACCAGGGAAAGCACCTAAGCTCC TGATCTATTCGGCATCCTACCGCAAAAGGGGAGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCTCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAGCGTACG	180

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
pAC23 (2F11)	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTCAGATAGCGAGTACTAATGTTGCCTGG TATCAGCAGAAACCAGGGAAAGCACCTAAGCTCC TGATCTATTGGGCATCCTACCGCTATAGTGGAGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCTCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAGCGTACG	181
H4E9 light chain	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTCAGAATGTGGGTACTAATGTTGCCTGG TATCAGCAGAAACCAGGGAAAGCACCTAAGCCCC TGATCTATTCGGCATCCTACCGCTACAGTGGAGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCTCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAGCGTACG	182
L2D2	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTCACAATGTGGGTACCAACGTTGCGTGG TATCAGCAGAAACCAGGGAAAGCACCTAAGCTCC TGATCTATTCGGCATCCCACCGGTACAGTGGAGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCTCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAGCGTACG	183
pAC6 (C1)	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTCAGATTATGGGTCCTAATGTTGCGTGG TATCAGCAGAAACCAGGGAAAGCACCTAAGCTCC TGATCTATTTGGCATCCTACCACGAAAGTGGAGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCTCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAGCGTACG	184
pAC7 (E10)	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTCAAATTGTGGGTACTAATGTTGCGTGG TATCAGCAGAAACCAGGGAAAGCACCTAAGCTCC TGATCTATTCGGCATCCCACCGTCCCAGTGGAGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCTCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAGCGTACG	185

CONSTRUCT	NUCLEOTIDE SEQUENCE	SEQ ID NO
pAC12 (H7)	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTCAGAAGGTGCTTACTAATGTTGCGTGG TATCAGCAGAAACCAGGGAAAGCACCTAAGCTCC TGATCTATTTGGCATCCTACCGCTACAGTGGAGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCTCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAGCGTACG	186
pAC13 (H11)	GATATCCAGATGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTGGGAGACAGAGTCACCATCACTTGCA AGGCCAGTCAGACTGTGAGTGCTAATGTTGCGTGG TATCAGCAGAAACCAGGGAAAGCACCTAAGCTCC TGATCTACTTGGCATCCTACCGCTACAGAGGAGTC CCATCAAGGTTCAGTGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAA GATTTCGCAACTTACTACTGTCACCAATATTACAC CTATCCTCTATTCACGTTTGGCCAGGGCACCAAGC TCGAGATCAAGCGTACG	187

Expression Vectors and Host Cells

[0108] In one aspect, the present invention is directed to an expression vector and/or a host cell that comprises one or more isolated polynucleotides of the present invention. For example, the host cell or expression vector comprises any one or more of the polynucleotides or polynucleotides encoding the polypeptides, ABMs and/or variant ABMs described above in the sections entitled "Anti-CEA Antigen Binding Molecules" and "Polypeptides and Polynucleotides of Anti-CEA ABMs." In another aspect, the present invention is directed to a method of producing an ABM that specifically binds membrane-bound human CEA, the method comprising: culturing a host cell comprising one or more isolated polynucleotides of the present invention or an expression vector comprising one or more isolated polynucleotides of the present invention in a medium under conditions allowing the expression of said one or more polynucleotide, wherein said one or more polynucleotides encodes one or more polypeptides that form part of the ABM; and recovering said ABM, wherein said ABM or a portion thereof binds the same epitope as, or is capable of competing for binding with the murine monoclonal antibody PR1A3.

[0109] Generally, any type of cultured cell line can be used to express the ABM of the present invention. In a preferred embodiment, CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, other mammalian cells, yeast cells, insect cells, or plant cells are used as the background cell line to generate the engineered host cells of the invention.

[0110] In a specific embodiment, the host cell or expression vector comprises one or more polynucleotides encoding an ABM that is a variant antibody or a fragment thereof having the binding specificity of the murine PR1A3 antibody; for example, the ABM binds the same epitope as that of PR1A3 or is capable of competing with the PR1A3 antibody for binding to the antigen. In a preferred embodiment, the antibody is affinity matured. The affinity matured antibody generally has improved binding affinity than that of the reference antibody from which the affinity matured antibody derived from. In another preferred embodiment, the antibody has desirable therapeutic properties including but not limited to: strong binding affinity for the CEA antigen, in particular, membrane-bound CEA, while having substantially no cross-reactivity against soluble CEA; an ability to induce cell lysis of CEA-expression cells *in vitro* and *ex-vivo,* preferably in a dose-dependent manner; an ability to inhibit CEA mediated cell adhesion *in vitro*; an ability to inhibit tumor tissue growth and/or induce tumor tissue regression in tumor models in mice (*e.g.*, xenograft mouse). In another preferred embodiment, the variant antibody or fragment thereof comprises a human Fc.

[0111] In one embodiment, one or several polynucleotides encoding an ABM of the present invention may be expressed under the control of a constitutive promoter or, alternately, a regulated expression system. Suitable regulated expression systems include, but are not limited to, a tetracycline-regulated expression system, an ecdysone-inducible expression system, a lac-switch expression system, a glucocorticoid-inducible expression system, a temperature-inducible promoter system, and a metallothionein metal-inducible expression system. If several different nucleic acids encoding an ABM of the present invention are comprised within the host cell system, some of them may be expressed under the control of a constitutive promoter, while others are expressed under the control of a regulated promoter. The maximal expression level is considered to be the highest possible level of stable polypeptide expression that does not have a significant adverse effect on cell growth rate, and will be determined using routine experimentation. Expression levels are determined by methods generally known in the art, including Western blot analysis using an antibody specific for the ABM or an antibody specific for a peptide tag fused to the ABM; and Northern blot analysis. In a further alternative, the polynucleotide may be operatively linked to a reporter gene; the expression levels of an ABM disclosed herein are determined by measuring a signal correlated with the expression level of the reporter gene. The reporter gene may be transcribed together with the nucleic acid(s) encoding said ABM as a single mRNA molecule; their respective coding sequences may be linked either by an internal ribosome entry site (IRES) or by a cap-independent translation enhancer (CITE). The reporter gene may be translated together with at least one nucleic acid encoding an ABM disclosed herein such that a single polypeptide chain is formed. The nucleic acids encoding an ABM of the present invention may be operatively linked to the reporter gene under the control of a single promoter, such that the nucleic acid encoding the ABM and the reporter gene are transcribed into an RNA molecule which is alternatively spliced into two separate messenger RNA (mRNA)

molecules; one of the resulting mRNAs is translated into said reporter protein, and the other is translated into the ABM.

[0112] Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of an ABM binding the same epitope as that of the murine PR1A3 antibody along with appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. *See*, for example, the techniques described in Maniatis *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory, N.Y. (1989) and Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Associates and Wiley Interscience, N.Y (1989).

[0113] A variety of host-expression vector systems may be utilized to express the coding sequence of the ABMs of the present invention. Preferably, mammalian cells are used as host cell systems transfected with recombinant plasmid DNA or cosmid DNA expression vectors containing the coding sequence of the protein of interest and the coding sequence of the fusion polypeptide. Most preferably, CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, other mammalian cells, yeast cells, insect cells, or plant cells are used as host cell system. Some examples of expression systems and selection methods are described in the following references and references cited therein: Borth *et al.*, *Biotechnol. Bioen.* 71(4):266-73 (2000-2001), in Werner *et al., Arzneimittelforschung/Drug Res.* 48(8):870-80 (1998), in Andersen and Krummen, *Curr. Op. Biotechnol.* 13:117-123 (2002), in Chadd and Chamow, *Curr. Op. Biotechnol.* 12:188-194 (2001), and in Giddings, *Curr. Op. Biotechnol.* 12: 450-454 (2001).

[0114] In alternate embodiments, other eukaryotic host cell systems may be used, including yeast cells transformed with recombinant yeast expression vectors containing the coding sequence of an ABM of the present invention, such as the expression systems taught in U.S. Pat. Appl. No. 60/344,169 and WO 03/056914 (methods for producing human-like glycoprotein in a non-human eukaryotic host cell) (the contents of each of which are incorporated by reference in their entirety); insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the coding sequence of an ABM binding the same epitope as that of the murine PR1A3 antibody or is capable of competing with PR1A3 for antigen binding; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing the coding sequence of the ABM of the invention including, but not limited to, the expression systems taught in U.S. Pat. No. 6,815,184 (methods for expression and secretion of biologically active polypeptides from genetically engineered duckweed); WO 2004/057002 (production of glycosylated proteins in bryophyte plant cells by introduction of a glycosyl transferase gene) and WO 2004/024927 (methods

of generating extracellular heterologous non-plant protein in moss protoplast); and U.S. Pat. Appl. Nos. 60/365,769, 60/368,047, and WO 2003/078614 (glycoprotein processing in transgenic plants comprising a functional mammalian GnTIII enzyme) (the contents of each of which is herein incorporated by reference in its entirety); or animal cell systems infected with recombinant virus expression vectors (*e.g.*, adenovirus, vaccinia virus) including cell lines engineered to contain multiple copies of the DNA encoding a chimeric ABM binding the same epitope as that of the murine PR1A3 antibody either stably amplified (CHO/dhfr) or unstably amplified in double-minute chromosomes (*e.g.*, murine cell lines). In one embodiment, the vector comprising the polynucleotide(s) encoding the ABM of the invention is polycistronic. Also, in one embodiment the ABM discussed above is an antibody or a fragment thereof. In a preferred embodiment, the ABM is an affinity matured antibody.

[0115] Stable expression is generally preferred to transient expression because it typically achieves more reproducible results and also is more amenable to large-scale production; however, it is within the skill of one in the art to determine whether transient expression is better for a particular situation. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with the respective coding nucleic acids controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows selection of cells which have stably integrated the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines.

[0116] A number of selection systems may be used, including, but not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA 48*:2026 (1962)), and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*:817 (1980)) genes, which can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler *et al.*, *Natl. Acad. Sci. USA 77*:3567 (1989); O'Hare *et al.*, *Proc. Natl. Acad. Sci. USA 78*:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA 78*:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin *et al.*, *J. Mol. Biol. 150*:1 (1981)); and hygro, which confers resistance to hygromycin (Santerre *et al.*, *Gene 30*:147 (1984) genes. Recently, additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. USA 85*:8047 (1988)); the glutamine synthase system; and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor,

2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, *in*: *Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory ed. (1987)).

[0117] The present invention is further directed to a method for modifying the glycosylation profile of the ABMs of the present invention that are produced by a host cell, comprising expressing in said host cell a nucleic acid encoding an ABM of the invention and a nucleic acid encoding a polypeptide with a glycosyltransferase activity, or a vector comprising such nucleic acids. Genes with glycosyltransferase activity include β(1,4)-N-acetylglucosaminyltransferase III (GnTII), α-mannosidase II (ManII), β(1,4)-galactosyltransferase (GalT), β(1,2)-N-acetylglucosaminyltransferase I (GnTI), and β(1,2)-N-acetylglucosaminyltransferase II (GnTII). In one embodiment, a combination of genes with glycosyltransferase activity are expressed in the host cell (e.g., GnTIII and Man II). Likewise, the method also encompasses expression of one or more polynucleotide(s) encoding the ABM in a host cell in which a glycosyltransferase gene has been disrupted or otherwise deactivated (e.g., a host cell in which the activity of the gene encoding α1-6 core fucosyltransferase has been knocked out). In another embodiment, the ABMs of the present invention can be produced in a host cell that further expresses a polynucleotide encoding a polypeptide having GnTIII activity to modify the glycosylation pattern. In a specific embodiment, the polypeptide having GnTIII activity is a fusion polypeptide comprising the Golgi localization domain of a Golgi resident polypeptide. In another preferred embodiment, the expression of the ABMs of the present invention in a host cell that expresses a polynucleotide encoding a polypeptide having GnTIII activity results in ABMs with increased Fc receptor binding affinity and increased effector function. Accordingly, in one embodiment, the present invention is directed to a host cell comprising (a) an isolated nucleic acid comprising a sequence encoding a polypeptide having GnTIII activity; and (b) an isolated polynucleotide encoding an ABM of the present invention, such as a chimeric, primatized or humanized antibody that binds human CEA. In a preferred embodiment, the polypeptide having GnTIII activity is a fusion polypeptide comprising the catalytic domain of GnTIII and the Golgi localization domain is the localization domain of mannosidase II. Methods for generating such fusion polypeptides and using them to produce antibodies with increased effector functions are disclosed in U.S. Provisional Pat. Appl. No. 60/495,142 and U.S. Pat. Appl. Publ. No. 2004/0241817, the entire contents of which are expressly incorporated herein by reference. In a particular embodiment, the modified ABM produced by the host cell has an IgG constant region or a fragment thereof comprising the Fc region. In another particular embodiment the ABM is a humanized antibody or a fragment thereof comprising an Fc region.

[0118] The ABMs with altered glycosylation produced by the host cells of the invention typically exhibit increased Fc receptor binding affinity and/or increased effector function as a result of the modification of the host cell (e.g., by expression of a glycosyltransferase gene). Preferably, the increased Fc receptor binding affinity is increased binding to a Fcγ activating receptor, such as the

FcγRIIIa receptor. The increased effector function is preferably an increase in one or more of the following: increased antibody-dependent cellular cytotoxicity, increased antibody-dependent cellular phagocytosis (ADCP), increased cytokine secretion, increased immune-complex-mediated antigen uptake by antigen-presenting cells, increased Fc-mediated cellular cytotoxicity, increased binding to NK cells, increased binding to macrophages, increased binding to polymorphonuclear cells (PMNs), increased binding to monocytes, increased crosslinking of target-bound antibodies, increased direct signaling inducing apoptosis, increased dendritic cell maturation, and increased T cell priming.

Generation and Use of ABMs Having Increased Effector Function Including Antibody-Dependent Cellular Cytotoxicity

[0119] In one aspect, the present invention provides glycoforms of ABMs (e.g., variant ABMs) binding the same epitope as that of the murine PR1A3 antibody and having increased effector function, including antibody-dependent cellular cytotoxicity. Glycosylation engineering of antibodies has been previously described. See, *e.g.*, U.S. Patent No. 6,602,684, incorporated herein by reference in its entirety. Methods of producing ABMs from host cells that have altered activity of genes involved in glyocsylation are also described herein in detail (See, e.g, preceding section entitled "Expression Vectors and Host Cells"). Increases in ADCC of the ABMs of the present invention is also achieved by increasing affinity of the antigen binding molecule for membrane-bound CEA, for example by affinity maturation or other methods of improving affinity (*see* Tang *et al., J. Immunol.* 2007, 179:2815-2823). Combinations of these approaches are also encompassed by the present invention.

[0120] Clinical trials of unconjugated monoclonal antibodies (mAbs) for the treatment of some types of cancer have recently yielded encouraging results. Dillman, *Cancer Biother. & Radiopharm. 12*:223-25 (1997); Deo *et al.*, *Immunology Today 18*:127 (1997). A chimeric, unconjugated IgG1 has been approved for low-grade or follicular B-cell non-Hodgkin's lymphoma. Dillman, *Cancer Biother. & Radiopharm. 12*:223-25 (1997), while another unconjugated mAb, a humanized IgG1 targeting solid breast tumors, has also showed promising results in phase III clinical trials. Deo *et al.*, *Immunology Today 18*:127 (1997). The antigens of these two mAbs are highly expressed in their respective tumor cells and the antibodies mediate potent tumor destruction by effector cells *in vitro* and *in vivo*. In contrast, many other unconjugated mAbs with fine tumor specificities cannot trigger effector functions of sufficient potency to be clinically useful. Frost *et al.*, *Cancer 80*:317-33 (1997); Surfus *et al.*, *J. Immunother. 19*:184-91 (1996). For some of these weaker mAbs, adjunct cytokine therapy is currently being tested. Addition of cytokines can stimulate antibody-dependent cellular cytotoxicity (ADCC) by increasing the activity and number of circulating lymphocytes. Frost *et al.*, *Cancer 80*:317-33 (1997); Surfus *et al.*, *J. Immunother. 19*:184-91 (1996). ADCC, a lytic attack on targeted cells, is triggered upon binding of leukocyte receptors to the constant region (Fc) of antibodies. Deo *et al.*, *Immunology Today 18*:127 (1997).

[0121] A different, but complementary, approach to increase ADCC activity of unconjugated IgG1s is to engineer the Fc region of the antibody. Protein engineering studies have shown that FcγRs interact with the lower hinge region of the IgG CH2 domain. Lund *et al.*, *J. Immunol. 157*:4963-69 (1996). However, FcγR binding also requires the presence of oligosaccharides covalently attached at the conserved Asn 297 in the CH2 region. Lund *et al.*, *J. Immunol. 157*:4963-69 (1996); Wright and Morrison, *Trends Biotech. 15*:26-31 (1997), suggesting that either oligosaccharide and polypeptide both directly contribute to the interaction site or that the oligosaccharide is required to maintain an active CH2 polypeptide conformation. Modification of the oligosaccharide structure can therefore be explored as a means to increase the affinity of the interaction.

[0122] An IgG molecule carries two N-linked oligosaccharides in its Fc region, one on each heavy chain. As any glycoprotein, an antibody is produced as a population of glycoforms which share the same polypeptide backbone but have different oligosaccharides attached to the glycosylation sites. The oligosaccharides normally found in the Fc region of serum IgG are of complex bi-antennary type (Wormald *et al.*, *Biochemistry 36*:130-38 (1997), with a low level of terminal sialic acid and bisecting N-acetylglucosamine (GlcNAc), and a variable degree of terminal galactosylation and core fucosylation. Some studies suggest that the minimal carbohydrate structure required for FcγR binding lies within the oligosaccharide core. Lund *et al.*, *J. Immunol. 157*:4963-69 (1996)

[0123] The mouse- or hamster-derived cell lines used in industry and academia for production of unconjugated therapeutic mAbs normally attach the required oligosaccharide determinants to Fc sites. IgGs expressed in these cell lines lack, however, the bisecting GlcNAc found in low amounts in serum IgGs. Lifely *et al.*, *Glycobiology 318*:813-22 (1995). In contrast, it was recently observed that a rat myeloma-produced, humanized IgG1 (CAMPATH-1H) carried a bisecting GlcNAc in some of its glycoforms. Lifely *et al.*, *Glycobiology 318*:813-22 (1995). The rat cell-derived antibody reached a similar maximal *in vitro* ADCC activity as CAMPATH-1H antibodies produced in standard cell lines, but at significantly lower antibody concentrations.

[0124] The CAMPATH antigen is normally present at high levels on lymphoma cells, and this chimeric mAb has high ADCC activity in the absence of a bisecting GlcNAc. Lifely *et al.*, *Glycobiology 318*:813-22 (1995). In the N-linked glycosylation pathway, a bisecting GlcNAc is added by GnTIII. Schachter, *Biochem. Cell Biol. 64*:163-81 (1986).

[0125] Previous studies used a single, antibody-producing CHO cell line that was previously engineered to express, in an externally-regulated fashion, different levels of a cloned GnTIII enzyme gene (Umaña, P., *et al., Nature Biotechnol. 17*:176-180 (1999)). This approach established for the first time a rigorous correlation between expression of a glycosyltransferase (e.g., GnTIII) and the ADCC activity of the modified antibody. Thus, the invention contemplates a variant ABM (e.g., an affinity matured ABM) that binds the same epitope as that of the murine PR1A3 antibody, comprising an Fc region or region equivalent to an Fc region having altered glycosylation resulting from

changing the expression level of a glycosyltransferase gene in the ABM-producing host cell. In a specific embodiment, the change in gene expression level is an increase in GnTIII activity. Increased GnTIII activity results in an increase in the percentage of bisected oligosaccharides, as well as a decrease in the percentage of fucose residues, in the Fc region of the ABM. This antibody, or fragment thereof, has increased Fc receptor binding affinity and increased effector function

[0126] The present invention is also directed to a method for producing an ABM of the present invention having modified oligosaccharides, comprising (a) culturing a host cell engineered to express at least one nucleic acid encoding a polypeptide having glycosyltransferase activity under conditions which permit the production of an ABM according to the present invention, wherein said polypeptide having glycosyltransferase activity is expressed in an amount sufficient to modify the oligosaccharides in the Fc region of said ABM produced by said host cell; and (b) isolating said ABM. In one embodiment, the polypeptide having glycosyltransferase activity is GnTIII. In another embodiment, there are two polypeptides having glycosyltransferase activity. In a particular embodiment, the two peptides having glycosyltransferase activity are GnTIII and ManII. In another embodiment, the polypeptide having glycosltransferase activity is a fusion polypeptide comprising the catalytic domain of GnTIII. In a more specific embodiment, the fusion polypeptide further comprises the Golgi localization domain of a Golgi resident polypeptide. Preferably, the Golgi localization domain is the localization domain of mannosidase II or GnTI. Alternatively, the Golgi localization domain is selected from the group consisting of: the localization domain of mannosidase I, the localization domain of GnTII, and the localization domain of α 1-6 core fucosyltransferase. The ABMs produced by the methods of the present invention have increased Fc receptor binding affinity and/or increased effector function. Generally, the increased effector function is one or more of the following: increased Fc-mediated cellular cytotoxicity (including increased antibody-dependent cellular cytotoxicity), increased antibody-dependent cellular phagocytosis (ADCP), increased cytokine secretion, increased immune-complex-mediated antigen uptake by antigen-presenting cells, increased binding to NK cells, increased binding to macrophages, increased binding to monocytes, increased binding to polymorphonuclear cells, increased direct signaling inducing apoptosis, increased crosslinking of target-bound antibodies, increased dendritic cell maturation, or increased T cell priming. The increased Fc receptor binding affinity is preferably increased binding to Fc activating receptors such as FcγRIIIa. In a particularly preferred embodiment the ABM is a humanized antibody or a fragment thereof.

[0127] In one embodiment, the percentage of bisected N-linked oligosaccharides in the Fc region of the ABM is at least about 10% to about 100%, specifically at least about 50%, more specifically, at least about 60%, at least about 70%, at least about 80%, or at least about 90-95% of the total oligosaccharides. In yet another embodiment, the antigen binding molecule produced by the methods of the invention has an increased proportion of nonfucosylated oligosaccharides in the Fc region as a

result of the modification of its oligosaccharides by the methods of the present invention. In one embodiment, the percentage of nonfucosylated oligosaccharides is at least about 20% to about 100%, specifically at least about 50%, at least about 60% to about 70%, and more specifically, at least about 75%. The nonfucosylated oligosaccharides may be of the hybrid or complex type. In yet another embodiment, the antigen binding molecule produced by the methods of the invention has an increased proportion of bisected oligosaccharides in the Fc region as a result of the modification of its oligosaccharides by the methods of the present invention. In one embodiment, the percentage of bisected oligosaccharides is at least about 20% to about 100%, specifically at least about 50%, at least about 60% to about 70%, and more specifically, at least about 75%. In a particularly preferred embodiment, the ABM produced by the host cells and methods of the invention has an increased proportion of bisected, nonfucosylated oligosaccharides in the Fc region. The bisected, nonfucosylated oligosaccharides may be either hybrid or complex. Specifically, the methods of the present invention may be used to produce antigen binding molecules in which at least about 10% to about 100%, specifically at least about 15%, more specifically at least about 20% to about 50%, more specifically at least about 20% to about 25%, and more specifically at least about 30% to about 35% of the oligosaccharides in the Fc region of the antigen binding molecule are bisected, nonfucosylated. The ABMs of the present invention may also comprise an Fc region in which at least about 10% to about 100%, specifically at least about15%, more specifically at least about 20% to about 25%, and more specifically at least about 30% to about 35% of the oligosaccharides in the Fc region of the ABM are bisected hybrid nonfucosylated.

[0128] In another embodiment, the present invention is directed to an antigen binding molecule (e.g., a variant ABM) that is capable of competing with the PR1A3 antibody for membrane-bound human CEA is engineered to have increased effector function and/or increased Fc receptor binding affinity, produced by the methods of the invention. The increased effector function can include, but is not limited to one or more of the following: increased Fc-mediated cellular cytotoxicity (including increased antibody-dependent cellular cytotoxicity), increased antibody-dependent cellular phagocytosis (ADCP), increased cytokine secretion, increased immune-complex-mediated antigen uptake by antigen-presenting cells, increased binding to NK cells, increased binding to macrophages, increased binding to monocytes, increased binding to polymorphonuclear cells, increased direct signaling inducing apoptosis, increased crosslinking of target-bound antibodies, increased dendritic cell maturation, or increased T cell priming. In a preferred embodiment, the increased Fc receptor binding affinity is increased binding to an Fc activating receptor, most preferably FcγRIIIa. In one embodiment, the antigen binding molecule is an antibody, an antibody fragment containing the Fc region, or a fusion protein that includes a region equivalent to the Fc region of an immunoglobulin. In a particularly preferred embodiment, the antigen binding molecule is a humanized affinity matured antibody.

[0129] The present invention further provides methods for the generation and use of host cell systems for the production of glycoforms of the ABMs of the present invention, having increased Fc receptor binding affinity, preferably increased binding to Fc activating receptors, and/or having increased effector functions, including antibody-dependent cellular cytotoxicity. The glycoengineering methodology that can be used with the ABMs of the present invention has been described in greater detail in U.S. Pat. No. 6,602,684, U.S. Pat. Appl. Publ. No. 2004/0241817 A1, U.S. Pat. Appl. Publ. No. 2003/0175884 A1, Provisional U.S. Patent Application No. 60/441,307 and WO 2004/065540, the entire contents of each of which is incorporated herein by reference in its entirety. The ABMs of the present invention can alternatively be glycoengineered to have reduced fucose residues in the Fc region according to the techniques disclosed in U.S. Pat. Appl. Pub. No. 2003/0157108 (Genentech), or in EP 1 176 195 A1 , WO 03/084570, WO 03/085119 and U.S. Pat. Appl. Pub. Nos. 2003/0115614, 2004/093621, 2004/110282, 2004/110704, 2004/132140 (Kyowa). The contents of each of these documents are herein incorporated by reference in their entireties. Glycoengineered ABMs of the invention may also be produced in expression systems that produce modified glycoproteins, such as those taught in U.S. Pat. Appl. Pub. No. 60/344,169 and WO 03/056914 (GlycoFi, Inc.) or in WO 2004/057002 and WO 2004/024927 (Greenovation), the contents of each of which are hereby incorporated by reference in their entirety.

Generation of Cell Lines for the Production of
Proteins with Altered Glycosylation Pattern

[0130] In one aspect, the present invention provides host cell expression systems for the generation of the ABMs of the present invention having modified glycosylation patterns. In particular, the present invention provides host cell systems for the generation of glycoforms of the ABMs of the present invention having an improved therapeutic value. Therefore, the invention provides host cell expression systems selected or engineered to express a polypeptide having a glycosyltransferase activity. In a specific embodiment, the glycosyltransferase activity is a GnTIII activity. In one embodiment, the polypeptide having GnTIII activity is a fusion polypeptide comprising the Golgi localization domain of a heterologous Golgi resident polypeptide. Specifically, such host cell expression systems may be engineered to comprise a recombinant nucleic acid molecule encoding a polypeptide having GnTIII, operatively linked to a constitutive or regulated promoter system.

[0131] In one specific embodiment, the present invention provides a host cell that has been engineered to express at least one nucleic acid encoding a fusion polypeptide having GnTIII activity and comprising the Golgi localization domain of a heterologous Golgi resident polypeptide. In one aspect, the host cell is engineered with a nucleic acid molecule comprising at least one gene encoding a fusion polypeptide having GnTIII activity and comprising the Golgi localization domain of a heterologous Golgi resident polypeptide.

[0132] Generally, any type of cultured cell line, including the cell lines discussed above, can be used as a background to engineer the host cell lines of the present invention. In a preferred embodiment, CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, other mammalian cells, yeast cells, insect cells, or plant cells are used as the background cell line to generate the engineered host cells of the invention.

[0133] The invention is contemplated to encompass any engineered host cells expressing a polypeptide having glycosyltransferase activity, e.g., GnTIII activity, including a fusion polypeptide that comprises the Golgi localization domain of a heterologous Golgi resident polypeptide as defined herein.

[0134] One or several nucleic acids encoding a polypeptide having glycosyltransferase activity, e.g., GnTIII activity, may be expressed under the control of a constitutive promoter or, alternately, a regulated expression system. Such systems are well known in the art, and include the systems discussed above. If several different nucleic acids encoding fusion polypeptides having glycosyltransferase activity, e.g., GnTIII activity, and comprising the Golgi localization domain of a heterologous Golgi resident polypeptide are comprised within the host cell system, some of them may be expressed under the control of a constitutive promoter, while others are expressed under the control of a regulated promoter. Expression levels of the fusion polypeptides having glycosyltransferase activity, e.g., GnTIII activity, are determined by methods generally known in the art, including Western blot analysis, Northern blot analysis, reporter gene expression analysis or measurement of glycosyltransferase activity, e.g., GnTIII activity. Alternatively, a lectin may be employed which binds to biosynthetic products of the GnTIII, for example, E_4-PHA lectin. Alternatively, a functional assay which measures the increased Fc receptor binding or increased effector function mediated by antibodies produced by the cells engineered with the nucleic acid encoding a polypeptide with glycosyltransferase activity, e.g., GnTIII activity, may be used.

Identification of Transfectants or Transformants that
Express the Protein Having a Modified Glycosylation Pattern

[0135] The host cells which contain the coding sequence of a variant ABM (e.g., a humanized, affinity matured ABM) that is capable of competing with the PR1A3 antibody for antigen binding and which express the biologically active gene products may be identified by at least four general approaches; (a) DNA-DNA or DNA-RNA hybridization; (b) the presence or absence of "marker" gene functions; (c) assessing the level of transcription as measured by the expression of the respective mRNA transcripts in the host cell; and (d) detection of the gene product as measured by immunoassay or by its biological activity.

[0136] In the first approach, the presence of the coding sequence of a variant ABM that is capable of competing with the PR1A3 antibody and/or the coding sequence of the polypeptide having glycosyltransferase (e.g.,GnTIII) activity can be detected by DNA-DNA or DNA-RNA hybridization

using probes comprising nucleotide sequences that are homologous to the respective coding sequences, respectively, or portions or derivatives thereof.

[0137] In the second approach, the recombinant expression vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, resistance to methotrexate, transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the coding sequence of the ABM of the invention, or a fragment thereof, and/or the coding sequence of the polypeptide having glycosyltransferase (e.g.,GnTIII) activity are inserted within a marker gene sequence of the vector, recombinants containing the respective coding sequences can be identified by the absence of the marker gene function. Alternatively, a marker gene can be placed in tandem with the coding sequences under the control of the same or different promoter used to control the expression of the coding sequences. Expression of the marker in response to induction or selection indicates expression of the coding sequence of the ABM of the invention and/or the coding sequence of the polypeptide having glycosyltransferase (e.g.,GnTIII) activity.

[0138] In the third approach, transcriptional activity for the coding region of the ABM of the invention, or a fragment thereof, and/or the coding sequence of the polypeptide having glycosyltransferase (e.g.,GnTIII) activity can be assessed by hybridization assays. For example, RNA can be isolated and analyzed by Northern blot using a probe homologous to the coding sequences of the ABM of the invention, or a fragment thereof, and/or the coding sequence of the polypeptide having glycosyltransferase (e.g.,GnTIII) activity or particular portions thereof. Alternatively, total nucleic acids of the host cell may be extracted and assayed for hybridization to such probes.

[0139] In the fourth approach, the expression of the protein products can be assessed immunologically, for example by Western blots, immunoassays such as radioimmuno-precipitation, enzyme-linked immunoassays and the like. The ultimate test of the success of the expression system, however, involves the detection of the biologically active gene products.

Therapeutic Applications and Methods of Using Anti-CEA of Antigen Binding Molecules

[0140] The invention is also directed to a method for targeting *in vivo* or *in vitro* cells expressing CEA. Cells that express CEA may be targeted for therapeutic purposes (e.g., to treat a disorder by targeting CEA-expressing cells for destruction by the immune system). In one embodiment, the present invention is directed to a method for targeting cells expressing CEA in a subject comprising administering to the subject a composition comprising an ABM of the invention. Cells that express CEA may also be targeted for diagnostic purposes (e.g., to determine if they are expressing CEA, either normally or abnormally). Thus, the invention is also directed to methods for detecting the presence of CEA or a cell expressing CEA, either *in vivo* or *in vitro*. One method of detecting CEA expression according to the present invention comprises contacting a sample to be tested, optionally

with a control sample, with an ABM of the present invention, under conditions that allow for formation of a complex between the ABM and CEA. The complex formation is then detected (e.g., by ELISA or other methods known in the art). When using a control sample with the test sample, any statistically significant difference in the formation of ABM-CEA complexes when comparing the test and control samples is indicative of the presence of CEA in the test sample.

[0141] In one aspect, ABMs of the present invention can be used target cells *in vivo* or *in vitro* that express CEA. The cells expressing CEA can be targeted for diagnostic or therapeutic purposes. In one aspect, the ABMs of the present invention can be used to detect the presence of CEA in a sample. CEA is abnormally expressed (e.g., overexpressed) in many human tumors compared to non-tumor tissue of the same cell type. Thus, the ABMs of the invention are particularly useful in the prevention of tumor formation, eradication of tumors and inhibition of tumor growth or metastasis. The ABMs of the invention also act to arrest the cell cycle, cause apoptosis of the target cells (e.g., tumor cells), and inhibit angiogenesis and/or differentiation of target cells. The ABMs of the invention can be used to treat any tumor expressing CEA. Particular malignancies that can be treated with the ABMs of the invention include, but are not limited to, colorectal cancer, non-small cell lung cancer, gastric cancer, pancreatic cancer and breast cancer.

[0142] The anti-CEA ABMs disclosed herein can be used alone to inhibit tumor growth or kill tumor cells. For example, the anti-CEA ABMs can bind to CEA that is on the membrane or cell surface of cancerous cells and elicit, e.g., ADCC or other effector mediated killing of the cancerous cells. The anti-CEA ABMs can be humanized, specifically, affinity matured, more specifically, glycoengineered and affinity matured.

[0143] The ABMs can alternatively be used alone in order to block the activity of the CEA antigen, particularly by physically interfering with its binding of another compound. For example, the antigen binding molecules can be used to block CEA mediated cell-adhesion.

[0144] The anti-CEA ABMs of the invention are administered to a mammal, preferably a human, in a pharmaceutically acceptable dosage form such as those discussed below, including those that may be administered to a human intravenously as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intra-cerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The ABMs also are suitably administered by intra tumoral, peritumoral, intralesional, or perilesional routes, to exert local as well as systemic therapeutic effects. The intraperitoneal route is expected to be particularly useful, for example, in the treatment of colorectal tumors.

[0145] For the treatment of disease, the appropriate dosage of ABM will depend on the type of disease to be treated, the severity and course of the disease, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The ABM is suitably administered to the patient at one time or over a series of treatments.

[0146] The present invention provides a method for selectively killing tumor cells expressing CEA. This method comprises reacting the antigen binding molecules or the conjugates (e.g., the immunotoxin) of the invention with said tumor cells. These tumor cells may be from a human carcinoma including colorectal carcinoma, non-small cell lung carcinoma (NSCLC), gastric carcinoma, pancreatic carcinoma and breast carcinoma.

[0147] In one embodiment, the present invention provides a method inhibiting CEA-mediated cell adhesion of a tumor cell. This method comprises contacting said tumor cell with the antigen binding molecules or the conjugates of the invention. These tumor cells may be from human cells, including colorectal cancer cells, non-small cell lung cancer cells (NSCLC), gastric cancer cells, pancreatic cancer cells and breast cancer cells.

[0148] Additionally, this invention provides a method of treating carcinomas (for example, human carcinomas) *in vivo*. This method comprises administering to a subject a pharmaceutically effective amount of a composition containing at least one of the antigen binding molecules or the immunoconjugates (e.g., the immunotoxin) of the invention.

[0149] In a further aspect, the invention is directed to a method for treating cancers characterized by CEA over-expression, including but not limited to colorectal cancer cells, NSCLC (non-small cell lung cancer), gastric cancer cells, pancreatic cancer cells and breast cancer cells, by administering a therapeutically effective amount of the humanized and affinity matured antigen binding molecules or variant antigen binding molecules disclosed herein.

[0150] In a further embodiment, the invention is directed to a method for inducing tumor tissue regression in a subject using the humanized and affinity matured antigen binding molecules or variant antigen binding molecules disclosed herein. Non-limiting examples of the tumor tissue includes colorectal tumor, non-small cell lung tumor, gastric tumor, pancreatic tumor and breast tumor. In a particular embodiment, the tumor tissue is a colorectal tumor.

[0151] In accordance with the practice of this invention, the subject may be a human, equine, porcine, bovine, murine, canine, feline, and avian subjects. Other warm blooded animals are also included in this invention.

[0152] The invention further provides methods for inhibiting the growth of tumor cells, treating a tumor in a subject, and treating a proliferative type disease in a subject. These methods comprise administering to the subject an effective amount of the composition of the invention.

[0153] In another aspect, the invention is directed to the use of the humanized and affinity matured antigen binding molecules or variant antigen binding molecules disclosed herein for the manufacture of a medicament for treating a disease related to abnormal CEA expression. In a particular embodiment, the disease is a cancer that overexpresses CEA, including but not limited to colorectal tumor, non-small cell lung tumor, gastric tumor, pancreatic tumor and breast tumor. In a particular embodiment, the tumor is a colorectal tumor.

Compositions, Formulations, Dosages, and Routes of Administration

[0154] In one aspect, the present invention is directed to pharmaceutical compositions comprising the ABMs of the present invention and a pharmaceutically acceptable carrier. The present invention is further directed to the use of such pharmaceutical compositions in the method of treatment of disease, such as cancer, or in the manufacture of a medicament for the treatment of disease, such as cancer. Specifically, the present invention is directed to a method for the treatment of disease, and more particularly, for the treatment of cancer, the method comprising administering a therapeutically effective amount of the pharmaceutical composition of the invention.

[0155] In one aspect, the present invention encompasses pharmaceutical compositions, combinations and methods for treating human carcinomas, for example colorectal carcinoma. For example, the invention includes pharmaceutical compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of an antibody of the present invention and a pharmaceutically acceptable carrier.

[0156] The ABM compositions of the invention can be administered using conventional modes of administration including, but not limited to, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

[0157] In one aspect of the invention, therapeutic formulations containing the ABMs of the invention are prepared for storage by mixing an antibody 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.

[0158] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[0159] The most effective mode of administration and dosage regimen for the pharmaceutical compositions of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the compositions should be titrated to the individual patient. Nevertheless, an effective dose of the compositions of this invention will generally be in the range of from about 0.01 to about 2000 mg/kg.

[0160] The molecules described herein may be in a variety of dosage forms which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

[0161] The composition comprising an ABM of the present invention will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disease or disorder being treated, the particular mammal being treated,

the clinic condition of the individual patient, the cause of the disease or disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The therapeutically effective amount of the antagonist to be administered will be governed by such considerations.

[0162] The examples below explain the invention in more detail. The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. The present invention, however, is not limited in scope by the exemplified embodiments, which are intended as illustrations of single aspects of the invention only, and methods which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLES

[0163] Unless otherwise specified, references to the numbering of specific amino acid residue positions in the following Examples are according to the Kabat numbering system.

EXAMPLE 1

Generation of affinity maturation libraries

H1/H2 library

[0164] For generation of an affinity maturation library randomized in the HCDR1 and HCDR2 region, triplets encoding positions F32 G33 in CDR1 and positions W50 N52 T52a K52b T54 E56 T58 in CDR2 were randomized. In a first step, a DNA fragment (fragment 1) was amplified using pMS22 as a template and primers MS-43 and EAB-679 which contains the randomized CDR1 positions (Fig. 11 and Table 6). Using the same template, primers MS-56 and MS-52 amplified a second fragment (fragment 2) which has an overlapping region with the 3'end of fragment 1. Amplification conditions included an initial 5-min 94 °C incubation step followed by 25 cycles, each consisting of a 1-min 94 °C denaturation, a 1-min 55 °C annealing, and a 20-sec and 50-sec 72 °C elongation step, for fragment 1 and fragment2, respectively. A final 10-min 72 °C incubation step was performed at the end. Both fragments were purified on a agarose gel. An overlapping extension PCR with fragment 1 and 2 using primers MS-43 and EAB-680, which harboured randomized positions of CDR2, generated a fragment with both CDRs randomized (fragment 3). For the assembly of fragments 1 and 2, equimolar amounts of fragment 1 and fragment 2 were used. Amplification conditions included an initial 5-min 94 °C incubation step followed by 5 cycles without primers, each cycle consisting of a 1-min 94 °C denaturation, a 1-min 55 °C annealing, and a 40-sec 72 °C

elongation step. After the addition of the outer primers, 20 additional cycles were performed using the same parameters. A fourth fragment (fragment 4) which overlaps with the 3' region of fragment 3 was PCR-amplified using again pMS22 as a template and primers MS-55 and MS-52. After gel purification, a final overlap extension PCR using fragment 3 and 4 as templates and primers MS-43 and MS-52 generated a fragment containing CL and parts of VH. For this, equimolar amounts of fragment 3 and fragment 4 were used. Amplification conditions included an initial 5-min 94 °C incubation step followed by 5 cycles without primers, each cycle consisting of a 1-min 94 °C denaturation, a 1-min 55 °C annealing, and a 80-sec 72 °C elongation step. After the addition of the outer primers, 20 additional cycles were performed using the same parameters. The resulting fragment was then gel-purified and ligated with pMS22 after NcoI/NheI digestion.

TABLE 6

Primer	SEQ ID NO:	H1/H2 Library Primer Nucleotide Sequences
MS-43	123	CCAGCCGGCCATGGCCGATATCCAGATGACCCAGTCTCCATC
MS-52	124	GAAGACCGATGGGCCTTTGGTGCTAG
MS-55	125	GCAACATATGTTGAAGAGTTTAAGGGACGG
MS-56	126	ATGAACTGGGTGCGACAGGCCCCTG
EAB-679	127	CAGGGGCCTGTCGCACCCAGTTCAT**MNNAWA**CTCAGTGAAG GTGTATCCAGAAGCC
EAB-680	128	CCGTCCCTTAAACTCTTCAACATAGGTTGCCTCTCCAGTTTTG GTGTTTATCCATCCCATCCACTCAAGCCCTTGTCCAGG

Randomization of primers EAB-679 and EAB-680:
Underlined: 60% original base and 40% randomization as N

L1/L2 library

[0165] For the generation of an affinity maturation library randomized in the LCDR1 and LCDR2 region, triplets encoding positions Q27, N28, V29, G30 T31 N32 in CDR1 and positions Y49 S50 Y53 R54 Y55 S56 in CDR2 were randomized. In a first step, a DNA fragment (fragment 1) was amplified using pMS22 as a template and primers EAB-685 and EAB-681 which contains the randomized CDR1 positions (Fig. 12 and Table 6). Using the same template, primers EAB-686 and EAB-687 amplified a second fragment (fragment 2) which has an overlapping region with the 3'end of fragment 1. Amplification conditions included an initial 5-min 94 °C incubation step followed by 25 cycles, each consisting of a 1-min 94 °C denaturation, a 1-min 55 °C annealing, and a 60-sec 72 °C elongation step, for fragment 1 and fragment2, respectively. A final 10-min 72 °C incubation step was performed at the end. Both fragments were purified on a agarose gel. An overlapping extension PCR with fragment 1 and 2 using primers EAB-685 and EAB-682, which harboured randomized positions of CDR2, generated a fragment with both CDRs randomized (fragment 3). For the assembly of fragments 1 and 2, equimolar amounts of fragment 1 and fragment 2 were used. Amplification

conditions included an initial 5-min 94 °C incubation step followed by 5 cycles without primers, each cycle consisting of a 1-min 94 °C denaturation, a 1-min 55 °C annealing, and a 60-sec 72 °C elongation step. After the addition of the outer primers, 20 additional cycles were performed using the same parameters. A fourth fragment (fragment 4) which overlaps with the 3' region of fragment 3 was PCR-amplified using again pMS22 as a template and primers EAB-688 and EAB-687. After gel purification, a final overlap extension PCR using fragment 3 and 4 as templates and primers EAB-685 and EAB-687 generated a fragment containing VL and parts of CL. For this, equimolar amounts of fragment 3 and fragment 4 were used. Amplification conditions included an initial 5-min 94 °C incubation step followed by 5 cycles without primers, each cycle consisting of a 1-min 94 °C denaturation, a 1-min 55 °C annealing, and a 80-sec 72 °C elongation step. After the addition of the outer primers, 20 additional cycles were performed using the same parameters. This fragment was then ligated with pMS22 after HindIII/SacI digestion.

TABLE 7

Primer	**SEQ ID NO:**	**L1/L2 Library Primer Nucleotide Sequence**
EAB-685	129	CAGCTATGACCATGATTACGCCAAGCTTGCATGCAAATTC TATTTCAAGG
EAB-686	130	GTTGCGTGGTATCAGCAGAAACCAGGG
EAB-687	131	GCTCTTTGTGACGGGCGAGCTCAGGCCCTGATGG
EAB-688	132	GGAGTCCCATCAAGGTTCAGTGGCAGTGGATCTGG
EAB-681	133	CCTGGTTTCTGCTGATACCACGCAACATTAGTACCCACAT TCTGACTGGCCTTGCAAGTGATGGTGACTC
EAB-682	134	CTGCCACTGAACCTTGATGGGACTCCACTGTAGCGGTAG GATGCCGAATAGATCAGGAGCTTAGGTGCTTTCCCTGG

Randomization of primers EAB-681 and EAB-682:
Underlined: 60% original base and 40% randomization as N

H3 libraries

[0166] For the generation of affinity maturation libraries randomized in the HCDR3 region, triplets encoding positions W95, D96, F97, Y98, D99, Y100, V100a, E100b, A100c, and M100d were randomized in two different approaches: (1) randomization of the entire segment (H3 full library) or (2) individual randomization of each position resulting in ten sublibraries. Sublibraries containing clones with individually randomized positions were pooled after transformation into bacteria (H3 pooled library). For the randomization of the HCDR3 region, fragments were PCR-amplified using a primer that annealed in the 3'end of CL and primers that harbour the randomized sequences of HCDR3 (Fig. 13). An overlap extension PCR was then performed with a second fragment that overlaps with the 3' end of fragment 1, and comprises the end of VH and the 5' region of CH1. The

assembled fragments were then ligated into pMS22 after *Sac*I/*Nhe*I digestion. For the generation of the H3 pooled library, ten DNA fragments were separately PCR-amplified using each of primers AC7-AC16 in combination with primer EAB-749. For the generation of the L3 full library, primers AC17 and EAB-749 were used. Plasmid pMS22 was used as a template. Amplification conditions included an initial 5-min 94 °C incubation step followed by 25 cycles, each consisting of a 1-min 94 °C denaturation, a 1-min 55 °C annealing, and a 36-sec 72 °C elongation step, followed by a final 10-min 72 °C incubation step. This resulted in about 580 bp long fragments which were purified on an agarose gel. For the overlap extension PCR, a second fragment was amplified using either primer EAB-750 or EAB-751 in combination with EAB-752. While primer EAB-750 had an overlapping sequence with randomization primers AC7-11, EAB-751 shared sequence homologies with randomization primers AC12-17. Amplification conditions included an initial 5-min 94 °C incubation step followed by 25 cycles, each consisting of a 1-min 94 °C denaturation, a 1-min 55 °C annealing, and a 12-sec 72 °C elongation step, followed by a final 10-min 72 °C incubation step. The resulting fragments were about 180bp long. For the assembly of both fragments, equimolar amounts of fragment 1 and the corresponding fragment 2 were used. Amplification conditions included an initial 5-min 94 °C incubation step followed by 5 cycles without primers, each cycle consisting of a 1-min 94 °C denaturation, a 1-min 55 °C annealing, and a 60-sec 72 °C elongation step. After the addition of the outer primers EAB-749 and EAB-752, 20 additional cycles were performed using the same parameters. At the end, a final 10-min 72 °C incubation step was performed. The gel-purified fragments were then ligated into pMS22 after *Sac*I/*Nhe*I-digestion and purified ligations were transformed into TG1 bacteria by electropration..

TABLE 8

Primer	SEQ ID NO:	H3 Libraries--Primer Nucleotide Sequence
AC7	135	CCAGTAGTCCATAGCCTCCACGTAATCATAGAAGTC**MNN**TCT CGCACAGTAATACACGGCAGTG
AC8	136	CCAGTAGTCCATAGCCTCCACGTAATCATAGAA**MNN**CCATCT CGCACAGTAATACACGGCAGTG
AC9	137	CCAGTAGTCCATAGCCTCCACGTAATCATA**MNN**GTCCCATCT CGCACAGTAATACACGGCAGTG
AC10	138	CCAGTAGTCCATAGCCTCCACGTAATC**MNN**GAAGTCCCATCT CGCACAGTAATACACGGCAGTG
AC11	139	CCAGTAGTCCATAGCCTCCACGTA**MNN**ATAGAAGTCCCATCT CGCACAGTAATACACGGCAGTG
AC12	140	CGTGGTCCCTTGGCCCCAGTAGTCCATAGCCTCCAC**MNN**ATC ATAGAAGTCCCATCTCGCACAG
AC13	141	CGTGGTCCCTTGGCCCCAGTAGTCCATAGCCTC**MNN**GTAATC ATAGAAGTCCCATCTCGCACAG
AC14	142	CGTGGTCCCTTGGCCCCAGTAGTCCATAGC**MNN**CACGTAATC ATAGAAGTCCCATCTCGCACAG

AC15	143	CGTGGTCCCTTGGCCCCAGTAGTCCATMNNCTCCACGTAATC ATAGAAGTCCCATCTCGCACAG
AC16	144	CGTGGTCCCTTGGCCCCAGTAGTCMNNAGCCTCCACGTAATC ATAGAAGTCCCATCTCGCACAG
AC17	145	CGTGGTCCCTTGGCCCCAGTAGTC*C*AT*A*GC*C*TC*C*AC*G*TA*A*TC*A* TA*G*AA*G*TC*C*CATCTCGCACAGTAATACACGGCAG
EAB-749	146	CCATCAGGGCCTGAGCTCGCCCGTC
EAB-750	147	CGTGGAGGCTATGGACTACTGGGGCCAAGG
EAB-751	148	GACTACTGGGGCCAAGGGACCACGGTCAC
EAB-752	149	GGTCAGGGCGCCTGAGTTCCACG

Randomization of primer AC17:
Bolded and Italic: 60% original base and 40% randomization as M
Underlined: 60% original base and 40% randomization as N

L3 libraries

[0167] For the generation of affinity maturation libraries randomized in the CDR3 region of the light chain, triplets encoding positions Y91, Y92, T93, Y94, and L95a were either randomized throughout the segment (L3 full library) or individually resulting in five sublibraries. Sublibraries containing clones with individually randomized positions were pooled after transformation into bacteria (L3 pooled library). For the generation of the five sublibraries, five DNA fragments were PCR-amplified using each of primers AC1-AC5 in combination with primer MS43. For the generation of the L3 full library, primer combination AC6 and MS43 were used (Fig. 14). Plasmid pMS22 was used as a template. Amplification conditions included an initial 5-min 94 °C incubation step followed by 25 cycles, each consisting of a 1-min 94 °C denaturation, a 1-min 55 °C annealing, and a 25-sec 72 °C elongation step, followed by a final 10-min 72 °C incubation step. The resulting fragments which encompass positions 1-104 of the VL domain were purified on an agarose gel and used as a template for an additional PCR amplification. All reactions were performed with primer EAB-746 which has an overlapping sequence with the randomization primers and MS43 using the same conditions described above. The purified fragments as well as pMS22 were digested with NcoI/XhoI. For all five sublibraries, 0.5 µg insert were ligated with 0.5 µg pAC16. For the L3 full library, ligation was performed with 9.8 µg insert and 9.8 µg pMS22. Purified ligations were transformed into TG1 bacteria by electroporation.

TABLE 9

Primer	**SEQ ID NO:**	**L3 libraries--Primer Nucleotide Sequence**
AC1	150	GGTGCCCTGGCCAAACGTGAATAGAGGATAGGTGTA **MNN**TTGGTGACAGTAGTAAGTTGC
AC2	151	GGTGCCCTGGCCAAACGTGAATAGAGGATAGGT**MNN** ATATTGGTGACAGTAGTAAGTTGC
AC3	152	GGTGCCCTGGCCAAACGTGAATAGAGGATA**MNN**GTA ATATTGGTGACAGTAGTAAGTTGC

AC4	153	GGTGCCCTGGCCAAACGTGAATAGAGG**MNN**GGTGTA ATATTGGTGACAGTAGTAAGTTGC
AC5	154	GGTGCCCTGGCCAAACGTGAA**MNN**AGGATAGGTGTA ATATTGGTGACAGTAGTAAGTTGC
AC6	155	GGTGCCCTGGCCAAACGTGAA**T**AGAGG**A**TA**G**GT**G**TA ATATTGGTGACAGTAGTAAGTTGC
EAB-746	156	CGCTTGATCTCGAGCTTGGTGCCCTGGCCAAACGTG
MS-43	123	CCAGCCGGCCATGGCCGATATCCAGATGACCCAGTCT CCATC

Randomization of primer AC6:
Bolded and Italic: 60% original base and 40% randomization as M
Underlined: 60% original base and 40% randomization as N

Generation of the antigens

[0168] Because both murine and humanized PR1A3 antibodies recognize only membrane bound but not shed soluble human CEA, a recombinant chimeric protein which contains the epitope of PR1A3 was generated for in vitro affinity maturation of humanized PR1A3 (SEQ ID NO:7 and 8). Generation of this hybrid protein was performed as described in Steward et al., 1999. In brief, DNA sequence of the B domain of human biliary glycoprotein (BGP) was replaced with the sequence of the human CEA-B3 domain, which contains the epitope of PR1A3. As a result, the sequence encodes a hybrid protein which comprises the N and A1 domains of BGP, the B3 domain of CEA and the A2 domain of BGP (N-A1-B3-A2, huNABA). This fusion product was then either linked to the Fc portion of human IgG1 (huNABA-Fc) (Steward et al., *Cancer Immunol Immunother,* 47:299-306, 1999) or fused to a sequence encoding the precision protease cleavage site, an avi tag and a (His)6 tag (huNABA-avi-his) (SEQ ID NO:158). huNABA-Fc was purified from the supernatant of a stably transfected CHO cell line using a protein A column. huNABA-avi-his was transiently transfected into HEK 293 cells, stably expressing the EBV-derived protein EBNA. A simultaneously co-transfected plasmid encoding a biotin ligase allowed avi tag-specific biotinlylation in vivo. The protein was then purified by immobilized metal affinity chromatography (IMAC) followed by gel filtration.

SEQ ID NO:158 (huNABA-avi-his) pETR6592

QLTTESMPFNVAEGKEVLLLVHNLPQQLFGYSWYKGERVDGNRQI
VGYAIGTQQATPGPANSGRETIYPNASLLIQNVTQNDTGFYTLQVIK
SDLVNEEATGQFHVYPELPKPSISSNNSNPVEDKDAMAFTCEPETQD
TTYLWWINNQSLPVSPRLQLSNGNRTLTLLSVTRNDTGPYECEIQNP
VSANRSDPVTLNVTYGPDTPTISPPDSSYLSGANLNLSCHSASNPSPQ
YSWRINGIPQQHTQVLFIAKITPNNNGTYACFVSNLATGRNNSIVKSI
TVSALSPVVAKPQIKASKTTVTGDKDSVNLTCSTNDTGISIRWFFKN
QSLPSSERMKLSQGNITLSINPVKREDAGTYWCEVFNPISKNQSDPIM
LNVNYNALPQENLINVDLEVLFQGPGSGLNDIFEAQKIEWHEARAH
HHHHH

Affinity maturation of humanized PR1A3

[0169] Generation of affinity-matured humanized PR1A3 Fabs was carried out by phage display using standard protocols (Silacci *et al*, *Proteomics*, 5(9):2340-2350, 2005). Selections with all affinity maturation libraries were carried out in solution according to the following procedure: 1. binding of ~ 1012 phagemid particles of each affinity maturation libraries to 100nM biotinylated huNABA-avi-his for 0.5 h in a total volume of 1ml, 2. capture of biotinylated huNABA-avi-his and specifically bound phage particles by addition of 5.4×10^7 streptavidin-coated magnetic beads for 10 min, 3. washing of beads using 5-10x 1ml PBS/Tween20 and 5-10x 1ml PBS, 4. elution of phage particles by addition of 1ml 100mM TEA (triethylamine) for 10 min and neutralization by adding 500ul 1M Tris/HCl pH 7.4 and 5. re-infection of exponentially growing *E. coli* TG1 bacteria, infection with helper phage VCSM13 and subsequent PEG/NaCl precipitation of phagemid particles to be used in subsequent selection rounds. Selections were carried out over 3-5 rounds using either constant or decreasing (from 10^{-7}M to $2x10^{-9}$M) antigen concentrations. In round 2, capture of antigen: phage complexes was performed using neutravidin plates instead of streptavidin beads. Specific binders were identified by ELISA as follows: 100 ul of 10nM biotinylated huNABA-avi-his per well were coated on neutravidin plates. Fab-containing bacterial supernatants were added and binding Fabs were detected via their Flag-tags by using an anti-Flag/HRP secondary antibody. ELISA-positive clones were bacterially expressed as soluble Fab fragments in 96-well format and supernatants were subjected to a kinetic screening experiment by SPR-analysis using BIACORE T100. Clones expressing Fabs with the highest affinity constants were identified and the corresponding phagemids were sequenced.

Purification of Fabs and measurement of the kinetic parameters

[0170] For the exact analysis of the kinetic parameters, Fabs were purified from bacterial cultures. A 500 ml culture was inoculated and induced with 1mM IPTG at an OD600 0.9. Bacteria were incubated at 25 °C overnight and harvested by centrifugation. After the incubation of the resuspended pellet for 20 min in 25 ml PPB buffer (30 mM Tris-HCl pH8, 1mM EDTA, 20% sucrose), bacteria were centrifuged again and the supernatant was harvested. This incubation step was repeated once with 25 ml of a 5 mM $MgSO_4$ solution. The supernatants of both incubation steps were pooled, filtered and loaded on a IMAC column (His gravitrap, GE Healthcare). Subsequently, the column was washed with 40 volumes. After the elution (500 mM NaCl, 500 mM Imidazole, 20 mM NaH_2PO_4 pH 7.4) the eluate was re-buffered using PD10 columns (GE Healthcare). The kinetic parameters of the purified Fabs were then studied by SPR-analysis in a dilution row that ranged from 200 nM to 6.25 nM.

EXAMPLE 2

[0171] The PR1A3 antibody was chimerized to have a human IgG1/kappa constant region, and expressed using the GylcoMab technology in order to have a high degree of afucosylated sugars in the Fc. The glycoengineered and non-glycoengineered antibodies were compared at a effector to target ratio of 25:1. The maximal amount of antibody dependent target cell killing was doubled by glycoengineering of the Fc region (Figure 2). A further increase in cell killing was achieved by increasing the effector to target ratio (Figure 2).

[0172] PR1A3 was humanized using frameworks identical to human germline sequences. The IMGT sequence IGHV7-4-1*02 (Accession No. X62110) was the acceptor for VH humanized and IMGT_hVK_1_39 (Accession No. X59315) was the acceptor for VL humanization. A humanized PR1A3 antibody comprising a heavy chain variable region construct CH7A and a light chain variable region construct CL1A showed satisfactory binding to human colon carcinoma cells as measured by flow cytometry (Figure 3).

[0173] Affinity maturation of PR1A3 by phage display was performed using standard protocols as described in detail in Example 1, herein. The parent humanized PR1A3 antibody that was used for affinity maturation comprises a heavy chain variable region construct CH7A and a light chain variable region construct CL1A. Tables 3-6 below show the libraries used for affinity maturation. For the L1/L2 library, positions Valine 29, Alanine50, or Serine51 within the CDRs were kept constant. For the H1/H2 library, positions Isoleucine51, Glycine55, or Alanine57 within the CDRs were kept constant (Figures 4 and 5).

[0174] An affinity matured heavy chain variable region construct, CH7A rF9, and an affinity matured light chain variable region construct, CL1A rH11, were paired with the parent light chain variable region construct and the heavy chain variable region construct, respectively, and with each other. All antibodies were converted into human IgG1/kappa and binding to the CEA-positive cell-line MKN45 was measured by flow cytometry. Antibodies comprising either one affinity matured heavy or light chain variable regions or both affinity matured heavy or light chain variable regions showed improved binding characteristic as compared to the humanized parent antibody (Figure 6). Figures 6, 10 and 15 show several examples where the matured light and heavy chains independently contribute to increased affinity. The parental antibody CH7A CL1A has the lowest signal intensity, as well as the highest EC50 value in Figures 6 and 15. The matured light chain shifts the EC50 values to lower numbers, whereas the matured heavy chains (rF9 in Figure 6, and rB9 in Figure 15) shift the total fluorescence signal intensity in a flow-cytometry measurement. Figure 10 shows the individual contributions of heavy and light chain measured by Biacore methodology. The combination of these two chains increases the affinity even further.

[0175] The binding affinities of the affinity matured heavy and light chain CDRs were determined by Biacore and listed in Table 10 below.

TABLE 10

SEQ ID NO	CDR-H3 (randomized residues are underlined selected residues in bold)	Affinity (determined by Biacore)	Construct
25	W D F Y D Y V E A M D Y	3681 nM	PMS22
26	W D F Y **H** Y V E A M D Y	586 nM	1C8
27	W D F **V** D Y V E A M D Y	1893 nM	3E1
28	W D F Y **W** Y V E A M D Y	746 nM	2D7
33	W D F **A H** Y **F Q T** M D	59 nM	Affinity Matured CDRH-3
34	W D F **A Y** Y **F Q T** M D	44 nM	Affinity Matured CDRH-3
35	W D F **A Y** Y **L** E A M D	69 nM	Affinity Matured CDRH-3
29	W D**A F E** Y V **K** A **L** D Y	26 nM	H3 Full (5) 19
30	W D F **F E** Y **F K T** M D Y	51 nM	H3 Full (5) 8
31	W D F **F Y** Y V **Q T** M D Y	81 nM	H3 Full (5) 28
33	W D F **S Y** Y V E A M D Y	132 nM	H3 Full (5) 27
	CDR-H1 and CDR-H2 randomized residues are underlined selected residues in bold		
1 and 13	EFGMN and WINTKTGEATYVEEFKG	3681 nM	pMS22
1 and 14	EFGMN and WINTKTGEATY**I**EEFKG	402 nM	H4E9
1 and 15	EFGMN and WINTK**S**GEATYVEEFKG		pAC14 (B9)
2 and 15	E**Y**GMN and WINTK**S**GEATYVEEFKG		pAC15 (F9)
3 and 16	E**YS**MN and **Y**INTK**N**GEA**N**YVEEFKG		H1/H2 (5) 2
2 and 17	E**Y**GMN and WINTK**N**GEATY**I**EEFKG		H1/H2 (5) 11
1 and 16	E**F**GMN and **Y**INTK**N**GEA**N**YVEEFKG		H1/H2 (5) 13
2 and 16	E**Y**GMN and **Y**INTK**N**GEA**N**YVEEFKG		H1/H2 (5) 14
5 and 13	EFGM**S** and WINTKTGEATYVEEFKG	26 nM	H3 Full (5) 19
	CDR-L1 and CDR-L2 randomized residues are underlined selected residues in bold		
36 and 46	QNVGTN and YSASYRYS	3681 nM	pMS22

37 and 47	**A**NVG**N**N and Y**L**ASNLSG	250 nM	pAC21 (3A1)
38 and 48	**K**NVGTN and Y**L**ASY**PQI**	700 nM	pAC19 (2C6)
39 and 49	**AA**VGT**Y** and YSASYR**KR**	220 nM	pAC18 (2F1)
40 and 50	Q**YAS**TN and Y**W**ASYRYS	290 nM	pAC23 (2F11)
36 and __	QNVGTN and **P**LI-YSASYRYS	402 nM	H4E9
41 and 51	**H**NVGTN and YSAS**H**RYS	2255 nM	L2D2
42 and 52	Q**IMGP**N and YLASY**HE**S		pAC6 (C1)
43 and 53	Q**I**VGTN and YSAS**H**R**P**S		pAC7 (E10)
44 and 54	Q**K**V**L**TN and Y**L**ASYRYS		pAC12 (H7)
45 and 55	QT**VSA**N and Y**L**ASYRY**R**		pAC13 (H11)
	CDR-L3 randomized residues are underlined selected residues in bold		
56	H Q Y Y T Y P L F T		pMS22

[0176] Table 11, below, summarizes the affinity constants of the various affinity matured antibody sequences. The parental antibody PR1A3 is listed as well as several light chain and heavy chain combinations of matured and non matured sequences. All values were obtained by Biacore technology by measuring the association (k_{on}) and dissociation (k_{off}) rate constants of the various soluble antibody constructs in Fab format on a Biacore chip with immobilized NABA-avi-his reagent (SEQ ID NO 158) as the antigen. The affinity constant is labeled with KD.

Table 11: Kinetic Analysis of Affinity Matured Clones

CLONE NAME	CHAIN	MONOVALENT AFFINITY	BIVALENT AFFINITY
PR1A3	wt/wt	k_{on}: $6.74x10^{3}$ 1/Ms; k_{off}: $2.48x10^{-2}$ 1/s; KD $3681x10^{-9}$M	k_{on}: $2.82x10^{5}$ 1/Ms; k_{off}: $5.52x10^{-4}$ 1/s; KD: $2x10^{-9}$M
1C8	hc/wt	k_{on}: $12.9x10^{3}$ 1/Ms; k_{off}: $0.76x10^{-2}$ 1/s; KD $586x10^{-9}$M	k_{on}: $4.67x10^{5}$ 1/Ms; k_{off}: $3.24x10^{-4}$ 1/s; KD: $0.693x10^{-9}$M
H4E9	hc/wt	k_{on}: $5.22x10^{3}$ 1/Ms; k_{off}: $0.21x10^{-2}$ 1/s; KD $402x10^{-9}$M	k_{on}: $2.92x10^{5}$ 1/Ms; k_{off}: $2.04x10^{-3}$ 1/s; KD: $0.7x10^{-9}$M
H3 Full (5) 19	hc/wt	k_{on}: $54.2x10^{3}$ 1/Ms; k_{off}: $0.13x10^{-2}$ 1/s; KD $24x10^{-9}$M	k_{on}: $9.02x10^{5}$ 1/Ms; k_{off}: $1.75x10^{-4}$ 1/s; KD: $0.19x10^{-9}$M
H3 Full (5) 8	hc/wt	k_{on}: $27.3x10^{3}$ 1/Ms; k_{off}: $0.14x10^{-2}$ 1/s; KD $51x10^{-9}$M	N/D

3A1	wt/ lc	k_{on}: $46.8x10^{3}$ 1/Ms; k_{off}: $1.17x10^{-2}$ 1/s; KD $250x10^{-9}$M	k_{on}: $2.42x10^{5}$ 1/Ms; k_{off}: $3.64x10^{-4}$ 1/s; KD: $1.5x10^{-9}$M
2F1	wt/ lc	k_{on}: $95.7x10^{3}$ 1/Ms; k_{off}: $2.07x10^{-2}$ 1/s; KD $220x10^{-9}$M	k_{on}: $4.23x10^{5}$ 1/Ms; k_{off}: $4.10x10^{-4}$ 1/s; KD: $0.952x10^{-9}$M
5L1A10	hc/wt	k_{on}: $15.6x10^{3}$ 1/Ms; k_{off}: $0.09x10^{-2}$ 1/s; KD $59x10^{-9}$M	N/D
5HFF12	hc/wt	k_{on}: $20.8x10^{3}$ 1/Ms; k_{off}: $0.09x10^{-2}$ 1/s; KD $44x10^{-9}$M	N/D
M4F1	hc/wt	k_{on}: $25.7x10^{3}$ 1/Ms; k_{off}: $0.17x10^{-2}$ 1/s; KD 69x10-9M	N/D
H4E9 x 2F1	hc/lc	k_{on}: $36.4x10^{3}$ 1/Ms; k_{off}: $0.35x10^{-2}$ 1/s; KD $96x10^{-9}$M	k_{on}: $4.23x10^{5}$ 1/Ms; k_{off}: $1.91x10^{-4}$ 1/s; KD: $0.452x10^{-9}$M
H4E9 x 3A1	hc/lc	N/D	k_{on}: $2.46x10^{5}$ 1/Ms; k_{off}: $1.36x10^{-4}$ 1/s; KD: $0.55x10^{-9}$M
1C8 x 2F1	hc/lc	k_{on}: $68.1x10^{3}$ 1/Ms; k_{off}: $0.87x10^{-2}$ 1/s; KD $128x10^{-9}$M	k_{on}: $9.68x10^{5}$ 1/Ms; k_{off}: $6.36x10^{-4}$ 1/s; KD: $0.66x10^{-9}$M
1C8 x 3A1	hc/lc	N/D	k_{on}: $2.89x10^{5}$ 1/Ms; k_{off}: $2.57x10^{-4}$ 1/s; KD: $0.888x10^{-9}$M
H3 Full (5) 19 x 2F1	hc/lc	k_{on}: $206x10^{3}$ 1/Ms; k_{off}: 0.25x10-2 1/s; KD: $12.2x10^{-9}$M	k_{on}: $1.76x10^{6}$ 1/Ms; k_{off}: $2.84x10^{-4}$ 1/s; KD: $0.16x10^{-9}$M
H3 Full (5) 8 x 2F1	hc/lc	N/D	k_{on}: $9.93x10^{5}$; 1/Ms; k_{off}: $2.71x10^{-4}$ 1/s; KD: $0.28x10^{-9}$M

What Is Claimed Is:

1. An antigen binding molecule (ABM) comprising a humanized, affinity-matured antigen binding domain comprising one or more complementarity determining regions (CDRs), wherein said antigen binding domain specifically binds membrane-bound human carcinoembryonic antigen (CEA), and wherein said antigen binding domain binds the same epitope as, or is capable of competing for binding with the murine monoclonal antibody PR1A3.

2. The ABM of claim 1, wherein said ABM has at least about 2-fold to about 10-fold greater affinity for CEA than the parent ABM that has not been affinity-matured.

3. The ABM of claim 1 or claim 2, wherein said ABM binds to human CEA with a K_D value lower than about 1 μM to about 0.001 nM.

4. The ABM claim 3, wherein said affinity-matured antigen binding molecule binds with a K_D value lower than about 100 nM.

5. The ABM of any one of claims 1-4, wherein said ABM has no substantial cross-reactivity against soluble CEA.

6. The ABM of any one of claims 1-5, comprising at least one heavy or light chain CDR 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:8, SEQ ID NO:10, SEQ ID NO:12, 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, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, 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, SEQ ID NO:45, SEQ ID NO:46, 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, SEQ ID NO:55, and SEQ ID NO:56, or a combination thereof.

7. The ABM of claim 6, wherein said at least one CDR is a heavy chain CDR.

8. The ABM of any one of claims 1-7, wherein said antigen binding domain 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, and

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:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35.

9. The ABM of any one of claims 1-8, wherein said antigen binding domain 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.

10. The ABM of claim 9, wherein said heavy chain variable region comprises:

SEQ ID NO:9 ,

SEQ ID NO:15, and

SEQ ID NO:25; and

wherein said light chain variable region comprises:

SEQ ID NO:45,

SEQ ID NO:55, and

SEQ ID NO:56.

11. The ABM of any one of claims 1-5, wherein said antigen binding domain comprises a heavy chain variable region comprising the sequence of:

QVQLVQSGSELKKPGASVKVSCKASGYTFTEX^{1}X^{2}MX^{3}WVRQAPGQ
GLEWMGX^{4}INTKX^{5}GEAX^{6}YX^{7}EEFKGRFVFSLDTSVSTAYLQISSLK
AEDTAVYYCARWDX^{8}X^{9}X^{10}YX^{11}X^{12}X^{13}X^{14}DYWGQGTTVTVSS

wherein X^1 is Y or F; X^2 is S or G; X^3 is N or S; X^4 is W or Y; X^5 is N, T or S; X^6 is T or N; X^7 is V or I; X^8 is F or A; X^9 is Y, A, V, F or S; X^{10} is D, H, W, E, or Y; X^{11} is V, L or F; X^{12} is E, K or Q; X^{13} is A or T; and X^{14} is M or L.

12. The ABM of claim 11, wherein said heavy chain variable region comprises the sequence of SEQ ID NO 107.

13. The ABM of any one of claims 1-5, wherein said antigen binding domain comprises a light chain variable region comprising the sequence of:

DIQMTQSPSSLSASVGDRVTITCKASX^{15}X^{16}X^{17}X^{18}X^{19}X^{20}VAWYQQKP
GKAPKX^{21}LIYX^{22}ASX^{23}X^{24}X^{25}X^{26}GVPSRFSGSGSGTDFTLTISSLQPED
FATYYCHQYYTYPLFTFGQGTKLEIK

wherein X^{15} is Q, A, K, or H; X^{16} is N, A, Y, I, K, T, or F; X^{17} is V, A, G, or M; X^{18} is G, S, T, or L; X^{19} is T, N, P, or A; X^{20} is N or Y; X^{21} is P or L; X^{22} is S, L, or W; X^{23} is Y, N, or H; X^{24} is R, L, P, or H; X^{25} is Y, S, Q, K, E, F, or P; and X^{26} is S, G, I, or R.

14. The ABM of claim 13, wherein said light chain variable region comprises the sequence of SEQ ID NO 108.

15. The ABM of any one of claims 1-14, wherein said antigen binding domain comprises a heavy chain variable region comprising the sequence of SEQ ID NO:107, and a light chain variable region comprising the sequence of SEQ ID NO:108.

16. The ABM of any one of claims 1-15, wherein said ABM comprises at least one framework region derived from a human germline immunoglobulin gene.

17. The ABM of claim 16, wherein said ABM comprises heavy chain framework regions FR1-3 of SEQ ID NO:102 and light chain framework regions FR1-3 of SEQ ID NO:106.

18. A variant antigen binding molecule (ABM) comprising an antigen binding domain comprising one or more complementarity determining regions (CDRs), wherein said antigen binding domain specifically binds membrane-bound human carcinoembryonic antigen (CEA), wherein said variant ABM comprises at least one amino acid substitution in at least one CDR that contributes to increased binding affinity of the variant ABM compared to a parent ABM, wherein the parent ABM comprises at least one CDR of the murine PR1A3 antibody, wherein said variant ABM binds the same epitope as, or is capable of competing with the PR1A3 antibody for binding to membrane-

bound CEA, and wherein said variant ABM does not comprise the sequence of any one of SEQ ID NOs:1-4.

19. The variant ABM of claim 18, wherein said variant ABM has no substantial cross-reactivity to soluble CEA.

20. The variant ABM of claim 18 or claim 19, wherein said variant ABM has at least about 2-fold to about 10-fold greater binding affinity for CEA than said parent ABM.

21. The variant ABM of any one of claims 18-20, wherein said variant ABM binds human CEA with a binding affinity of K_D lower than about 1μM to about 0.001 nM.

22. The variant ABM of any one of claims 18-20, wherein said variant ABM has a K_D lower than about 100 nM.

23. The variant ABM of any one of claims 18-22, wherein said parent ABM comprises all six CDRs of the murine PR1A3 antibody, and wherein said variant ABM comprises one or more amino acid substitutions in said at least two CDRs.

24. The variant ABM of any one of claims 18-23, wherein said variant ABM comprises at least one CDR 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:8, SEQ ID NO:10, SEQ ID NO:12, 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, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, 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, SEQ ID NO:45, SEQ ID NO:46, 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, SEQ ID NO:55, and SEQ ID NO:56, or a combination thereof.

25. The variant ABM of claim 24, wherein said at least one CDR is a heavy chain CDR.

26. The variant ABM of claim 24 or claim 25, wherein said antigen binding domain 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, and

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:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35.

27. The variant ABM of any one of claims 24-26, wherein said antigen binding domain comprises a heavy chain variable region comprising the sequence of SEQ ID NO:107.

28. The variant ABM of any one of claims 18-27, wherein said antigen binding domain 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.

29. The variant ABM of claim 28, wherein said antigen binding domain comprises a light chain variable region comprising the sequence of SEQ ID NO:108.

30. The variant ABM of any one of claims 24-29, wherein said antigen binding domain comprises:

SEQ ID NO:9,

SEQ ID NO:15, and

SEQ ID NO:25; and

wherein said light chain variable region comprises:

SEQ ID NO:45,

SEQ ID NO:55, and

SEQ ID NO:56.

31. The variant ABM of claim 30, wherein said antigen binding domain comprises a heavy chain variable region comprising the sequence of SEQ ID NO:107, and a light chain variable region comprising the sequence of SEQ ID NO:108.

32. The variant ABM of any one of claims 18-24, wherein said variant ABM comprises at least one framework region derived from a human germline immunoglobulin gene.

33. The variant ABM of claim 32, wherein said variant ABM comprises heavy chain framework regions FR1-3 of SEQ ID NO:102 and light chain framework regions FR1-3 derived from SEQ ID NO:106.

34. The ABM of any one of claims 1-17 or the variant ABM of any one of claims 18-33, wherein said ABM or said variant ABM comprises an Fc region.

35. The ABM or the variant ABM of claim 34, wherein said Fc region is a human IgG Fc region.

36. The ABM or the variant ABM of claim 34 or claim 35, wherein Fc region is a glycoengineered Fc region.

37. The ABM or the variant ABM of claim 36, wherein at least about 20% to about 100% of the N-linked oligosaccharides in said Fc region are nonfucosylated.

38. The ABM or the variant ABM of claim 36, wherein at least about 20% to about 100% of the N-linked oligosaccharides in said Fc region are bisected.

39. The ABM or the variant ABM of claim 36, wherein at least about 20% to about 50% of the N-linked oligosaccharides in said Fc region are bisected, nonfucosylated.

40. The ABM or the variant ABM of any one of claims 36-39, wherein said ABM or the variant ABM has at least one increased effector function.

41. The ABM or the variant ABM of claim 40, wherein said at least one increased effector function is selected from the group consisting of: 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.

42. The ABM or the variant ABM of claim 41, wherein said increased effector function is increased ADCC or increased binding to NK cells.

43. The ABM or the variant ABM of any one of claims 34-42, wherein said ABM or variant ABM has an increase in ADCC of at least about 40% to about 100% as compared to said parent antigen binding molecule.

44. The ABM of any one of claims 1-17, or the variant ABM of any one of claims 18-33, wherein said ABM or said variant ABM is an antibody or fragment thereof selected from the group consisting of: a whole antibody, an scFv fragment, an Fv fragment, an F(ab')2 fragment, a minibody, a diabody, a triabody, and a tetrabody.

45. The ABM of any one of claims 1-17, or 34-44, or the variant ABM of any one of claims 18-44, wherein said ABM or said variant ABM increases maximum survival time in an animal model of disease by at least from about 10 to about 120 days compared to said parent ABM.

46. The ABM or variant ABM of claim 45, wherein said animal model of disease is a mouse tumor xenograft model using LS174T human colorectal carcinoma cells.

47. The ABM of any one of claims 1-17, or 34-46, or the variant ABM of any one of claims 18-43, wherein said ABM or said variant ABM increases ADCC by at least about 10% to about 100% in an *in vitro* cytotoxicity assay.

48. The ABM of any one of claims 1-17, or 34-47, or the variant ABM of any one of claims 18-43, wherein said ABM or said variant ABM is at least from about 10-fold to about 1000-fold more potent at inducing ADCC at a given concentration compared to the murine PR1A3 antibody.

49. The ABM or variant ABM of claim 48, wherein ADCC is measured by *an in vitro* cytotoxicity assay.

50. An isolated polypeptide comprising an antigen binding domain that specifically binds membrane-bound CEA, wherein said antigen binding domain comprises one or more CDR sequences

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, SEQ ID NO:12, 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, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, 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, SEQ ID NO:45, SEQ ID NO:46, 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, SEQ ID NO:55, and SEQ ID NO:56, or a combination thereof.

51. An isolated polypeptide comprising a first sequence and a second sequence, wherein said first sequence comprises one or more CDR sequences 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, SEQ ID NO:12, 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, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35; and

wherein said second sequence comprises one or more CDR sequences 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, SEQ ID NO:45, SEQ ID NO:46, 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, SEQ ID NO:55, and SEQ ID NO:56; and wherein said polypeptide specifically binds membrane-bound CEA.

52. An isolated polypeptide comprising a sequence that is at least about 80% to about 100% identical to a sequence selected from the group consisting of: SEQ ID NOs:101, 105, 107, 108, and 188-216, wherein said polypeptide specifically to binds membrane-bound CEA.

53. A humanized antigen binding molecule specific for membrane-bound CEA comprises the polypeptide of any one of claims 45-52.

54. The humanized antigen binding molecule of claim 53, wherein said humanized antigen binding molecule has no substantial cross-reactivity to soluble CEA.

55. An antibody that specifically binds to membrane-bound CEA, said antibody comprising the heavy chain variable region of SEQ ID NO:107 and the light chain variable region of SEQ ID NO:108, wherein said antibody has increased ADCC compared to the murine PR1A3 antibody.

56. The antibody of claim 55, wherein said antibody comprises an Fc region that has been glycoengineered.

57. The antibody of claim 55 or 56, wherein said antibody has no substantial cross-reactivity to soluble CEA.

58. The antibody of any one of claims 55-57, wherein said antibody increases maximum survival time in an animal model of disease by at least from about 10 to about 120 days compared to the murine PR1A3 antibody.

59. The antibody of any one of claims 55-58, wherein said antibody increases ADCC by at least about 10% to about 100% in an *in vitro* cytotoxicity assay.

60. An isolated polynucleotide encoding the polypeptide of any one of claims 45-52.

61. A composition comprising a first isolated polynucleotide sequence and a second isolated polynucleotide sequence, wherein said first isolated polynucleotide sequence is at least about 80% to about 100% identical to a sequence selected from the group consisting of: SEQ ID NOs:113, 119, and 159-177, and wherein said second isolated polynucleotide sequence is at least about 80% to about 100% identical to a sequence selected from the group consisting of SEQ ID NOs:117, 120, and 178-187, wherein said polynucleotides encode polypeptides that specifically to bind membrane-bound CEA.

62. The composition of claim 61, wherein said first isolated polynucleotide is SEQ ID NO: 119, and wherein said second isolated polynucleotide is SEQ ID NO:120.

63. The composition of claim 61, wherein said first isolated polynucleotide encodes the polypeptide of SEQ ID NO: 107, and wherein said second isolated polynucleotide encodes the polypeptide of SEQ ID NO:108.

64. An isolated polynucleotide encoding a polypeptide specific for membrane-bound CEA, wherein said polynucleotide comprises one or more heavy chain CDR-encoding sequences selected from the group consisting of SEQ ID NOs:57-77.

65. The isolated polynucleotide of claim 64, wherein said polynucleotide comprises a sequence that is at least about 80% to about 100% identical to SEQ ID NO:119.

66. An isolated polynucleotide encoding a polypeptide specific for membrane-bound CEA, wherein said polynucleotide comprises one or more light chain CDR-encoding sequences selected from the group consisting of: SEQ ID NOs:78-98.

67. The isolated polynucleotide of claim 66, wherein said polynucleotide comprises a sequence that is at least about 80% to about 100% identical to SEQ ID NO:120.

68. A vector comprising the polynucleotide of any one of claims 60-67.

69. The vector of claim 68, wherein said vector is a cloning vector or an expression vector.

70. The vector of claim 68, wherein said vector is a vector suitable for making a phage display library.

71. A host cell comprising the vector of any one of claims 68-70.

72. A host cell comprising a first isolated polynucleotide and a second isolated polynucleotide, wherein said first isolated polynucleotide comprises a sequence selected from the group consisting of SEQ ID NOs:113, 119, and 159-177, and wherein said second isolated polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO:8 and SEQ ID NOs:117, 120, and 178-187.

73. A method of producing an ABM that specifically binds membrane-bound human CEA, said method comprising:

(a) culturing the host cell of claim 71 or claim 72 in a medium under conditions allowing the expression of said polynucleotide, wherein said polynucleotide encodes a polypeptide that forms part of said ABM; and

(b) recovering said ABM,

wherein said ABM or a portion thereof binds the same epitope as, or is capable of competing for binding with the murine monoclonal antibody PR1A3.

74. A composition comprising the ABM of any one of claims 1-17 or 34-49, the variant ABM of any one of claims 18-49, or the antibody of any one of claims 55-59 and a pharmaceutically acceptable carrier.

75. A method of inducing cell lysis of a tumor cell, said method comprising contacting said tumor cell with the ABM of any one of claims 1-17 or 34-49, the variant ABM of any one of claims 18-49, the antibody of any one of claims 55-59, or the composition of claim 74.

76. The method of claim 75, wherein said tumor cell is selected from the group consisting of colorectal cancer cells, NSCLC (non-small cell lung cancer), gastric cancer cells, pancreatic cancer cells and breast cancer cells.

77. The method of claim 75, wherein said cell lysis is induced by antibody dependent cell cytotoxicity of said ABM, variant ABM.

78. A method of diagnosing disease in a subject, said method comprising administering to said subject an effective amount a diagnostic agent, wherein said diagnostic agent comprises the ABM any one of claims 1-17 or 34-49, the variant ABM of any one of claims 18-49, or the antibody of any one of claims 55-59 and a label that allows detection of a complex of said diagnostic agent and CEA.

79. The method of claim 78, wherein said label is an imaging agent.

80. A method of treating a subject having a cancer that abnormally expresses CEA, said method comprising administering to said subject a therapeutically effective amount of the ABM of any one of claims 1-17 or 34-49, the variant ABM of any one of claims 18-49, the antibody of any one of claims 55-59, or the composition of claim 74.

81. 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 of any one of claims 1-17 or 34-49, the variant ABM of any one of claims 18-49, the antibody of any one of claims 55-59, or the composition of claim 74

82. The method of claim 80 or 81, wherein said cancer is selected from the group consisting of colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, pancreatic cancer and breast cancer.

83. A method of inducing in a subject regression of a tumor that abnormally expresses CEA, said method comprising administering to said subject a therapeutically effective amount of the ABM of any one of claims 1-17 or 34-49, the variant ABM of any one of claims 18-49, the antibody of any one of claims 55-59, or the composition of claim 74.

84. The method of claim 83, wherein said tumor is a cancer selected from the group consisting of colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, pancreatic cancer and breast cancer.

85. The method of any one of claims 80-84, wherein said ABM or said variant ABM is administered in combination with chemotherapy or radiation therapy.

86. The method of any one of claims 80-85, wherein said ABM or said variant ABM or said composition is administered with a chemotherapeutic agent.

87. The method of any one of claims 78-86, wherein said subject is a human.

88. A method of inhibiting CEA-mediated cell adhesion of a tumor cell that abnormally expresses CEA, said method comprising contacting said tumor cell with an effective amount of the ABM of any one of claims 1-17 or 34-49, the variant ABM of any one of claims 18-49, the antibody of any one of claims 55-59, or the composition of claim 74.

89. Use of the ABM of any one of claims 1-17 or 34-49, the variant ABM of any one of claims 18-49, the antibody of any one of claims 55-59, or the composition of claim 74 in the manufacture of a medicament for treating a subject having a cancer that abnormally expresses CEA.

90. The use of claim 89, wherein said cancer is selected from the group consisting of colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, pancreatic cancer and breast cancer.

91. The isolated polynucleotide of claim 64, wherein said polynucleotide comprises:

a) a sequence selected from the group consisting of: SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:61; and

b) a sequence selected from the group consisting of: SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66; and

c) a sequence selected from the group consisting of: SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77.

92. The isolated polynucleotide of claim 91, wherein said polynucleotide comprises:
SEQ ID NO:58;
SEQ ID NO:64; and
SEQ ID NO:67.

93. The isolated polynucleotide of claim 91, wherein said polynucleotide comprises the sequence of SEQ ID NO:119.

94. The isolated polynucleotide of claim 66, wherein said polynucleotide comprises:

a) a sequence selected from the group consisting of: SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87; and

b) a sequence selected from the group consisting of: SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97; and

c) a sequence of SEQ ID NO:98.

95. The isolated polynucleotide of claim 94, wherein said polynucleotide comprises:
SEQ ID NO:87;
SEQ ID NO:97; and
SEQ ID NO:98.

96. The isolated polynucleotide of claim 94, wherein said polynucleotide comprises the sequence of SEQ ID NO:120.

97. The invention as described hereinabove.